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Increasing Ambient GABA Levels in the Hippocampus to Improve Cognition in a Mouse Model of Schizophrenia: A Research Protocol

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Abstract

Introduction: Schizophrenia is a complex psychiatric disorder characterized by cognitive, positive, and negative symptoms, with cognitive impairments significantly affecting learning, memory, and executive functions. The hippocampus, particularly the CA1 region, is a critical area of interest due to its role in learning and memory and its dysfunction in schizophrenia. This protocol explores the effects of increasing ambient Gamma-Aminobutyric Acid (GABA) levels in the CA1 region on cognitive functions in a Methylazoxymethanol Acetate (MAM) model of schizophrenia.

Methods: Utilizing a two-by-two factorial design, male Sprague-Dawley rats undergo either a sham or MAM treatment, followed by a control or GABA modulation intervention. The study will employ GABA transporter inhibitor Tiagabine to increase ambient GABA levels and assess its impact on neuronal excitability and cognitive function. Behavioural assessments will include the Morris water maze and the Attentional set-shifting test to evaluate spatial memory and cognitive flexibility. Histological examinations and electrophysiological measurements will provide insights into structural and functional changes in the hippocampus.

Results: The results are expected to demonstrate neurodevelopmental disruptions in MAM-treated offspring and improvements in cognitive performance following GABA modulation. Enhanced spatial memory and cognitive flexibility are anticipated in MAM-treated rats with GABA modulation, as evidenced by performance in the Morris water maze and Attentional set-shifting test. Electrophysiological recordings are likely to show increased tonic inhibition in the CA1 region.

Discussion: This study aims to extend the understanding of tonic inhibition in schizophrenia treatment. By focusing on ambient GABA modulation, the research could offer novel insights into therapeutic strategies for schizophrenia, especially in enhancing cognitive functions. However, the translational applicability of intracerebral administration of uptake inhibitors like Tiagabine poses a significant challenge, highlighting the need for future research in more clinically viable methods of GABA modulation.

Conclusion: This study was designed to contribute to schizophrenia research, particularly in understanding cognitive impairments and developing potential therapeutic strategies targeting the GABAergic system. The proposed findings could pave the way for novel treatments, improving the quality of life for individuals with schizophrenia.

Keywords schizophrenia; ambient GABA; α5GABAA; tiagabine; hippocampus; CA1; MAM; MWM; AST

Introduction

Schizophrenia is a psychiatric disorder characterized by a spectrum of symptoms that can be broadly categorized into cognitive, positive, and negative domains [1]. Positive symptoms, often the most recognizable, include hallucinations, delusions, and thought disorders [2]. Conversely, negative symptoms are represented by social withdrawal, lack of motivation, and a flat or inappropriate mood [2, 3]. The cognitive impairments associated with schizophrenia significantly affect learning, memory, and executive function, leading to challenges in daily functioning and reduced quality of life [2-4]. In Canada alone, over 360,000 individuals grapple with some form of schizophrenia [5]. Worldwide, schizophrenia affects nearly 24 million people, presenting a significant challenge to both affected individuals and the broader healthcare community [6].

The hippocampus, which is crucial for learning and memory processes, plays a consistent role in schizophrenia [7]. In schizophrenia, hippocampal dysfunction is manifested by abnormalities in both the structure and function of this region, leading to difficulties in forming and retrieving memories, which are core cognitive symptoms of the disorder [7, 10]. One such hippocampal change is the disrupted balance between excitatory and inhibitory neurotransmission [8, 9]. GABA, or γ-aminobutyric acid, is the primary inhibitory neurotransmitter in the brain, playing a crucial role in balancing excitatory and inhibitory signals to ensure optimal neural function [1, 12]. The concentration of

GABA in the brain's extracellular space is crucial for tonic inhibition—a form of inhibition that sets the baseline excitability of neurons and is important for cognition [13]. Central to GABA's function are the GABAA receptors, pentameric ion channels designed for the selective passage of chloride ions [14, 15]. Among the various GABAA receptor subunits, the α5 subunit, predominantly found in the hippocampus, stands out for its role in mediating tonic inhibition [16]. Research indicates decreased hippocampal feed-forward and tonic GABA-mediated inhibition in schizophrenia models, leading to cognitive deficits [17]. This decrease is often accompanied by increased hippocampal excitability, which has been linked to the cognitive deficits observed in the disorder [4].

Studies have suggested that in schizophrenia, alterations in ambient GABA levels, especially in the CA1 region of the hippocampus, contribute to the hyperexcitability of neurons [18]. This alteration in GABA levels may be related to changes in the synthesis, release, uptake, and degradation of GABA, as well as to the function of GABAA receptors, particularly those containing the α5 subunit (α5GABAA receptors) [19-21]. GABA transporters, specifically GABA transporter-1 (GAT-1), play a critical role in regulating extracellular GABA levels by GABA reuptake from the synaptic cleft back into neurons and glial cells [22]. To increase ambient GABA, one could consider inhibiting these transporters, thereby elevating extracellular GABA levels.

The MAM model of schizophrenia, involving prenatal exposure to methylazoxymethanol acetate (MAM), has emerged as a prominent tool in understanding the developmental origins and progression of hippocampal dysfunction in schizophrenia [23]. This model induces neurodevelopmental disruptions that lead to long-term alterations in hippocampal structure and function, paralleling aspects of the human condition [23, 24]. Importantly, studies utilizing the MAM model have consistently demonstrated hippocampal hyperexcitability [25]. Interestingly, two papers have shown that increasing tonic inhibition in this model, using pharmacological or genetic methods targeting α5GABAA receptors, improves cognition [26, 27]. These studies indicate that increasing tonic potential has therapeutic potential in treating cognitive symptoms of schizophrenia.

This protocol proposes another strategy to increase tonic inhibition in the MAM model. To expand our understanding of the neurobiological underpinnings of schizophrenia and its pathology, this protocol will evaluate whether increasing ambient GABA levels in the hippocampal CA1 region can reduce cognitive deficits in the MAM model of schizophrenia.

Methods

Model & Study Design

Male offspring of pregnant Sprague-Dawley rats were selected for this study, as commonly used in schizophrenia research [23, 26, 28]. Animals underwent either a sham procedure or MAM treatment on gestational day 17. For the MAM treatment, pregnant Sprague-Dawley rats received a single intraperitoneal injection of methylazoxymethanol acetate (MAM, 25 mg/kg), a procedure designed to induce neurodevelopmental disruptions in the offspring that mimic aspects of schizophrenia, including long-term alterations in brain structure and neurotransmitter systems [23, 26]. This treatment leads to significant changes in the hippocampus and prefrontal cortex, mimicking the structural and functional brain abnormalities observed in humans with schizophrenia, such as ventricular enlargement and reduced gray matter volume [23]. Furthermore, MAM treatment disrupts the dopaminergic, glutamatergic, and GABAergic neurotransmitter systems, reflecting the complex neurochemical imbalances associated with schizophrenia [23, 26, 28]. All subsequent experiments were conducted once animals reached adulthood (PND 56). A two-by-two factorial design was employed, with one factor being treatment (sham vs. MAM) and the other being intervention (control vs. GABA modulation).

Control dams were administered with equivalent volumes of saline. After injection, dams were monitored for any immediate adverse reactions for 4-6 days, and offspring were observed from birth to adulthood (PND 56) [23]. Monitoring included daily weight checks and assessments of general recovery behaviours such as activity levels, grooming, feeding, and social interactions with littermates [23]. Behavioural assessments also included the righting reflex, negative geotaxis, auditory startle response, and social play behaviour to evaluate neurodevelopmental progress [23, 26]. Through these comprehensive evaluations, the MAM model provides a robust framework for studying the neurodevelopmental underpinnings of schizophrenia and testing potential interventions aimed at ameliorating cognitive and behavioural deficits associated with the disorder.

To modulate ambient GABA levels within the CA1 hippocampal region, the GABA transporter inhibitor Tiagabine was administered, specifically targeting the GABA transporter-1 [29]. This approach was designed to inhibit GABA reuptake, thereby increasing the extracellular concentration of GABA and enhancing tonic inhibition [29, 30]. Tiagabine was administered intracerebrally at a concentration of 5 µM in a volume of 0.5-1 µl using a microinfusion pump for precise control. Administration was conducted via intracerebral infusion using precise stereotaxic methods, targeting the CA1 region of the hippocampus. The infusion was performed over 5-10 minutes to ensure adequate diffusion and minimize backflow.

Morris Water Maze

Following interventions, all rats underwent cognitive function tests using the Morris Water Maze (MWM) for spatial learning and memory [31]. In the MWM test, a circular pool of 1.8 m in diameter and 0.6 m deep, filled with opaque water maintained at 22-25°C, was utilized [31, 32]. The pool contained a small, circular platform, 10-15

cm in diameter, submerged 1-2 cm below the water surface in one of its quadrants. Large, distinct visual cues were placed on the walls surrounding the pool for spatial guidance [32, 33]. During the training phase, from Day 1 to Day 5, each rat underwent four trials per day, with varying starting positions to prevent path bias. Rats were placed in the pool and timed for their ability to locate the submerged platform, recording latency, distance travelled, and swim speed using an automated tracking system. If a rat failed to find the platform within 60 seconds, it was guided to it, allowing a brief observation of the spatial cues. This phase aimed to train the rats to use visual cues to navigate to the platform, with learning indicated by decreased latency across trials [33]. On Day 6, a probe trial was conducted by removing the platform and allowing rats to swim for 60 seconds, measuring the time spent in the quadrant previously containing the platform. This assessed the rats' memory retention, with more time spent in the target quadrant indicating better spatial memory [32, 33]. The reversal phase, from Day 7 to Day 9 followed by another probe trial on Day 10, involved relocating the platform to a different quadrant and retraining rats to find the new location. This part of the test evaluated cognitive flexibility and the ability to relearn, with performance metrics like latency and swim speed compared to the initial training phase [33]. Figure 1 provides a general visual representation of the MWM Test.

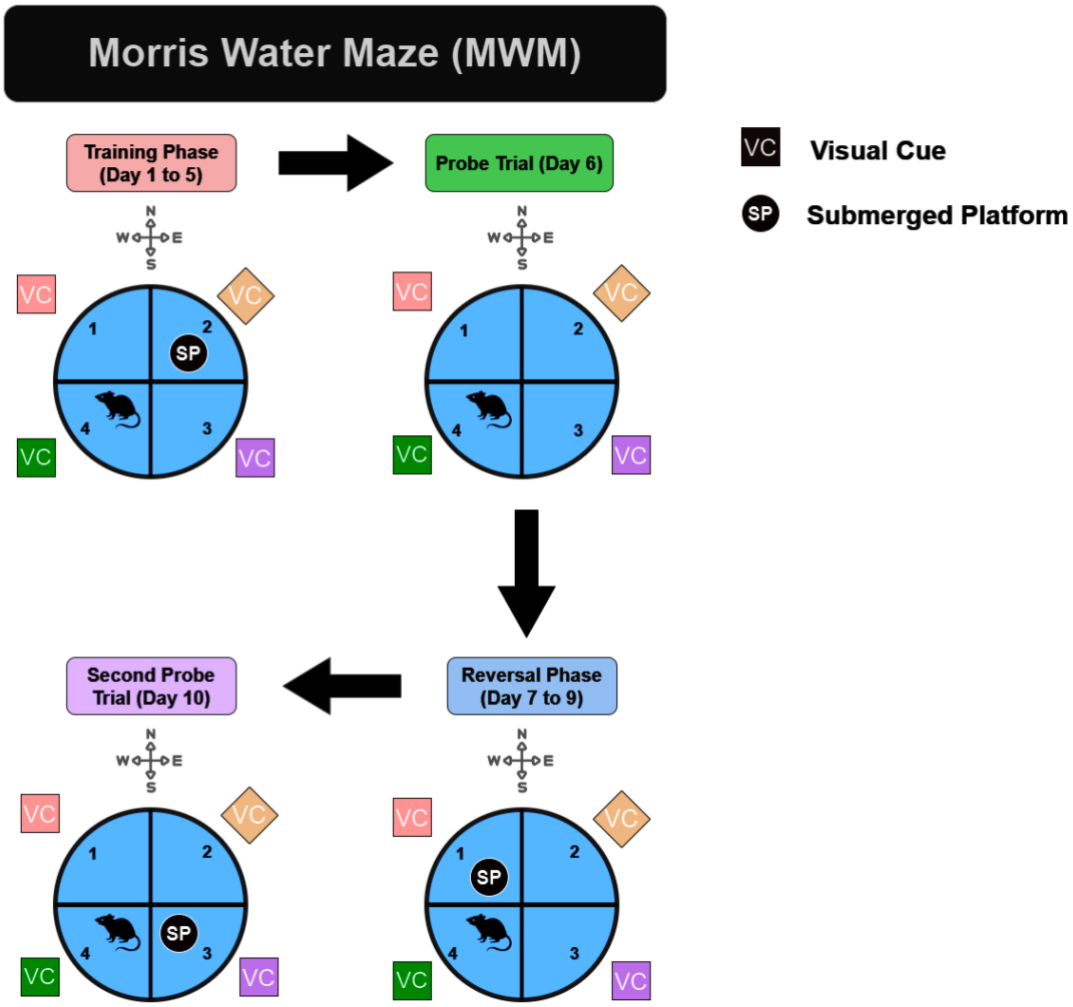

Figure 1. Setup of the Morris Water Maze (MWM) test. The diagram depicts a circular pool (blue circle), 1.8 meters in diameter and 0.6 meters deep, filled with opaque water at a temperature of 22-25°C. A small, circular platform (in red), submerged 1-2 cm below the surface, is positioned in one of the pool's quadrants, serving as the goal for navigation. Around the pool, large visual cues (represented by green, red, purple and yellow squares) on the walls provide spatial guidance. The diagram outlines the test's phases, including the training phase, where rats locate the submerged platform with varied starting positions, aiming to prevent path bias. Performance is monitored via an automated tracking system, capturing metrics such as latency, distance travelled, and swim speed. The subsequent probe trial, where the platform is removed to assess memory retention, and the reversal phase, testing cognitive flexibility and relearning by relocating the platform, are also represented. Created using Diagrams.net.

Attentional Set-Shifting Test

The attentional set-shifting test (AST) was used to evaluate attention and cognitive flexibility, as performed by others [26, 34]. In this assay, rats dug in two bowls, one containing a food reward. Through the test, rats learned to respond to a relevant sensory dimension associated with the food reward, while ignoring an irrelevant dimension. Over numerous trials, variations within the protocol assessed cognitive flexibility [26]. Rats were restricted to a diet of 12 g of food per day for one week before the start of the assessment. Using cereal as a reward, rats were trained to dig for food in two bowls within the AST chamber, with cues along two dimensions: odour (essential oil applied to the rim of the bowl) and digging medium. Rats were then tested through multiple stages, where six consecutive correct trials were required to reach the next stage. Within each trial, rats had to make correct sensory discrimination to dig in the food bowl containing the food reward. The first stage was simple discrimination (SD), where only the relevant sensory dimension was present. Half the rats discriminated between two odours, and the other half between two digging media. The next stage was compound discrimination (CD), where the irrelevant dimension (digging media or odour, respectively) was introduced, but the rule to find the reward remained the same as in SD. Stage 3 was reversal learning (RL1), where the odours and media remained the same, and the relevant dimension was unchanged, but the rule was flipped: the negative cue from SD now became the positive cue. The fourth stage was an intra-dimensional shift (IDS), where the odours and media were all changed, but the same sensory dimension as SD remained relevant. The subsequent stage was another reversal (RL2), using the same odours and media as the IDS stage. The final stage was an extra-dimensional set-shift (EDS), where all new odours and media were introduced again, but the relevant sensory dimension was now switched: rats that were initially discriminating between odours now had to attend to digging media, and vice versa [26]. Performance was measured based on the number of trials taken to reach the criterion (i.e., six consecutive correct choices) in each stage, where a higher number of trials at a new stage indicated poorer cognitive flexibility [26]. Figure 2 provides a general visual representation of the AST protocol.

Histology

Following the completion of behavioural assessments, rats were euthanized, and their brain tissues were extracted for histological examination [35]. Brains were fixed in a 4% paraformaldehyde solution, followed by cryoprotection in a sucrose solution [35]. The preserved brains were then sectioned using a cryostat into slices approximately 40 µm thick [36, 37]. Nissl staining was employed to assess neuronal density and structural integrity within the hippocampus, which is crucial for revealing neuronal populations [38]. Additionally, Fluoro-Jade staining was used to identify and quantify degenerating neurons, providing insights into the extent of neuronal damage due to MAM exposure [39].

To further test the hypotheses regarding the role of GABAergic signalling in the MAM model of schizophrenia, histological analyses were expanded to include staining for GAT1 (GABA transporter 1) and Type A GABA Receptor Alpha5-Subunit(α5 GABAA). These specific stainings are critical for assessing the levels and distribution of GAT1 and α5 GABAA in the hippocampus, elements vital for understanding the impact of MAM treatment on GABAergic neurotransmission [40, 41]. The inclusion of GAT1 staining allows for the examination of GABA reuptake mechanisms, while α5 GABAA receptor staining provides insights into the modulation of tonic inhibition and its contribution to the cognitive functions affected in schizophrenia [42, 43].

Quantitative microscopic analysis of these stained sections assessed parameters like neuronal density, the integrity of hippocampal structures, and specifically, the alterations in α5 GABAA and GAT1 expression [42, 43]. This comprehensive histological analysis was used to verify that MAM treatment disrupts hippocampal development and affects GABAergic signalling components, as reported by others [43, 44]. The findings from GAT1 and α5 GABAA staining are expected to significantly contribute to the understanding of GABAergic dysfunction in the MAM model of schizophrenia, offering critical insights into the neurobiological mechanisms underlying the disorder and potential therapeutic targets [42-44].

Quantitative measures of GAT1 and α5 GABAA levels in the hippocampus across all treatment groups (Sham-Control, Sham-Tiagabine, MAM-Control, MAM-Tiagabine) were introduced to assess any alterations in their expression due to MAM treatment or Tiagabine intervention. A rabbit polyclonal anti-GAT1 antibody and a mouse monoclonal anti-α5 GABAA receptor subunit antibody were utilized at a dilution of 1:500 for both. A solution containing 5% normal goat serum and 0.3% Triton X-100 in PBS was used to block non-specific antibody binding, ensuring that the primary antibodies would bind specifically to their target antigens without background interference. Primary antibodies against GAT1 and α5 GABAA were incubated overnight at 4°C, at a dilution of 1:500 in PBS containing 1% BSA and 0.3% Triton X-100, to achieve optimal binding to their respective antigens. This was followed by extensive washing in PBS to remove any unbound antibodies, ensuring the specificity of the staining. The secondary antibodies, conjugated with fluorescent dyes, were then applied at a dilution of 1:1000 for 2 hours at room temperature, providing a bright and distinct signal for the targeted proteins.

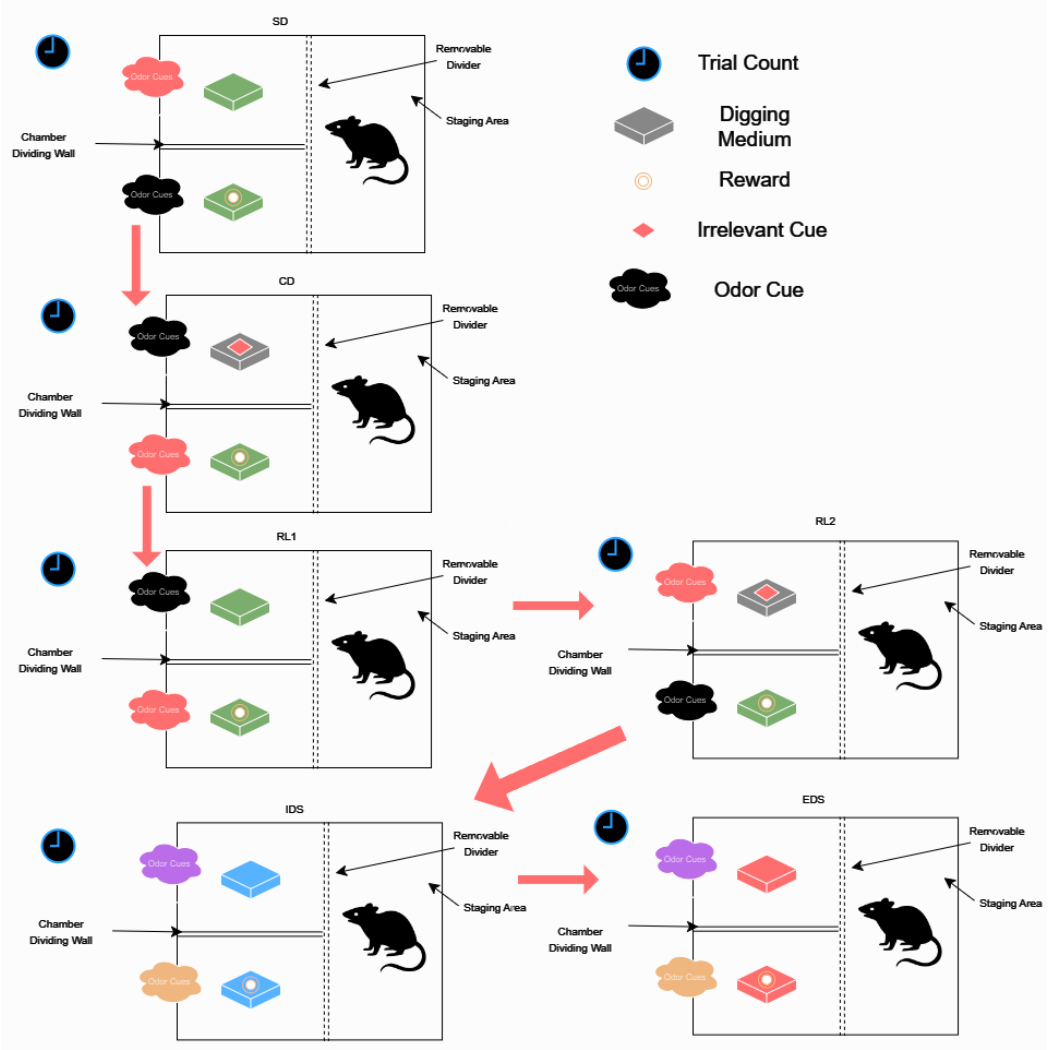

Figure 2. The sequential stages of the Attentional Set-Shifting Test (AST) used to evaluate attention and cognitive flexibility in rats. The diagram is organized as a flowchart, detailing the progression through six critical stages of the test: Simple Discrimination (SD), Compound Discrimination (CD), Reversal Learning 1 (RL1), Intra-Dimensional Shift (IDS), Reversal Learning 2 (RL2), and Extra-Dimensional Set-Shift (EDS). Each stage is visually distinguished by sensory cues (odor and digging medium), with icons indicating odor clouds for olfactory cues and varied color textures for digging media. Only one of the two bowls in each stage contains a food reward, symbolized by a sphere piece icon. The complexity of the test increases as rats are required to discriminate based on changing relevant and irrelevant cues, with performance measured by the number of trials to reach six consecutive correct choices. Red arrows guide the viewer through the test sequence, illustrating the cognitive flexibility and attentional shifts required at each stage. A legend explains the symbols used, ensuring clarity in understanding the test's components and objectives. Created using Diagrams.net.

Imaging was conducted using a confocal microscope, equipped with a 40x oil objective lens, which enabled the high-resolution visualization of GAT1 and α5 GABAA distribution and expression levels within the hippocampal structure. The use of oil immersion facilitated the capture of detailed images with high numerical aperture, significantly enhancing the resolution and clarity of the fluorescent signals. Z-stacks were acquired through the regions of interest, and maximum intensity projections were generated to illustrate the precise localization and relative abundance of GAT1 and α5 GABAA throughout the hippocampus.

To further elucidate the effects of Tiagabine on hippocampal function, this study employs a dual approach in its administration. Tiagabine is administered systemically to animals to assess its overall impact on behaviour and cognitive functions. In parallel, for direct assessment of Tiagabine's effects on tonic inhibition within the hippocampal neurons, the drug will be applied locally during electrophysiological recordings. This dual approach allows for a comprehensive evaluation of Tiagabine's efficacy and mechanism of action in modulating GABAergic signalling.

Measuring Tonic Inhibition

Whole-cell voltage-clamp recording was used to measure the magnitude of tonic inhibition, modified from techniques by others [26]. Coronal brain slices (300 µm) containing the hippocampus were prepared in ice-cold artificial cerebrospinal fluid (aCSF) and then allowed to recover in aCSF for at least one hour. Slices were then moved to a recording chamber and continually perfused with aCSF. Pyramidal neurons of the CA1 were patched in whole-cell configuration using a high-chloride intracellular solution in the recording pipette. Patched cells were voltage-clamped at -70 mV until a stable baseline holding current was reached. To study the effect of GABA uptake inhibition on tonic inhibition, aCSF containing Tiagabine (5 µM) was then perfused into the recording chamber. The subsequent shift in the holding current was quantified, allowing measurement of the increase in tonic current due to the inhibition of GABA uptake. After the application of aCSF containing Tiagabine (5 µM) and observing the resultant shift in holding current, a wash-out procedure will be implemented. This involves replacing the Tiagabine-containing aCSF with fresh Tiagabine-free aCSF, allowing the brain slices to return to baseline conditions over a 20-30 minute period. Monitoring the reversal of Tiagabine's effects provides insight into the drug's temporal impact on tonic inhibition and the potential for restoring normal neural excitability.

Animal Selection for immunohistochemistry (IHC) focusing on GAT1 transporter and GABAa5 receptor levels, versus those undergoing electrophysiological analysis, will be based on behavioural test outcomes. This ensures a comprehensive examination of the relationship between behavioural phenotypes and underlying neurobiological changes. Animals exhibiting varying degrees of behavioural responses will be equally represented in both IHC and electrophysiological groups to address potential disparities in tonic inhibition and receptor expression [45]. This approach guarantees a nuanced understanding of how GABA modulation impacts cognitive functions across different behavioural profiles, ensuring that the study comprehensively tests the hypothesis that modulating ambient GABA levels could improve cognitive outcomes in a MAM model of schizophrenia [46]. Differences in behavioural scores will be analyzed to determine if variations in tonic inhibition or GABAa5 and GAT1 expression correlate with specific behavioural outcomes, providing deeper insight into the neurobiological mechanisms underlying schizophrenia-like symptoms.

Incorporating Control Groups

To accurately assess the impact of Tiagabine on tonic inhibition and to establish a comprehensive understanding of its therapeutic potential, the study design includes several control groups. Specifically, both MAM-treated and wild-type (non-treated) animals will be included, with subsets receiving either systemic Tiagabine administration or no drug intervention. This arrangement yields four experimental groups: MAM-treated with Tiagabine, MAM-treated without Tiagabine, wild-type with Tiagabine, and wild-type without Tiagabine. Such a design ensures a thorough evaluation of Tiagabine's effects across different baseline conditions of hippocampal excitability and allows for the comparison of its effects in the presence and absence of neurodevelopmental disruptions induced by MAM treatment.

Electrophysiology

This study incorporated analyses of long-term depression (LTD) and long-term potentiation (LTP) in CA1 pyramidal neurons to enhance our comprehension of synaptic plasticity within the context of schizophrenia and the impact of Tiagabine treatment. Designed to provide insights into the effects of Tiagabine on the delicate balance between LTP and LTD, as well as on short-term potentiation, these experiments aimed to acquire robust and reliable data by recording from at least 5 to 6 cells per animal. This approach captured a comprehensive range of responses to the experimental conditions. High-frequency stimulation (HFS) at a protocol of 100 Hz for 1 second was employed to induce LTP, while low-frequency stimulation (LFS) consisting of 1 Hz for 15 minutes was applied to trigger LTD. Additionally, paired-pulse ratio (PPR) experiments, which involved applying two stimuli at inter-stimulus intervals ranging from 25 to 500 ms, were conducted to assess changes in the probability of presynaptic neurotransmitter release, a critical factor in synaptic efficiency and plasticity [47].

The experimental setup required two specialized solutions: the artificial cerebrospinal fluid (aCSF) and a high chloride internal solution, each tailored to replicate specific physiological conditions crucial for accurate electrophysiological recordings. The aCSF, containing 124 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM glucose, 2 mM $CaCl_2$, and 1 mM $MgCl_2$, with its pH adjusted to 7.4, served as the medium mimicking the brain's extracellular fluid. It was continuously oxygenated with a 95% O_2 and 5% CO_2 gas mixture, simulating the in vivo blood supply to the brain

For the intracellular recordings, a high chloride internal solution composed of 130 mM CsCl, 10 mM HEPES, 0.6 mM EGTA, 5 mM QX-314-Cl, 4 mM ATP-Mg, and 0.4 mM GTP-Na was introduced, with the pH fine-tuned to 7.3 using CsOH. This solution's high chloride content increased intracellular chloride conductance, thereby enhancing the recording clarity of GABAA receptor-mediated inhibitory postsynaptic potentials [48]. The addition of ATP and GTP was crucial for sustaining the cellular energy state and ensuring the G-protein-mediated signalling pathways were intact during recordings [49, 50]. Recording pipettes, carefully pulled and calibrated, had a resistance of 3-5 MΩ when filled with high chloride internal solution, ensuring the fidelity and reliability of the recordings.

In Vivo Microdialysis for Ambient GABA Level Measurement

In the in vivo microdialysis procedure for ambient GABA level measurement, rats were anesthetized and then securely mounted in a stereotaxic frame for precise positioning [44, 51]. A dual-function microdialysis probe was carefully inserted into the CA1 region of the hippocampus, designed to collect extracellular fluid samples and administer substances directly to the targeted brain region [52]. To ensure a constant delivery of Tiagabine to the target area and avoid diluting the GABA concentration, which could interfere with hypothesis testing, the infusion was conducted at a steady rate. Simultaneously, the microdialysis probe was meticulously calibrated to maintain the balance between perfusing the area with aCSF and preserving the natural extracellular environment. Extracellular fluid samples were collected over 20-minute intervals for a total duration of 2 hours. Each sample volume was carefully measured to a quantity (e.g., 1-2 µL per interval) that allows for precise analysis without significantly altering the ambient GABA concentration. This methodical approach facilitated monitoring ambient GABA levels before, during, and after the infusion of Tiagabine, ensuring that any observed changes in GABA concentration accurately reflect the effects of Tiagabine rather than procedural artifacts. The collected samples were then analyzed using high-performance liquid chromatography (HPLC), offering a direct and precise quantification of the changes in extracellular GABA concentration induced by Tiagabine.

Statistical Analysis

Data will be analyzed using a two-way ANOVA to examine the effects of treatment (MAM vs. Sham) and intervention (GABA modulation vs. Control) on cognitive and electrophysiological outcomes. Tukey's HSD will be performed for post-hoc analysis of group differences. Statistical significance will be set at $p<0.05$. All analyses will be conducted using R.

Results

Model Verification

Following the administration of MAM on gestational day 17, neurodevelopmental disruptions in the offspring are expected to be observed, resembling aspects of schizophrenia. As histological analyses show, these disruptions are expected to manifest as altered hippocampal structure and neuronal density [53]. Specifically, Nissl and Fluoro-Jade staining should reveal changes in neuronal organization and indications of neuronal degeneration, respectively, confirming the successful establishment of the MAM model [54]. The administration of Tiagabine could significantly elevate ambient GABA levels, as measured by in vivo microdialysis and HPLC analysis [55, 56]. This elevation in GABA should correlate with an increase in tonic inhibition, demonstrated by enhanced tonic current amplitude in whole-cell voltage-clamp recordings of pyramidal neurons. These results will illustrate the effectiveness of Tiagabine in increasing GABAergic signalling within the hippocampus.

Further histological analysis targeting GAT1 and α5 GABAA, crucial for regulating extracellular GABA levels and tonic inhibition, respectively, is expected to reveal significant changes in their expression across all treatment groups [40, 57]. This would provide direct evidence of how the interventions influenced the hippocampal GABAergic system, illustrating the specific impact of Tiagabine and MAM treatment on key components of GABA regulation.

Additional Electrophysiology Findings

As part of examining synaptic plasticity within the context of schizophrenia and the effect of Tiagabine treatment, detailed electrophysiological studies were conducted. These studies focused on measuring the magnitude of tonic inhibition and the balance between LTP and LTD in CA1 pyramidal neurons [58]. The results from these studies are expected to reveal that, compared to sham-treated controls, MAM-treated rats exhibited a significant decrease in tonic inhibition, which was effectively countered by Tiagabine administration, as evidenced by the enhanced tonic current amplitude in whole-cell voltage-clamp recordings [47]. Furthermore, LTP and LTD experiments are expected to provide insights into synaptic plasticity, showing that Tiagabine treatment helped to restore the balance between LTP and LTD disrupted by the MAM treatment [32, 33]. These results would not only support the efficacy of Tiagabine in modulating GABAergic signalling but also underscore its potential therapeutic benefits for cognitive deficits associated with schizophrenia.

Tiagabine Improves Spatial Memory after MAM

The MWM test will determine whether increasing ambient GABA improves spatial memory following MAM treatment. As reported by others, MAM is known to impair memory, evidenced by longer latencies to reach the platform during training and reduced time spent in the target quadrant during the probe trial [31-33]. The MAM-treated rats are anticipated to exhibit these deficits. However, with Tiagabine treatment, improvement in these measures suggests enhanced spatial memory. Specifically, MAM rats treated with Tiagabine should show shorter latencies to reach the platform and increased time in the target quadrant, indicating mitigation of MAM-induced memory impairments.

Tiagabine Improves Cognitive Flexibility after MAM

Further, in assessing cognitive flexibility using the MWM reversal phase and the AST, the MAM treatment is expected to promote deficits in adapting to new information or rules. These deficits will likely manifest as longer latencies to learn the new platform location in the MWM and increased trials to criterion in the more challenging stages of the AST [26, 34]. Conversely, MAM rats treated

with Tiagabine, are expected to observe improved performance in these tasks, demonstrating enhanced cognitive flexibility. Tiagabine treatment should facilitate quicker adaptation to new platform locations in the MWM and fewer trials to reach criteria in the AST stages, particularly in extra-dimensional shifts and reversal learning.

Discussion

This protocol, inspired by the work of Donegan et al. (2019) and Gill et al. (2011), explores the effects of increasing ambient GABA levels in the CA1 hippocampal region on cognitive functions in a MAM model of schizophrenia [26, 27]. The study aims to extend the understanding of tonic inhibition's role in schizophrenia by targeting ambient GABA levels, anticipating that this approach substantially improves cognitive performance within the MAM model [26, 27].

Findings are expected to align with existing research on cognitive impairment in schizophrenia, offering a novel perspective on treatment strategies [16, 26, 27]. The MWM test likely shows significant improvements in cognitive performance among MAM-treated rats following ambient GABA modulation, particularly in spatial learning and memory, thus validating the role of GABA in hippocampal-dependent processes [23, 28, 55]. Furthermore, the AST provides insights into cognitive flexibility and reversal learning in the MAM model of schizophrenia [26,43]. If hypotheses are confirmed, these findings underscore the critical role of GABAergic signalling in schizophrenia and hippocampal dysfunction [10, 26]. This research could pave the way for novel therapeutic strategies, leading to more targeted and effective treatments for cognitive symptoms in schizophrenia.

Building on the existing literature, the study extends the understanding of tonic inhibition in schizophrenia treatment [26, 27]. Validating treatments that target tonic inhibition, this study introduces a novel approach by increasing ambient GABA levels. However, translating these findings into patient treatment, particularly using uptake inhibitors like Tiagabine, raises questions about safety and specificity [56, 57].

Understanding gene expression changes is crucial because of their impact on neurotransmitter systems, synaptic formation, and neural circuitry, directly affecting behaviour and cognitive functions [59]. Techniques such as RNA sequencing can identify key genes and pathways that are dysregulated, offering insights into the genetic underpinnings of schizophrenia [60]. Simultaneously, examining synaptic plasticity, which encompasses the structural and functional changes in synapses responsible for learning and memory, is essential [17, 25]. This involves assessing processes like LTP and LTD through electrophysiological measurements and imaging studies [61]. These investigations could reveal how shifts in GABAergic signalling affect synaptic strength and structure, influencing cognitive processes. Ultimately, integrating findings from gene expression studies with analyses of synaptic plasticity will illuminate how molecular alterations contribute to the complex behavioural and electrophysiological phenotypes observed in schizophrenia, guiding the development of targeted therapeutic strategies.

Conclusions

The main intent of publishing this protocol is to offer a research avenue for investigating the potential of modulating ambient GABA levels to improve cognitive functions in schizophrenia. This research is important to the field as it provides a novel approach to understanding and treating the cognitive deficits associated with schizophrenia, focusing on the GABAergic system within the hippocampal CA1 region.

This study opens new research questions, particularly regarding the translation of our findings from animal models to human treatments. The challenges lie in the safety and applicability of treatments like Tiagabine in humans, underscoring the need for future research in this area. Future research should also explore systemic pharmacological approaches to modulating ambient GABA levels and investigate the sex-specific effects [62], and responses to GABA modulation in schizophrenia [16]. Additionally, there is a need to delve into the molecular mechanisms underlying the observed behavioural and electrophysiological changes, such as changes in gene expression or synaptic plasticity.

Overall, this protocol represents a step towards developing new interventions to improve the quality of life for individuals affected by schizophrenia. It highlights the importance of continued research efforts to unravel the multifaceted nature of this disorder and develop effective treatments.

List of Abbreviations Used

aCSF: artificial cerebrospinal fluid
ANOVA: analysis of variance
AST: attentional set-shifting test
CD: compound discrimination
EDS: extra-dimensional set-shift
GABA: gamma-aminobutyric acid
GABAA: gamma-aminobutyric acid type A
α5 GABAA: type A GABA receptor alpha5-subunit
GAT-1: GABA transporter-1
HPLC: high-performance liquid chromatography
HSD: honestly significant difference
IDS: intra-dimensional shift
IHC: immunohistochemistry
MAM: methylazoxymethanol acetate
MWM: Morris water maze
PND: postnatal day
RL1: reversal learning 1
RL2: reversal learning 2
SD: simple discrimination
SEM: standard error of the mean

Conflicts of Interest
The author declares that they have no conflict of interest.

Ethics Approval and/or Participant Consent
As this document is a research proposal and is not currently being implemented, it does not require ethics approval at this time.

Authors' Contributions
IM: Substantial contributions to the conception and design of the study, acquisition, analysis, and interpretation of research for the study; drafted the manuscript and revised it critically for important intellectual content; gave final approval of the version to be published.

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References
[1] Luvsannyam E, Jain MS, Pormento MKL, Siddiqui H, Balagtas ARA, Emuze BO, et al. Neurobiology of schizophrenia: A comprehensive review. Cureus (Palo Alto, CA). 2022 Apr 8;14(4):e23959. https://doi.org/10.7759/cureus.23959
[2] Picchioni MM, Murray RM. Schizophrenia. BMJ. 2007-07-12;335(7610):91. https://doi.org/10.1136/bmj.39227.616447.be
[3] Correll CU, Schooler NR. Negative symptoms in schizophrenia: A review and clinical guide for recognition, assessment, and treatment. Neuropsychiatric Disease and Treatment. 2020 Jan 1;16:519-34. https://doi.org/10.2147/NDT.S225643
[4] Jahangir M, Zhou J, Lang B, Wang X. GABAergic system dysfunction and challenges in schizophrenia research. Frontiers in Cell and Developmental Biology. 2021 May 14;9:663854. https://doi.org/10.3389/fcell.2021.663854
[5] The Schizophrenia Society of Canada and The Schizophrenia Society of Canada Foundation 2020-2021 Annual Report. [Internet]. The Schizophrenia Society of Canada. [cited 2023 Dec 1]. Available from: https://schizophrenia.ca/annual-reports-audits/
[6] Schizophrenia [Internet]. World Health Organization. [cited 2023 Dec 2]. Available from: https://www.who.int/news-room/fact-sheets/detail/schizophrenia#:~:text=Magnitude%20and%20impact,300%20people%20(0.32%25)%20worldwide
[7] Heckers S, Konradi C. Hippocampal neurons in schizophrenia. Journal of Neural Transmission. 2002 May 1;109(5-6):891-905. https://doi.org/10.1007/s007020200073
[8] Uhlhaas PJ, Singer W. Abnormal neural oscillations and synchrony in schizophrenia. Nature reviews. Neuroscience. 2010 Feb 1;11(2):100-13. https://doi.org/10.1038/nrn2774
[9] Fisahn A, Neddens J, Yan L, Buonanno A. Neuregulin-1 modulates hippocampal camma oscillations: Implications for schizophrenia. Cerebral Cortex. 2009 Mar 1;19(3):612-8. https://doi.org/10.1093/cercor/bhn107
[10] Lieberman JA, Girgis RR, Brucato G, Moore H, Provenzano F, Kegeles L, et al. Hippocampal dysfunction in the pathophysiology of schizophrenia: A selective review and hypothesis for early detection and intervention. Molecular Psychiatry. 2018 Aug 1;23(8):1764-72. https://doi.org/10.1038/mp.2017.249
[11] Sigel E, Steinmann ME. Structure, function, and modulation of GABAA receptors. The Journal of Biological Chemistry. 2012 Nov 23;287(48):40224-31. https://doi.org/10.1074/jbc.R112.386664
[12] Huang T, Lin Y, Hsiao C, Wang L, Ajibola M, Abdulmajeed W, et al. Differential expression of GABAA receptor subunits δ and α6 mediates tonic inhibition in parvalbumin and somatostatin interneurons in the mouse hippocampus. Frontiers in Cellular Neuroscience. 2023 Jul 20;17:1146278. https://doi.org/10.3389/fncel.2023.1146278
[13] Turi GF, Wittmann G, Lechan RM, Losonczy A. Ambient GABA modulates septo-hippocampal inhibitory terminals via presynaptic GABAb receptors. Neuropharmacology. 2015 Jan;88:55-62. https://doi.org/10.1016/j.neuropharm.2014.10.005
[14] Magnin E, Francavilla R, Amalyan S, Gervais E, David LS, Luo X, et al. Input-specific synaptic location and unction of the α5 GABA A receptor subunit in the mouse CA1 hippocampal neurons. The Journal of Neuroscience. 2019 Jan 30;39(5):788-801. https://doi.org/10.1523/JNEUROSCI.0567-18.2018
[15] Duan J, Pandey S, Li T, Castellano D, Gu X, Li J, et al. Genetic deletion of GABAA receptors reveals distinct requirements of neurotransmitter receptors for GABAergic and glutamatergic synapse development. Frontiers in Cellular Neuroscience. 2019 Jun 4;13:217. https://doi.org/10.3389/fncel.2019.00217
[16] Xu M, Wong AHC. GABAergic inhibitory neurons as therapeutic targets for cognitive impairment in schizophrenia. Acta Pharmacologica Sinica. 2018 May 1;39(5):733-53. https://doi.org/10.1038/aps.2017.172

[17] Gisabella B, Bolshakov VY, Benes FM, Costa E. Regulation of synaptic plasticity in a schizophrenia model. Proceedings of the National Academy of Sciences. 2005 Sep 13;102(37):13301-6. https://doi.org/10.1073/pnas.0506034102
[18] Teleanu RI, Niculescu A, Roza E, Vladâcenco O, Grumezescu AM, Teleanu DM. Neurotransmitters—Key factors in neurological and neurodegenerative disorders of the central nervous system. International Journal of Molecular Sciences. 2022 May 25;23(11):5954. https://doi.org/10.3390/ijms23115954
[19] Marques TR, Ashok AH, Angelescu I, Borgan F, Myers J, Lingford-Hughes A, et al. GABA-A receptor differences in schizophrenia: a positron emission tomography study using [11C]Ro154513. Molecular Psychiatry. 2021 Jun 1;26(6):2616-25. https://doi.org/10.1038/s41380-020-0711-y
[20] Glykys J, Mody I. Hippocampal network hyperactivity after selective reduction of tonic inhibition in GABA A receptor alpha5 subunit-deficient mice. Journal of Neurophysiology. 2006 May;95(5):2796-807. https://doi.org/10.1152/jn.01122.2005
[21] Scimemi A. Structure, function, and plasticity of GABA transporters. Frontiers in Cellular Neuroscience. 2014 Jun 17;8:161. https://doi.org/10.3389/fncel.2014.00161
[22] Motiwala Z, Aduri NG, Shaye H, Han GW, Lam JH, Katritch V, et al. Structural basis of GABA reuptake inhibition. Nature (London). 2022 Jun 23;606(7915):820-6. https://doi.org/10.1038/s41586-022-04814-x
[23] Lodge DJ. The MAM rodent model of schizophrenia. Current Protocols in Neuroscience. 2013 Apr;63(1):9.43.1-9.43.7. https://doi.org/10.1002/0471142301.ns0943s63
[24] Hradetzky E, Sanderson TM, Tsang TM, Sherwood JL, Fitzjohn SM, Lakics V, et al. The methylazoxymethanol acetate (MAM-E17) rat model: Molecular and functional effects in the hippocampus. Neuropsychopharmacology. 2012 Jan 1;37(2):364-77. https://doi.org/10.1038/npp.2011.219
[25] Sanderson TM, Cotel M, O'Neill MJ, Tricklebank MD, Collingridge GL, Sher E. Alterations in hippocampal excitability, synaptic transmission and synaptic plasticity in a neurodevelopmental model of schizophrenia. Neuropharmacology. 2012 Mar;62(3):1349-58. https://doi.org/10.1016/j.neuropharm.2011.08.005
[26] Donegan JJ, Boley AM, Yamaguchi J, Toney GM, Lodge DJ. Modulation of extrasynaptic GABAA alpha 5 receptors in the ventral hippocampus normalizes physiological and behavioral deficits in a circuit specific manner. Nature Communications. 2019 Jun 27;10(1):1-12. https://doi.org/10.1038/s41467-019-10800-1
[27] Gill KM, Lodge D, Cook JM, Aras S, Grace AA. A novel α5GABAAR-positive allosteric modulator reverses hyperactivation of the dopamine system in the mam model of schizophrenia. Neuropsychopharmacology (New York, N.Y.). 2011 May 11;36(9):1903-11. https://doi.org/10.1038/npp.2011.76
[28] Lodge DJ, Grace AA. Aberrant hippocampal activity underlies the dopamine dysregulation in an animal model of schizophrenia. The Journal of Neuroscience. 2007 Oct 17;27(42):11424-30. https://doi.org/10.1523/JNEUROSCI.2847-07.2007
[29] Borden LA, Murali Dhar TG, Smith KE, Weinshank RL, Branchek TA, Gluchowski C. Tiagabine, SK&F 89976-A, CI-966, and NNC-711 are selective for the cloned GABA transporter GAT-1. European Journal of Pharmacology. 1994 Oct 14;269(2):219-24. https://doi.org/10.1016/0922-4106(94)90089-2
[30] Sałat K, Podkowa A, Mogilski S, Zaręba P, Kulig K, Sałat R, et al. The effect of GABA transporter 1 (GAT1) inhibitor, tiagabine, on scopolamine-induced memory impairments in mice. Pharmacol Rep. 2015 Dec;67(6):1155-62. https://doi.org/10.1016/j.pharep.2015.04.018
[31] Folley BS, Astur R, Jagannathan K, Calhoun VD, Pearlson GD. Anomalous neural circuit function in schizophrenia during a virtual Morris water task. NeuroImage (Orlando, Fla.). 2010 Feb 15;49(4):3373-84. https://doi.org/10.1016/j.neuroimage.2009.11.034
[32] Skarsfeldt T. Differential effect of antipsychotics on place navigation of rats in the Morris water maze. A comparative study between novel and reference antipsychotics. Psychopharmacology. 1996 Mar 1;124(1-2):126-33. https://doi.org/10.1007/BF02245612
[33] Nunez J. Morris water maze experiment. Journal of Visualized Experiments. 2008 Sep 24,(19). https://doi.org/10.3791/897
[34] Gastambide F, Cotel MC, Gilmour G, O'Neill MJ, Robbins TW, Tricklebank MD. selective remediation of reversal learning deficits in the neurodevelopmental MAM model of schizophrenia by a novel mGlu5 positive allosteric modulator. Neuropsychopharmacology (New York, N.Y.). 2012 Mar 1;37(4):1057-66. https://doi.org/10.1038/npp.2011.298
[35] Amin SN, Younan SM, Youssef MF, Rashed LA, Mohamady I. A histological and functional study on hippocampal formation of normal and diabetic rats. F1000 Research. 2013;2:151. https://doi.org/10.12688/f1000research.2-151.v1

[36] Potts EM, Coppotelli G, Ross JM. Histological-based stainings using free-floating tissue sections. Journal of Visualized Experiments. 2020 Aug 25,(162). https://doi.org/10.3791/61622
[37] Rajamohamedsait HB, Sigurdsson EM. Histological staining of amyloid and pre-amyloid peptides and proteins in mouse tissue. In: Amyloid Proteins. Totowa, NJ: Humana Press; 2012. p. 411-24. https://doi.org/10.1007/978-1-61779-551-0_28
[38] Kjonigsen LJ, Lillehaug S, Bjaalie JG, Witter MP, Leergaard TB. Waxholm space atlas of the rat brain hippocampal region: Three-dimensional delineations based on magnetic resonance and diffusion tensor imaging. NeuroImage. 2015 Mar 1;108:441-9. https://doi.org/10.1016/j.neuroimage.2014.12.080
[39] Schmued LC, Albertson C, Slikker W. Fluoro-Jade: A novel fluorochrome for the sensitive and reliable histochemical localization of neuronal degeneration. Brain Research. 1997 Mar 14;751(1):37-46. https://doi.org/10.1016/S0006-8993(96)01387-X
[40] Gill K, Grace A. The role of α5 GABAA receptor agonists in the treatment of cognitive deficits in schizophrenia. Curr Pharm Des 2014; 20: https://doi.org/10.2174/13816128196661312161146l2
[41] de Jonge JC, Vinkers CH, Hulshoff Pol HE, Marsman A. GABAergic mechanisms in schizophrenia: Linking postmortem and in vivo studies. Front Psychiatry 2017; 8. https://doi.org/10.3389/fpsyt.2017.00118
[42] Featherstone RE, Burton CL, Coppa-Hopman R, Rizos Z, Sinyard J, Kapur S, et al. Gestational treatment with methylazoxymethanol (MAM) that disrupts hippocampal-dependent memory does not alter behavioural response to cocaine. Pharmacology, Biochemistry and Behavior. 2009 Oct 1;93(4):382-90. https://doi.org/10.1016/j.pbb.2009.05.010
[43] Flagstad P, Mørk A, Glenthøj BY, van Beek J, Michael-Titus AT, Didriksen M. Disruption of neurogenesis on gestational day 17 in the rat causes behavioral changes relevant to positive and negative schizophrenia symptoms and alters amphetamine-induced dopamine release in nucleus accumbens. Neuropsychopharmacology. 2004 Nov 1;29(11):2052-64. https://doi.org/10.1038/sj.npp.1300516
[44] Darvesh AS, Carroll RT, Geldenhuys WJ, Gudelsky GA, Klein J, Meshul CK, et al. In vivo brain microdialysis: Advances in neuropsychopharmacology and drug discovery. Expert Opinion on Drug Discovery. 2011 Feb;6(2):109-27. https://doi.org/10.1517/17460441.2011.547189
[45] Lee V, Maguire J. The impact of tonic GABAA receptor-mediated inhibition on neuronal excitability varies across brain region and cell type. Front Neural Circuits. 2014; 8. https://doi.org/10.3389/fncir.2014.00003
[46] Könen T, Karbach J. Analyzing individual differences in intervention-related changes. Adv Methods Pract Psychol Sci. 2021; 4: https://doi.org/10.1177/2515245920979172
[47] Branco T, Staras K, Darcy KJ, Goda Y. Local dendritic activity sets release probability at hippocampal synapses. Neuron. 2008; 59: 475–485. https://doi.org/10.1016/j.neuron.2008.07.006
[48] Succol F, Fiumelli H, Benfenati F, Cancedda L, Barberis A. Intracellular chloride concentration influences the GABAA receptor subunit composition. Nat Commun. 2012; 3: 738. https://doi.org/10.1038/ncomms1744
[49] Gulati S, Jin H, Masuho I, Orban T, Cai Y, Pardon E et al. Targeting G protein-coupled receptor signaling at the G protein level with a selective nanobody inhibitor. Nat Commun. 2018; 9. https://doi.org/10.1038/s41467-018-04432-0
[50] Sperlágh B. ATP-mediated signaling in the nervous system. In: Handbook of Neurochemistry and Molecular Neurobiology. Springer US: Boston, MA. 2008, pp 227–254. https://doi.org/10.1007/978-0-387-30382-6_10
[51] Zhang S, Takeda Y, Hagioka S, Takata K, Aoe H, Nakatsuka H, et al. Measurement of GABA and glutamate in vivo levels with high sensitivity and frequency. Brain Research Protocols. 2005 Feb 1;14(2):61-6. https://doi.org/10.1016/j.brainresprot.2004.03.005
[52] Westerink BH, Drijfhout WJ, vanGalen M, Kawahara Y, Kawahara H. The use of dual-probe microdialysis for the study of catecholamine release in the brain and pineal gland. Advances in Pharmacology. 1998;42:136-40. https://doi.org/10.1016/S1054-3589(08)60714-0
[53] Harrison PJ. The hippocampus in schizophrenia: A review of the neuropathological evidence and its pathophysiological implications. Berlin: Springer. Jun 1, 2004. https://doi.org/10.1007/s00213-003-1761-y
[54] Schmued LC, Hopkins KJ. Fluoro-Jade B: A high affinity fluorescent marker for the localization of neuronal degeneration. Brain Research. 2000 Aug 25;874(2):123-30. https://doi.org/10.1016/S0006-8993(00)02513-0
[55] Halonen T, Nissinen J, Jansen JA, Pitkänen A. Tiagabine prevents seizures, neuronal damage and memory impairment in experimental status epilepticus. European Journal of Pharmacology. 1996 Mar 28;299(1):69-81. https://doi.org/10.1016/0014-2999(95)00835-7
[56] Bauer J, Cooper-Mahkorn D. Tiagabine: Efficacy and safety in partial seizures - Current status. Neuropsychiatric Disease and Treatment. 2008 Aug 1;4(4):731-6. https://doi.org/10.2147/ndt.s833

[57] Inglefield JR, Perry JM, Schwartz RD. Postischemic inhibition of GABA reuptake by tiagabine slows neuronal death in the gerbil hippocampus. Hippocampus. 1995;5(5):460-8. https://doi.org/10.1002/hipo.450050508
[58] Milior G, Di Castro MA, Sciarria LP, Garofalo S, Branchi I, Ragozzino D et al. Electrophysiological properties of CA1 pyramidal neurons along the longitudinal axis of the mouse hippocampus. Sci Rep. 2016; 6. https://doi.org/10.1038/srep38242
[59] Flavell SW, Greenberg ME. Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. Annu Rev Neurosci. 2008; 31: 563–590. https://doi.org/10.1146/annurev.neuro.31.060407.125631
[60] Wu Y, Zhang C-Y, Wang L, Li Y, Xiao X. Genetic insights of schizophrenia via single cell RNA-sequencing analyses. Schizophr Bull. 2023; 49: 914–922. https://doi.org/10.1093/schbul/sbad002
[61] Jiang F, Bello ST, Gao Q, Lai Y, Li X, He L. Advances in the electrophysiological recordings of long-term potentiation. Int J Mol Sci. 2023; 24: 7134. https://doi.org/10.3390/ijms24087134
[62] Li X, Zhou W, Yi Z. A glimpse of gender differences in schizophrenia. Gen Psych. 2022 Aug 1;35(4): e100823. https://doi.org/10.1136/gpsych-2022-100823

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