

Upgrade Proposal

Julia Marcinkowska

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).

The NMDA hypofunction hypothesis of schizophrenia proposes that decreased activity of NMDA receptors has a key role in the development of schizophrenia pathology. The affected NMDA receptors are primarily localised at GABAergic fast-spiking PV interneurons¹; where decreased activity of PV interneurons causes a disinhibition of their activity on pyramidal neurons, disrupting the excitation-inhibition (E/I) balance, and leading to increased excitation. MR spectroscopy studies report increased glutamate in the ACC, hippocampus, and other medial temporal cortical regions²⁻⁴. Hyperactivity in the hippocampus is observed in the early stages in schizophrenia, as well as in people at clinical high risk of schizophrenia that subsequently develop the disorders, suggesting that increased glutamatergic activity in this region might be implicated in the development of the pathology at early stages of the disorder, and later contribute to hyperdopaminergia in the striatum. This is consistent with the observations that administration of NMDA antagonists like phencyclidine and ketamine induces behaviours comparable to all three schizophrenia symptom dimensions (positive, negative, and cognitive symptoms)⁵, and repeated administration results in increased release of DA in rodent striatum⁶, suggesting that hyperdopaminergia is caused by decreased NMDA activity^{7,8}. The role of NMDA hypofunction in the early stages of schizophrenia makes it an important target of translational research.

Alterations in synaptic function have also been implicated in the aetiology of schizophrenia⁹ and are thought to be related to the E/I imbalance. Excitotoxicity caused by increased glutamatergic activity might be one of the contributing factors in the reduction in synaptic connections in schizophrenia¹⁰. Post-mortem studies report decreased levels of synaptic proteins¹¹, supporting the theory of alteration in synaptic density. Recent advantages in PET imaging have enabled in-vivo imaging of synaptic density. By using a radiotracer selective for the synaptic vesicle glycoprotein 2A (SV2A), localised in the pre-synaptic terminals, SV2A density can be a proxy for measuring synaptic density. Human SV2A PET studies reported decreased levels of SV2A in people with schizophrenia^{12,13} and an altered relationship glutamate and synaptic function¹⁴. In healthy participants there is a positive correlation between SV2A density and

glutamate in ACC and hippocampus, but no significant correlations were found in schizophrenia¹⁴, suggesting disrupted relationship between glutamate release and synaptic function.

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)¹⁵. 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 hyperactivity¹⁶ where E/I imbalance is understood to originate. Preliminary results from a clinical trial published at clinicaltrials.gov (NCT03129360)¹⁷ 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 glutamate¹⁸; evidence from preclinical studies on epilepsy suggests that it might restore E/I imbalance by increasing the vesicular release of GABA¹⁹. On the other hand there is also evidence for that LEV could be inhibiting glutamate release²⁰⁻²³. There is very little studies of the effects of LEV on glutamate release in-vivo, and none in schizophrenia.

Aims and objectives

The aim of my project is to examine the relationship between synaptic connectivity and glutamatergic function in schizophrenia by comparing 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 vesicles²⁴, therefore I expect that LEV will decrease the amount of glutamate released from presynaptic terminals.

Similarly 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. LEV

decreases EPSC in a frequency-dependent and activity-dependent manner²¹, and it's been proposed that it preferentially acts on hyperactive synapses^{24,25}. Therefore I think that a greater decrease in glutamate will be observed in participants with schizophrenia than healthy controls.

Lastly, I believe there will be no change in symptoms. Evidence suggests that LEV could potentially normalise some changes associated with the aetiology of schizophrenia, such as normalising the E/I imbalance, hippocampal hyperactivity¹⁶ and hyperperfusion¹⁷; however I don't believe that a single dose is enough to yield observable effects on symptoms.

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).

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 ($[^{11}\text{C}]\text{UCB-J } V_T$) in the ACC in patients with schizophrenia¹³, and altered relationship between glutamate and SV2A density in the hippocampus¹⁴.
- 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 in 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.

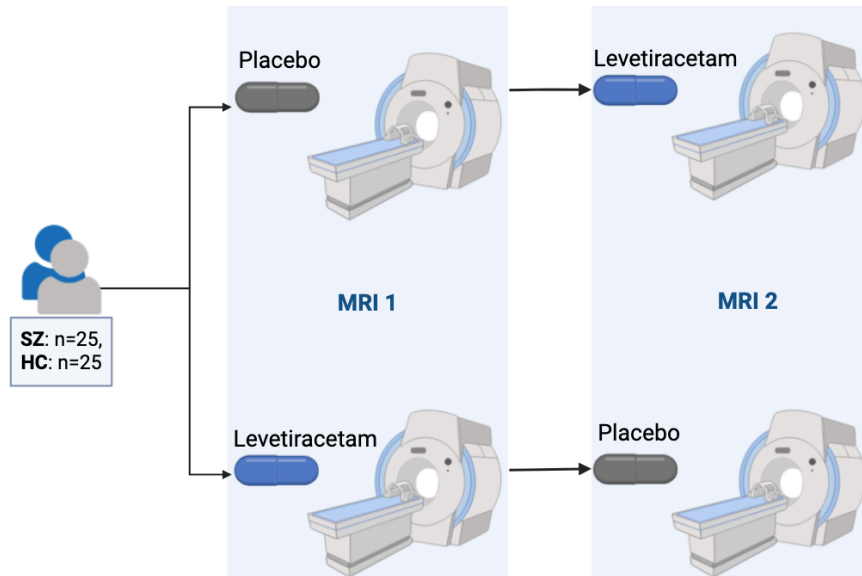


Figure 1: Study design: participants are randomised into the order in which they receive the placebo and levetiracetam (HC= healthy controls, SZ=participants with schizophrenia)

Analysis

Change in glutamate

- MRS data processing will be done in Osprey, and values corrected for partial volume effects 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 participants 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 add a plot with our data in the final version of the proposal)

Source: [Plots](#)

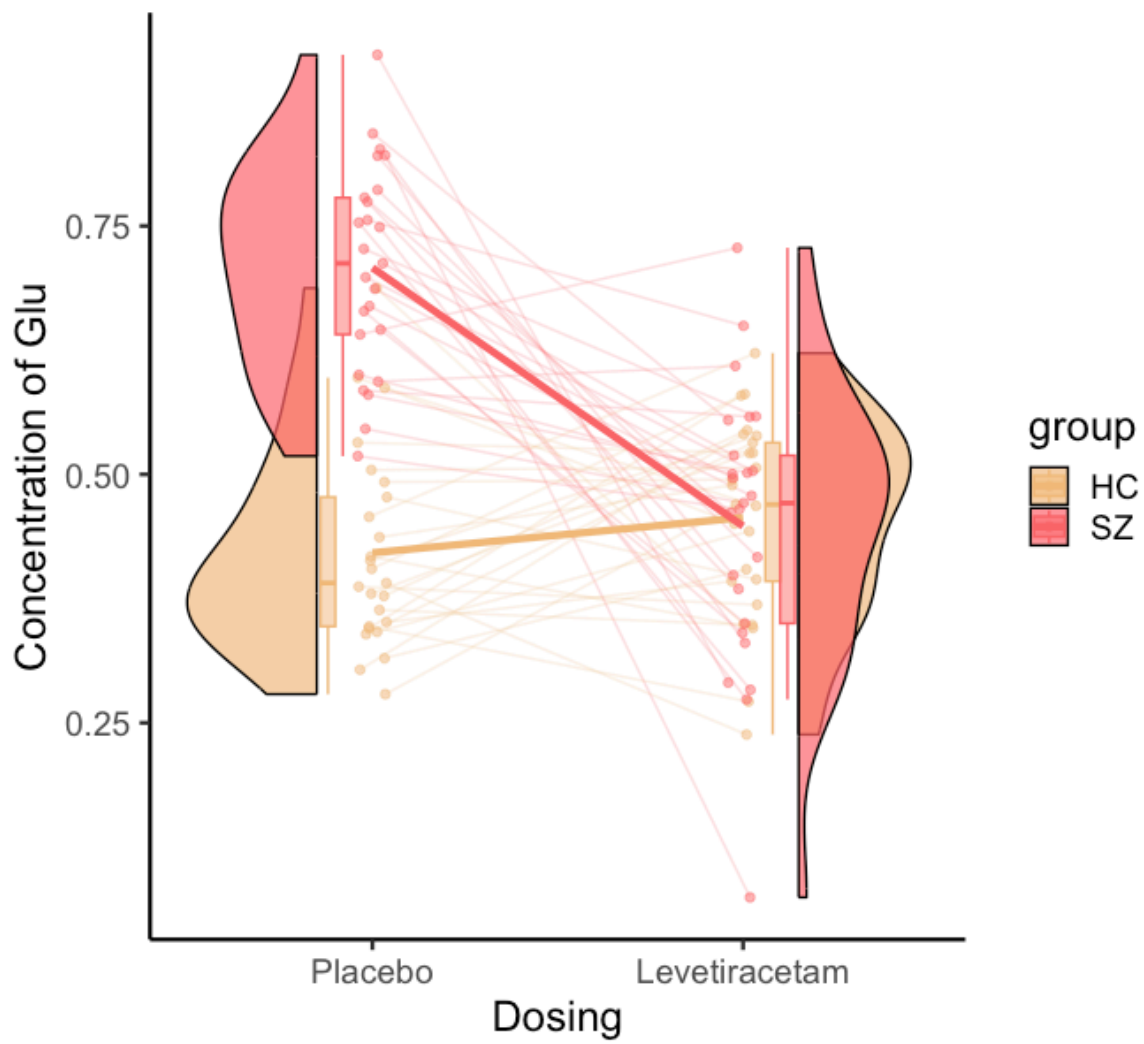


Figure 2: Comparison of Glu levels change between placebo and levetiracetam in HC and SZ

Change in symptoms

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

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: 11 (44%)
- HC: 5 (20%)
- **Total: 16 (32%)**

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.

Planned future work

- Continuing recruitment
- Set up MRS preprocessing pipeline
- ?

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^{12,13}, 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.

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

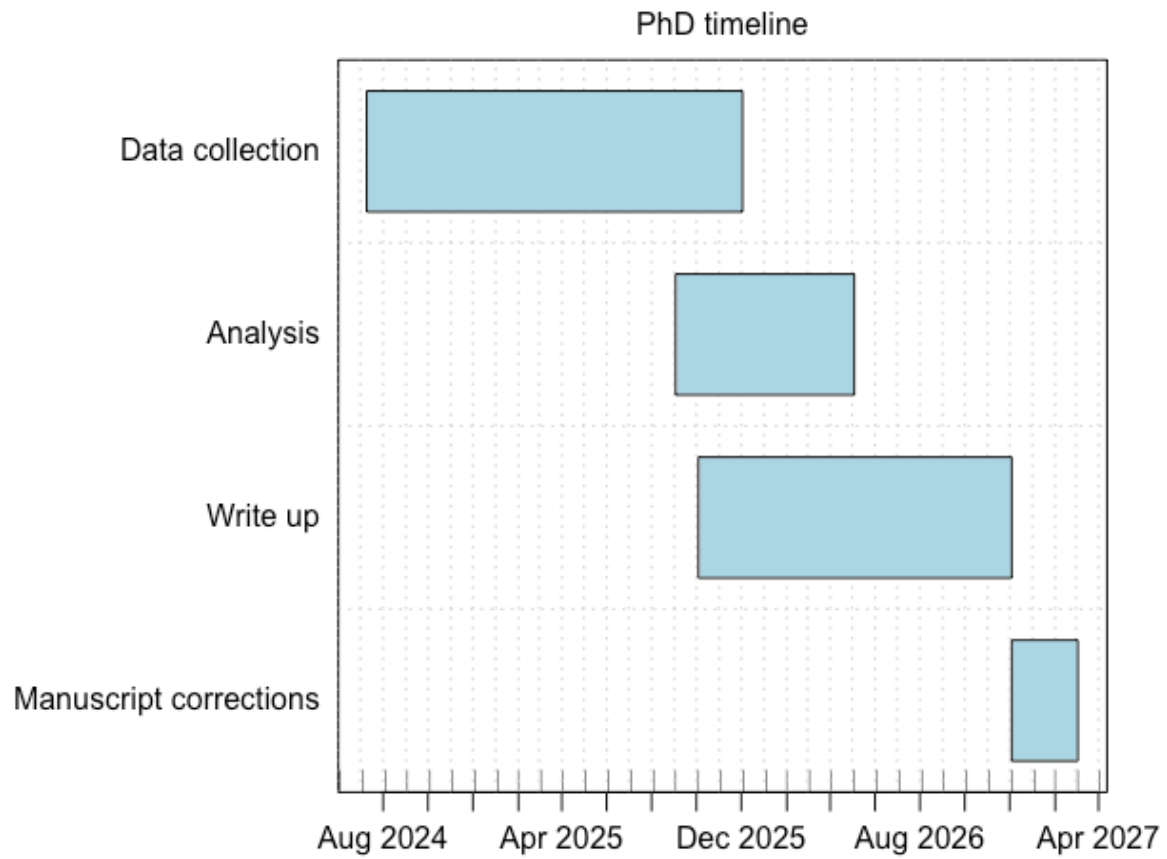


Figure 3: Gantt chart of planned work during my PhD

Source: [Plots](#)

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