

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).

Glutamatergic transmission is thought to be disrupted 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 regions³⁻⁶. Although several studies report seemingly contradictory results of decreased glutamate in schizophrenia^{3,7}, the discrepancies might be due to several confounding factors such as duration of illness, exposure to medication, or region of interest⁶. Glutamate levels are associated with illness severity and treatment response³. Antipsychotic medication decreases glutamate levels⁷⁻⁹. Non-responders have higher levels of glutamate at baseline^{10,11} and post-treatment¹¹. One study reported that increased glutamate in the ACC at baseline in non-responders was associated with lower likelihood of responding to treatment¹².

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 interneurons¹³, 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.

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)¹⁴, and repeated administration results in increased release of DA in rodent striatum¹⁵, suggesting that hyperdopaminergia is caused by decreased NMDA activity^{1,2}. 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 schizophrenia¹⁶ 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 schizophrenia¹⁷. Post-mortem studies report decreased levels of synaptic proteins¹⁸, 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^{19,20} and an altered relationship glutamate and synaptic function²¹. In healthy participants there is a positive correlation between SV2A density and glutamate in ACC and hippocampus, but no significant correlations were found in schizophrenia²¹, 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)²². 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 hyperactivity²³ where E/I imbalance is understood to originate. Preliminary results from a clinical trial published at clinicaltrials.gov (NCT03129360)²⁴ 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 glutamate²⁵; evidence from preclinical studies on epilepsy suggests that it might restore E/I imbalance by increasing the vesicular release of GABA²⁶. On the other hand there is also evidence for that LEV could be inhibiting glutamate release²⁷⁻³⁰. 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 vesicles³¹, 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 manner²⁸, and it's been proposed that it preferentially acts on hyperactive synapses^{31,32}. 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 etiology of schizophrenia, such as normalising the E/I imbalance, hippocampal hyperactivity²³ and hyperperfusion²⁴; 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).

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-group⁹.

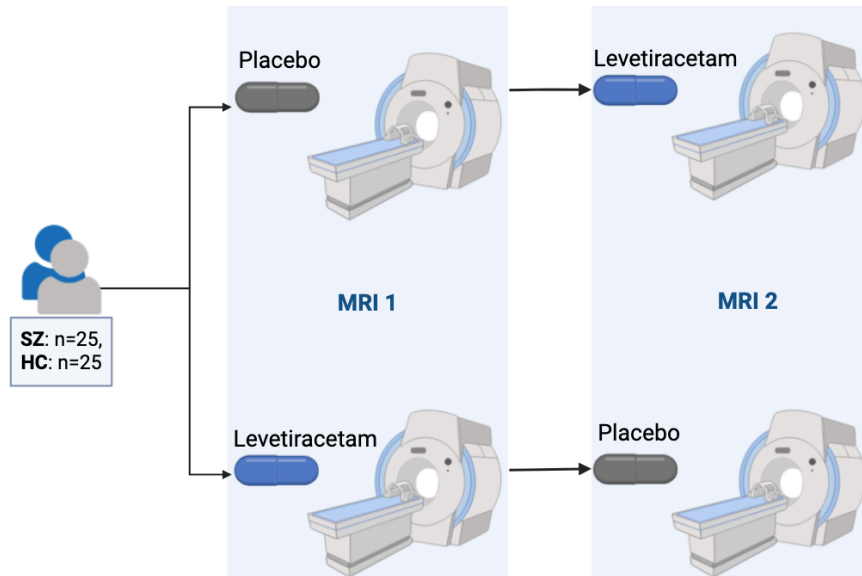


Figure 1: Study design: participants are randomised into the order in which they receive the placebo and levetiracetam (HC= healthy controls, SZ=participants with schizophrenia)

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 schizophrenia²⁰, and altered relationship between glutamate and SV2A density in the hippocampus²¹.
- 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.

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](#)

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.

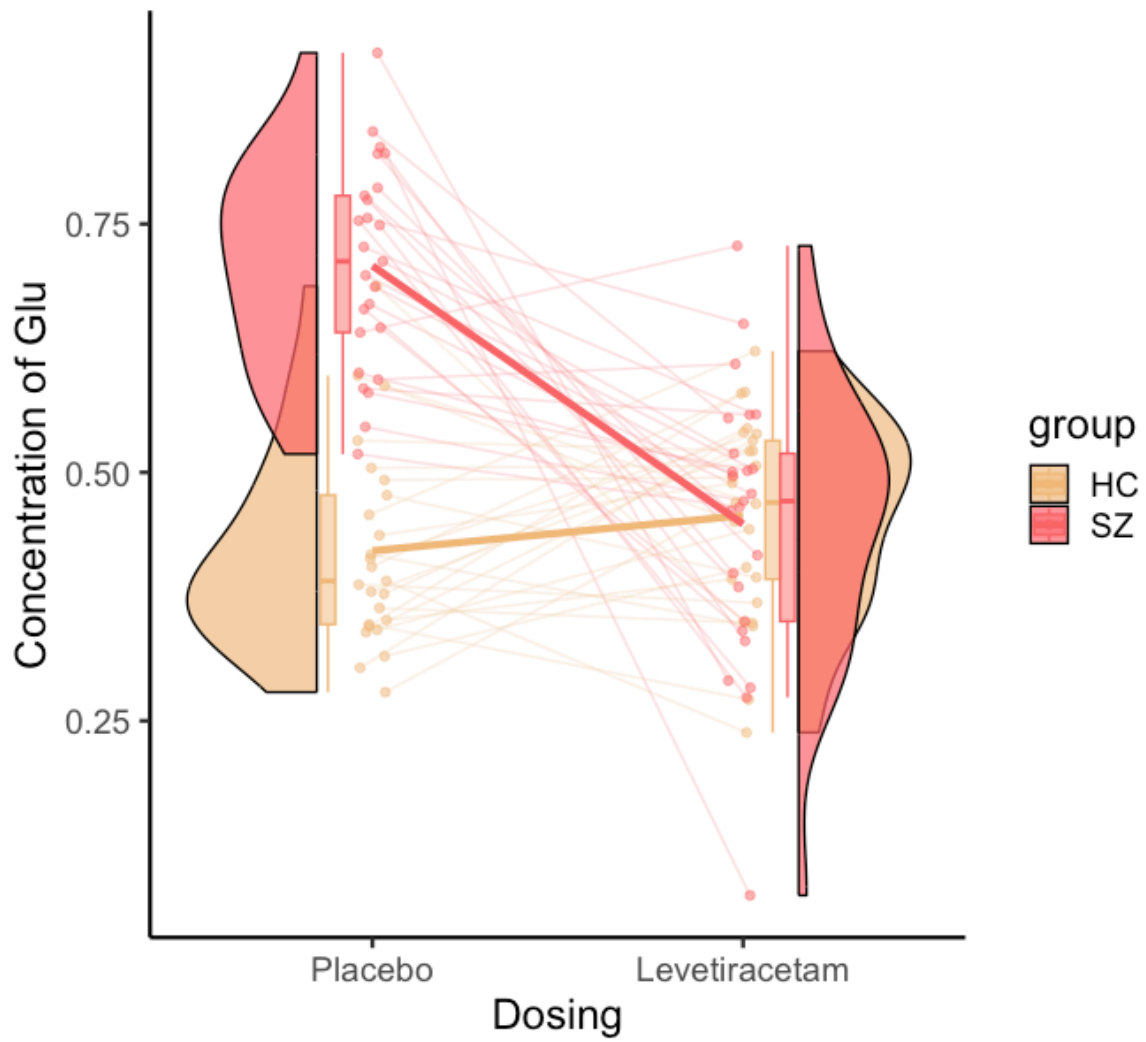


Figure 2: Comparison of Glu levels change between placebo and levetiracetam in HC and SZ

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^{19,20}, 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

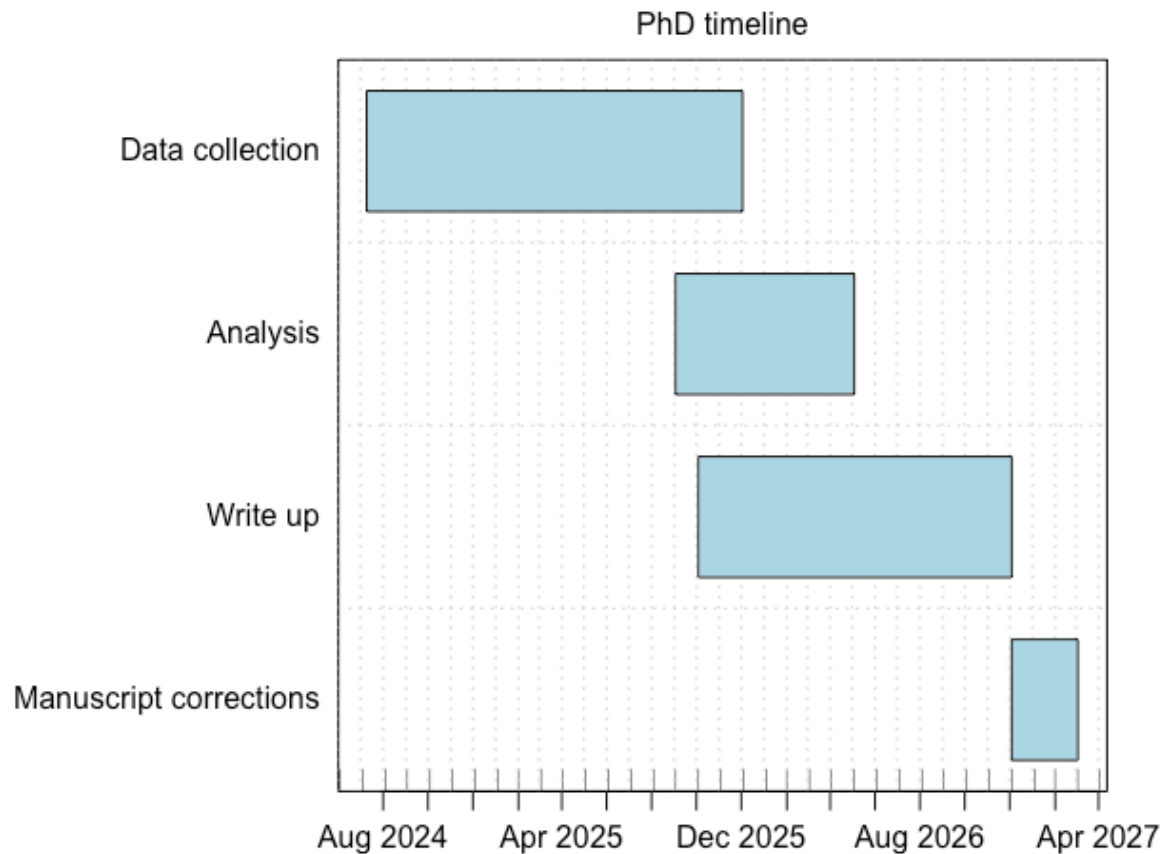


Figure 3: Gantt chart of planned work during my PhD

Source: [Plots](#)

References

1. Grace, A. A. Dopamine System Dysregulation by the Hippocampus: Implications for the Pathophysiology and Treatment of Schizophrenia. *Neuropharmacology* **62**, 1342–1348. ISSN: 00283908. <https://linkinghub.elsevier.com/retrieve/pii/S0028390811001912> (Mar. 2012).
2. Grace, A. A. Dysregulation of the Dopamine System in the Pathophysiology of Schizophrenia and Depression. *Nature Reviews Neuroscience* **17**, 524–532. ISSN: 1471-003X, 1471-0048. <https://www.nature.com/articles/nrn.2016.57> (Aug. 2016).
3. Merritt, K. & Egerton, A. Nature of Glutamate Alterations in Schizophrenia: A Meta-Analysis of Proton Magnetic Resonance Spectroscopy Studies. *Schizophrenia Bulletin* **43**, S84–S84. ISSN: 0586-7614, 1745-1701. <https://academic.oup.com/schizophreniabulletin/article-lookup/doi/10.1093/schbul/sbx021.223> (suppl_1 Mar. 1, 2017).
4. Kraguljac, N. V., White, D. M., Reid, M. A. & Lahti, A. C. Increased Hippocampal Glutamate and Volumetric Deficits in Unmedicated Patients With Schizophrenia. *JAMA Psychiatry* **70**, 1294. ISSN: 2168-622X. <http://archpsyc.jamanetwork.com/article.aspx?doi=10.1001/jamapsychiatry.2013.2437> (Dec. 1, 2013).
5. Marsman, A. *et al.* Glutamate in Schizophrenia: A Focused Review and Meta-Analysis of 1H-MRS Studies. *Schizophrenia Bulletin* **39**, 120–129. ISSN: 1745-1701, 0586-7614. <https://academic.oup.com/schizophreniabulletin/article-lookup/doi/10.1093/schbul/sbr069> (Jan. 2013).
6. Nakahara, T. *et al.* Glutamatergic and GABAergic Metabolite Levels in Schizophrenia-Spectrum Disorders: A Meta-Analysis of 1H-magnetic Resonance Spectroscopy Studies. *Molecular Psychiatry* **27**, 744–757. ISSN: 1359-4184, 1476-5578. <https://www.nature.com/articles/s41380-021-01297-6> (Jan. 2022).
7. Jeon, P. *et al.* Progressive Changes in Glutamate Concentration in Early Stages of Schizophrenia: A Longitudinal 7-Tesla MRS Study. *Schizophrenia Bulletin Open* **2**, sgaa072. ISSN: 2632-7899. <https://academic.oup.com/schizbullopen/article/doi/10.1093/schizbullopen/sgaa072/6126062> (Jan. 1, 2021).
8. Bojesen, K. B. *et al.* Treatment Response after 6 and 26 Weeks Is Related to Baseline Glutamate and GABA Levels in Antipsychotic-Naïve Patients with Psychosis. *Psychological Medicine* **50**, 2182–2193. ISSN: 0033-2917, 1469-8978. https://www.cambridge.org/core/product/identifier/S0033291719002277/type/journal_article (Oct. 2020).
9. Egerton, A. *et al.* Effects of Antipsychotic Administration on Brain Glutamate in Schizophrenia: A Systematic Review of Longitudinal 1H-MRS Studies. *Frontiers in Psychiatry* **8**, 66. ISSN: 1664-0640. <http://journal.frontiersin.org/article/10.3389/fpsy.2017.00066/full> (Apr. 28, 2017).

10. Fan, L. *et al.* Glutamatergic Basis of Antipsychotic Response in First-Episode Psychosis: A Dual Voxel Study of the Anterior Cingulate Cortex. *Neuropsychopharmacology* **49**, 845–853. ISSN: 0893-133X, 1740-634X. <https://www.nature.com/articles/s41386-023-01741-x> (Apr. 2024).
11. Reyes-Madrigal, F. *et al.* Striatal Glutamate, Subcortical Structure and Clinical Response to First-Line Treatment in First-Episode Psychosis Patients. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **113**, 110473. ISSN: 02785846. <https://linkinghub.elsevier.com/retrieve/pii/S0278584621002323> (Mar. 2022).
12. Egerton, A. *et al.* Response to Initial Antipsychotic Treatment in First Episode Psychosis Is Related to Anterior Cingulate Glutamate Levels: A Multicentre 1H-MRS Study (OPTiMiSE). *Molecular Psychiatry* **23**, 2145–2155. ISSN: 1359-4184, 1476-5578. <https://www.nature.com/articles/s41380-018-0082-9> (Nov. 2018).
13. Nakazawa, K., Jeevakumar, V. & Nakao, K. Spatial and Temporal Boundaries of NMDA Receptor Hypofunction Leading to Schizophrenia. *npj Schizophrenia* **3**, 7. ISSN: 2334-265X. <https://www.nature.com/articles/s41537-016-0003-3> (Feb. 3, 2017).
14. Beck, K. *et al.* Association of Ketamine With Psychiatric Symptoms and Implications for Its Therapeutic Use and for Understanding Schizophrenia: A Systematic Review and Meta-analysis. *JAMA Network Open* **3**, e204693. ISSN: 2574-3805. <https://jamanetwork.com/journals/jamanetworkopen/fullarticle/2766225> (May 21, 2020).
15. Balla, A. Continuous Phencyclidine Treatment Induces Schizophrenia-Like Hyperreactivity of Striatal Dopamine Release. *Neuropsychopharmacology* **25**, 157–164. ISSN: 0893133X. [https://www.nature.com/doifinder/10.1016/S0893-133X\(01\)00230-5](https://www.nature.com/doifinder/10.1016/S0893-133X(01)00230-5) (Aug. 2001).
16. Howes, O. D. & Onwordi, E. C. The Synaptic Hypothesis of Schizophrenia Version III: A Master Mechanism. *Molecular Psychiatry*. ISSN: 1359-4184, 1476-5578. <https://www.nature.com/articles/s41380-023-02043-w> (Apr. 11, 2023).
17. Glantz, L. A., Gilmore, J. H., Lieberman, J. A. & Jarskog, L. F. Apoptotic Mechanisms and the Synaptic Pathology of Schizophrenia. *Schizophrenia Research* **81**, 47–63. ISSN: 09209964. <https://linkinghub.elsevier.com/retrieve/pii/S0920996405003683> (Jan. 2006).
18. Osimo, E. F., Beck, K., Reis Marques, T. & Howes, O. D. Synaptic Loss in Schizophrenia: A Meta-Analysis and Systematic Review of Synaptic Protein and mRNA Measures. *Molecular Psychiatry* **24**, 549–561. ISSN: 1359-4184, 1476-5578. <https://www.nature.com/articles/s41380-018-0041-5> (Apr. 2019).
19. Radhakrishnan, R. *et al.* In Vivo Evidence of Lower Synaptic Vesicle Density in Schizophrenia. *Molecular Psychiatry* **26**, 7690–7698. ISSN: 1359-4184, 1476-5578. <https://www.nature.com/articles/s41380-021-01184-0> (Dec. 2021).
20. Onwordi, E. C. *et al.* Synaptic Density Marker SV2A Is Reduced in Schizophrenia Patients and Unaffected by Antipsychotics in Rats. *Nature Communications* **11**, 246. ISSN: 2041-1723. <http://www.nature.com/articles/s41467-019-14122-0> (Dec. 2020).

21. Onwordi, E. C. *et al.* The Relationship between Synaptic Density Marker SV2A, Glutamate and N-acetyl Aspartate Levels in Healthy Volunteers and Schizophrenia: A Multimodal PET and Magnetic Resonance Spectroscopy Brain Imaging Study. *Translational Psychiatry* **11**, 393. ISSN: 2158-3188. <https://www.nature.com/articles/s41398-021-01515-3> (July 17, 2021).
22. Wang, M., Jiang, L. & Tang, X. Levetiracetam Is Associated with Decrease in Subclinical Epileptiform Discharges and Improved Cognitive Functions in Pediatric Patients with Autism Spectrum Disorder. *Neuropsychiatric Disease and Treatment* **Volume 13**, 2321–2326. ISSN: 1178-2021. <https://www.dovepress.com/levetiracetam-is-associated-with-decrease-in-subclinical-epileptiform-peer-reviewed-article-NDT> (Aug. 2017).
23. Roeske, M. J. *et al.* Modulation of Hippocampal Activity in Schizophrenia with Levetiracetam: A Randomized, Double-Blind, Cross-over, Placebo-Controlled Trial. *Neuropsychopharmacology*. ISSN: 0893-133X, 1740-634X. <https://www.nature.com/articles/s41386-023-01730-0> (Oct. 13, 2023).
24. Goff, D. *Levetiracetam in Early Psychosis* version 1.9. NCT03129360 (2020). <https://clinicaltrials.gov/study/NCT03129360?id=NCT03129360&limit=10&rank=1&tab=results>.
25. Contreras-García, I. J. *et al.* Levetiracetam Mechanisms of Action: From Molecules to Systems. *Pharmaceuticals* **15**, 475. ISSN: 1424-8247. <https://www.mdpi.com/1424-8247/15/4/475> (Apr. 2022).
26. Luz Adriana, P. M. *et al.* Effect of Levetiracetam on Extracellular Amino Acid Levels in the Dorsal Hippocampus of Rats with Temporal Lobe Epilepsy. *Epilepsy Research* **140**, 111–119. ISSN: 09201211. <https://linkinghub.elsevier.com/retrieve/pii/S0920121117304710> (Feb. 2018).
27. Contreras-García, I. J. *et al.* Synaptic Vesicle Protein 2A Expression in Glutamatergic Terminals Is Associated with the Response to Levetiracetam Treatment. *Brain Sciences* **11**, 531. ISSN: 2076-3425. <https://www.mdpi.com/2076-3425/11/5/531> (Apr. 23, 2021).
28. Meehan, A. L., Yang, X., Yuan, L.-L. & Rothman, S. M. Levetiracetam Has an Activity-dependent Effect on Inhibitory Transmission. *Epilepsia* **53**, 469–476. ISSN: 0013-9580, 1528-1167. <https://onlinelibrary.wiley.com/doi/10.1111/j.1528-1167.2011.03392.x> (Mar. 2012).
29. Yang, X.-F., Weisenfeld, A. & Rothman, S. M. Prolonged Exposure to Levetiracetam Reveals a Presynaptic Effect on Neurotransmission. *Epilepsia* **48**, 1861–1869. ISSN: 0013-9580, 1528-1167. <https://onlinelibrary.wiley.com/doi/10.1111/j.1528-1167.2006.01132.x> (Oct. 2007).
30. Lee, C.-Y., Chen, C.-C. & Liou, H.-H. Levetiracetam Inhibits Glutamate Transmission through Presynaptic P/Q-type Calcium Channels on the Granule Cells of the Dentate Gyrus. *British Journal of Pharmacology* **158**, 1753–1762. ISSN: 0007-1188, 1476-5381. <https://bpspubs.onlinelibrary.wiley.com/doi/10.1111/j.1476-5381.2009.00463.x> (Dec. 2009).

31. Meehan, A. L., Yang, X., McAdams, B. D., Yuan, L. & Rothman, S. M. A New Mechanism for Antiepileptic Drug Action: Vesicular Entry May Mediate the Effects of Levetiracetam. *Journal of Neurophysiology* **106**, 1227–1239. ISSN: 0022-3077, 1522-1598. <https://www.physiology.org/doi/10.1152/jn.00279.2011> (Sept. 2011).
32. Löscher, W., Gillard, M., Sands, Z. A., Kaminski, R. M. & Klitgaard, H. Synaptic Vesicle Glycoprotein 2A Ligands in the Treatment of Epilepsy and Beyond. *CNS Drugs* **30**, 1055–1077. ISSN: 1172-7047, 1179-1934. <http://link.springer.com/10.1007/s40263-016-0384-x> (Nov. 2016).