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Dopamine and Glutamate in Antipsychotic-Responsive Compared With Antipsychotic-Nonresponsive Psychosis: A Multicenter Positron Emission Tomography and Magnetic Resonance Spectroscopy Study (STRATA)

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The variability in the response to antipsychotic medication in schizophrenia may reflect between-patient differences in neurobiology. Recent cross-sectional neuroimaging studies suggest that a poorer therapeutic response is associated with relatively normal striatal dopamine synthesis capacity but elevated anterior cingulate cortex (ACC) glutamate levels. We sought to test whether these measures can differentiate patients with psychosis who are antipsychotic responsive from those who are antipsychotic nonresponsive in a multicenter cross-sectional study. 1H-magnetic resonance spectroscopy (1H-MRS) was used to measure glutamate levels (Glu corr) in the ACC and in the right striatum in 92 patients across 4 sites (48 responders [R] and 44 nonresponders [NR]). In 54 patients at 2 sites (25 R and 29 NR), we additionally acquired 3,4-dihydroxy-6-[18F]fluoro-L-phenylalanine (6F-DOPA) positron emission tomography (PET) to index striatal dopamine function (K1, min−1). The mean ACC Glu corr was higher in the NR than the R group after adjustment for age and sex ($P_{1,80} = 4.27; P = .04$). This was associated with an area under the curve for the group discrimination of 0.59. There were no group differences in striatal dopamine function or striatal Glu corr. The results provide partial further support for a role of ACC glutamate, but not striatal dopamine synthesis, in determining the nature of the response to antipsychotic medication. The low discriminative accuracy might be improved in groups with greater clinical separation or increased in future studies that focus on the antipsychotic response at an earlier stage of the disorder and integrate other candidate predictive biomarkers. Greater harmonization of multicenter PET and 1H-MRS may also improve sensitivity.

Key words: 1H-MRS/PET/antipsychotic response, treatment resistance/schizophrenia

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Introduction

The degree to which symptoms of schizophrenia will improve with antipsychotic medication is extremely variable. For some patients, antipsychotics can be very effective in improving symptoms. However, a majority of patients experience only a partial improvement,\(^1\)\(^3\) and around a third of all patients meet criteria for treatment-resistant schizophrenia (TRS), for which the only recommended antipsychotic is clozapine.\(^4\)\(^6\) The difficulty of identifying TRS by clinical criteria, combined with a reluctance to prescribe clozapine, leads to a delay in clozapine initiation during which time patients are exposed to ineffective medications and symptoms are active and disabling.\(^7\)

There is an initial indication that delay in clozapine prescription is associated with a worse response when clozapine is eventually prescribed.\(^8\)

Emerging biological and epidemiological evidence suggests that antipsychotic nonresponsive illness could be categorically distinct from antipsychotic responsive illness.\(^9\)\(^-\)\(^14\) Elucidating the pathophysiology of antipsychotic nonresponse could identify new targets for drug development and could also enable the development of predictive biomarkers to identify such patients early in the illness, allowing treatment with clozapine to begin earlier.

A prominent neurochemical hypothesis of schizophrenia centers on elevated dopamine synthesis and release in the striatum, arising from increased activity in mesostriatal dopaminergic neurons.\(^15\) The blockade of striatal D\(_2\) dopamine receptors is considered a critical feature of antipsychotic efficacy.\(^16\) While the response may require a threshold level of D\(_2\) occupancy, in antipsychotic nonresponsive schizophrenia, symptoms may persist despite high levels of D\(_2\) blockade.\(^7\)\(^,\)\(^16\) This raises the possibility that antipsychotic nonresponsive patients have a different pathophysiology that is not addressed by D\(_2\) blockade.\(^19\)

Recently, molecular imaging studies have shown that striatal dopamine synthesis capacity is lower in TRS relative to that in patients who respond to antipsychotics.\(^20\)\(^,\)\(^21\) In longitudinal studies, higher levels of striatal D\(_2\) occupancy by dopamine\(^22\)\(^,\)\(^23\) and striatal dopamine synthesis capacity\(^24\) are associated with a greater response to antipsychotic treatment. Thus, biomarkers of striatal hyperdopaminergia may be predictive of an increased likelihood to respond to first-line (D\(_2\) blocking) antipsychotic treatment.

If TRS is not associated with abnormal striatal dopamine synthesis capacity, then the pathophysiology probably lies elsewhere. One possibility is that TRS arises due to abnormal glutamatergic signaling, particularly in cortical areas.\(^25\) A series of cross-sectional studies have indicated that poor antipsychotic response is associated with a higher level of glutamate metabolites in the anterior cingulate cortex (ACC)\(^14\)\(^,\)\(^26\)\(^-\)\(^28\) relative to levels in patients who have shown a good response or healthy volunteers. In first-episode psychosis, a higher level of ACC glutamate is predictive of a worse response to antipsychotic treatment.\(^29\)

Higher frontal glutamate metabolites are also predictive of a poor response following reintiation of antipsychotic treatment.\(^30\) In the striatum, glutamate metabolites may be elevated at illness onset\(^31\)\(^,\)\(^32\) but the relationship with the antipsychotic response is less clear.\(^27\)\(^,\)\(^28\)\(^,\)\(^33\)\(^-\)\(^35\) These observations may be particularly important in the context of the substantial efforts to develop glutamatergic drugs for schizophrenia, as they may suggest that glutamate modulation may be more effective in TRS than in antipsychotic-responsive patients.

So far, cross-sectional studies of dopaminergic\(^20\)\(^-\)\(^23\) or glutamatergic\(^14\)\(^,\)\(^26\)\(^-\)\(^28\)\(^,\)\(^33\)\(^,\)\(^36\) function in relation to antipsychotic response have been single-center studies that have recruited relatively small and homogenous patient cohorts. A key step in scaling this research toward developing predictive biomarkers for future stratified clinical trials is to test for these associations in a larger, more clinically representative patient sample and to determine the accuracy of group discrimination. The main aim of the current study was, therefore, to determine if glutamate levels in the ACC and striatum and striatal dopamine synthesis capacity differentiate antipsychotic nonresponsive from antipsychotic responsive psychosis in a multicenter cross-sectional sample. We hypothesized that, compared with the antipsychotic-responsive group, antipsychotic nonresponse would be characterized by lower striatal dopamine synthesis capacity and higher glutamate levels in the striatum and ACC. A secondary aim was to investigate relationships between ACC and striatal glutamate and striatal dopamine synthesis capacity in the same individuals.

Methods

Regulatory Approvals

The study had NHS Research Ethics Committee (15/LO/0038) and Administration of Radioactive Substances Advisory Committee (630/3764/32558) approvals. Participation required the provision of written informed consent.

Participants

Study participants were recruited and assessed across 4 UK sites: King’s College London (KCL), University of Manchester (UoM), University of Edinburgh (UoE), and Cardiff University (CU). Inclusion criteria required that participants were aged between 18 and 65, met Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria for schizophrenia or schizoaffective disorder, and were able to understand and consent to the study procedures. Exclusion criteria included currently meeting International Classification of Diseases (ICD) criteria for harmful substance misuse, or psychotic disorder secondary to substance misuse, pregnancy, previous severe head injury involving loss of consciousness for >5 minutes, and for Magnetic Resonance Imaging (MRI) images, a history of severe head injury involving loss of consciousness, current neuroleptic medication, inability to follow instructions, or a condition that might limit MRI image quality.

Study procedures. Exclusion criteria included currently meeting International Classification of Diseases (ICD) criteria for harmful substance misuse, or psychotic disorder secondary to substance misuse, pregnancy, previous severe head injury involving loss of consciousness for >5 minutes, and for Magnetic Resonance Imaging (MRI)
Definition of Antipsychotic Responder and Antipsychotic Nonresponder groups

Antipsychotic Responders (R) were defined as having had (1) treatment with only 1 antipsychotic drug since illness onset, or, if there were any treatment changes, then these were due to adverse effects as opposed to nonresponse; (2) a CGI-SCH severity score of <4; (3) a PANSS total score of <60; and (4) a compliance rating scale (CRS) score >3.

Antipsychotic nonresponders (NR) were defined as having (1) documented treatment with at least 2 antipsychotics for >4 weeks each, at doses above the minimum therapeutic doses as defined by the British National Formulary; (2) a CGI-SCH severity score of >3; (3) a PANSS total score of at least 70; and (4) a CRS of >3. The targets for participant enrollment differed by site, but each site aimed to recruit a 1:1 ratio of R and NR.

Proton Magnetic Resonance Spectroscopy (1H-Magnetic Resonance Spectroscopy)

Glutamate levels were measured using 1H-magnetic resonance spectroscopy (1H-MRS) at 3 tesla at all 4 sites (see supplementary Methods). Non-rotated 1H-MRS voxels were positioned in the ACC (20 × 20 × 20 mm³; supplementary figure 1) and in the right striatum (20 × 20 × 20 mm³; supplementary figure 2). Spectra were acquired using Point RESolved Spectroscopy (PRESS, echo time = 35 ms; repetition time = 2000 ms; 128 averages, bandwidth/sample frequency ±2500 Hz, complex points = 4096), and analyzed in LCModel version 6.3-1L using a standard LCModel basis set. Representative spectra are provided in supplementary figure 3. Metabolite estimates were water-referenced. Gannet software (version 2.0, http://www.gabamrs.com/) co-registered the 1H-MRS voxel to the corresponding T1-weighted image to determine the voxel tissue composition. Metabolite values were corrected for voxel tissue content using the formula:

$$M_{corr} = M \ast (WM + 1.21 \ast GM + 1.55 \ast CSF) / (WM + GM)$$

where M is the uncorrected metabolite concentration, and WM, GM, and CSF indicate the percentages of tissue type in the voxel. Further details are provided in the supplementary information. The primary outcome variable was Glx. For completeness, data for glutamate plus glutamine (Glx), are also presented.

Quality of 1H-MRS was determined by a review of LCModel estimates of spectral line width and signal-to-noise ratio. Spectra were excluded under any of the following criteria: (1) absence of corresponding unsuppressed water acquisition; (2) compared with the overall mean for the voxel across all sites and participants, spectral line width was 2 standard deviations above; or (3) spectral signal-to-noise ratio was 2 standard deviations below. Individual metabolite concentration estimates associated with Cramér Rao lower bounds (CRLB) > 20% were excluded. We relied on these quality control procedures to identify and exclude any datasets potentially corrupted by motion or other artifacts.

3,4-Dihydroxy-6-[18F]Fluoro-L-Phenylalanine Positron Emission Tomography

Striatal dopamine function was measured using 3,4-dihydroxy-6-[18F]fluoro-L-phenylalanine (18F-DOPA) PET. The study acquired 18F-DOPA PET scans in participants who had also participated in 1H-MRS, at 2 sites (KCL and the UoM). To reduce the formation of radiolabeled 18F-DOPA metabolites, participants received carbidopa (150 mg) and entacapone (400 mg) orally 1 hour before 18F-DOPA imaging. Thirty seconds after the start of PET image acquisition, approximately 150 MBq of 18F-DOPA was administered by bolus intravenous injection. Emission data were acquired in list mode over the 95-minute period immediately post-injection.

Head movement was corrected for by frame-by-frame realignment using mutual information image registration. An 18F-DOPA template, together with a striatal atlas and cerebellum were nonlinearly normalized to each PET summation image in Statistical Parametric Mapping version 12 (http://www.fil.ion.ucl.ac.uk/spm) running in Matlab 2015b (Mathworks Inc.). This process allows automatic placement of volumes of interest (VOI) on individual PET images. The rate constant for the uptake of 18F-DOPA in the striatum (K1

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$$K_1$$ was calculated using graphical analysis adapted for a reference tissue input function, using the cerebellum as the reference region. We investigated $$K_1$$ across the whole
to evaluate the accuracy of ACC Glu levels in the R and NR groups returned an area under curve (AUC) of 0.59 (figure 2). Subsequent empirical cut-point estimation returned an optimal cut point of 0.98, which was associated with a Youden index of 0.22, sensitivity = 0.73, specificity = 0.49, and AUC of 0.61. There were no significant correlations between ACC Glu and PANSS scores or CPZÉ dose (N = 86; r = −0.04 to .15).

There was no association between striatal Glu_\text{corr} and Glx_\text{corr} and age (N = 83; Glu_\text{corr} r = −0.13, P = 0.25; Glx_\text{corr} r = −0.09, P = .44), or sex (Glu_\text{corr} T_{81} = 0.30; P = .77; Glx_\text{corr} T_{81} = 1.76; P = .08). There were also no significant effects of current tobacco or cannabis use or antipsychotic CPZÉ dose (P > .07). There was no between-group difference in striatal Glu_\text{corr} (F_{1,81} = 0.96; P = .33) or Glx_\text{corr} (F_{1,81} = 1.39; P = .24) (table 3), or significant correlations between striatal Glu_\text{corr} or Glx_\text{corr} and PANSS scores or CPZÉ dose (N = 83; r = −.17 to .11). Site differences were present across 1H-MRS data (supplementary tables 3 and 4; supplementary Results).

Striatal Dopamine Function

Striatal 18F-DOPA K_i values were not associated with age (N = 54; r = .07; P = .61), sex (T_{52} = 0.66; P = .51), tobacco (F_{2,52} = .17; P = .85), cannabis use (F_{1,52} = 1.20; P = .28), or antipsychotic CPZÉ dose (r = .06; P = .67). K_i values did not differ between the R and NR groups (F_{1,52} = 1.24; P = .27) (table 3, figure 1). ROC analysis of whole striatal 18F-DOPA K_i in antipsychotic R and NR returned an AUC of 0.59. K_i values were not associated with PANSS scores or CPZÉ dose (r = −.01 to .13).

Relationships Between Glutamate and Dopamine

There was no main effect of ACC Glu_\text{corr} on striatal 18F-DOPA K_i (F_{1,80} = 1.03; P = .31), but the interaction between ACC Glu_\text{corr} and group was significant (F_{1,50} = 6.53; P = .01). This was related to a positive relationship between ACC Glu_\text{corr} and striatal 18F-DOPA K_i in NR (N = 29; r = .37; P = .05) but not in R (N = 25; r = −.31; P = .13). Striatal Glu_\text{corr} was negatively associated with striatal 18F-DOPA K_i across the whole brain.

Glutamate Metabolite Levels

ACC Glu_\text{corr} and Glx_\text{corr} were related to age (N = 86; Glu_\text{corr} r = −.21; P = .05, Glx_\text{corr} r = −.27; P = .01) (supplementary figure 4), and sex (mean ± s.d. Glu_\text{corr} male: 0.10 ± 0.94; female: −0.52 ± 1.04; T_{84} = 2.21; P = .03; Glx_\text{corr} male: 0.11 ± 0.93; female: −0.56 ± 1.09; T_{84} = 2.40; P = .01). There were no significant effects of current tobacco or cannabis use or antipsychotic CPZÉ dose (P > .14). The NR group had significantly higher ACC Glu_\text{corr} levels compared with the R group after adjustment for age and sex (main effect of group: Glu_\text{corr} F_{1,81} = 4.99; P = .03; η^2 = 0.06; table 3, figure 1). A similar result at threshold levels of significance was detected for Glx_\text{corr} (F_{1,81} = 3.92; P = .05; η^2 = 0.05; table 3, figure 1). Interactions between group and age or group and sex did not show any evidence of an effect. After excluding participants who were currently taking benzodiazepines (n = 10) or antidepressants (n = 14), the effects of group on Glu_\text{corr} remained borderline significant (P = .04 and P = .07, respectively). The effect of group was not significant in unadjusted analysis (Glu_\text{corr} F_{1,84} = 2.37; P = .13; Glx_\text{corr} F_{1,84} = 1.77; P = .19; table 3). ROC analysis of non-adjusted ACC Glu_\text{corr} levels in the R and NR groups returned an area under curve (AUC) of 0.59 (figure 2). Subsequent empirical cut-point estimation returned an optimal cut point of 0.98, which was associated with a Youden index of 0.22, sensitivity = 0.73, specificity = 0.49, and AUC of 0.61. There were no significant correlations between ACC Glu_\text{corr} and PANSS scores or CPZÉ dose (N = 86; r = −0.04 to .15).
Dopamine, Glutamate, and Antipsychotic Response

Discussion

The main aim of this study was to test whether measures of dopamine synthesis capacity in the striatum [20,21] and glutamate concentration (Glu corr) in the ACC [14,26] could differentiate patients with antipsychotic-nonresponsive from antipsychotic-responsive schizophrenia. In line with our hypothesis, we found that ACC mean Glu corr was higher in the NR compared with the R group, which was significant when age and sex were included in the model. There were no between-group differences in striatal dopamine function nor in striatal Glu corr. These results are partially consistent with previous evidence that the degree of antipsychotic response in schizophrenia may be related to ACC glutamate concentration [14,26-30] but not with evidence linking response to striatal dopamine function [20-24]. The AUC for both glutamate and dopamine measures indicated low discriminative accuracy. This indicates that these measures alone are unlikely to be sufficiently sensitive to identify chronic patients with antipsychotic nonresponsive from responsive illness in routine clinical practice.

The higher mean ACC Glu corr in NR is broadly consistent with cross-sectional [14,26-28] and prospective studies [29,30] associating higher levels of ACC glutamatergic

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Table 1. Clinical and Demographic Characteristics of the 'H-MRS Sample

<table>
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<tr>
<th>Sample size</th>
<th>48</th>
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<tr>
<td>Age (years)</td>
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<td>28.9 ± 7.5</td>
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<td>Ethnicity</td>
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<td>3</td>
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<tr>
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<td>Schizoaffective disorder</td>
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<td>Amisulpride</td>
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<td>CPZE mg/day</td>
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<td>Duration of illness</td>
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<td>17/324</td>
<td>.59</td>
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<td>Cannabis ever Y/N</td>
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<td>.25</td>
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<tr>
<td>Cannabis current Y/N</td>
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<td>PANSS positive</td>
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<td>PANSS negative</td>
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<tr>
<td>PANSS total</td>
<td>52.7 ± 6.7</td>
<td>86.7 ± 8.8</td>
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Note: Data are expressed as mean ± standard deviation unless otherwise specified. 'H-MRS, 'H-magnetic resonance spectroscopy; CNS, central nervous system; CPZE, chlorpromazine equivalent dose; PANSS, Positive and Negative Syndrome Scale. Current cannabis use was defined as use within the last 7 days. P values relate to independent samples t-tests, Chi square, or Fisher’s exact test as appropriate. There were no significant group differences in clinical or demographic characteristics other than in PANSS scores.

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sample ($F_{1,50} = 4.97; P = .03$), and there was no group by striatal Glu corr interaction ($F_{1,50} = 2.02; P = .16$).
Table 2. Clinical and Demographic Characteristics of the $^{18}$F-DOPA PET Sample

<table>
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<th>Antipsychotic Responder</th>
<th>Antipsychotic Nonresponder</th>
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<td>Sample size</td>
<td>25</td>
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<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.8 ± 9.6</td>
<td>30.0 ± 8.3</td>
<td>.86</td>
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<tr>
<td>Sex male/female</td>
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<td>24/5</td>
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<td>156.06 ± 15.93</td>
<td>155.01 ± 14.89</td>
<td>.81</td>
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Note: Data are expressed as mean ± standard deviation unless otherwise specified. $^{18}$F-DOPA PET, 3,4-dihydroxy-6-[18F]fluoro-L-phenylalanine positron emission tomography; CNS, central nervous system; CPZE, chlorpromazine equivalent dose; PANSS, Positive and Negative Syndrome Scale. Current cannabis use was defined as use within the last 7 days. $P$ values relate to independent samples $t$-tests, Chi square, or Fisher’s exact test as appropriate. There were no significant group differences in clinical or demographic characteristics other than in PANSS scores.

metabolites with a poor antipsychotic response. However, these studies differ in the glutamate measurement (glutamate or Glx) or Glu ratios (to creatine), sometimes corrected for voxel tissue composition. In addition, 2 studies did not detect differences in ACC glutamate metabolites between a TRS and antipsychotic R group, and 1 found ACC Glx, but not glutamate, was elevated in patients with ultra-resistant schizophrenia (URS) compared with healthy volunteers, but not in TRS or URS compared with antipsychotic responders. Together with the current findings, the overall literature not only may indicate an association between elevated ACC glutamatergic metabolites and antipsychotic nonresponse but also suggests that effect sizes may be small and influenced by methodological factors and sample characteristics. In terms of biological mechanism, one explanation is that patients who are less likely to respond to treatment exhibit greater elevations in frontal glutamate metabolites, potentially linked to a greater degree of N-methyl-D-aspartate (NMDA) receptor or gamma-aminobutyric acid (GABA)ergic dysfunction resulting from genetic or developmental mechanisms. In addition, antipsychotic medication could have less impact on frontal glutamatergic dysfunction in those who respond poorly to treatment. In the striatum, the lack of group difference in Glu$_{corr}$ is consistent with 2 recent cross-sectional studies examining TRS to first-line antipsychotic responders or healthy volunteers. This could indicate that elevations in striatal...
glutamate at illness onset\textsuperscript{31,32} are reduced during antipsychotic treatment\textsuperscript{34} irrespective of the response category. Alternatively, as we also observed no group difference in striatal 18F-DOPA $K_{\text{cer}}$, these findings may indicate that the participants selected for our samples did not markedly differ in the overall striatal pathophysiology.

In the 54 patients with dopamine measures evaluated across 2 sites, there was no group difference in striatal 18F-DOPA $K_{\text{cer}}$, indicating similar levels of presynaptic dopamine synthesis and storage capacity. This finding differs from previous smaller studies that have associated increased striatal dopamine function with a good antipsychotic response.\textsuperscript{30-34} Using the same 18F-DOPA PET method, we reported lower striatal $K_{\text{cer}}$ in 12 patients with TRS compared with 12 antipsychotic responders.\textsuperscript{20} In another study comparing a TRS group currently taking clozapine with antipsychotic-responsive patients, the resistant group again had lower $K_{\text{cer}}$ than the responders.\textsuperscript{21} In first-episode psychosis, striatal $K_{\text{cer}}$ was positively related to subsequent antipsychotic response.\textsuperscript{24} Lower

\begin{table}[h!]
\centering
\caption{Glutamate and Dopamine Measures in the Antipsychotic Responder and Antipsychotic Nonresponder Groups\textsuperscript{1}}
\begin{tabular}{llll}
\hline
 & Antipsychotic Responder & Antipsychotic Nonresponder & ES, or GLM, Group & GLM: Group, Age, and Sex \\
\hline
\hline
\textsuperscript{1}H-MRS glutamate (Glu\textsubscript{m}) & & & & \\
Anterior cingulate cortex & & & & \\
KCL & 19.39 ± 3.56 (16) & 20.29 ± 2.63 (18) & $d = 0.29$ & \\
UoM & 13.74 ± 1.75 (17) & 14.28 ± 1.59 (15) & $d = 0.74$ & \\
UoE & 12.57 ± 1.18 (7) & 13.33 ± 1.90 (5) & $d = 0.72$ & \\
CU & 11.65 ± 1.68 (5) & 11.57 ± 2.22 (3) & $d = 0.04$ & \\
Overall & $-0.15 ± 1.05$ (45) & $0.17 ± 0.88$ (41) & $F_{1,84} = 2.37; P = .13; \eta^2 = 0.03$ & $F_{1,81} = 4.99; P = .03; \eta^2 = 0.06$ \\
Right striatum & & & & \\
KCL & 10.78 ± 1.47 (16) & 11.07 ± 1.62 (18) & $d = 0.19$ & \\
UoM & 8.35 ± 1.12 (17) & 7.89 ± 1.01 (15) & $d = 0.43$ & \\
UoE & 8.67 ± 1.13 (5) & 7.41 ± 1.21 (4) & $d = 1.08$ & \\
CU & 9.00 ± 3.15 (4) & 8.58 ± 1.43 (4) & $d = 0.17$ & \\
Overall & $0.10 ± 1.00$ (42) & $-0.11 ± 0.96$ (41) & $F_{1,84} = 1.77; P = .19; \eta^2 = 0.02$ & $F_{1,81} = 3.92; P = .05; \eta^2 = 0.05$ \\
\hline
\hline
\textsuperscript{1}H-MRS Glx (Glx\textsubscript{m}) & & & & \\
Anterior cingulate cortex & & & & \\
KCL & 25.86 ± 5.28 (16) & 26.85 ± 4.28 (18) & $d = 0.21$ & \\
UoM & 19.67 ± 2.56 (17) & 20.17 ± 1.87 (15) & $d = 0.22$ & \\
UoE & 18.59 ± 1.61 (7) & 19.62 ± 1.87 (5) & $d = 0.59$ & \\
CU & 14.82 ± 2.15 (5) & 15.36 ± 2.14 (3) & $d = 0.34$ & \\
Overall & $-0.13 ± 1.06$ (45) & $0.15 ± 0.88$ (41) & $F_{1,84} = 1.39; P = .24; \eta^2 = 0.01$ & \\
Right striatum & & & & \\
KCL & 15.08 ± 2.65 (16) & 14.27 ± 3.15 (18) & $d = 0.29$ & \\
UoM & 13.34 ± 1.81 (17) & 12.89 ± 2.33 (15) & $d = 0.22$ & \\
UoE & 16.69 ± 5.84 (5) & 12.70 ± 3.20 (4) & $d = 0.85$ & \\
CU & 11.75 ± 3.53 (4) & 14.10 ± 3.41 (3) & $d = 0.68$ & \\
Overall & $0.13 ± 0.91$ (42) & $-0.13 ± 1.04$ (40) & $F_{1,84} = 2.41; P = .12; \eta^2 = 0.05$ & \\
\hline
\hline
\textsuperscript{18}F-DOPA PET $K_{\text{cer}}$ & & & & \\
Whole striatum & & & & \\
KCL & 0.0125 ± 0.0095 (11) & 0.0128 ± 0.0010 (16) & $d = 0.04$ & \\
UoM & 0.0139 ± 0.0013 (14) & 0.0143 ± 0.0010 (13) & $d = 0.34$ & \\
Overall & $-0.18 ± 1.05$ (25) & $0.12 ± 0.93$ (29) & $F_{1,52} = 2.41; P = .12; \eta^2 = 0.02$ & \\
Sensorimotor striatum & & & & \\
KCL & 0.0126 ± 0.0011 (11) & 0.0149 ± 0.0010 (16) & $d = 2.19$ & \\
UoM & 0.0149 ± 0.0016 (14) & 0.0156 ± 0.0001 (13) & $d = 0.52$ & \\
Overall & $-0.25 ± 0.96$ (25) & $0.16 ± 0.95$ (29) & $F_{1,52} = 1.39; P = .24; \eta^2 = 0.01$ & \\
Associative striatum & & & & \\
KCL & 0.0126 ± 0.0010 (11) & 0.0128 ± 0.0010 (16) & $d = 0.2$ & \\
UoM & 0.0136 ± 0.0014 (14) & 0.0138 ± 0.0010 (13) & $d = 0.16$ & \\
Overall & $-0.13 ± 1.07$ (25) & $0.09 ± 0.95$ (29) & $F_{1,52} = 0.62; P = .44; \eta^2 = 0.01$ & \\
Limbic striatum & & & & \\
KCL & 0.0122 ± 0.0010 (11) & 0.0128 ± 0.0010 (16) & $d = 0.6$ & \\
UoM & 0.0136 ± 0.0012 (14) & 0.0139 ± 0.0010 (13) & $d = 0.3$ & \\
Overall & $-0.14 ± 1.05$ (25) & $0.12 ± 0.99$ (29) & $F_{1,52} = 0.86; P = .36; \eta^2 = 0.02$ & \\
\hline
\end{tabular}
\end{table}

\textit{Note:} Data are presented by site and as overall Z-score. Data are expressed as mean ± standard deviation (number of observations). ES, effect size; GLM, general linear model; \textsuperscript{1}H-MRS, \textsuperscript{1}H-magnetic resonance spectroscopy; \textsuperscript{18}F-DOPA PET, 3,4-dihydroxy-6-[\textsuperscript{18}F]fluoro-L-phenylalanine positron emission tomography; KCL, King’s College London; UoM, University of Manchester; UoE, University of Edinburgh; CU, Cardiff University.
availability of D₂ receptors for radiotracer binding, which may reflect increased D₂ occupancy by dopamine, was also associated with subsequent response to 6 weeks of treatment with amisulpride. A dopamine depletion study also indicated that higher levels of synaptic dopamine are predictive of a good antipsychotic response.

Within cortico-striatal networks, counterbalancing pathways and feedback loops regulate neurotransmitter balance. For example, glutamate release can both increase and decrease dopamine levels, and dopamine receptor activation modulates glutamate release, and dopamine neurons may co-release glutamate. In NR only, ACC Glu<sub>corr</sub> was positively correlated to striatal 18F-DOPA K<sub>cer</sub><sup>-1</sup>. In contrast, striatal Glu<sub>corr</sub> and striatal K<sub>cer</sub><sup>-1</sup> were negatively correlated across the whole sample. We previously found that ACC glutamate and striatal K<sub>cer</sub><sup>-1</sup> were negatively correlated in patients with early psychosis and no significant relationship between these variables in healthy controls. Correlations between striatal glutamate and striatal K<sub>cer</sub><sup>-1</sup> have not previously been investigated in patients but are positively correlated in healthy volunteers. Together these findings could suggest that glutamate-dopamine relationships may change with illness onset, progression, or antipsychotic response. One potential mechanism may involve alterations in the balance between the opposing influences of direct and indirect glutamatergic projections from the cortex to mesostriatal dopamine neurons. This interpretation could be further examined in animal models and in longitudinal patient studies over the course of antipsychotic treatment.

Relative to previous research, a strength of the current study is the large sample size, which reduces the risk of false-positive findings. There are also design differences compared with previous studies that may contribute to the lack of group difference in dopamine measures and the marginal group difference in ACC glutamate measures. The criteria used to define the antipsychotic NR and R groups may have led to less clinical separation of these groups than in our previous 18F-DOPA PET and 1H-MRS studies. In the current study, the R group criteria allowed a higher level of symptom severity, while the NR group was less symptomatic and met fewer of the criteria for establishing treatment resistance.

Further strengths of our study include the establishment of collaborative multicenter 1H-MRS and PET imaging in the UK, which allowed us to achieve a large sample size for both 1H-MRS and 18F-DOPA PET imaging. With a view toward developing predictive biomarkers for stratified clinical trials, we formally assessed the accuracy of these measures for classifying antipsychotic response and nonresponse. In our previous multicenter 1H-MRS study in first-episode psychosis, our a priori outcome variable was glutamate in ratio to creatinine. In the current multicenter study, we were able to correct glutamate estimates for voxel tissue composition (Glu<sub>corr</sub>, our primary outcome variable) by applying the same software (Gannet) to extract voxel tissue fractions.
in data acquired across different MRI systems. This has the advantage that potential influences of voxel creatine content (otherwise often used as an internal standard) are avoided (see supplementary Discussion).

Our study also has several limitations. It is not possible to establish the proportion of the NR group that would meet Treatment Response and Resistance In Psychosis (TRRIP) consensus requirements for “TRS” as we did not include a prospective trial of antipsychotic medication or collect objective evidence of adherence. As we only collected clinical data at a single time-point, we did not establish the stability of R/NR status. These factors could have led to a less clinical separation between the R and NR groups and reduced our ability to observe differences in glutamate or dopamine measures. While the R and NR groups did not differ in duration of illness or current antipsychotic dose, the inclusion of patients who had been taking antipsychotic medication for some time may have influenced both 18F-DOPA \( K_{\text{corr}} \) and \( ^1\text{H}-\text{MRS} \) glutamate values. The absence of a healthy control group means that we are unable to interpret \( ^1\text{H}-\text{MRS} \) glutamate and 18F-DOPA \( K_{\text{corr}} \) values in comparison to what may be expected in psychiatrically healthy individuals. Neither the \( ^1\text{H}-\text{MRS} \) glutamate nor 18F-DOPA PET dopamine imaging measures specifically index neurotransmission. \( ^1\text{H}-\text{MRS} \) estimates the total amount of intracellular glutamate in the voxel, including neurons as well as other cell types. 18F-DOPA is used to index presynaptic dopamine synthesis and storage capacity rather than dopamine release. Previous studies of glutamate in relation to antipsychotic response/nonresponse at a field strength of 3 tesla have detected differences in glutamate or Glx.\(^{14,26-30}\) Although glutamate values obtained with short TE PRESS at 3 tesla are routinely reported and published, glutamate can be difficult to reliably quantify without specialized sequences. Despite the fitting methods, the glutamate signal is likely to include some contamination from glutamine and macromolecules and this may vary across the site. As for other imaging modalities, there was between-scanner variation in both \( ^1\text{H}-\text{MRS} \) and PET data, which will have reduced the sensitivity of our study. Although we did not detect significant site by group interactions, we cannot exclude the possibility that scanner variation impacted our results. Between-scanner variation is discussed further in the supplementary Discussion.

The results highlight the importance of considering age and sex effects in future studies of glutamate in schizophrenia. A lower level of ACC Glu \(_{\text{corr}} \) in older patients with schizophrenia is consistent with other reports.\(^{59,72}\) There is some evidence that age-related decline in ACC glutamate\(^{58}\) is greater in schizophrenia than in healthy aging,\(^{66,72}\) although other studies have reported similar rates of ACC glutamate decrease in patients and healthy volunteers.\(^{59,73}\) Our finding of higher ACC Glu \(_{\text{corr}} \) levels in male compared with female participants is less clear due to the relatively small number of female participants.

In conclusion, our findings support previous research linking increases in ACC glutamate to a poor antipsychotic response. However, the poor group discrimination suggests that glutamate \( ^1\text{H}-\text{MRS} \) or 18F-DOPA measures alone cannot distinguish between antipsychotic responsive and nonresponsive groups after a mean of 5–6 years of illness. Multicenter, cross-platform \( ^1\text{H}-\text{MRS} \) and PET studies are rare, and in future studies, sensitivity may be improved through greater harmonization. It is also possible that glutamatergic and dopaminergic markers may have more predictive power earlier in the course of the disorder before the potentially confounding effects of treatment and illness duration have taken effect. They may also have increased predictive power in combination with other factors that may associate with antipsychotic response, such as clinical and demographic measures,\(^{11-13}\) brain network connectivity,\(^{74}\) genetic factors,\(^{75,76}\) and blood measures.\(^{77}\) We plan to address these issues in future studies.

Supplementary Material
Supplementary material is available at Schizophrenia Bulletin.

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Data Availability
At the time of submission, the data governance frameworks are being put in place to make a fully anonymized version of the data available to the wider research community via TranSMART data sharing platform: https://transmartfoundation.org/, which will be hosted at the MRC eMedLab: https://www.emedlab.ac.uk/. To apply...
for access to the data, please contact J.H.M. at james.maccabe@kcl.ac.uk.

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