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The association between N-Methyl-D-Aspartate Receptor availability and glutamate levels: a multi-modal PET-MR brain imaging study in first episode psychosis and healthy controls

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Abstract

Background:
Evidence from post-mortem studies and in vivo imaging studies suggests there may be reduced N-Methyl-D-Aspartate Receptor (NMDAR) levels in the hippocampus in patients with schizophrenia. Other studies have reported increased glutamate in striatum in schizophrenia patients. It has been hypothesised that NMDAR hypofunction leads to the disinhibition of glutamatergic signalling, however, this has not been tested in vivo.

Methods:
In this study we investigated the relationship between hippocampal NMDAR and striatal glutamate using simultaneous Positron Emission Tomography-Magnetic Resonance (PET-MR) imaging. We recruited 40 volunteers to this cross-sectional study; 21 patients with schizophrenia, all in their first episode of illness, and 19 healthy controls.

We measured hippocampal NMDAR availability using the PET ligand $[^{18}\text{F}]\text{GE179}$. This was indexed relative to whole brain as the distribution volume ratio (DVR). Striatal glutamatergic indices (glutamate and Glx) were acquired simultaneously, using combined PET-MR proton magnetic resonance spectroscopy ($^1\text{H}$-MRS).

Results:
A total of 33 individuals (15 healthy controls, 18 patients) were included in the analyses (mean [SD] age of controls, 27.31(4.68) years; mean [SD] age of patients, 24.75 (4.33), 27 male and 6 female). We found an inverse relationship between hippocampal DVR and striatal glutamate levels in people with first-episode psychosis (rho = -0.74, p <0.001) but not in healthy controls (rho = -0.22, p = 0.44).

Conclusion:
This study suggests lower NMDAR availability in the hippocampus may drive increased striatal glutamate levels in patients with schizophrenia. Further work is required to determine whether these findings may yield new targets for drug development in schizophrenia.
Introduction

Schizophrenia is a chronic mental illness with a lifetime prevalence of approximately 1%. (McCutcheon et al., 2020) It is the third most disabling illness worldwide. (Üstün et al., 1999) Treatments are ineffective in about one third of patients (Howes et al., 2017; Beck et al., 2019) and are poorly tolerated because of cardiometabolic and other side-effects. (Howes et al., 2004; Mitchell et al., 2013; Pillinger, Beck, Gobjila, et al., 2017; Pillinger, Beck, Stubbs, et al., 2017) Cognitive symptoms are particularly resistant to treatment. (Goff, Hill and Barch, 2011) This highlights the need to understand the pathophysiology of schizophrenia in order to develop new treatment approaches. (Kaar et al., 2020)

Multiple lines of evidence indicate that glutamatergic dysregulation is a key aspect of the pathophysiology of schizophrenia. (McCutcheon, Krystal and Howes, 2020) Healthy control studies show that the administration of NMDAR antagonists such as ketamine induces schizophreniform symptoms (Beck et al., 2020) and increases brain glutamate (Stone et al., 2012) and glutamine (Rowland et al., 2005) levels, suggesting a link between NMDAR hypofunction and increased brain glutamate levels. (Moghaddam et al., 1997; Lorrain et al., 2003; Iltis et al., 2009; Chowdhury et al., 2012) Genetic, (Consortium et al., 2014) post-mortem (Rubio, Drummond and Meador-Woodruff, 2012) and animal studies (Belforte et al., 2010; Korotkova et al., 2010) also implicate N-Methyl-D-Aspartate Receptor (NMDAR) hypofunction in schizophrenia. Post-mortem data report reduced MK801 binding (Beneyto et al., 2007) and lower mRNA and protein levels of the essential NMDAR subunit, NR1, in the hippocampus. (Vrajová et al., 2010) Moreover, in vivo evidence from SPECT and PET studies show lower binding of NMDAR selective tracers in the hippocampus relative to the whole brain. (Pilowsky et al., 2006; Beck et al., 2021)

Striatal dysfunction is strongly implicated in the pathophysiology of schizophrenia. (McCutcheon, Abi-Dargham and Howes, 2019) For example, abnormalities in striatal neural transmission directly contribute not only to the positive and negative psychotic symptoms (McCutcheon, Abi-Dargham and Howes, 2019) but also abnormal cognitive processing observed in schizophrenia. (Simpson, Kellendonk and Kandel, 2010; Goodroe, Starnes and Brown, 2018; Borgan, O’Daly, et al., 2019) The striatum receives extensive glutamatergic projections from the hippocampus. (Selden et al., 1994; Grace and Gomes, 1999) and evidence shows that hippocampal dysfunction directly influences striatal function. (Lodge and Grace, 2011; Grace, 2012; Modinos et al., 2015, 2021) Magnetic Resonance Spectroscopy studies provide evidence for increased glutamate levels in the striatum of patients with schizophrenia. (Merritt et al., 2016; Nakahara et al., 2021) consistent
with the hypothesis that NMDAR hypofunction in the hippocampus results in increased glutamate levels in the striatum in schizophrenia. However, it remains unknown if hippocampal NMDAR levels are associated with striatal glutamate levels in patients with schizophrenia. We sought to test this hypothesis by using combined PET-MR imaging to simultaneously measure both NMDAR availability in the hippocampus and glutamate levels in the striatum. In addition, given the evidence to support the involvement of fronto-striatal interactions in schizophrenia (McCutcheon et al., 2021) and to assess the specificity of the relationship between hippocampal NMDAR and striatal glutamate we included an exploratory analysis to determine if there is an association between frontal NMDAR availability and striatal glutamate levels.

Materials and Methods
Approval was obtained by the West London & GTAC Research Ethics Committee (reference: 16/LO/0130) and the Administration of Radioactive Substances Advisory Committee. Volunteers demonstrated capacity and provided written informed consent to participate. We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines for case-control studies. (von Elm et al., 2008)

A total of 32 patients were screened for eligibility, 25 were eligible, and 21 were included in the study. 36 healthy volunteers were screened for eligibility as controls, 34 were eligible, and 19 were included in the study. Participants who were eligible, but were not included in the study, withdrew following screening prior to scanning.

A total of 40 individuals participated, including 21 patients (n = 12 antipsychotic-free and n = 9 treated with antipsychotics) and 19 healthy controls. The PET but not MRS data for 19 patients and 18 controls have been published previously. (Beck et al., 2021)

Patients with First Episode Psychosis (FEP) were recruited from Early Intervention services in London, United Kingdom. Data were collected from November 7th 2016 to August 2nd 2019. Inclusion criteria were as follows: a diagnosis of a DSM-IV psychotic disorder according to the Structured Clinical Interview of DSM-IV-TR Axis I Disorders-Patient Edition, (First, M. B., Spitzer, R. L., Gibbon M., and Williams, 2002) fulfilling criteria for having a first episode of psychosis, (Breitborde, Srihari and Woods, 2009) and less than 5 years’ illness duration.
For comparison, a sample of healthy control subjects was included. They were matched to the patients on the basis of age (+/- 3 years) and sex. Their inclusion criteria were as follows: no current or lifetime history of Axis I Disorder as determined by the Structural Clinical Interview of DSM-IV-TR Axis I Disorders-Patient Edition.\(^{(\text{First, M. B., Spitzer, R.L, Gibbon M., and Williams, 2002})}\)

Exclusion criteria for all volunteers were as follows: history of significant head trauma (such as loss of consciousness > 1 minute or requiring hospital admission), dependence on illicit substances or alcohol, positive urine drug test (SureScreen Diagnostics, Derby, UK) for substances that might affect NMDAR (e.g. stimulants) on the day of scanning, medical comorbidity (other than minor illnesses), current use (within 3 months) of any of the following drugs which may interfere with NMDAR; antidepressants,\(^{(\text{McGinnity et al., 2015})}\) mood stabilizers (Zeise, Kasparow and Ziegglänsberger, 1991; O’Donnell et al., 2003; Basselin et al., 2006) benzodiazepines, (Van Sickle et al., 2002) and contraindications to scanning (such as pregnancy).

Participants with psychosis were classified as antipsychotic-free if they had been free from antipsychotic treatment for at least 6 weeks (oral) or 6 months for depot formulations.\(^{(\text{Jauhar et al., 2019})}\) Antipsychotic-naïve was defined as never having had antipsychotic treatment.

**Clinical and Demographic Variables**

Current age and illness duration were recorded (see Table 1). Clinical symptom severity was determined using the Positive and Negative Syndrome Scale (PANSS).\(^{(\text{Kay, Fiszbein and Opler, 1987})}\) Psychotropic medication histories were recorded, and equivalent chlorpromazine doses were calculated using the method reported by Leucht et al.\(^{(\text{Leucht et al., 2014})}\)

**PET-MR scanning**

**PET acquisition**

All participants underwent a dynamic, continuous 90-minute simultaneous PET-MR acquisition after a bolus injection of \(^{18}\text{F}\)GE-179 (mean [SD], 140.31 [9.04] MBq) using a 3T PET/MR scanner (Biograph mMR, Siemens Healthcare, Erlangen, Germany). In parallel to PET/MR imaging, continuous arterial sampling using an MR compatible blood sampler (http://www.swisstrace.ch/blood-sampler-twilite.html) was performed for the first 16 minutes followed by 6 discrete samples. A T1-weighted structural Magnetization Prepared Rapid Gradient-Echo (MP-RAGE) image was acquired for co-registration. At the end of the session, a separate low dose CT scan (140 kV, 10 mA, helical acquisition) of the subject’s head was acquired on a GE Discovery DST 710 PET/CT (GE Healthcare, Chicago, Illinois,
United States of America), and used for tissue attenuation correction during the PET image reconstruction.

**1H-MRS acquisition:**

MRI data were acquired in the same scanning session as the PET data using the combined PET-MR. A sagittal 3D T1-weighted structural MRI scan (MP-RAGE) was acquired to enable 1H-MRS voxel prescription and segmentation. Proton MRS spectra were acquired for the right striatum. The striatal voxel (8ml - 20 x 20 x 20 mm) was placed at the lower end of the right dorsal caudate. It was located 3mm dorsal to the anterior commissure, including the maximum amount of grey matter, with dorsal extension (thickness) of 2 cm (Figure 1) based on a previous study in first-episode psychosis.(De La Fuente-Sandoval et al., 2013)

Proton MRS spectra (Point RESovled Spectroscopy, echo time 30 ms, repetition time 3000 ms, number of acquisition points 2048, acquisition bandwidth 2500 Hz, averages 128) were acquired. CHEmically Selective Suppression (CHESS) was used to supress the water signal. Water unsuppressed data were acquired with a separate acquisition with 16 averages. Shimming and water suppression were optimized before each scan using vendor automated adjustments. The spectra were further manually shimmed to achieve full width at half maximum < or equal 20 Hz (calculated based on magnitude spectra). Glutamate signal at 3T includes some contribution from glutamine, of about 10-15%. (Snyder and Wilman, 2010)

**Analysis of PET data**

NMDAR availability was determined as the $[^{18}F]$GE-179 volume of distribution ($V_r$, cm$^3$/mL) calculated using the standard 2-tissue compartmental modelling method with a metabolite-corrected arterial plasma input function. Prior to kinetic modelling, all the individual PET data underwent the same image processing pipeline to measure and correct for subject motion, segment brain tissues and extract $[^{18}F]$GE179 tracer activity in the main regions of interest. For comparison with previous results,(Pilowsky et al., 2006) PET analysis was performed using the distribution volume ratio (DVR). The DVR was calculated for the hippocampus and frontal cortex using the whole brain as the normative region. Full details of the PET acquisition and analysis are in a previous publication.(Beck et al., 2021)
Analysis of $^1$H-MRS data

LC-model version 6.3-1L was used to estimate the water-scaled glutamate (primary outcome measure) and Glx concentrations. (‘LCModel’, no date) Eddy current correction was applied. Spectra were inspected visually, and metabolite analyses were restricted to Cramer-Rao lower bounds (CRLB) for glutamate ≤ 20 % and signal-to-noise ratio ≥ 10. The CRLB is a reliability indicator, as it is a lower bound estimate of the standard deviation of the estimated concentration. Percentage standard deviation values for CRLB were taken from the LCModel output. SPM 12 and Gannet 3.1 were used to identify the amounts of white matter, grey matter, and CSF in the $^1$HMRS voxel prescribed in the striatum. The following correction (Pretzsch et al., 2019) was subsequently applied to correct for CSF content within the voxel (where $M$ = raw metabolite value, GM = Grey matter, WM = White Matter, CSF = Cerebrospinal fluid). T1 and T2 corrections were not applied for the tissue water, except for assuming $T2 = 80$ ms for tissue water.

$$M_{corr} = \frac{M \ast (1.207 \ast GM + WM + 1.548 \ast CSF)}{(1 - CSF)}$$

Statistical Analysis

Statistical analysis was done with R version 3.3.2 and SPSS version 22 (IBM Corp), with significance set at $p < 0.05$ (two-tailed).

Data normality was assessed using the Shapiro-Wilk test. Demographics, and experimental variables were compared across groups using independent-samples t-tests for parametric and Mann-Whitney tests for non-parametric data.

To test our main hypothesis that hippocampal NMDAR availability is negatively associated with increased glutamate in the striatum we conducted a Spearman’s correlation as the hippocampal NMDAR data were not normally distributed. For completeness, we also conducted this correlation with Glx. As exploratory analyses we looked at the association between NMDAR availability in the frontal cortex and glutamatergic indices, again using Spearman’s correlation due to non-normally distributed data. We included two measures of NMDAR availability in the analysis (DVR and $V_T$). DVR is our primary outcome measure as it had been used as the primary outcome in a prior study (Pilowsky et al., 2006) and because we found greater noise in the $V_T$ values, which showed more than double the variability seen with DVR (coefficients of variation for the hippocampus in
healthy controls were 18.2% and 7.1% respectively). (McGinnity et al., 2014; Beck et al., 2021) However, we include $V_f$ as well for completeness. Where there were significant relationships, we conducted exploratory sub-group analyses to investigate if current antipsychotic treatment influenced the relationships.

To determine whether striatal glutamate or Glx levels were higher in patients compared to controls, an independent samples t-test was used. To compare group differences in hippocampal and frontal cortex DVR Mann-Whitney tests were used as data were not normally distributed. Independent t-tests and Mann-Whitney tests were used, as appropriate, for comparing group key markers of spectral quality (Cramer-Rao Lower Bound, Full Width at Half Maximum, Signal-to-Noise Ratio, Gray Matter %, White Matter%, Cerebrospinal Fluid %).

**Results**

**Descriptive Data**

Of the 40 individuals who participated, four healthy controls were excluded due to technical reasons (failure to acquire data due to a malfunction of the arterial line (n=1); poor quality proton MRS spectra (CRLB values >20% and/or signal-to-noise values < 10 in relation to the concentration of glutamate) (n=2), and poorly located striatal voxel (n=1)). Three patients were excluded due to a positive urine test for cocaine (n=1), PET quality control failure - coefficient of variation for $V_f$ estimate >20% (n=1); or poor-quality proton MRS spectra (n=1). Therefore, the analysis of the association between NMDAR availability (hippocampus or frontal cortex) and striatal glutamate levels included 15 healthy controls and 18 patients (11 antipsychotic free), mean age 25.91(4.61), 27 male and 6 females. There was no significant difference found for age ($t_{31} = 1.61, p = 0.11$). Degree of medication exposure did not correlate with striatal glutamate or Glx concentrations (glutamate $r = 0.38, p = 0.40$, Glx $r = 0.42, p = 0.36$), hippocampal DVR ($r = -0.13, p = 0.79$), or frontal DVR ($r = -0.18, p = 0.70$). Descriptive data for this sample are presented in Table 1.
Table 1. Clinical and demographic variables

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy Volunteers (n = 15)</th>
<th>Patients with FEP (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean (SD)</td>
<td>24.75 (4.33)</td>
<td>27.31 (4.68)</td>
</tr>
<tr>
<td>Sex, No. male/female</td>
<td>12/3</td>
<td>15/3</td>
</tr>
<tr>
<td>Diagnosis, schizophrenia (n)/ schizoaffective disorder (n)</td>
<td>18/0</td>
<td>18/0</td>
</tr>
<tr>
<td>Illness duration, months, mean (SD)</td>
<td>21.72 (15.82)</td>
<td>8/3/7</td>
</tr>
<tr>
<td>Antipsychotic free/ naïve/ antipsychotic treated (n)</td>
<td>3/1/2/1</td>
<td></td>
</tr>
<tr>
<td>Current antipsychotic medication (n): Aripiprazole:Apiprazole&amp;Olanzapine:Risperidone:Lurasidone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For patients taking antipsychotic treatment; chlorpromazine equivalent dose (mg/d), mean (SD)</td>
<td>401.91 (177.34)</td>
<td></td>
</tr>
<tr>
<td>PANSS positive score, mean (SD) *</td>
<td>19.33 (4.41)</td>
<td></td>
</tr>
<tr>
<td>PANSS negative score, mean (SD) *</td>
<td>19.28 (5.59)</td>
<td></td>
</tr>
<tr>
<td>PANSS general score, mean (SD) *</td>
<td>36.83 (7.46)</td>
<td></td>
</tr>
<tr>
<td>PANSS total score, mean (SD) *</td>
<td>75.44 (11.39)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FEP, first-episode psychosis; Shaded box, not applicable; PANSS, Positive and Negative Syndrome Scale; SD=standard deviation; n=number

Scores range for total PANSS=53-88, general PANSS subscale: 25-46, positive subscale of the PANSS:12-27; negative subscale of the PANSS: 8-28; Higher scores indicate greater symptom severity.

Proton Magnetic Resonance Spectroscopy

Example spectra are provided in Figure 2.(Prescot et al., 2011) Voxel segmentation and spectral quality are shown in eTable 1.

Differences in NMDAR availability and glutamatergic indices between patients and healthy controls

Hippocampal NMDAR DVR was not normally distributed (p = 0.01). We found a significant difference in hippocampal NMDAR DVR between the patients (Mean Rank = 12.83) and healthy controls (Mean Rank = 22.0; Mann Whitney test U = 60.00; z = -2.71, p = 0.006), as previously reported in a partially overlapping sample.(Beck et al., 2021) eTable2 and eResults 1 in supplementary information give the full results.

Striatal glutamate and Glx data were normally distributed (p = 0.32, p = 0.55, respectively). There were no significant differences between the glutamate or Glx levels in the striatum in patients relative to controls (glutamate p = 0.88, Glx p = 0.99).
Correlation between NMDAR availability (hippocampus and frontal cortex) & glutamate/Glx levels in the striatum

Hippocampus

We found a significant negative correlation between hippocampal NMDAR availability (DVR) and striatal glutamate in the patient group (rho = -0.74, p < 0.001, Figure 3) but not in healthy controls (rho = -0.22, p = 0.44). The same relationship was observed when antipsychotic-free and antipsychotic treated subsets of patients were looked at individually (rho = -0.75, p = 0.008, rho = -0.86, p = 0.01, respectively, eFigure1 in supplementary information).

There was also a significant negative correlation between hippocampal NMDAR availability and striatal Glx in the patient group (rho = -0.56, p = 0.02) but not in the healthy controls (rho = -0.32, p = 0.25). When the patient subgroup analysis was done the negative correlation was significant in the antipsychotic-treated group (rho = -0.82, p = 0.02) and in the antipsychotic free group (rho = -0.61, p = 0.05).

There were no significant correlations between striatal glutamate or Glx and NMDAR VT. eResults2 in supplementary information give the full results for VT.

Frontal Cortex

There were no significant correlations between striatal glutamate or Glx and NMDAR availability (DVR or VT) in the frontal cortex in any group. eResults3 in supplemental information give the full results.

Discussion:

Our main finding is an inverse relationship between relative hippocampal NMDAR availability and striatal glutamate levels in people with first-episode psychosis. To our knowledge, this is the first in vivo study to assess NMDAR availability and glutamatergic measures in both healthy controls and patients with schizophrenia. The use of simultaneous PET-MR acquisition reduces a number of potential confounding factors linked to temporal separation of data acquisition, such as effects of biological variation, or symptom fluctuation.

Our finding that lower hippocampal NMDAR availability is associated with higher striatal glutamate levels is consistent with the hypothesis that NMDAR dysfunction in the hippocampus disrupts striatal neurotransmission in schizophrenia.(Olney et al., 1995; Stone, Morrison and Pilowsky, 2007)

Our finding of significantly lower NMDAR availability in the hippocampus is consistent with our prior
finding in a larger sample that included the patients reported here. (Beck et al., 2021) It adds to other evidence for hippocampal dysfunction in schizophrenia. (Lieberman et al., 2018; Borgan, Laurikainen, et al., 2019; Nour et al., 2021; Onwordi et al., 2021)

**Interpretation and implications for the pathophysiology of psychosis**

Our finding that lower hippocampal NMDAR availability is associated with higher striatal glutamate levels provides further evidence that hippocampal dysfunction is linked to the function of the striatum in psychosis. (Stone et al., 2010; Lieberman et al., 2018; Adams et al., 2020) However, it should be recognised that this does not preclude the involvement of other circuit dysfunction in the illness as well. (McCutcheon et al., 2021)

Our findings are in line with both pre-clinical and clinical studies which have shown that NMDAR blockade induced by ketamine can increase striatal activity, (Breier et al., 1998; Kokkinou et al., 2017) and other evidence for altered hippocampal-basal ganglia relationships in patients with first episode psychosis (Schwarz et al., 2022) and individuals at clinically high risk of psychosis. (Modinos et al., 2020; Allen et al., 2021) NMDAR blockade can also increase striatal dopamine, however, we were unable to measure dopamine levels in this study. (Kegeles et al., 2000; Vollenweider et al., 2000; Kokkinou, Ashok and Howes, 2017) Thus, it would be useful for future studies to determine if NMDAR levels in patients are associated with striatal levels of other neurotransmitters in addition to glutamate to determine neurochemical specificity. We did not find a significant relationship between NMDAR availability in the frontal cortex and striatal glutamate, despite evidence that this circuit maybe abnormal in schizophrenia, (Van Den Heuvel et al., 2010) (McCutcheon et al., 2021) this could suggest the association between NMDAR availability and striatal glutamate may be specific to certain brain circuits.

**Strengths and Limitations**

Strengths include that our patient sample involved people in their first episode of illness, many of whom were antipsychotic free/naive. Therefore, illness duration and chronic antipsychotic treatment are unlikely to have influenced the observed association. Furthermore, we find that the significant negative association between hippocampal NMDAR availability and striatal glutamate levels remained present when only the antipsychotic-free group was analysed. However, this is not
proof of a causal relationship, and further studies are needed to longitudinally investigate treatment effect.

We did not see a difference in striatal glutamate or Glx between patients or healthy controls, in contrast to previous studies. (Merritt et al., 2016; Nakahara et al., 2021) This may be due to type II error, as our study was not powered to detect case-control differences in MRS glutamate measures. Alternatively, this may be due to a proportion of the sample taking antipsychotic medication. A recent meta-analysis found that the standardized mean difference of glutamate levels in the basal ganglia were negatively associated with the proportion of medicated patients in the study sample. (Nakahara et al., 2021) A Longitudinal study has also shown that antipsychotics can reduce striatal glutamate levels in schizophrenia. (De La Fuente-Sandoval et al., 2013) There is also meta-analytic evidence that lower glutamate levels may be associated with antipsychotic exposure in other brain areas. (Merritt et al., 2021)

Glutamate signal at 3T includes some contribution from glutamine, of about 10-15%. Scanning at higher field strengths is needed to better separate signals from glutamate and glutamine. (Snyder and Wilman, 2010) There is a relatively low spatial resolution for MRS and it is not possible to differentiate between intracellular and extracellular glutamate concentrations.

Vt and DVR measures are not able to differentiate between specific and non-specific binding and so we are not able to exclude the possibility that our findings are influenced by alterations in non-specific binding. (Beck et al., 2021) There is evidence that [18F]GE-179 has nanomolar affinity for the PCP site of the NMDAR channel and low affinity for other CNS receptors, indicating it is specific to the NMDAR. (Vibholm et al., 2020) Furthermore, there is evidence that electrical stimulation of the NMDAR results in an increase in the uptake of [18F] GE-179 and that this uptake is blocked by the administration of ketamine and is sensitive to changes in the NMDAR. (Vibholm et al., 2021) However, there are also studies in rodents and primates which have not used this stimulation method and do not find that NMDAR antagonists such as ketamine were able to block [18F] GE-179 binding. (Schoenberger et al., 2018) However, these studies co-administered anaesthetic agents which may interact with the NMDAR and, consequently, alter the sensitivity of these approaches to detect changes. (McGinnity et al., 2018) (Sander, Schoenberger and Hooker, 2018) Thus, whilst the in vitro evidence shows the tracer is specific to the NMDAR, there is some inconsistency in the in vivo evidence, potentially due to the use of anaesthetic agents that interact with NMDAR. Further work is
needed to confirm this. A blocking study using an NMDAR selective drug would also be useful to
determine the proportion of the GE-179 signal that is specific to NMDAR in patients.

The relationship between $[^{18}F]$GE179 availability in the hippocampus, as measured by DVR, and
 glutamate concentration is strongly and significantly negative in patients. However, although a
similar negative relationship is seen with the $V_f$ measure, it is not significant. This maybe because the
$V_f$ measure has more than double the variability of DVR in controls, reducing the sensitivity to detect
relationships. Still, even though the DVR measure is more sensitive, it must be kept in mind that it is
not necessarily the most accurate method of measuring NMDAR availability.

Conclusions
These findings suggest that lower NMDAR availability in the hippocampus is associated with higher
striatal glutamate levels in patients with schizophrenia. This supports the theory that hippocampal
NMDAR hypofunction leads to striatal glutamatergic dysfunction in schizophrenia.

Figure Legends:
Figure 1 – Voxel placement in the right dorsal caudate
Figure 2- Example of proton magnetic resonance spectroscopy spectra from the right striatum
(LCModel output). Raw data are shown in black, and fitted data are shown in red. The metabolite
signal assignments are based on Prescot et al. (61) Cre, creatine, Glx glutamate + glutamine, NAA, N-
acetyl aspartate.
Figure 3- Relationship of $[^{18}F]$ GE179 tracer uptake in the hippocampus, as measured by DVR, and
striatal glutamate concentration in A) First Episode Psychosis and B) Healthy Controls

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Author Contribution

Conceptualisation: KB and ODH. Data curation: KB. Formal analysis: KB, MV, DL, JS, SB, RAM, BS, CJM, JD and FB. Funding acquisition: KB and ODH. Investigation: KB, CJM, RAM, TP, TS, RN, SJ, NS and SK. Methodology: KB, ODH, AH, MV, JD, RN, CJM, FT, DL and JS. Project administration: KB and ODH. Resources: KB, OH and AH. Supervision: AH, ODH and JS. Validation: DL and MV. Visualisation: KB. Writing- original draft: KB. Writing-review and editing: all authors.

Conflicts of Interest

KB, AA, BS, JD, NS, TS, FT, DL have no conflicts of interest. MV has received consulting honoraria from GSK. CJM has received fees from GE Healthcare Ltd but neither he nor any of his family have ever been employed by the organisation; nor does he or any of his family have holdings / a financial stake in GE Healthcare Ltd. SK received travel expenses for a scientific meeting from Autifony Ltd. RM has received honoraria for lectures from Otsuka and is an investigator on research funded by Neurocrine. Dr Pillinger has participated in educational speaker meetings organised by Lundbeck, Otsuka, Sunovion, Schwabe Pharma and Recordati. Dr Neji is an employee of Siemens Healthcare. In the last 3 years, JMS has been PI on research studies sponsored by Takeda and Protexin. He has received an honorarium from Janssen for attending an advisory board meeting. SJ has received honoraria for educational talks given for Sunovian. KCL has received honoraria for educational talks SJ has given for
Lundbeck. SJ is Co PI on a drug trial for Alkermes. FB became an employee at COMPASS Pathways plc after completion of this work. This work is unrelated to COMPASS Pathways plc. AH has received honoraria or consultation fees from Imperial Innovations and is a shareholder in Imperial Innovations, and has participated in educational speaker meetings organised by Siemens. ODH is a part-time employee of H Lundbeck A/s (obtained post after completion of this project) and has received investigator-initiated research funding from and/or participated in advisory/speaker meetings organised by Angellini, Autifony, Biogen, Boehringer-Ingelheim, Eli Lilly, Heptares, Global Medical Education, Invicro, Jansenn, Lundbeck, Neurocrine, Otsuka, Sunovion, Rand, Recordati, Roche and Viatris/Mylan. Neither Prof Howes or his family have holdings/a financial stake in any pharmaceutical company. Prof Howes has a patent for the use of dopaminergic imaging.

References


‘LCModel’ (no date) http://s-provencher.com/lcmodel.shtml (November 19, 2019).


