Frontal Glutamate and γ-Aminobutyric Acid Levels and Their Associations With Mismatch Negativity and Digit Sequencing Task Performance in Schizophrenia

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IMPORTANCE Auditory mismatch negativity (MMN) is a biomarker for schizophrenia thought to reflect glutamatergic N-methyl-D-aspartate receptor function and excitatory-inhibitory neurotransmission balance. However, the association of glutamate level with MMN has not been directly examined in patients with schizophrenia, to our knowledge.

OBJECTIVE To investigate the contributions of glutamate and γ-aminobutyric acid (GABA) to MMN and digit sequencing task (DST) performance, an assessment of verbal working memory, in schizophrenia.

DESIGN, SETTING, AND PARTICIPANTS Fifty-three control participants from the community and 45 persons with schizophrenia from outpatient clinics completed an electroencephalographic session for MMN, magnetic resonance spectroscopy for glutamate and GABA, and a DST. The study dates were July 2011 to May 2014, and the dates of our analysis were May 2014 to August 2015.

MAIN OUTCOMES AND MEASURES Glutamate, GABA, the ratio of glutamine to glutamate, MMN amplitude, and DST. Structural equation modeling was used to test the effects of neurochemistry and MMN amplitude on DST performance.

RESULTS The 45 persons with schizophrenia were a mean (SD) of 37.7 (12.8) years and the control participants were 37.1 (13.1) years. The schizophrenia group had a mean (SD) of 14.7 (12.1) years of illness. Mismatch negativity amplitude ($F = 4.39, P = .04$) and glutamate ($F = 9.69, P = .002$) were reduced in the schizophrenia group. Smaller MMN amplitude was significantly associated with lower GABA level ($P = .008$), lower glutamate level ($P = .05$), and higher ratio of glutamine to glutamate ($P = .003$). Reduced MMN amplitude was linked to poor verbal working memory in schizophrenia ($P = .002$). Modeling revealed that a proxy of glutamatergic function, indexed by the ratio of glutamine to glutamate, influenced a path from the ratio of glutamine to glutamate to MMN to verbal working memory ($P = .38$ [root-mean-square error of approximation, $P < .001$] by $\chi^2$ test), supporting the contention that MMN serves as an intermediate biomarker linking glutamatergic function to DST performance in schizophrenia.

CONCLUSIONS AND RELEVANCE The role of glutamate and GABA in MMN and verbal working memory deficits in schizophrenia has been frequently debated. These data provide in vivo evidence that support glutamatergic and GABAergic regulation of MMN and verbal working memory function in schizophrenia.
Auditory mismatch negativity (MMN) is a negative electrical wave recorded by electroencephalography in response to new vs ongoing auditory inputs and is a replicated biomarker for schizophrenia.\textsuperscript{1,4} Mismatch negativity is thought to index an auditory trace memory function that automatically detects a mismatch between a new stimulus in the background of the ongoing stimuli\textsuperscript{5-8} and has been linked to the glutamatergic N-methyl-d-aspartate (NMDA) receptor and excitatory-inhibitory neurotransmission functions.\textsuperscript{9} However, the long-standing assumption of a glutamatergic contribution to abnormal MMN in schizophrenia has not been directly examined in this disorder, to our knowledge. A link between γ-aminobutyric acid (GABA) and MMN in schizophrenia is even less clear.

Mismatch negativity is generated primarily through the supratemporal auditory cortex, although the frontal cortex may also have a role by serving as top-down modulation of MMN.\textsuperscript{10} This modulation has been interpreted in several ways, including attention switching, inhibition, salience change detection, and predictive coding of the auditory deviance detection.\textsuperscript{7,11,12} The underlying molecular mechanism of the MMN generation is strongly linked to glutamatergic NMDA receptors in the auditory cortex.\textsuperscript{9,13,14} Whether a frontal glutamatergic mechanism is linked to MMN generation has not been studied to date, although the frontal mechanisms of MMN, such as predictive coding, likely require top-down excitatory-inhibitory signaling. In this context, potential GABAergic effects on MMN were studied through administration of GABA compounds, but the findings were not consistent.\textsuperscript{15-17} How endogenous GABA may influence MMN has not been reported, to our knowledge. We hypothesized that abnormal MMN in schizophrenia is in part due to aberrant frontal glutamate-GABA biochemistry. Using proton magnetic resonance spectroscopy (MRS), we measured anterior cingulate and medial frontal lobe levels of glutamate, glutamine, and GABA in vivo and determined how they relate to the MMN abnormality observed in schizophrenia.

In healthy control individuals, larger P300a amplitude at the electrode site frontal zero (FZ) was associated with a higher anterior cingulate ratio of glutamine to glutamate.\textsuperscript{18} For MMN, besides the supratemporal auditory cortex as the primary source, the right inferior frontal,\textsuperscript{10,11,19} left inferior frontal,\textsuperscript{10,20} medial frontal and orbitofrontal,\textsuperscript{21,22} and anterior cingulate\textsuperscript{23-25} have been implicated as potential sources of MMN generation. Studies\textsuperscript{21-26} of MMN source localization in schizophrenia implicate the anterior cingulate as the most consistent frontal location. Furthermore, MMN measured at the electrode site FZ typically yields the largest schizophrenia-control difference in MMN.\textsuperscript{2,22} Therefore, this study investigated the association between anterior cingulate and medial frontal MRS measures and MMN measured at FZ, but it should be noted that multiple brain region generators contribute to MMN at this electrode.

Aberrant MMN has also been proposed to reflect auditory working memory deficits in schizophrenia.\textsuperscript{9,28,29} Impaired working memory is considered a core cognitive deficit in schizophrenia.\textsuperscript{10,3} Flutamatergic and GABAergic pathways are the essential regulatory mechanism for working memory.\textsuperscript{31,32} The present study tested the hypothesis that an MMN link to verbal working memory performance, as assessed with the digit sequencing task (DST), in schizophrenia is regulated by glutamate and GABA levels. γ-Aminobutyric acid levels were assessed using an improved macromolecule suppression MRS technique.\textsuperscript{33} Glutamate levels, including the ratio of glutamine to glutamate, were assessed with an MRS technique optimized for the detection of these metabolites.\textsuperscript{34-36} The ratio of glutamine to glutamate is thought to reflect glutamatergic neurotransmission whereby the ratio of conversion from glutamine to glutamate may index glutamatergic function.\textsuperscript{37} In schizophrenia, a higher ratio of glutamine to glutamate has been found in the cerebrospinal fluid\textsuperscript{38} and brain tissue measured with MRS in some studies\textsuperscript{39-42} but not in another study.\textsuperscript{43} The study\textsuperscript{44} reporting an association between P300a and the anterior cingulate ratio of glutamine to glutamate in healthy control individuals further supports the proposal of studying the association between MMN and the ratio of glutamine to glutamate because P300a and MMN are variants of oddball paradigms. P300a is an active oddball paradigm,\textsuperscript{18} and MMN is a passive oddball paradigm.\textsuperscript{44} Therefore, we combined evoked potential and improved MRS techniques and used structural equation modeling to better understand the influence of glutamate and GABA on the MMN and DST assessment of verbal working memory in schizophrenia.

### Methods

#### Participants

Fifty-three control participants and 45 persons with schizophrenia spectrum disorder completed this study. Participants were 18 to 65 years old, with no current or past neurological disorder or major medical conditions and with no recent substance abuse. Persons with schizophrenia spectrum disorder were diagnosed as having schizophrenia (n = 40) or schizoaffective disorder (n = 5) as determined with the Structured Clinical Interview for DSM-IV-TR, Patient Edition. Controls had no past or present Axis I psychiatric disorder as determined with the Structured Clinical Interview for DSM-IV-TR, Non-patient Edition. Age and sex were frequency matched between patients and controls. We also attempted to frequency match the smoking status between patients and controls. The patient group comprised individuals with early and chronic schizophrenia, with a mean (SD) length of illness of 14.7 (12.1) years. The Brief Psychiatric Rating Scale (BPRS) was used to assess psychopathology in the patients. The cognitive functions assessed were verbal working memory (using a DST\textsuperscript{45}) and processing speed (using the digit symbol coding subtest of the Wechsler Adult Intelligence Scale III\textsuperscript{46}). Deficits in working memory\textsuperscript{47-49} and processing speed\textsuperscript{50,51} are considered the cognitive domains most affected in schizophrenia. Participant characteristics are listed in Table 1. The study dates were July 2011 to May 2014, and the dates of our analysis were May 2014 to August 2015. Additional participant and study details are given in the eMethods in the Supplement. The study was approved by the University of Maryland, Baltimore County, Institutional Review Board. All participants provided written informed consent before participation in the study.
The event-related potential and MRS methods have been described previously,\textsuperscript{33,36,52} and details are given in the Methods in the Supplement. Briefly, the event-related potential was recorded using a 64-channel system\textsuperscript{2} (SynAmps; Compumedics Neuroscan) at a 1-kHz sampling rate with bandpass at 0.1 to 200 Hz. The paradigm was a passive duration-deviant oddball task.\textsuperscript{52} Standard and deviant trials were averaged separately, followed by subtraction of the 2 mean waveforms.Mismatch negativity was scored by peak detection within a poststimulus window of 100 to 225 milliseconds by an automatic algorithm, followed by visual inspection to verify correct placement of each marker for peak detection. Magnetic resonance scanning was conducted on a 3-T imaging system (Tim Trio; Siemens) equipped with a 32-channel head coil. Spectra were acquired with a short-echo sequence for detection of glutamatergic measures\textsuperscript{36} and a spectral editing sequence for detection of GABA with minimal macromolecule contamination.\textsuperscript{21} Metabolites were quantified with available tool kits (LCModel; LCModel Inc\textsuperscript{53} and freely available GANNET\textsuperscript{54}) and corrected for the proportion of voxel gray, white, and cerebrospinal fluid tissue proportions\textsuperscript{55} and then reported in institutional units (Table 2). Figure 1 shows voxel location, representative spectra, and MMN waveforms.

### Event-Related Potential and MRS
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### Statistical Analysis
Demographic variables were analyzed with \( \chi^2 \) test for categorical data. Continuous variables for between-group comparisons, including verbal working memory, processing speed, MMN, and MRS measures, were analyzed with analysis of variance. The group comparisons were repeated using age, sex, and smoking status. For MRS measures, voxel cerebrospinal fluid, white matter, and gray matter volume proportions were used as covariates. Pearson product moment correlations were conducted to examine the association between MMN and MRS measures with specific hypotheses regarding associations between MMN and glutamate and the ratio of glutamine to glutamate. The associations between the ratio of glutamine to glutamate, GABA, MMN, and DST performance were examined with structural equation modeling. All tests were 2 tailed, and significance was set at \( P \leq .05 \) except for nonhypothesized tests, for which a Bonferroni correction was applied.

### Results

#### Participant Characteristics
Demographic, clinical, and cognitive characteristics of participants are listed in Table 1. Persons with schizophrenia had significantly lower scores for DST verbal working memory...
Table 2. MMN and MRS Measures

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>F Score</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMN Measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude, μV</td>
<td>−0.8 (1.4)</td>
<td>4.39</td>
<td>.04b</td>
</tr>
<tr>
<td>Latency, ms</td>
<td>180.1 (32.6)</td>
<td>1.20</td>
<td>.27</td>
</tr>
<tr>
<td>MRS metabolites, IU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>8.6 (1.0)</td>
<td>9.69</td>
<td>.002b</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2.2 (0.4)</td>
<td>0.57</td>
<td>.45</td>
</tr>
<tr>
<td>Ratio of glutamine:glutamate</td>
<td>0.26 (0.06)</td>
<td>0.62</td>
<td>.43</td>
</tr>
<tr>
<td>GABA</td>
<td>0.87 (0.18)</td>
<td>1.25</td>
<td>.26</td>
</tr>
<tr>
<td>N-acetylaspartate</td>
<td>9.8 (1.0)</td>
<td>6.98</td>
<td>.01b</td>
</tr>
<tr>
<td>Choline</td>
<td>1.9 (0.3)</td>
<td>0.01</td>
<td>.94</td>
</tr>
<tr>
<td>Creatine</td>
<td>9.1 (0.8)</td>
<td>3.67</td>
<td>.06</td>
</tr>
<tr>
<td>Myo-inositol</td>
<td>6.8 (0.9)</td>
<td>0.53</td>
<td>.47</td>
</tr>
<tr>
<td>Glutathione</td>
<td>2.1 (0.3)</td>
<td>2.66</td>
<td>.11</td>
</tr>
<tr>
<td>MRS Voxels, % Volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>11.5 (2.6)</td>
<td>5.34</td>
<td>.02b</td>
</tr>
<tr>
<td>White matter</td>
<td>36.1 (3.5)</td>
<td>5.77</td>
<td>.02b</td>
</tr>
<tr>
<td>Gray matter</td>
<td>52.2 (2.9)</td>
<td>1.13</td>
<td>.29</td>
</tr>
</tbody>
</table>

Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; GABA, γ-aminobutyric acid; MMN, mismatch negativity; MRS, magnetic resonance spectroscopy; NA, not applicable.

* Covariates were age, sex, and smoking status. Covariates also included the percentage of voxel cerebrospinal fluid, white matter, and gray matter for MRS metabolites only.

** Statistically significant.

(P = .02) and processing speed (P = .001) compared with the control group. There were no significant differences in age, sex, or smoking status between groups.

**MMN, Ratio of Glutamine to Glutamate, and GABA**

The schizophrenia group showed significantly reduced MMN amplitude (P = .04) but not latency (P = .27) compared with controls. Glutamate levels were significantly lower in the schizophrenia group compared with the control group (P = .002), but GABA levels and the ratio of glutamine to glutamate were not significantly different between groups (P > .05 for both). Reanalyses of group comparisons with inclusion of the covariates did not change the presence or absence of statistical significance. Group means for MMN and MRS metabolite measurements and statistics are listed in Table 2.

The association between glutamate and MMN amplitude was statistically significant in schizophrenia, such that higher glutamate levels were associated with larger (more negative) MMN amplitude (r = −0.28, P = .05) (Figure 2B). The smaller ratio of glutamine to glutamate was related to larger MMN amplitude in patients with schizophrenia (r = 0.45, P = .003) (Figure 2A). When considering only cases with glutamine fits with estimated standard deviations (Cramer-Rao lower bounds) less than 20%, the ratio of glutamine to glutamate remained significantly related to MMN (r = 0.46, P = .01). Higher GABA levels were also associated with greater MMN amplitude (r = −0.39, P = .008) (Figure 2C). Therefore, the ratio of glutamine to glutamate and GABA were both significantly associated with MMN but in the opposite direction (Figure 2A and C). These statistically significant associations were not observed in the control group (P > .05 for all) (Figure 2B, D, and F), although an exploration of the MMN vs ratio of glutamine to glutamate data in controls suggested an inverted U relationship (Figure 2B).

**Cognitive Correlates**

Greater MMN amplitude was significantly related to better DST verbal working memory (r = 0.46, P = .002) but not processing speed (r = .06) in the schizophrenia group. These correlations were not significant in controls (P > .40 for all). Higher GABA level was significantly correlated with better DST verbal working memory (r = 0.40, P = .009) and processing speed (r = 0.33, P = .03) in patients with schizophrenia but not in controls (r < 0.22, P > .16 for both). The ratio of glutamine to glutamate was not significantly correlated with DST verbal working memory or processing speed in either group (r < 0.25, P > .15 for both).

**Structural Equation Modeling**

A leading theory conceptualizing MMN involvement in the cognitive deficit in schizophrenia is that aberrant MMN may reflect auditory working memory deficits in schizophrenia, which could be optimal because of altered glutamatergic NMDA receptor function or interplay between glutamatergic and GABAergic neurotransmission.9 Figure 3A shows the model with the best fit for the schizophrenia group (P = .38 [RMSEA, P < .001] by χ² test; AIC, 26.78). This model supports the contention that the ratio of glutamine to glutamate has an effect on DST performance that is mediated by MMN. This model also showed that GABA and the ratio of glutamine to glutamate in schizophrenia were strongly inversely correlated. Comparatively, the same model for the control group (Figure 3B) was
also a good fit ($P = .26$ [RMSEA, $P = .05$] by $\chi^2$ test; AIC, 27.28) but not as good as that for the schizophrenia group. This model showed that both GABA and the ratio of glutamine to glutamate contributed significantly to MMN amplitude in the control group, but they did not substantially contribute to DST performance. Therefore, glutamatergic function as indexed by the ratio of glutamine to glutamate appeared to have a different role in patients with schizophrenia vs controls, with the data supporting the hypothesis that MMN had a significant role in mediating glutamatergic function and DST performance only in schizophrenia.

Not all aspects of the modeled paths differed between the schizophrenia and control groups because GABA levels had a similar and modest direct effect on DST performance in both cohorts (Figure 3). A full model with direct paths from the ratio of glutamine to glutamate to verbal working memory and other alternative models did not fit as well and are shown in eFigure 1, eFigure 2, and eFigure 3 in the Supplement.

**Correlations With Clinical Symptoms and Antipsychotic Medication Use**

Chlorpromazine equivalents were not significantly correlated with MMN amplitude ($P = .48$), glutamine ($P = .99$), glutamate ($P = .12$), ratio of glutamine to glutamate ($P = .32$), or GABA ($P = .60$). Smaller MMN amplitude was significantly related to more BPRS negative symptoms ($r = 0.30, P = .045$) but not BPRS positive symptoms or total score ($P > .20$ for both). Glutamate, glutamine, ratio of glutamine to glutamate, and GABA were not significantly correlated with BPRS negative, positive, or total scores ($P > .15$ for all). Smaller MMN amplitude was significantly related to longer duration of illness ($r = 0.36, P = .02$).

**Other MRS Metabolites**

The stimulated-echo acquisition mode MRS sequence also provided data to explore associations between MMN and the following prominent metabolites: total $N$-acetylaspartate, $myo$-inositol, glutathione, total creatine, and total choline (Table 2).
lists the group means [SDs]). There were no significant correlations between these metabolites and MMN except for creatine in controls ($r = 0.32$, $P = .02$), which did not survive a Bonferroni correction for 10 tests. This exploration further implies the specificity of associations found between the ratio of glutamine to glutamate, GABA, and MMN in schizophrenia.

Discussion

To our knowledge, this investigation is the first study to test the association of in vivo glutamate and GABA levels and MMN in schizophrenia. The results showed that smaller MMN amplitude in schizophrenia was significantly associated with lower GABA level, lower glutamate level, and higher ratio of glutamine to glutamate. Modeling of the data revealed that glutamatergic function as indexed by the ratio of glutamine to glutamate influenced a path from the ratio to MMN to DST assessment of verbal working memory, supporting the hypothesis that MMN serves as an intermediate biomarker linking glutamatergic function to verbal working memory in schizophrenia.

The underlying NMDA receptor mechanism of MMN generation was initially identified in nonhuman primate investigations with applications of NMDA receptor agonists and an-
tions of glutamin to glutamate. Another interpretation is that a knockdown of this enzyme leads to elevated frontal lobe 
but also found that several other frontal and cingulate sources are associated with MMN and working memory in schizophrenia.
This result illustrates the need to examine the glutamatergic, MMN, and working memory hypothesis in other frontal and temporal areas. In healthy humans, other frontal areas besides the auditory cortex, such as the right inferior frontal cortex, are often linked to the MMN component. However, in schizophrenia, the anterior cingulate and medial frontal region are commonly associated with MMN source localization. Therefore, the MRS location may partially explain why the finding was stronger in the schizophrenia group compared with healthy controls.

There was a significant correlation between GABA and verbal working memory ($r = 0.40$, $P = .009$) in patients with schizophrenia. Impaired working memory is a key cognitive deficit in schizophrenia. GABA levels are lower in patients with schizophrenia compared with healthy controls. GABA closely interacts with the ratio of glutamate to glutamine, and after accounting for this ratio, GABA was no longer a significant contributor to the MMN-DST verbal working memory path in schizophrenia. This finding suggests that the GABA effect on MMN could be mediated through a glutamatergic mechanism, which would be consistent with animal data showing that GABA antagonist effects on MMN were reversed by NMDA receptor antagonism. Overall, while not identical, many predictions of the glutamatergic and GABAergic involvement in MMN in schizophrenia are supported herein by MRS measures of endogenous neurotransmitters.

The anterior cingulate and medial frontal cortex is the frontal location that is most consistently reported to be associated with MMN generation in schizophrenia. However, because signals from the electrode FZ reflect contributions from wider sources, such as the auditory cortex, this finding should be interpreted with caution. A recent MMN source localization study confirmed the involvement of the anterior cingulate but also found that several other frontal and cingulate sources are associated with MMN and working memory in schizophrenia. This result illustrates the need to examine the glutamatergic, MMN, and working memory hypothesis in other frontal and temporal areas. In healthy humans, other frontal areas besides the auditory cortex, such as the right inferior frontal cortex, are often linked to the MMN component. However, in schizophrenia, the anterior cingulate and medial frontal region are commonly associated with MMN source localization. Therefore, the MRS location may partially explain why the finding was stronger in the schizophrenia group compared with healthy controls.

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The study investigated a large sample and applied a structural equation model to test direct and indirect effects of neurochemistry on MMN and DST verbal working memory. Limitations of the study include that only one brain region was assessed with MRS. Several other frontal locations, as well as the auditory cortex, are associated with the generation of MMN. Additional research is needed to investigate other regions, especially the auditory cortex, associated with MMN. The MMN measured at FZ was not designed to assess contributions from temporal vs frontal brain regions, limiting the anatomically specific MRS-MMN interpretation. The inclusion fit criterion for glutamate was liberal, but inclusion of only those participants with standard fit criteria did not change the results. Finally, despite the lack of correlation with chlorpromazine, there remains the potential confound of antipsychotic medication use on the findings.

Conclusions

To our knowledge, this investigation is the first study to show a significant association between in vivo glutamatergic measurements, MMN, and verbal working memory in schizophrenia. The modeling supports the contention that MMN may index the glutamatergic contribution to verbal working memory performance in schizophrenia. γ-Aminobutyric acid level was also involved but appeared to be more modest and indirect. These data provide strong support for the involvement of the glutamatergic system in MMN and verbal working memory function in schizophrenia.

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Study concept and design: Rowland, Hong.

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REFERENCES


Gray JA, Roth BL. Molecular targets for treating cognitive dysfunction in schizophrenia. Schizophr Bull. 2007;33(3):1100-1119.

Volk DW, Austin MC, Pierry JN, Sampson AR, Lewis DA. Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical y-amino butyric acid neurons in subjects with schizophrenia. Arch Gen Psychiatry. 2000;57(3):237-245.


