Upgrade Proposal

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Background

Summary of current state of the field and context within which the research is located, covering current theory/state of the evidence and referring to relevant literature (500-1,000 words).

Glutamatergic transmission is thought to be dusrupted in the early stages of schizophrenia, contributing to the development of aberrant dopaminergic signalling[1,2], and leading to excitation-inhibition (E/I) imbalance. MR spectroscopy studies report increased glutamate in the ACC, hippocampus, and other medial temporal cortical regions of people with schizophrenia[3–6]. Glutamate levels are associated with illness severity and treatment response[3]. Antipsychotic medication decreases glutamate levels[7–9], however non-responders have higher levels of glutamate at baseline[10,11] and post-treatment[11], suggesting that modulation of glutamatergic signalling is an important for the effectiveness of pharmacological treatments. The NMDA hypofunction hypothesis of schizophrenia proposes that decreased activity of NMDA receptors at GABAergic fast-spiking PV interneurons[12] causes a disinhibition of their activity on pyramidal neurons, disrupting the excitation-inhibition (E/I) balance, and leading to increased excitation. In the early stages of the disorder, increase in glutamate drives hippocampal hyperactivty, which later contributes to hyperdopaminergia in the striatum[1,2,13]. The role of glutamate dysfuction in symptoms is consistent with the observation thtat NMDA antagonists like phencyclidine and ketamine induces behaviours comparable to all three schizoprenia symptom dimensions (positive, negative, and cognitive symptoms)[14], and repeated administration results in increased release of dopamine in rodent striatum[13], making it an important target of translationsal research.

Alterations in synaptic function have been implicated in the disruption of E/I balance in schizophrenia[15]. In particular synaptic vesicle glycoprotein 2A (SV2A), a protein located on synaptic vesicles, in the presynaptic terminals, is thought to be related to glutamatergic dysfunction in schizophrenia. SV2A plays a role in neurotransmitter release, and although the exact mechanisms of actions are not known, it seems to affect excitatory and inhibitory signalling differently. The distribution of SV2A in synaptic terminals varies depending on the brain region and type of neuron (excitatory/inhibitory)[16,17] and it seems to be preferentially associated with GABAergic rather than glutamatergic neurons[18]. Across different stages of maturation, the expression of SV2A with GABA and glutamate transporters differed across

hippocampal regions and neuron types, and the colocalisation varied different stages of rat development[16], suggesting that SV2A might have a role in maturation of the E/I balance across development. In line with this, altering SV2A expression in mice was found to affect the E/I balance[19]. In animal models studies, the exposure to environmental factors associated with schizophrenia risk has resulted in decrease in synaptic markers[20]. Postmortem studies report decreased number of presynaptic[21], but also post-synaptic[22] markers in people with schizophrenia. Human SV2A PET studies reported decreased levels of SV2A in people with schizophrenia[23,24], which seem to appear later in the disease progression[25], and an altered relationship glutamate and synaptic function[26]; in healthy participants there is a positive correlation between SV2A density and glutamate in ACC and hippocampus, but no significant correlations were found in schizophrenia[26], suggesting there's a disrupted relationship between glutamate release and synaptic function. While SV2A has a role in maintaining E/I balance, conversly it is also likely that excitotoxicity caused by excess glutamate leads to synapse loss, observed as decreased SV2A in schizophrenia[27].

Levetiracetam (LEV) is an anticonvulsant drug that selectively binds to SV2A, and works by normalising the excitation inhibition imbalance in epilepsy. It was also found to be helpful in treating subclinical epileptiform discharges in autism spectrum disorder (ASD)[28]. Since schizophrenia is also associated with E/I imbalance, the effects of LEV could be useful in studying schizophrenia aetiology, and could offer a translational potential. So far only one study tested the effects of LEV in schizophrenia; their findings suggesting that LEV can normalise hippocampal hyperactivity[29] where E/I imbalance is understood to originate. Preliminary results from a clinical trial published at clinicaltrials.gov (NCT03129360)[30] show decreased hippocampal CBF 2h after administering LEV to people with early psychosis, however statistical significance of the difference between group means was not reported. It is not clear whether its action is due to increase in the release of GABA or decrease in glutamate[31]. Evidence from preclinical studies on epilepsy suggests that it might restore E/I imbalance by increasing the vesicular release of GABA[32]. On the other hand there is also evidence for that LEV could be inhibiting glutamate release[33–36], this would be in line with animal studies which have shown that LEV decreases neurotransmission by decreasing the amount of available synaptic vesicles[37]. It's also possible that LEV affects excitatory and inhibitory neurotransmission differently when the E/I balance is disrupted compared to healthy mammals. Evidence from studies looking at the effect of LEV in epilepsy suggest that the magnitude of the effect differs when there is an imbalance between excitation and inhibition. LEV decreases EPSC in a frequency-depedent and activity-dependent manner[34], and it's been proposed that it preferentially acts on hyperactive synapses[37,38].

There is very little studies of the effects of LEV on glutamate release in-vivo, and none in schizophrenia. To my knowledge no studies have examined the effects of LEV on glutamate and on symptoms after a single dose, which is a gap in knowledge that I aim to address with my project. Previous PET studies have consistently reported decreased SV2A density in the ACC and the hippocampus[23,24], therefore I will examine glutamate levelts in these two regions.

Aims and objectives

The aim of my project is to examine the relationship between synaptic connectivity and glutamatergic function in schizophrenia by commparing the change in glutamate levels after administration of LEV in healthy controls and people with schizophrenia.

I will aim to answer the following questions:

1. Does modulating SV2A lead to lower glutamate levels in healthy people?
2. Does modulating SV2A lead to lower glutamate levels in people with schizophrenia? Is the change different to that in healthy controls?
3. Does modulating SV2A lead to change in symptoms in schizophrenia?

Hypotheses under investigation

I hypothesise that administration of LEV in healthy people will lead to decreased glutamate levels. Animal studies have shown that LEV decreases neurotransmission by decreasing the amount of available synaptic vesicles[37], therefore I expect that LEV will decrease the amount of glutamate released from presynaptic terminals.

Similarily I think that a decrease in glutamate will be observed in participants with schizophrenia. Evidence from studies looking at the effect of LEV in epilepsy suggest that the magnitude of the effect differs when there is an imbalance between excitation and inhibition, therefore I think that a greater decrease in glutamate will be observed in participants with schizophrenia than healthy controls.

Lastly, I believe that LEV will improve symptoms of schizophrenia. Evidence suggests that LEV could potentially normalise some changes associated with the eatiology of schizophrenia, such as normalising the E/I imbalance, hippocampal hyperactivity[29] and hyperperfusion[30]. One clinical trial reported a small decrease in PANSS scores across all three symptom domains, as well as improvement in cognitive functon following 8 weeks of LEV[39]. However, no studies examined the effect of LEV on symptoms after a single dose.

Methodology

Study design and data collection

Study design

- Single-blind, randomised, placebo-controlled trial with cross-over design.
- Participants undergo two MRI scans- one after taking placebo and the other taking levetiracetam.

- They are randomised to the order in which they receive them.
- The recruitment target is 50 participants: 25 healthy controls (HC) and 25 people with schizophrenia (SZ).

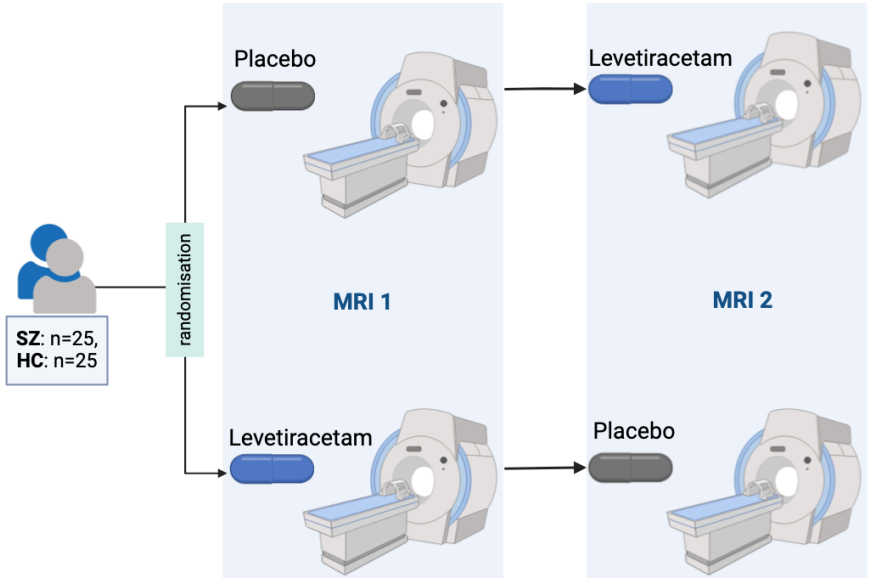


Figure 1: Study design: participants are randomised into the order in which they receive the placebo and levetiracetam (HC= healthy controls, SZ=participatns with schizophrenia)

Sample size

Based on previous MRS studies measuring glutamate changes following antipsychotic treatment, a sample size of 24 would be required to achieve 80% power within-group[9].

Measuring glutamate

- Glutamate levels in the ACC and the Hippocampus using single voxel spectroscopy (svs) PRESS sequence. The choice of those regions was based on previous findings of decreased SV2A density ([11C]UCB-J V_T) in the ACC in patients with schizophrenia[24], and altered relationship between glutamate and SV2A density in the hippocampus[26].
- I will also report Glx levels to verify if similar differences are observed compared to glutamate signal (This is due to limited ability to separate glutamine and glutamate using the PRESS sequence at 3T).

Behavioural measures

- Change is symptoms is assessed using Positive and Negative Syndrome Scale (PANSS).
- PANSS is administered at the screening appointment, and before and after every scan, to assess any change in symptoms related to levetiracetam.

Analysis

Change in glutamate

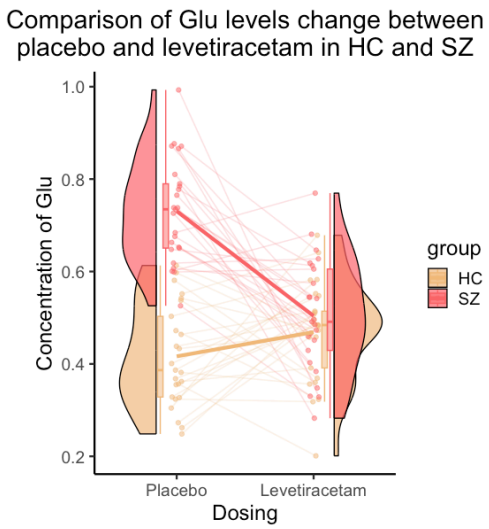
- MRS data processing will be done in Osprey, and values corrected for partial volume efects for concentration of Glu (and Glx) will be extracted for each participant's scans.
- To compare the changes in levels of glutamate between participants with schizophrenia and healthy controls I will do a 2x2 ANOVA.
- I will also compare the concentration of glutamate at baseline between healthy controls and particiapnts with schizophrenia.
- I will compare the effect of levetiracetam on Glx levels in healthy controls (HC) and patients with schizophrenia (SZ). This will be visualised on a raincloud plot such as the one below.
- Below is example of data visualisation using a raincloud plot. The data used in this graph is made up.
- I will also visualise the change in glutamate after administration of LEV. Below is an example graph based on the currently available data (HC: n=3, SZ n=6)

Change in symptoms

- PANSS score for each symptom group and overall PANSS score will be calculated for all participants.

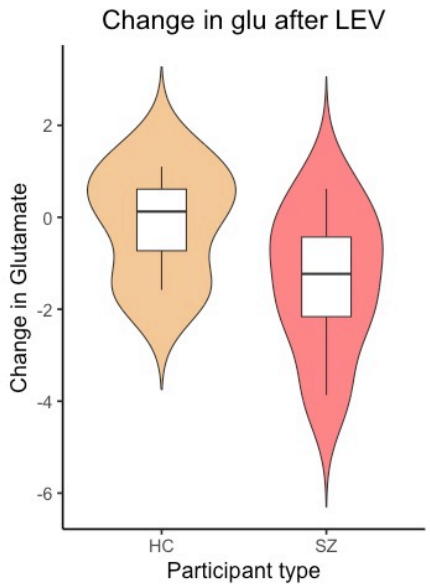
Power calculation

Based on pprevious findings it was calculated that a sample size of 25 participants in each participant group will be sufficient to achieve the power of 0.8.



(a) Comparison of Glu levels change between placebo and levetiracetam in HC and SZ

Source: Plots



(a) Change in glutamate

Progress made to date, including pilot work, if applicable

Progress with recruitment

So far the number of participants that completed the screening & baseline, and both MRI appointments in each group is:

- SZ: 12 (48%)
- HC: 6 (24%)
- **Total: 16 (36%)**

I have been working on opening new research sites at 3 NHS trusts in north London, where we will get support with recruitment from the local research delivery teams. The first research site (CNWL) is expected to open by the end of September 2024.

Progress with analyses

I am working on the setting up the data analysis pipeline for the MRS data. I have been learning Osprey and wrote the code that I continue to re-run once new data appears and troubleshoot any errors that come up.

Contribution to existing knowledge.

How the research will form a distinct contribution to existing knowledge on the subject and afford evidence of originality shown by discovery of new facts or exercise of independent critical power

This project will be the first one to examine the effects of modulating SV2A with levetiracetam on glutamate levels in schizophrenia. It was previously shown that SV2A density is decreased in schizophrenia[23,24], and that there is an altered relationship between Glu and SV2A in schizophrenia. The present study will provide more insight into the relationship between glutamate levels and synaptic density in schizophrenia. Such findings might have translational potential-

Personal share in investigations

Where work is done in conjunction with the supervisor and/or with collaborators or other students, a statement of the candidate's own personal share in the investigations

I am jointly responsible for recruitment/data collection with other student. I will do my analysis and write up independently.

Planned future work and timeline for the remainder of studies.

- **Data collection**: October 2023 - Jan 2025
- **Data analysis**: May 2024 - June 2026
- **Write up**: November 2025 - January 2027
- **Corrections to the manuscript**: January 2027 - April 2027
- **Thesis submission**: May 2027
- **Viva**: July 2027

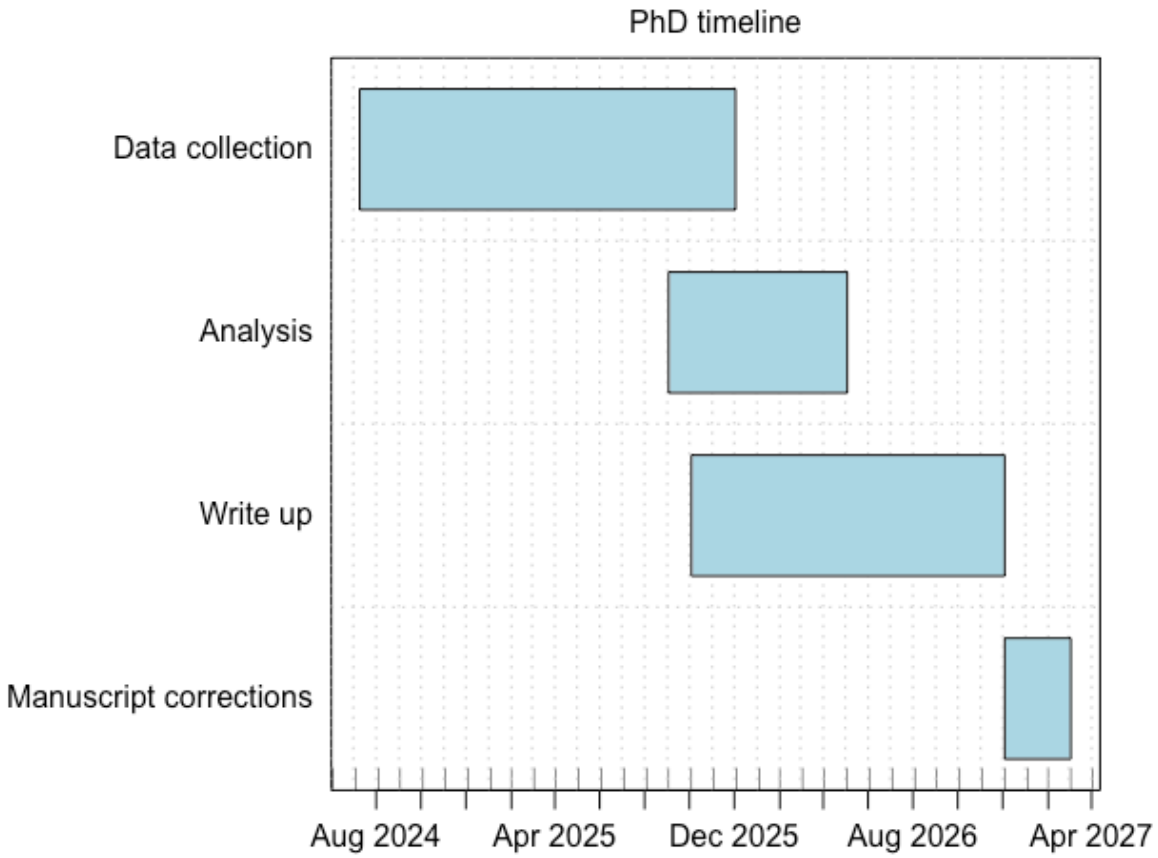


Figure 4: Gantt chart of planned work during my PhD

Source: Plots

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