The Relationship Between Aberrant Neuronal Activation in the Pregenual Anterior Cingulate, Altered Glutamatergic Metabolism, and Anhedonia in Major Depression

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Context: Major depressive disorder (MDD) is characterized by diverse metabolic and functional abnormalities that occur in, among other regions, the pregenual anterior cingulate cortex (pgACC), a cortical region linked to anhedonia.

Objectives: To contextualize metabolic, functional, and clinical parameters and thus to reveal cellular mechanisms related to anhedonia.

Design: The pgACC was investigated using a combined functional magnetic resonance imaging and magnetic resonance spectroscopic approach. Negative blood oxygenation level–dependent (BOLD) activations in the pgACC were assessed during emotional stimulation. Quantitative J-resolved magnetic resonance spectroscopy in the pgACC enabled simultaneous determination of glutamine, glutamate, N-acetylaspartate, glucose, and γ-aminobutyric acid concentrations. Subjective emotional intensity ratings as well as various clinical parameters were determined.

Setting: The patients were recruited and evaluated in the Department of Psychiatry, University of Zurich, while the measurements were performed in the Institute of Biomedical Engineering, University of Zurich and the Technical University Zurich.

Participants: Nineteen unmedicated patients with MDD and 24 healthy subjects.

Main Outcome Measures: Reduced glutamine levels and lower functional responses in pgACC in anhedonic depressed patients were expected to be the predominant effect of abnormal glutamatergic transmission. It was further tested if, among patients, the ratings of emotional intensity on visual stimulation predicted the amount of metabolic and functional alterations in terms of reduced relative metabolite concentrations and BOLD changes.

Results: Patients with highly anhedonic MDD show decreased glutamine but normal glutamate and γ-aminobutyric acid concentrations, with glutamine concentrations being dissociated from glucose concentrations. Glutamate and N-acetylaspartate concentrations in pgACC correlate with negative BOLD responses induced by emotional stimulation in MDD; whereas in healthy subjects, negative BOLD responses correlate with γ-aminobutyric acid instead. Negative BOLD responses as well as glutamate and N-acetylaspartate concentrations correlate with emotional intensity ratings, an anhedonia surrogate, in those with MDD but not in healthy subjects.

Conclusion: Aberrant neuronal activation patterns of the pgACC in anhedonic depression are related to deficits of glutamatergic metabolism.

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lowing for glial glutamate (Glu) reuptake and its conversion to glutamine (Gln).\(^\text{18}\) Consequential altered metabolic processes are visible via the assessments of neuronal and glial markers by quantitative in vivo magnetic resonance spectroscopy (MRS). Specifically, there is converging evidence for reduced N-acetylaspartate (NAA) levels and total concentration of Glu and Gln (Glx) in the pgACC.\(^\text{19-21}\) while γ-amino butyric acid (GABA) levels remain unchanged.\(^\text{22}\) Glutamatergic dysfunction in MDD is further supported by pharmacological benefits of Glu-modulating substances.\(^\text{15,23}\)

Based on these findings, it might first be postulated that altered functional BOLD response in the pgACC is predicted by concomitant changes of NAA and Glu concentrations, indicating affected neuronal integrity as an excitotoxic result of reduced glial Glu uptake from the synaptic cleft. As a consequence, a regional hypometabolism can be hypothesized, which might be reflected by reduced amplitudes of NBRs in the pgACC.\(^\text{11,24}\) Second, reduced glial conversion of Glu to Gln in patients with MDD would result in the primary effect of decreased Gln, while global reductions of Glu might be partially masked by the same effect. However, farther downstream, effects for, eg, GABA, Glu, or glucose (Glc) had to be tested owing to alternative compensation mechanisms. Third, given the prevalent role of pgACC in processing hedonic values of external stimuli, both functional and metabolic markers are likely to predict reduced ratings of subjective emotional intensity in patients with MDD.

In contrast to previous studies,\(^\text{20,22}\) the use of 2-dimensional J-resolved MRS allowed for separate quantification of Glu and Gln along with simultaneous assessment of GABA, Glc, and NAA concentrations.\(^\text{25,26}\) This metabolic dimension had to be aligned with the assessment of functional responses on emotional stimuli using fMRI. Finally, metabolic and functional markers had to be related to clinical measures of the depressive state.

**METHODS**

**SUBJECTS**

Subjects with an acute MDD episode from the inpatient department of psychiatry at the University of Zurich were recruited if they scored 24 or higher on the 21-item Hamilton Depression Rating Scale\(^\text{27}\) and the 21-item Beck Depression Inventory (BDI)\(^\text{28}\) (separately).\(^\text{28}\) Exclusion criteria were major medical illness, history of seizures, head trauma with loss of consciousness, an abnormal clinical laboratory test result, and pregnancy. Specific psychiatric exclusion criteria consisted of atypical forms of depression, suicidal ideation, any additional psychiatric disorder, history of substance abuse or dependence, and electroconvulsive therapy in the previous 6 months. Healthy subjects without any psychiatric, neurologic, or medical illness were self-referred from online study advertisements. The study was approved by the University of Zurich’s institutional review board, and all subjects gave written informed consent before screening. All subjects were right-handed. All subjects underwent fMRI and MRS on subsequent days (at the same time) in random sequence. Groups consisted of 19 subjects with depression (mean age, 40.0 years; 8 males) and 24 control subjects (mean age, 34.6 years; 6 males). Groups did not differ significantly by age (t test, \(P = .07\) or sex (χ² test, \(P = .35\)). The mean Hamilton Depression Rating Scale score was 33.1 (standard deviation [SD], 7.1); the mean BDI score was 29.9 (SD, 4.9); and the mean anhedonia score was 17.31 (SD, 3.4) in the depression group. To measure anhedonia, we used a previously demonstrated 3-factor solution of the BDI\(^\text{29,30}\) with the factors anhedonia, somatic complaints, and negative self-focus. Anhedonia scores were then used for clinical subgroup analyses. Patients had been free of psychotropic medication for at least 1 week prior to scanning (mean, 9.1 days [SD, 7.9 days]); prior medications included only citalopram, paroxetine, and mirtazapine. The mean duration of the current depressive episode was 15.8 weeks (SD, 16.2 weeks), and the mean number of previous depressive episodes was 1.8 (SD, 2.2).

**fMRI STIMULATION PARADIGM**

The fMRI paradigm has been described elsewhere in full detail.\(^\text{9,11}\) The subjects were asked to view photographs from the International Affective Picture System\(^\text{13}\) with positive and negative valence. The International Affective Picture System photographs had to be passively watched (picture viewing) or, in the picture judgment conditions, judged according to their valence (positive or negative). In 50% of the trials, the pictures were preceded by an expectancy period of 8 to 11.5 seconds, which indicated the type of task associated with the picture that would be subsequently presented. After the presentation of each picture, a fixation cross was shown (6-8 seconds), which allowed the subjects to recover from the previous emotional stimulation; this period also served as a baseline condition to distinguish between positive and negative BOLD responses.\(^\text{24}\) A total of 158 trials were presented in 6 runs with 79 trials for the picture viewing conditions and 79 trials for the picture judgment conditions, resulting in a total scan time of 37 minutes. The different types of International Affective Picture System photographs and viewing/judgment tasks were pseudorandomized within and across the 6 runs. Prior to the experimental session, the subjects were familiarized with the paradigm by completing a test run of 10 trials.

Immediately after the fMRI session, a selection of pictures was presented a second time. Each picture was followed by a task period, which consisted of an emotional intensity rating, valence rating, and recognition test. The mean of the 3 ratings was calculated for each subject.

**fMRI DATA ACQUISITION AND ANALYSIS**

Measurements were performed on a Philips Intera 3-T whole-body magnetic resonance unit equipped with an 8-channel head coil. Functional time series were acquired with a sensitivity-encoded single-shot echo-planar sequence. The following acquisition parameters were used in the fMRI protocol: echo time = 35 milliseconds, field of view = 22 cm, acquisition matrix = 80 × 80, interpolated to 128 × 128, voxel size = 2.75 × 2.75 × 4 mm\(^3\), and sensitivity-encoded acceleration factor \(R = 2.0\). Using a midsagittal scout image, 32 contiguous axial slices were placed along the anterior-posterior commissure plane covering the entire brain with a repetition time of 3000 milliseconds (TR = 82°). The first 3 acquisitions were discarded owing to T1 saturation effects. A 3-dimensional T1-weighted anatomical scan was obtained for structural reference.

Data were analyzed using the SPM2 software package (Wellcome Trust Center for Neuroscience, London, England). Functional data were corrected for differences in slice acquisition time, realigned to the first volume, corrected for motion artifacts, mean-adjusted by proportional scaling, spatially normalized, and smoothed using a 8-mm full-width-at-half-maximum gaussian kernel. The time series were high pass-
filtered to eliminate low-frequency components (filter width, 128 seconds) and adjusted for systematic differences across trials. Statistical analysis was performed by modeling the different conditions convolved with a hemodynamic response function as explanatory variables within the context of the general linear model on a voxel-by-voxel basis using motion parameters as additional regressors. Region of interest (ROI) analyses were performed for correlations of obtained neurotransmitter levels and NBRs. Group effects were tested using individual contrast images of all subjects in a random-effects model, allowing inference to a general population. For the ROI analysis, effect sizes (percentage of signal change) for the different conditions were extracted for each subject separately using MarsBaR\textsuperscript{33} for the predefined region (pgACC). Mean signal changes of voxels within this ROI were extracted. Signal changes were calculated relative to the mean signal intensity of this ROI across the whole experiment.

**MRS DATA ACQUISITION AND ANALYSIS**

In each subject, single-voxel proton MRS data were acquired at rest from a volume of interest of 20 mm $\times$ 25 mm $\times$ 35 mm = 17.5 mL, which was placed in the right pgACC (Figure 1). Magnetic resonance spectroscopic acquisitions were performed using a Philips Intera 3T whole-body magnetic resonance unit (Philips Healthcare, Best, the Netherlands) equipped with a transmit-receive volume coil. To enable unambiguous, separate, and simultaneous quantification of GABA, Gln, Glu, Glc, and NAA, data were acquired using a maximum echo–sampled 2-dimensional J-resolved point-resolved spectroscopy (JPRESS) sequence,\textsuperscript{20} which encodes the J coupling along the indirect spectral dimension by acquiring data with multiple echo times (eFigure 1, http://www.archgenpsychiatry.com). This approach allows for a significant reduction of spectral overlap by spreading multiplets along 2 frequency axes. The echo times for the JPRESS experiment ranged from 31 to 229 milliseconds, with a step size of 2 milliseconds, a phase cycling of 4 for each echo time, a bandwidth in the direct dimension of 2 kHz, and 2048 sample points. Using 100 encoding steps and 4 averages per encoding step at a repetition time of 2500 milliseconds, the acquisition time for 1 voxel added up to 16 minutes. The sequence was preceded by water suppression using frequency-selective excitation and gradient spoofing followed by adiabatic frequency-selective rephasing and gradient spoofing. The 2-dimensional JPRESS data were quantified using ProFit,\textsuperscript{25} a 2-dimensional fitting procedure, which applies the full amount of prior knowledge by fitting a linear combination of simulated 2-dimensional basis metabolite spectra for the following 19 brain metabolites: alanine, ascorbic acid, aspartate, creatine, GABA, Gln, Glu, glycine, glycerolphosphorylcholine, glutathione, lactate, myo-inositol, NAA, N-acetylaspartylglutamate, phosphorylcholine, phosphonylthanolamine, scyllo-inositol, and taurine. Simulation of the basis metabolite spectra was performed with GAMMA.\textsuperscript{34} Cramer-Rao lower bounds,\textsuperscript{35} an estimate of the fitting error, were used as a quality criterion to exclude data sets with unreliable quantification results. Hence, analyses of group effects and correlational interdependence were restricted to subjects who met strict quality criteria to indicate reliable spectral quantification (Cramer-Rao lower bounds < 20\%)\textsuperscript{36} for each metabolite. This resulted in reduced and different sample sizes for distinct substances, since some metabolites give rise to more prominent resonance lines than others owing to differences in absolute concentrations and coupling behavior. In addition, covariance coefficients were determined in order to exclude systematic correlations due to spectral overlap for reported physiological correlations between metabolites (eFigure 1). Because determination of absolute metabolite concentrations in millimolars requires a reliable T1 and T2 relaxation correction, while relaxation times of coupled metabolites are hardly known for spectroscopy at 3 T, all metabolite concentrations are given relative to creatine levels. Creatine was proven to be an appropriate internal reference for the ProFit analysis in MDD as previously suggested\textsuperscript{21}; absolute creatine concentrations in patients with MDD and healthy volunteers (mean [SD], MDD, n = 19: 6.32 [1.20] mM; healthy volunteers, n = 24: 6.30 [0.96] mM; P < .05) were determined by using the internal water reference method. Additionally, recorded 1-dimensional point-resolved spectroscopy spectra (echo time = 30 milliseconds, 128 averages, 5-minute acquisition time) (eFigure 1) from the same volume of interest were analyzed by LCModel (S.W. Provencher, PhD).

**STATISTICAL ANALYSIS**

For whole-brain exploration of NBR, clusters of deactivation were identified with a global height threshold of $P < .001$ (corrected for family-wise errors). To detect between-group differences, BOLD contrast images and individual metabolite concentrations of all subjects of each group (all subjects with MDD

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**Figure 1.** Glutamine (Gln) in major depressive disorder (MDD). A, Placement of the magnetic resonance spectroscopic voxel (orange frame, upper panel) in the anterior cingulate cortex. Functional magnetic resonance imaging analysis revealed decreased negative blood oxygenation level–dependent responses in patients with MDD within this region compared with controls (difference map, $P < .001$, uncorrected; lower panel). B, Mean Gln concentrations (relative to creatine [Cr]) ± standard error of the mean measured in the magnetic resonance spectroscopic voxel depicted in A in MDD patients with high anhedonia (MDDHA) (n = 5) and low anhedonia (MDDLA) (n = 5) and healthy controls (n = 14). C, In healthy controls, Gln and glucose (Glc) levels (relative to Cr) show a significant correlation ($r = 0.686$, $P < .01$), whereas in patients with MDD, Gln and Glc levels are uncorrelated.
and those with high or low anhedonia scores vs control subjects) were included in 2-sample t tests (threshold, \( P < .001 \), uncorrected; extent voxel threshold of at least 5 contiguous voxels for fMRI analysis). Signal changes from the fMRI experiment within the pgACC volume of interest, metabolite levels of distinct components at rest for the same region, individual intensity rating scores, and the anhedonia subscore were correlated with each other by applying Pearson correlation analysis (SPSS, version 12; SPSS Inc, Chicago, Illinois); statistical significance was set at \( P < .05 \). Significant correlations were controlled for confounding effects of age by partial correlation analyses were calculated separately for healthy controls (n=22; −0.18 [0.08]) in the pgACC based on a whole-brain analysis (peak coordinates \( x, y, z = 10 \) for patients with MDD (n=19; mean [SD], 2.04 [0.62]) and thus was significantly higher (\( P = .02, t = 2.57 \)) than in controls (n=13; 1.35 [0.46]). Just as for Glu concentrations, the mean Glc to Gln ratio for the MDDhA group (n=5; 1.77 [0.92]) was found to be in between those for MDDhA patients and controls, but differences failed to reach statistical significance (eFigure 2B).

Because Gln is also an indirect precursor for GABA synthesis inside neurons, the strong and highly significant correlation between GABA and Glc (n=10; \( r = .775 \), \( P = .01 \)) found in controls (eFigure 2C) becomes weaker and fails to reach significance in patients with MDD (n=8; \( r = .666 \), \( P = .07 \)). However, the GABA to Gln ratio shows a significant correlation with Glc in patients with MDD (n=7; \( r = .864 \), \( P = .01 \)) (Figure 2D), which is not present in controls (n=9; \( r = −.132 \), \( P = .74 \)).

DECREASED NBR PREDICTED BY Glu AND NAA LEVELS IN MDD

Patients and healthy controls showed significant NBRs in the region of interest, the pgACC, during viewing and judgment of emotional pictures compared with when at rest (\( P < .001 \), family-wise–corrected). Negative BOLD responses were significantly smaller (\( P < .001 \), uncorrected; \( k > 10 \)) for patients with MDD (n=19; mean [SD], percent signal change, 0.12 [0.07]) compared with controls (n=22, −0.18 [0.08]) in the pgACC based on a whole-brain analysis (peak coordinates \( x, y, z \), respectively, in Montreal Neurological Institute space: −12, 52, and −2) as well as on an analysis restricted to our ROI that was defined by the MRS single voxel placed in the anterior cingulate cortex (\( P < .05 \)) (Figure 1A and

### RESULTS

ANHEDONIA-DEPENDENT DECREASE IN Gln CONCENTRATION AND DISSOCIATION OF Gln FROM Glc LEVELS IN MDD

Based on patients’ mean anhedonia scores (n=16; mean [SD], 17.31 [3.40]), a comparison of Gln levels (relative to creatine; see the “Methods” section) revealed significantly lower Gln concentrations (\( P = .01, r = 2.92 \)) (Figure 1B) for MDD patients with anhedonia scores higher than the mean value (MDDhA) (n=5; Gln concentration, 0.20 [0.05]) when compared with healthy controls (n=14; Gln=0.34 [0.15]). Although the data also suggested group differences in Glc levels between MDD patients with anhedonia scores lower than the mean value (MDDIA) (n=5; Glc, 0.27 [0.09]) and MDDhA (Figure 1B) and between MDDhA patients and controls, these differences failed to reach statistical significance (\( P = .22 \) and \( P = .23 \), respectively). In contrast to Glm levels, no group differences could be found between those with MDDIA or MDDhA and controls for concentrations of GABA, Glu, Glc, or NAA (Table and eTable) or any other metabolite (eFigure 2).

A strong correlation between controls’ Glc and Gln levels were found (n=12; \( r = 0.686 \), \( P = .01 \)) (Figure 1C), while this correlation was absent in patients with MDD (n=10; \( P = .30 \)). The same was true for Gln relative to Glu concentrations; the ratio of Gln to Glu was exclusively found to correlate with Glc concentrations in controls (n=11; \( r = 0.63 \), \( P = .03 \)) but not in patients with MDD (n=10; \( P = .70 \)) (eFigure 2A). Given the dissociation of Glu-Gln conversion from Glc levels in patients with depression, the Gln deficit (Figure 1B) is also reflected by the ratio of Gln to Glc, which yields the greatest value for the MDDhA group (n=5; mean [SD], 2.04 [0.62]) and thus was significantly higher (\( P = .02, t = 2.57 \)) than in controls (n=13; 1.35 [0.46]). Just as for Glu concentrations, the mean Glc to Gln ratio for the MDDhA group (n=5; 1.77 [0.92]) was found to be in between those for MDDhA patients and controls, but differences failed to reach statistical significance (eFigure 2B).

### Table. Group Comparisons of Metabolite Levels

<table>
<thead>
<tr>
<th>Measure</th>
<th>Controls (Mean [SD])</th>
<th>Patients With MDD (Mean [SD])</th>
<th>Patients With MDDhA (Mean [SD])</th>
<th>Patients With MDDIA (Mean [SD])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr, mM</td>
<td>6.30 (0.96)</td>
<td>6.32 (1.20)</td>
<td>6.22 (0.75)</td>
<td>6.29 (0.63)</td>
</tr>
<tr>
<td>Gln/Cr ratio</td>
<td>0.34 (0.15)</td>
<td>0.25 (0.10)</td>
<td>0.20 (0.05)</td>
<td>0.27 (0.08)</td>
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<tr>
<td>Glu/Cr ratio</td>
<td>1.35 (0.13)</td>
<td>1.34 (0.20)</td>
<td>1.27 (0.15)</td>
<td>1.38 (0.20)</td>
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<tr>
<td>GABA/Cr ratio</td>
<td>0.21 (0.07)</td>
<td>0.21 (0.08)</td>
<td>0.21 (0.13)</td>
<td>0.20 (0.05)</td>
</tr>
<tr>
<td>Glc/Cr ratio</td>
<td>0.45 (0.27)</td>
<td>0.40 (0.14)</td>
<td>0.38 (0.18)</td>
<td>0.42 (0.13)</td>
</tr>
<tr>
<td>NAA/Cr ratio</td>
<td>1.50 (0.15)</td>
<td>1.47 (0.14)</td>
<td>1.45 (0.15)</td>
<td>1.51 (0.13)</td>
</tr>
</tbody>
</table>

Abbreviations: Cr, creatine; GABA, \( \gamma \)-aminobutyric acid; Glc, glucose; Gln, glutamine; Glu, glutamate; MDD, major depressive disorder; MDDhA, major depressive disorder with anhedonia scores lower than the mean; MDDIA, major depressive disorder with anhedonia scores lower than the mean; NAA, N-acetylaspartate.

\( a \) Twenty-four controls; 19 patients with MDD; 9 patients with MDDhA; and 7 patients with MDDIA.

\( b \) Fourteen controls; 12 patients with MDD; 5 patients with MDDhA; and 5 patients with MDDIA.

\( c \) Twenty-two controls; 17 patients with MDD; 8 patients with MDDhA; and 6 patients with MDDIA.

\( d \) Thirteen controls; 11 patients with MDD; 4 patients with MDDhA; and 3 patients with MDDIA.

\( e \) Eighteen controls; 13 patients with MDD; 6 patients with MDDhA; and 6 patients with MDDIA.
Figure 2A). Additional group effects were found in the dorsal posterior cingulate cortex (peak coordinates: 8, −10, and 46), and bilateral superior temporal gyrus (peak coordinates: 48, −30, and 8)/middle temporal gyrus (peak coordinates: −10, and 46), and bilateral superior temporal gyrus (peak coordinates: 8, −10, and 46). The ROI analysis of the MDDhA and MDDIA subgroups revealed that statistically significant differences in mean NBR (t = 2.81, P = .01) were only reached when the MDDhA group (n=9; mean [SD] percent signal change, 0.10 [0.07]) and healthy controls (n=22; −0.18 [0.08]) were compared. Similar to the Glu concentrations, mean NBR of the MDDIA group (n=13; −0.14 [0.81]) reached a level in between those of the MDDhA group and controls, but differences were not statistically significant (eFigure 3).

As previously reported by our group, NBRs are specifically predicted by GABA levels in the anterior cingulate cortex ROI (n=12; r=0.78, P =.001) in controls. In contrast to this finding, NBR variance between subjects cannot be explained by GABA levels (n=10; P =.48) in patients with MDD. Instead, patients’ NBRs correlate with Glu levels (n=12; r=0.64, P =.02) (Figure 2B and Figure 3), while no Glu-NBR correlation was found in controls (n=21; P =.30). Similar results were obtained for NAA; patients’ NAA levels significantly correlated with NBR (n=16; r=0.50, P =.02) (Figure 3 and eFigure 3B), while this correlation was absent in healthy controls. Correlations of NBR with metabolites remained significant when tested separately for picture judgment and picture viewing. In addition, NAA correlated positively with Glu in both patients with MDD (n=17; r=0.751, P <.001) (Figure 3 and eFigure 3C) and healthy controls (n=23; r=0.443, P =.03). However, the NAA-Glu correlation in patients with MDD is substantially stronger than that in controls. In contrast to Glu and NAA, Gln levels nonspecifically correlated with NBR in both patients with MDD (n=11; r=0.78, P =.005) and controls (n=13; r=0.75, P =.003).

EMOTIONAL INTENSITY, METABOLIC AND FUNCTIONAL MARKERS, AND ANHEDONIA

Within the patient group, a significant inverse correlation (n=12; r=−0.62, P =.03) between intensity scores and the BDI anhedonia subscore (Figure 3 and eFigure 4) was found, indicating that emotional intensity reports may serve as a reliable surrogate for the degree of anhedonia. This was supported by the direct analysis of MDD subgroups, which revealed that the MDDhA group (anhedonia score > 17) had significantly (t=4.75, P <.001) lower intensity ratings (n=7; mean [SD], 2.42 [0.36]) than the MDDIA group (n=5, anhedonia score < 17; 3.31 [0.24]). This was true for both positive and negative stimuli when tested separately. When compared with controls, a trend (t=1.95, P =.06) toward reduced intensity ratings is seen in the MDDhA group, but not in the MDDIA group (P =.25) (eFigure 4B). Global assessment revealed comparable mean ratings of subjective intensity of emotional pictures for patients with MDD (n=15; 2.82 [0.49]) and healthy controls (n=23; 2.93 [0.65]).

Given that emotional intensity was found to be a good measure of the degree of anhedonia in patients, direct
prediction by metabolic concentrations or functional BOLD responses of intensity ratings was tested. Patients’ intensity ratings were found to significantly correlate with both the negative BOLD amplitude in the anterior cingulate cortex ROI during the picture condition in our fMRI experiment (n = 14; r = 0.58, P = .03) and Glu concentrations for the same region (n = 12; r = 0.82, P = .001) (Figure 3 and eFigure 3C and D). This was not the case in healthy controls, who showed no correlation of intensity ratings with either NBR (n = 21; P = .63) or with levels of Glu (n = 20; P = 0.76). N-acetylaspertate also correlated with intensity ratings (n = 12; r = 0.64, P = .03) exclusively in patients with MDD (Figure 3 and eFigure 3D). While emotional intensity showed strong linear correlations with anhedonia, Glu, and NBR, the more global degree of anhedonia did not correlate with the biomarkers at the time of the experiment.

In the pgACC, a cortical region that is involved in mood regulation,7 substantial metabolic and functional changes were found in patients with MDD when compared with controls. In MDD, Glu and NAA concentrations in the pgACC were found to correlate with NBRs induced by emotional stimulation, which were generally reduced in patients with MDD compared with controls; whereas in healthy controls, NBRs correlated with GABA instead. In addition, reported emotional intensity ratings, which were found to be anhedonia surrogates, also correlate with Glu, NAA, and NBR in patients with MDD. A decrease of Gln concentration and a dissociation of Gln levels from resting state Glc concentrations in the pgACC of highly anhedonic patients with MDD was observed, while mean Glu, GABA, and Glc concentrations remained normal.

Glutamine is a direct precursor of the neuronal GABA and Glu neurotransmitters pools, but is itself synthesized from glial Glu, which is in fast exchange with tricarboxylic acid cycle intermediates (Figure 4). Thus, the reduction of total Gln in the pgACC of patients with MDD along with the absence of the Glu-Glc correlation suggest a reduced glial conversion of the excitotoxic neurotransmitter Glu to its precursor and nontoxic transport form Gln. This interpretation is further supported by a correlation of the Glu to Gln ratio with Glc in healthy controls, which is absent in MDD, and by a significant increase of the Glc to Gln ratio in those with MDD compared with controls. Our results regarding reduced Gln concentrations in the pgACC are in line with previous reports of reduced Glx (Glu and Gln) in the same cortical region20–22 but exceed these by specifying the deficit to be primarily related to Gln. In addition, the indicated distortion in Glu-Gln cycling was predicted by a previous postmortem study,16 which demonstrated decreased expression of glial glutamine synthetase, an enzyme that mediates the conversion of Glu into Gln inside astroglia in our region of interest (Brodmann area 24). This hypothesis might be proven by direct observation of Glu-Gln cycling using carbon 13–labeled MRS in the future.

The observed reduction of NBR upon emotional stimulation in the pgACC of patients with MDD complements previous studies about functional deficits in the medial prefrontal cortex.5,6,11 The correlations of NBR with Glu and NAA support the hypothesis that altered glutamatergic metabolism and neuronal viability might be related to the observed NBR reduction in patients with depression. Considering that most of the intracellular Glu pool as well as the acetyl moiety of NAA (its methyl group gives rise to the dominant resonance line at 2.0 ppm) are in fast turnover with tricarboxylic acid cycle intermediates,26,37 these metabolites can be taken as a measure for energy metabolism and hence for neuronal activation or function.38 Therefore, from the direction of the Glu-NBR and NAA-NBR correlations, a hypometabolism and thus reduced neuronal resting state activity in the pgACC can be concluded for patients with highly anhedonic MDD, who also show decreased NBRs upon emotional stimulation. This interpretation is in accordance with investigations that have compared the metabolism in this part of the anterior cingulate cortex in controls and patients with MDD using positron emission tomography. These positron emission tomography studies demonstrated that a hypometabolism of the pgACC predicts partial or no response to selective serotonin reuptake inhibitor treatment in patients with MDD7 as well as the dependence of NBRs from anaerobic glycolysis at rest and related baseline metabolic levels.24

In addition, recent postmortem results revealed lower prefrontal concentrations (Brodmann area 24) of the glial transporter enzymes SLCA1 and SLCA2, which mediate the Glu reuptake from the synaptic cleft (Figure 4).25 Because Glu accumulation in the synaptic cleft has an excitotoxic effect on glutamatergic neurons39 and oligodendrocytes, impairment of glial and neuronal function and, as a long-term consequence, changes in neuronal and glial density might result. This is in accordance with...
additional postmortem results that report reduced neuronal somal sizes and dendritic reshaping along with increased neuronal and decreased astroglial density. The observed hypometabolism could thus reflect reduced neuronal cross-linking due to a reduction in dendritic arborization and axonal loss caused by demyelination due to an excess of excitotoxic Glu in the synaptic cleft. However, Glu and NAA concentrations should rather be interpreted as functional state markers and not as trait markers that would indicate the progress of neurodegeneration. This is supported by the observation of complete recovery of NAA levels in multiple sclerosis, cerebral ischemia, and brain injury and by the role of Glu in brain energy metabolism. A causality of the 2 depicted enzymatic changes remains to be clarified: a reduction of Glu-Gln conversion would also consequently distort glial Glu uptake, while a primarily reduced glial Glu reuptake could also diminish the glial Glu pool and hence result in a down-regulation of Glu-Gln conversion enzymes. The absence of significant group differences for Glu levels might be explained by the balancing dependency of Glu synthesis from tricarboxylic acid cycle intermediates from distorted glial Glu to Gln conversion and Glu reuptake. 

In accordance with previous in vivo MRS studies using GABA editing techniques, GABA levels did not differ significantly between patients and controls, though Gln normally serves as a precursor to GABA. Because there is no evidence for direct impairment of the GABAergic system, primary deficits in glutamatergic metabolism may lead to the observed disentanglement of GABA levels from Glc levels as well as a distortion of the excitation-inhibition balance, rendering the absence of NBR modulation by GABA a secondary effect. 

In addition to the direct correlations between functional and metabolic markers, Glu and NAA levels as well as NBR also directly correlate with the reported subjective emotional intensity ratings of emotional pictures, which were found to be a good surrogate for anhedonia (Figure 3). Thus, the depicted deficits, including indices of hypometabolism, should be specified for patients with high anhedonia scores. Accordingly, the reduction of Gln levels and NBR as well as the increase of the Glc/Gln ratio are most prominent in highly anhedonic patients. Hence, the relevance of the proposed distortion in glial Glu-Gln cycling and Glu reuptake and the resulting functional and structural changes on a specific clinical pattern as potential biomarker become evident. Our findings should be limited to the investigated group of severely depressed patients with BDI scores greater than 24. Antidepressant effects of lithium (which facilitates Glu reuptake), ketamine (an N-methyl-D-aspartate-receptor antagonist), and lamotrigine (which decreases Glu release into the synaptic cleft), which were especially proven for treatment-resistant depression, would further support the suggested impact of glutamatergic aberrations for severe anhedonic MDD.

Previous inconsistencies regarding hypermetabolism and hypometabolism in the pgACC might be the result of inhomogeneous groups regarding severity and diversity of clinical symptoms and treatment response. Also, a specificity within anterior cingulate cortex subregions should be considered, as MDD is not only characterized by affected mood regulation, mainly related to the pgACC, but also by distortion of emotional attention and cognition and alterations in the vegetative and somatic nervous system. As a consequence of the variety of symptoms that reflect MDD, functional, metabolic, and structural changes inside an entire network of brain areas suggest a limbic-cortical dysregulation. It is thus necessary to extend the combined in vivo fMRI and 2-dimensional J-resolved MRS approach we presented to different sub-regions as well as to different subtypes of MDD.

In conclusion, a combined fMRI/MRS in vivo approach enabled us to integrate the clinical dimension of anhedonia into a functional/metabolic framework and to reveal cellular mechanisms related to altered brain function in MDD. Therefore, a change from a descriptive to a causal level of in vivo investigations of MDD in human subjects was attained. Specifically, downregulation of astroglial Gln synthesis from Glu and an impairment of the Glu reuptake from the synaptic cleft into astroglia in the pgACC of subjects with depression were demonstrated for the first time in vivo. The present study links these neurochemical alterations to a hypometabolism in the pgACC of highly anhedonic patients, which might be related to previously reported changes regarding neuronal somal cell size, dendritic arborization, as well as neuronal and glial cell density caused by an accumulation of excitotoxic Glu in the synaptic cleft. Our findings may guide future investigations on novel therapeutic targets. Based on the depicted link between measures of anhedonia and biomarkers of altered metabolism and function, the latter might be used as a supporting objective measure and possible predictor for therapy responses in MDD.

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Author Contributions: Drs Walter, Henning, and Grimm contributed equally to this work. Drs Walter and Henning jointly derived the reported interdependence of func-
tional, metabolic, and clinical parameters in MDD. Dr. Walter performed the statistical analysis. Dr. Henning conceived the neurochemical model and coanalyzed parts of the MRS data. Drs. Walter, Henning, and Grimm wrote the manuscript. Drs. Grimm and Boeker carried out the fMRI experiments and analyzed the fMRI data. Dr. Schulte designed and implemented the software for MRS data acquisition and quantification and performed the MRS data analysis. Drs. Schulte and Beck, and Ms. Schnepf carried out the MRS experiments. Drs. Dyda and Boesiger supervised the MRS protocol optimization and validation and revised the manuscript; Dr. Boesiger also provided the magnetic resonance scanner. Dr. Northoff designed the study and supervised the entire project. Drs. Boesiger, Northoff, and Boeker took the financial responsibility for the combined MRI/MRS study. Drs. Walter, Henning, Grimm, and Northoff had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Additional Information: The eFigures and eTable are available at http://www.archgenpsychiatry.com.

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To authors, reviewers, Archives and JAMA staff, and readers, it is with profound sadness that I announce the retirement of our editorial assistant, Mary Frances (“Fran”) MacNeil, for health reasons. Fran started as my secretarial assistant when I became the chairman of the Consolidated Department of Psychiatry at Harvard Medical School 18 years ago. She then assumed the position of editorial assistant for the Archives of General Psychiatry when I accepted the editorship in late 2001 after completing my term as chairman.

The transition in that first year was extremely taxing. Learning the system and cleaning up the inevitable backlog of manuscripts while new manuscripts were being submitted at a steady pace were daunting. The office was awash with paper, but Fran managed to make order out of what seemed to be chaos. My goal was to bring down the time from submission to the first decision and the time from acceptance to publication, which was nearly a year then, so that the Archives could compete for the best science. Within 2 years, we were able to make a decision within 4 weeks in more than 90% of the submissions, and the time from acceptance to publication fell to slightly over 6 months. Fran’s organizational skills and determination made these gains possible.

She shepherded the Archives as submissions, reviews, and all communications moved from being paper based to being fully electronic. The process initially seemed quite complicated but ultimately proved to be highly efficient. Thus, the Archives’ statistics improved further and its citation impact rose to 1.98 this last year.

Fran was born and raised in Inverness, Nova Scotia, Canada. She was the youngest of 6 children, with her nearest sibling being 11 years older. She graduated from the University of Alberta and then moved to Belmont, Massachusetts, where several of her aunts resided. She obtained a position in the legal department at McLean Hospital, a Harvard affiliate. She has worked at McLean for more than 40 years in several positions of responsibility. Her husband died of cancer when she was in her mid 40s, and she raised a son and a daughter alone. They are both now very successful professionals, each with 2 children.

Fran is a very formal person. Everyone was always addressed by his or her proper title (“Fran, you can call me Joe.” “Yes, Dr Coyle.”). Given the Archives’ rejection rate of more than 85%, there were a lot of unhappy authors. But, she always dealt with the authors and reviewers with great respect and patience, even if they were clearly in the wrong. Her greatest personal liability was her greatest asset for the Archives: she blamed herself if anything went wrong. As a consequence, her attention to detail was meticulous so that no mistakes would ever happen. While she was not shy, she clearly did not want to draw attention to herself. Yet, she had a rich sense of humor and could skewer the foibles of the academics with whom she was in constant contact. Every day would be punctuated by a few hearty laughs that lightened the pressure of dealing with the unremitting flow of manuscripts. She was intensely loyal to and proud of the Archives of General Psychiatry.

Fran will be greatly missed by the extended Archives family.

Joseph T. Coyle, MD
Editor