1. Introduction

Mounting evidence suggests that both dysfunction of glutamate neurotransmission and immune system alterations may contribute to the aetiology of schizophrenia, with substantial bodies of support emerging for both theories (Baumeister et al., 2014; Beck et al., 2016; Benros and Mortensen, 2015; Egerton et al., 2020; Meyer, 2014). It is also becoming clear that these pathological mechanisms may be linked, as an excessive...
activation of immune pathway may dysregulate glutamate concentrations, leading to behavioural alterations (Haroon et al., 2017; Miller et al., 2016). Most empirical research investigating this link between inflammation and glutamate dysfunction has been conducted in subjects with mood disorders (Haroon et al., 2017; Miller et al., 2016), whilst work on glutamate neurotransmission and immune system alternations in schizophrenia have largely proceeded along separate paths. Given the emerging relevance of both the glutamate and immune systems to the onset and treatment-responsivity of schizophrenia (Egerton et al., 2012, 2020, 2021, 2022; He et al., 2020; Iwata et al., 2019; Lin et al., 1998; Mondelli et al., 2015; Mouchlianitis et al., 2016; Nakahara et al., 2021; Tarumi et al., 2020; Zhang et al., 2004), it is important to understand the relationship between inflammation and glutamate in this condition.

The glutamate hypothesis of schizophrenia posits that increased activation of pyramidal glutamatergic neurons is caused by disinhibition of NMDA-regulated GABAergic inhibitory interneurons due to NMDA receptor hypofunction (Homayoun and Moghaddam, 2007; Lisman et al., 2008; Olney et al., 1999; Steiner et al., 2012). The concentration of glutamate in the human brain can be measured using proton magnetic resonance spectroscopy (1H-MRS). 1H-MRS meta-analyses find that, overall, in schizophrenia, glutamate metabolites may be elevated in the basal ganglia and reduced in the medial frontal cortex (mFC, including anterior cingulate cortex, ACC) (Marsman et al., 2013; Merritt et al., 2016, 2022; Nakahara et al., 2021; Smucny et al., 2021; Sydnor and Roalf, 2020). However, the extent and direction of glutamate abnormality may relate to illness stage, severity, medication effects, genetic and other factors (Bustillo et al., 2017; Merritt et al., 2016, 2021, 2022; Nakahara et al., 2021). Of particular interest are findings from some studies indicating that mFC/ACC glutamate metabolites may be increased in patients who show a poor compared to good response to antipsychotic treatment (Egerton et al., 2012, 2018, 2021; Iwata et al., 2019; Mouchlianitis et al., 2016; Szule et al., 2013; Tarumi et al., 2020). Overall, glutamate levels are more variable in schizophrenia than in healthy volunteers (Merritt et al., 2022). This could indicate differential influences of contributing mechanisms on glutamate levels, potentially including inflammation, which could also relate to treatment response.

Epidemiological studies have recognised infections and autoimmune diseases as risk factors for developing schizophrenia (Benrós and Mortensen, 2015; Canetta and Brown, 2012; Cullen et al., 2019), and immune-related gene polymorphisms have been associated with the disorder (Hudson and Miller, 2018). Altered levels of circulating pro- and anti-inflammatory cytokines in schizophrenia provide more direct evidence for immune dysfunction, with increased levels of proinflammatory cytokines present in the peripheral plasma and serum of prodromal (Stojanovic et al., 2014), first episode (Di Nicola et al., 2013; Zajkowska and Mondelli, 2014), acutely ill (Goldsmith et al., 2016) and chronic (Goelho et al., 2008; Miller et al., 2011) schizophrenia. Moreover, high levels of pro-inflammatory cytokies have also been associated with more severe symptoms at disease onset and after administration of antipsychotics (Chase et al., 2016; Frydecka et al., 2014; Lee et al., 2017; Miller et al., 2011; Stojanovic et al., 2014), and with worse antipsychotic response (Lin et al., 1998; Mondelli et al., 2015).

The convergence of inflammation and the glutamate system has most thoroughly been investigated in the context of major depressive disorder. For example, interferon-alpha, a common treatment for hepatitis C, increases glutamate concentration in the caudate and ACC, and this is congruent with the development of depressive symptoms (Haroon et al., 2014). Higher levels of peripheral inflammatory markers also predict worse antidepressant efficacy of the glutamate antagonist ketamine in individuals with major depressive disorder (Hashimoto, 2015; Walker et al., 2015). Furthermore, increased plasma levels of C-reactive protein (CRP), a nonspecific marker of inflammatory processes, has been associated with increased levels of glutamate in the caudate of individuals with depression (Haroon et al., 2016). Higher levels of IL-6 has also been associated with higher concentrations of glutamate in the dorsal ACC in adolescents with depression (Ho et al., 2021). There is thus evidence that inflammation may increase brain glutamate metabolites in patients with mood disorders and contribute to treatment resistance (Miller and Raison, 2016), but it is unknown whether this relationship is also observed in schizophrenia.

The primary aim of the current study is to determine whether peripheral cytokine levels are related to brain glutamate in patients with schizophrenia. We hypothesised that markers of increased peripheral inflammation would be associated with increased levels of brain glutamate. Secondly, given that both glutamate increases and peripheral inflammation may be more pronounced in antipsychotic non-responsive schizophrenia and that inflammation has been suggested to lead to treatment resistance depression through its effects on the glutamatergic system (Haroon and Miller, 2017), we hypothesised that the relationship between peripheral inflammation and brain glutamate levels would be stronger in antipsychotic non-responsive compared to antipsychotic responsive illness, reflecting greater activation of inflammatory-glutamate pathways.

2. Methods

2.1. Regulatory approvals

The study was approved by the NHS Research Ethics Committee (ref 15/LO/0038). All participants provided written informed consent.

2.2. Participants

Recruitment and assessment took place at King’s College London and Universities of Manchester, Edinburgh and Cardiff. Participants were 18–65 years of age and had DSM-5 diagnosis of schizophrenia or schizoaffective disorder and were able to understand and consent to the study procedures. Exclusion criteria were pregnancy, severe head injury, meeting ICD criteria for substance misuse or psychotic disorder secondary to substance misuse, treatment with clozapine in the last 3 months, or contraindications to MRI. Clinical diagnosis was confirmed using the MINI (Sheehan et al., 1998), and illness severity assessed using the Positive and Negative Syndrome Scale (Kay et al., 1987) and Clinical Global Impression scale for Schizophrenia (Haro et al., 2003).

2.3. Definition of antipsychotic responder & non-responder groups

Antipsychotic responders and antipsychotic non-responders were defined as described in Egerton et al. (2021). Briefly, 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 non-response; (2) a CGI-SCH severity score of < 4; (3) a PANSS total score of < 60 (Leuch et al., 2005); and (4) a compliance rating scale (CRS) score (Kemp et al., 1996) of > 3. Antipsychotic non-responders (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.

2.4. Proton magnetic resonance spectroscopy

Glutamate levels were measured using 1H-MRS at 3 Tesla on either a General Electric MR750 (Chicago, USA), Philips Achieva (Philips Healthcare, The Netherlands) or a Siemens Verio magnetic resonance system, as previously described (Egerton et al., 2021). Sagittal T1-weighted images were acquired to guide voxel positioning. Non-rotated voxels measuring 20 × 20 × 20 mm was positioned in the anterior cingulate cortex (ACC), and in the caudate. Further details and images of voxel positioning and spectral quality are provided in Egerton et al.
were run to test for any interaction between the effects of antipsychotic response status and cytokine concentrations on brain glutamate, including the same covariates as stated above. This analysis was also carried out for IL-8, because in an overlapping sample of participants to those included in the current study, higher levels of IL-8 was associated with a poor response to antipsychotic treatment (Enache et al., 2021).

3. Results

3.1. Subject demographics

Eighty participants completed 1H-MRS imaging and blood cytokine assays (40 treatment responders, 40 treatment non-responders). One subject was excluded due to very high C-reactive protein levels (CRP > 50), likely indicative of acute infection/injury. Nine subjects were excluded due to missing BMI values. Of the remaining 70 participants, 5 were excluded from the caudate analysis due to the 1H-MRS data failing quality control, and 2 were excluded from the ACC analysis due to the 1H-MRS data failing quality control. The final numbers for analyses were 68 for ACC (36 R, 32 NR), and 65 for caudate (33 R, 32 NR). The final study population is a subset of the participants in Egerton et al. (2021) and Enache et al. (2021). Characteristics of the study population are presented in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Responders (R)</th>
<th>Non-responders (NR)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>(n = 70)</td>
<td>(n = 37)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>(8.05)</td>
<td>(8.66)</td>
<td>0.999</td>
</tr>
<tr>
<td>Mule n</td>
<td>(82.86%)</td>
<td>(83.78%)</td>
<td>0.828</td>
</tr>
<tr>
<td>BMI</td>
<td>(4.87)</td>
<td>(4.89)</td>
<td>0.026 ^</td>
</tr>
<tr>
<td>Smoking</td>
<td>(58.57%)</td>
<td>(59.46%)</td>
<td>0.877 ^</td>
</tr>
<tr>
<td>Current</td>
<td></td>
<td></td>
<td>0.231 ^</td>
</tr>
<tr>
<td>Antipsychotic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>16</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>14</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Risperidone</td>
<td>10</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Aminopridie</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>7</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Paliperidone</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Clozipox</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Flupenidox</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Combination</td>
<td>7</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>CPBE</td>
<td>(7.03)</td>
<td>(7.28)</td>
<td>0.380</td>
</tr>
<tr>
<td>Age of Psychosis</td>
<td>24.64</td>
<td>25.30 (7.28)</td>
<td>0.380</td>
</tr>
<tr>
<td>Duration of Illness</td>
<td>4.70 (5.72)</td>
<td>4.46 (6.30)</td>
<td>0.071 ^</td>
</tr>
<tr>
<td>PANS Positive</td>
<td>16.90</td>
<td>12.00 (3.16)</td>
<td>&lt;0.001 ^</td>
</tr>
<tr>
<td>PANS Negative</td>
<td>17.24</td>
<td>13.38 (3.47)</td>
<td>&lt;0.001 ^</td>
</tr>
<tr>
<td>PANS General</td>
<td>34.31</td>
<td>27.35 (3.93)</td>
<td>=0.001 ^</td>
</tr>
<tr>
<td>PANS Total</td>
<td>68.46</td>
<td>52.73 (5.46)</td>
<td>=0.001 ^</td>
</tr>
</tbody>
</table>

Note: Continuous variables expressed as mean and standard deviation. Categorical variables expressed as number and percentage. p-Values for the comparisons between antipsychotic responders and non-responders were based on t-test (*), Mann-Whitney (c) and chi-squared (c) tests as appropriate. Bold indicate significant p values. There were no significant group differences in clinical or demographic characteristics other than in BMI and PANS scores. n: number of subjects, BMI: body mass index, CPBE: chlorpromazine equivalent dose, PANS: positive and negative syndrome Scale.
shown in Table 1.

3.2. Relationships between inflammatory markers and brain glutamate

Descriptive statistics are included in Table 2. Across all participants with schizophrenia, plasma IFN-γ concentration was positively correlated with glutamate concentrations in the caudate (r = 0.31, p = 0.02) Fig. 1. This relationship followed the same direction in the ACC but was only at trend level for significance (r = 0.22, p = 0.09). No other cytokines were significantly correlated with glutamate levels in the caudate or in the ACC. Covarying for CPZE did not meaningfully alter these results.

In this smaller subset of the participants reported in Egerton et al. (2021) and Enache et al. (2021), the group differences in ACC glutamate levels and IL-8 levels were non-significant.

3.3. Effect of antipsychotic response

The correlation coefficients between plasma IFN-γ concentration and caudate glutamate levels in antipsychotic responders (r = 0.25, p = 0.20) did not differ significantly from the correlation coefficient observed in antipsychotic non-responders (Fig. 2A, r = 0.18, p = 0.36): z = 0.2750, p = 0.783, CI: −0.4060 to 0.5351. There was also no group × IFN-γ interaction on caudate glutamate levels (β = −0.090, p = 0.85). The correlation coefficients between IL-8 concentrations and glutamate in the caudate differed significantly between the groups (Fig. 2B, z = −2.1895, p = 0.0286, CI: −0.9407 to 0.0532). There was a significant group × IL-8 interaction on caudate glutamate levels (β = −1.195, p = 0.048), with antipsychotic non-responders showing a positive correlation (r = 0.46, p = 0.01) not present in the antipsychotic responders (r = −0.07, p = 0.72). The groups did not differ significantly when looking at the association between IL-8 and glutamate in the ACC. Covarying for chlorpromazine equivalent dose did again not meaningfully alter these results.

4. Discussion

The primary aim of the current study was to test the hypotheses that markers of increased peripheral inflammation would be associated with increased levels of brain glutamate in schizophrenia. Our data showed a positive association between plasma IFN-γ levels and glutamate levels in the caudate, providing preliminary support for the convergence of immune and glutamatergic processes in schizophrenia. In the ACC, a similar pattern of association between IFN-γ and glutamate was observed, but below the threshold for statistical significance. Caudate or ACC glutamate levels were not associated with levels of the other analysed cytokines across the whole patient cohort. However, when assessing the antipsychotic responsive and antipsychotic non-responsive groups separately, peripheral levels of IL-8 and caudate glutamate concentrations were positively correlated in antipsychotic non-responders, but not in the antipsychotic responders. Together, these results provide initial evidence linking specific peripheral pro-inflammatory markers to caudate glutamate levels in schizophrenia and may suggest that these inflammatory-glutamatergic processes are most marked in those patients responding poorly to antipsychotic treatment.

Although previous studies have reported relationships between peripheral inflammation and other brain markers in schizophrenia, including associations with cortical thickness and volume (Jacomb et al., 2018; Kalmady et al., 2014; Mondelli et al., 2011; Wu et al., 2019; Zhang et al., 2016), neurocognitive impairment and psychomotor slowing (Goldsmith et al., 2020; Kogan et al., 2018; North et al., 2021), this is to our knowledge the first study to report a relationship between peripheral inflammation and brain glutamate in schizophrenia. Whilst inflammatory markers such as INF-α, CRP and IL-6 have been implicated in the interaction between inflammation and glutamate in depression (Felger et al., 2016; Haroon et al., 2014; Hashimoto, 2015; Ho et al., 2021; Walker et al., 2015), our main finding was a relationship between IFN-γ and brain glutamate across the whole patient cohort, which could potentially suggest some degree of specificity to schizophrenia.

Whilst our study does not allow for inference about cause-and-effect relationships due to being cross-sectional, it has been established that peripheral immune activation can influence brain function through several pathways. As reviewed by Miller et al. (2013), the humoral pathways include circulating cytokines passing through leaky regions of the blood brain barrier, and active transport of circulating cytokines into the brain via cytokine specific transporters. The neural route involves activation of cytokine receptors on afferent nerve fibres that then transduce signals to the brain, and the cellular route is whereby chemokines released by activated microglia and adhesion molecules expressed in the central nervous system can attract activated peripheral cell types including monocytes and T cells to the meninges and brain parenchyma. Immune mediators have been shown to significantly influence the extracellular concentration of glutamate by altering the balance between glutamate release from glial and immune cells (Haroon et al., 2017), and its clearance mechanisms (McCullumsmith and Sanacora, 2015). Inflammatory cytokines and their signalling pathways can also activate the kynurenine (KYN) pathway of tryptophan metabolism, which generates neuroactive metabolites which can also affect glutamate metabolism (Chiappelli et al., 2018). Concurrently, both IFN-γ and IL-8 have been shown to modulate glutamatergic synaptic transmission in preclinical experiments (Cui et al., 2012; Garg et al., 2009); therefore, while the exact mechanistic pathways underlying the positive relationships between IFN-γ and caudate glutamate levels in the whole patient cohort and IL-6 in antipsychotic non-responders are not yet fully defined, these findings are consistent with evidence linking increases in peripheral proinflammatory cytokines with glutamate function.

As little is known about the interplay between peripheral inflammation and brain glutamate in schizophrenia, it is valuable to view our results within the context of studies assessing these factors independently. In an overlapping sample of participants to those included in the current study, higher levels of IL-8 (Enache et al., 2021) was associated with poor response to antipsychotic treatment. Previous research has also implicated IL-8 in the prognosis and therapeutic response of individuals with schizophrenia, with higher baseline IL-8 levels being
associated with worse antipsychotic response (Zhang et al., 2004), and higher IL-8 may predicting less improvement in negative symptoms (He et al., 2020). Here we observed a positive relationship between IL-8 and caudate glutamate in the treatment non-responsive but not treatment responsive group. High serum levels of IFN-γ, alongside IL-6, have previously been reported to predict a poor response to antipsychotic medication after 12 weeks of treatment in patients with first episode psychosis (Mondelli et al., 2015), but we did not observe significant differences in the relationship between IFN-γ and brain glutamate between treatment responders and treatment non-responders in the current study. In an overlapping sample of participants to those included in the current study, higher levels of ACC glutamate (Egerton et al., 2021) were also associated with a poor response to antipsychotic treatment. Although caudate glutamate was not associated with antipsychotic response in this cohort (Egerton et al., 2021), some previous studies have found decreases in caudate glutamate during effective antipsychotic treatment (de la Fuente Sandoval et al., 2013; McQueen et al., 2021).

The relationship between IFN-γ and glutamate levels in the ACC showed a similar trend to that in the caudate, although it did not reach statistical significance. Compared to previous studies in depression finding associations between cytokines and dorsal ACC glutamate (Ho et al., 2021; Haroon et al., 2014) our ACC 1H-MRS voxel was positioned towards the more rostral perigenual ACC, and these regions may differ in both glutamate concentration and function (Li et al., 2022).

In the case of IL-8, we only observed a relationship with caudate glutamate in treatment non-responders, and there was no significant group difference between IL-8 and ACC glutamate, despite previous findings implicating ACC glutamate levels in treatment non-response (Egerton et al., 2012, 2018, 2021; Iwata et al., 2019; Mouchlianitis et al., 2016; Szulc et al., 2013; Tarumi et al., 2020). Previous research in depression has also emphasized the role of glutamate in the basal ganglia in the relationship with peripheral inflammation. For instance, Haroon et al. (2016) reported a significant association between peripheral inflammation and glutamate levels in the basal ganglia, but not in the ACC. In depression, the interplay between inflammation and the basal ganglia is suggested to be related to anhedonia and lack of motivation, and it is plausible that this rationale could extend to schizophrenia. Taken together, our results indicate a positive relationship between peripheral levels of IFN-γ and brain glutamate levels in schizophrenia, independent of antipsychotic response, whilst the relationship between IL-8 and caudate glutamate may be more specific to antipsychotic non-responsive illness.

Strengths of our study include the use of a standardised protocol to recruit a relatively large sample of patients across several sites in the UK. 1H-MRS acquisition sequences were harmonised across research sites, and metabolites estimated using the same analysis pipeline. We were also able to assay several cytokines previously implicated in schizophrenia. However, our study also has several limitations. As it was designed to investigate mechanisms underlying antipsychotic response in schizophrenia, it did not include a healthy control group. This means that we are unable to attribute the observed relationship between 1H-MRS acquisition sequences were harmonised across research sites, and metabolites estimated using the same analysis pipeline. We were also able to assay several cytokines previously implicated in schizophrenia. However, our study also has several limitations. As it was designed to investigate mechanisms underlying antipsychotic response in schizophrenia, it did not include a healthy control group. This means that we are unable to attribute the observed relationship between 1H-
MRS glutamate measures and IFN-γ values to schizophrenia specifically. Further work is needed to assess the diagnostic specificity of this relationship, with comparison to both a healthy control group, and mood disorders such as MDD, where a relationship between brain glutamate levels and peripheral inflammation has previously been reported (Haroon et al., 2014, 2016; Ho et al., 2021). As clinical data were only gathered at a single cross-sectional timepoint, we did not ascertain the stability or timing of treatment response / non-response, and as non-response to antipsychotic medication was not determined prospectively and did not include an objective evidence of adherence, we are unable to determine the proportion of the NR group that would meet consensus guidelines for treatment-resistant schizophrenia (Howes et al., 2017). These factors may have contributed to a less distinct clinical separation between the R and NR groups and our ability to observe differences in inflammatory-glutamate relationships in relation to response status. Additionally, all participants were currently taking antipsychotic medication which may influence both glutamate levels (Egerton et al., 2017; Merritt et al., 2022; Zahid et al., 2022) and peripheral immune markers (Baumeister et al., 2016; Romeo et al., 2018; Tourjman et al., 2013). Future research could determine whether the relationships between IFN-γ, IL-8 and glutamate are present in medication-naïve psychosis and the association with subsequent antipsychotic response. As most of our patient sample were male (82%), we were not able to investigate potential effects of sex, and women may show greater responses to inflammatory challenges (Moieni et al., 2015). Lastly, 1H-MRS measurements cannot differentiate between intracellular and extracellular glutamate (Duarte and Xin, 2019). However, 1H-MRS-assessed glutamate levels correlate with transcranial magnetic stimulation-based measures of cortical excitability, suggesting that 1H-MRS measures reflect neural glutamatergic activity (Stagg et al., 2011).

In conclusion, the current study provides initial support for a positive relationship between peripheral inflammation and brain glutamate levels in schizophrenia. Further work is needed to clarify the
mechanistic link between pro-inflammatory cytokines and brain glutamate concentrations, and to confirm whether inflammatory-glutamate mechanisms make a greater contribution to antipsychotic non-responsive schizophrenia.

Funding

This research was funded by the UK Medical Research Council (MRC), Stratified Medicines Initiative, reference MR/1017794/1 ‘STRATA’ Research at the London site was supported by the Department of Health via the National Institute for Health Research (NIHR) Specialist Biomedical Research Center for Mental Health award to South London and Maudsley NHS Foundation Trust (SLaM) and the Institute of Psychiatry, Psychology and Neuroscience at King’s College London. SFM is supported by the UK Medical Research Council [MR/N013700/1] and King’s College London member of the MRC Doctoral Training Partnership in Biomedical Sciences. VM is supported by MQ: Transforming Mental Health (Grant: MQBF/1 and MQBF/4). The views expressed are not necessarily those of the NHS, the NIHR, or the Department of Health.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data governance frameworks are being put in place to make a fully anonymized version of the data available to the wider research community. To apply for access, contact JHM at james.maccabe@kcl.ac.uk

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2023.05.005.

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Baumeister, D., Russell, A., Pariante, C.M., Mondelli, V., 2014. Inflammatory biomarker profile of inflammatory-glutamate mechanisms in schizophrenia. To apply for access, contact JHM at james.maccabe@kcl.ac.uk


