STUDIES OF BEHAVIOUR, HIPPOCAMPAL PLASTICITY AND MOLECULAR PATHWAYS IN A KETAMINE-BASED MODEL OF SCHIZOPHRENIA

MUHAMMAD HAZIM BIN ABDULLAH

UNIVERSITI SAINS MALAYSIA

2025

STUDIES OF BEHAVIOUR, HIPPOCAMPAL PLASTICITY AND MOLECULAR PATHWAYS IN A KETAMINE-BASED MODEL OF SCHIZOPHRENIA

by

MUHAMMAD HAZIM BIN ABDULLAH

Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science

May 2025

ACKNOWLEDGEMENT

Praise be to Allah the Almighty for His mercies. Although it has been challenging for me, with His Mercies and Blessings on me everything became easier and I was able to complete this thesis.

I would like to dedicate this work to my family, especially my parents and siblings; Abdullah Awang and Roselini Sallehuddin, Nur Amira Abdullah and Nur Asyiqin Abdullah for their endless support and unwavering faith in me. Next, I would like to give a special thanks to my supervisor, Assoc. Prof. Dr. Zurina Hassan for the chances and opportunity that have been given. I am grateful for the time that has been spent throughout the study in facilitating me with brilliant ideas and comments which helps me a lot in improving and completing my thesis. Not to forget, I would like to express my gratitude towards my co-supervisor, Dr. Simon Trent for his cooperation which helped me a lot, especially in planning and completing the experiments.

I am also grateful to Professor Dr. Vikneswaran Murugaiyah (Director Centre for Drug Research) for the facilities provided during this research project. My special appreciation goes to Dr Mohamad Anuar Ahad for his excellent guidance, especially with the experimental works. I am indebted for the knowledge and time that he spent throughout this study. Many thanks to the staff Centre for Drug Research who contributed to the success of this study.

Lastly, I would like to express my appreciation and thanks to Roselina Sallehuddin, Zulkifli Osman, Muhammad Faiz, Tan Ai Fein, Rima Atria, Mohamad Azmeer Effendy, Aiman Nadhirah, Nurul Husna and Suleiman Yunusa, for their contribution and for being part of my master degree journey.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
/	per
<	Less than
=	Equal
>	More than
^{o}C	Degree of Celsius
cm	Centimeters
g	Gram
Hz	Hertz
i.p.	Intraperitoneal
kDa	Kilodalton
kg	Kilogram
KHz	Kilohertz
m	Meter
M	Molar
mA	Milliampere
mg	Milligram
mg/mL	Milligram per millilitre
mg/kg	Miligram per kilogram
min	Minutes
mL	Milliliters
mm	Millimetres
ms	Millisecond

n	Number of samples
NO	Nitric Oxide
p	Probability value
sec	Second
U/mL	Unit per millilitre
µL	Microlitres
µm	Micrometre
x	Multiply
α	Alpha
γ	Gamma
δ	Delta
θ	Theta
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
AP	Anterior posterior
AR	Analytical reagent
ARASC	Animal Research and Service Centre
BDNF	Brain-derived neurotrophic factor
C	Sample concentration
CA	Cornu ammonis
CA1	Cornu ammonis 1
Ca^{2+}	Calcium
CA3	Cornu ammonis 3
CAMKIIα	Calcium/calmodulin kinases II alpha

cAMP	Cyclic adenosine monophosphate
CDK5	Cyclin-dependent kinase 5
Cl^-	Chloride
CNS	Central nervous system
CREB	cAMP response element-binding protein
ΔfosB	DeltafosB
DG	Dentate gyrus
E	East
EC	Entorhinal cortex
E-LTP	Early long-term potentiation
EPM	Elevated plus maze
ERK	Extracellular regulated kinase
fEPSP	field Excitatory postsynaptic potential
FST	Forced swim test
GABA	γ-aminobutyric acid
H_2O_2	Hydrogen peroxide
HRP	Horseradish peroxidase
I/O	Input/output
IgG	Immunoglobulin G
Ket	Ketamine
LDS	Lithium dodecyl sulphate
L-LTP	Late long-term potentiation
LTD	Long-term depression
LTP	Long-term potentiation

MAGUKs	membrane-associated guanylate kinases
Mg^{2+}	Magnesium
ML	Mediolateral
MWM	Morris water maze
N	North
NA	Noradrenaline
Na^{+}	Sodium
NMDA	N-methyl D-aspartate acid
NORT	Novel object recognition test
OFT	Open-field test
pH	Potential of hydrogen
PKA	Protein kinase
PKC	Protein kinase C
PPF	Paired pulse facilitation
PSD-95	Postsynaptic density protein 95
PVDF	Polyvinylidene fluoride
rcf	Relative centrifugal force
S	South
SD / σ	Standard deviation
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Standard error mean
SYNP	Synaptophysin
TBS	Theta-burst stimulation

TBST	Tris-buffered saline containing tween 20
TEMED	Tetramethylethylenediamine
USA	United State of America
USM	Universiti Sains Malaysia
V	Volt
VGCC	Voltage-gated calcium channel
v/v/v	volume per volume per volume
veh.	Vehicle
VH	Ventral hippocampus
W	West
w/v	Weigh per volume

LIST OF APPENDICES

KAJIAN MENGENAI TINGKAH-LAKU, KEPLASTIKAN HIPOKAMPAL DAN TAPAK JALAN MOLEKUL DI DALAM MODEL SKIZOFRENIA BERASASKAN KETAMIN

ABSTRAK

Skizofrenia adalah gangguan kesihatan mental yang kompleks, kronik dan berulang yang disertai dengan gejala positif, negatif dan kognitif. Ketamin, antagonis tidak kompetitif untuk reseptor n-metil, d-aspartat (NMDA) dicadangkan sebagai ubat yang sesuai untuk membangunkan aktiviti psikotik kerana sifat psikotomimetiknya yang kuat serta tahap neurotoksisiti yang berkurangan berbanding ubat-ubatan yang digunakan sebelum ini untuk membangunkan model skizofrenia. Walau bagaimanapun, terdapat jurang yang perlu diisi untuk membangunkan model skizofrenia yang baik menggunakan ketamin seperti dos dan tempoh optimum yang diperlukan serta julat kesan ke atas gejala seperti skizofrenia dalam model haiwan. Kajian ini bertujuan untuk mencirikan tingkah laku, keplastikan neuro dalam hippokampal dan laluan molekul dalam model skizofrenia yang berasaskan ketamin. Dos ketamin yang berbeza (10, 50 dan 100 mg/kg) telah diberikan kepada tikus untuk membangunkan model skizofrenia melalui intraperitoneal (ip). selama 10 hari. Untuk menilai kesan ketamin pada gejala seperti skizofrenia dalam model haiwan, beberapa kajian tingkah laku telah dilakukan. Kesan ketamin terhadap gejala positif boleh diwakili oleh hiperagitasi dan tingkahlaku hiperaktif yang dikaji dengan mengukur aktiviti lokomotor menggunakan ujian medan terbuka (OFT). Tambahan pula, kesan ketamin terhadap gejala negatif diperhatikan dengan menilai tingkah laku emosi, termasuk kebimbangan dan kemurungan menggunakan ujian renang paksa (FST) dan labirin tambah tinggi (EPM). Sementara itu, labirin air Morris (MWM) dilakukan

untuk memerhatikan kesan ke atas gejala kognitif melalui aktiviti berasaskan ingatan dan pembelajaran berkaitan ruang. Akhir sekali, untuk mengkaji keplastikan neuro hippokampal dalam model berasaskan ketamin, elektrofisiologi in vivo dilakukan diikuti dengan analisis blot barat untuk mengukur ekspresi molekul protein yang terlibat dalam keplastikan sinaptik. Penemuan kami menunjukkan bahawa pengambilan ketamin (10, 50 dan 100 mg/kg) selama 10 hari membawa kepada peningkatan aktiviti dalam parameter OFT termasuk aktiviti lokomotor, jumlah jarak perjalanan, jumlah aktiviti dan halaju min. Selain itu, ketamin (10, 50 dan 100 mg/kg) menyebabkan peningkatan ketara dalam tingkah laku seperti kebimbangan dalam EPM serta peningkatan tingkah laku seperti kemurungan dalam FST. Hasil keputusan juga menunjukkan bahawa ketamin (10, 50 dan 100 mg/kg) menjejaskan ingatan berkait ruang dalam tugas MWM dan menyebabkan defisit ingatan jangka panjang dalam model tikus semasa rakaman elektrofisiologi. Akhir sekali, penemuan dalam kajian ini juga telah mencadangkan bahawa ketamin 10, 50 dan 100 mg/kg telah mengurangkan ekspresi molekul protein yang terlibat dalam keplastikan sinaptik dan LTP dengan ketara. Kesimpulannya, pemberian ketamin secara kronik menggunakan 10, 50 dan 100 mg/kg mampu menyebabkan gejala seperti skizofrenia yang dapat diperhatikan dalam prestasi ujian tingkah laku. Kemerosotan yang diperhatikan dalam aktiviti LTP dan ekspresi protein isyarat molekul telah mencadangkan bahawa defisit kognitif yang diperhatikan berkemungkinan disebabkan oleh gangguan dalam keplastikan hippokampal dan laluan molekulnya.

STUDIES OF BEHAVIOUR, HIPPOCAMPAL PLASTICITY AND MOLECULAR PATHWAYS IN A KETAMINE-BASED MODEL OF SCHIZOPHRENIA

ABSTRACT

Schizophrenia is a complex, chronic and relapsing mental health disorder that presents with positive, negative and cognitive symptoms. Ketamine, a non-competitive antagonist for the n-methyl, d-aspartate (NMDA) receptor was suggested to be a suitable drug for developing psychotic activities due to its strong psychotomimetic properties as well as reduced level of neurotoxicity compared to previously used medications for developing schizophrenia model. However, there are gaps that need to be filled to develop a good model of schizophrenia using ketamine such as the optimum doses and duration required as well as the range of the effects on the schizophrenia-like symptoms in the animal model. The study aimed to characterise the behavioural, hippocampal neuroplasticity and molecular pathways in different doses of the ketamine-induced schizophrenia model. Different doses of ketamine (10, 50 and 100 mg/kg) were administered to the rats to develop a schizophrenia model via intraperitoneal (i.p). for 10 days. To assess the effects of ketamine on schizophrenia-like symptoms in the animal model, several behavioural studies were performed. The effects of ketamine on positive symptoms can be represented by hyperagitation and hyperactivity which were studied by measuring the locomotor activities using an open-field test (OFT). Furthermore, the effects of ketamine on negative symptoms were observed by evaluating the emotional behaviours, including anxiety and depression using the forced swim test (FST) and elevated plus maze (EPM). Meanwhile, the

Morris water maze (MWM) was performed to observe the effects on cognitive symptoms via spatial memory and learning related activities. Finally, to study the hippocampal neuroplasticity in the ketamine-based model, the in vivo electrophysiology was done followed by western blot analysis to measure the molecular expression of the proteins involved in synaptic plasticity process. Our findings indicated that ketamine administration (10, 50 and 100 mg/kg) for 10 days leads to increase activities in the OFT parameters including locomotor activity, total distance travelled, total activity and mean velocity. Moreover, ketamine (10, 50 and 100 mg/kg) caused significant increased in anxiety-like behaviours in the EPM as well as increased depression-like behaviours in FST. The result also demonstrated that ketamine (10, 50 and 100 mg/kg) affected spatial memory in MWM task and caused long-term memory deficits in the rat model during the electrophysiological recordings. Finally, the findings in this study have also suggested that ketamine 10, 50 and 100 mg/kg have significantly reduced the molecular expression of proteins involved in synaptic plasticity. In conclusion, chronic ketamine administration using 10, 50 and 100 mg/kg was able to induce positive, negative and cognitive symptoms of schizophrenia which observed in the behavioural performances. The impairments observed in the LTP activity and molecular signalling proteins expression have suggested that cognitive deficits observed can be caused by the interruption in the hippocampal plasticity and its molecular pathways.

CHAPTER 1

INTRODUCTION

According to World Health Organization (WHO), schizophrenia is a highly complex psychiatric disorder that affects about 0.32% of the world's population. Schizophrenia may lead to several dysfunctions that can be classified into positive, negative and cognitive symptoms. Due to the complex nature of the disease, the current medication and treatment have only focused on antipsychotic drugs for treating the psychotic symptoms and therapies to improve the quality of life. On top of that, the prognosis of the disease is based on the observable symptoms which made it more difficult for the treatment. Although there is still a lack of understanding of the neuropathology and psychopathology of schizophrenia, previous studies have indicated impairments of the neurotransmitter systems including the glutamatergic, dopaminergic and serotonergic systems as possible factors (Becker et al., 2003; Frohlich and Van Horn, 2014). Furthermore, several studies have associated schizophrenia to anomalies in glutamatergic receptor expression, subtypes and function. (Becker et al., 2003; Frohlich and Van Horn, 2014).

To gain better understanding especially on suitable medication and treatment for schizophrenia, researchers have been developing models that can represent the psychopathological appearance of those with schizophrenia. Several types of models have been used for studies related to schizophrenia which include developmental, pharmacologically-induced, lesion or genetic modification (Jones, Watson and Fone, 2011; Ang et al., 2021). Among the models, the pharmacologically induced schizophrenia models using n-methyl-d-aspartate (NMDA) receptor antagonist have been commonly used.

Ketamine, a non-competitive antagonist for NMDA receptor, causes receptor blockade and interrupts glutamatergic activity (Frohlich and Van Horn, 2014). Due to its strong psychotomimetic properties, ketamine has been shown to cause behavioural alterations and cognitive impairment similar to schizophrenia, making it a suitable agent for developing the schizophrenia model (Becker et al., 2003; Chatterjee et al., 2011). Throughout the years, researchers have been trying to develop a pharmacologically or drug induced model of schizophrenia using ketamine. However, so far, there are varieties of doses and treatment regimens that have been proposed. Moreover, the findings and presentation of symptoms in the previous models are diverse which indicates the incomplete development of the models that can fully represent the disease.

In this project, we have developed a pharmacology model of schizophrenia by repeated administration of different doses (10, 50 and 100 mg/kg) of NMDA receptor non-competitive agonist, ketamine and further evaluate the effects of ketamine on behaviour, electrophysiology and molecular synaptic plasticity. The doses used in this study are based on the previously used doses in studies using ketamine-based model for schizophrenia. To access the schizophrenia-like symptoms in the animal model, several behavioural tasks have been performed. Positive symptoms of schizophrenia have been associated with psychomotor agitation which can be observed by hyperactivity in the open field test (OFT). Meanwhile, to access the negative symptoms that can be represented by the emotional-associated behaviours in the ketamine-based model of schizophrenia, the elevated plus maze (EPM) and forced swim test (FST) were performed. Last but not least, to evaluate the cognitive symptoms in the model, the novel object recognition test for visual working memory

and Morris water maze (MWM) test for spatial working memory were performed. Since cognitive impairments in schizophrenia has been associated with impairments in memory and learning and hippocampal plasticity, the *in vivo* hippocampal plasticity study was performed as well as molecular study on harvested hippocampal tissue to observe several biomarkers related to molecular signalling and synaptic plasticity. The project aimed to evaluate the range of schizophrenia-like effects caused by ketamine antagonism on NMDA receptor as well as hippocampal plasticity and molecular synaptic pathways

1.2 Problem Statement

Schizophrenia often presents with symptoms categorised as positive, negative and cognitive. Since it is considered a complex psychotic disease, the available medication and treatment selection, prognosis and life functioning of people with schizophrenia are based on clinical signs (Galuppi et al., 2010; Patel et al., 2014). Thus, both clinical and pre-clinical studies are important to understand schizophrenia and for the development of drug interventions and strategizing the best possible diagnosis and prognosis of the disease. For clinical and progression of illness studies, developing a suitable animal model that can represent the pathophysiology and other aspects of the disease is crucial. Current models for schizophrenia available include developmental, genetic and pharmacology or drug-induced models. Since schizophrenia is closely associated with hypoglutamatergic activity of brain regions (Frohlich and Van Horn, 2014) as well as other aspects such as easier to conduct compared to other types of schizophrenia models, inducing schizophrenia using NMDA receptor antagonists such as Phencyclidine (PCP) and MK-801 has been the choice of previous researchers. A PCP derivative known as ketamine has recently shown great potential for the induction

of schizophrenia in animal models. However, there are still gaps that need to be filled to develop a good ketamine-induced schizophrenia model. For example, the current focus of ketamine model is to develop the psychotic symptoms rather than the overall schizophrenia-like symptoms. Moreover, some symptoms especially psychotic symptoms usually occur for an episode or a short duration of time and do not persist. On top of that, the optimum dose and treatment regime required to induce overall schizophrenia-like symptoms are still in questions, with different doses and treatment regimes have been proposed by the researchers in the past studies. In addition, the severity of cognitive symptoms and it effects on the patients' quality of life has highlighted the important of understanding the neurobiology and pathways leading to the impairments. Moreover, the cognitive symptoms in schizophrenia have been associated with impairments or deficits in working memory (Cirillo and Seidman, 2003; Simpson et al., 2010; Miyamoto and Nitta, 2014). Since memory is closely related to hippocampal plasticity, understanding the pathway and mechanism involved in memory deficits caused by schizophrenia is essential. Furthermore, there are past studies that have showed several associations between protein that involved in synaptic plasticity and molecular signalling with schizophrenia deficits in patients. However, there is no study so far that has explained and highlighted the possible relationships between cognitive symptoms in schizophrenia with hippocampal plasticity as well as protein and synaptic pathways involved. Thus, in this study, we aimed to develop a ketamine model of schizophrenia, observe the appearance of the symptoms and further explore the effects of ketamine-induced schizophrenia on hippocampal plasticity and its proteins pathway.

1.3 Research Hypothesis

Repeated ketamine administration causes the presentation of positive, negative and cognitive symptoms of schizophrenia in rat models that can be observed through behavioural studies. In addition, ketamine-induced schizophrenia in rat models causes a deficit in *in vivo* hippocampal neuroplasticity. Moreover, chronic injections (*i.p.*) of ketamine may cause changes in molecular signaling expression in the hippocampus of the rat model. Lastly, the action of ketamine is predicted to be dose dependent.

1.4 Research Objectives

1.4.1 General Objective

To study the effects of different doses of ketamine on behaviour, hippocampal plasticity and molecular expressions in ketamine-based model of schizophrenia.

1.4.2 Specific objectives

1. To determine the doses of ketamine in chronic treatment induced schizophrenia model.
2. To study the positive symptoms via locomotor activities using open field test (OFT) in acute and chronic ketamine-induced schizophrenia rats.
3. To observe the negative symptoms via emotional (anxiety and depression) related behaviour of chronic ketamine-induced schizophrenia rats in elevated plus maze (EPM) and forced swim test (FST).
4. To measure the spatial and recognition/visual memory and learning of chronic ketamine-induced schizophrenia rats in Morris water maze (MWM) test and novel object recognition test (NORT).

5. To elucidate the effects of schizophrenia on *in vivo* hippocampal neuroplasticity in a ketamine-based model of schizophrenia.
6. To evaluate the molecular synaptic signalling proteins expressions that regulates synaptic plasticity in the hippocampus of ketamine-induced schizophrenia rats

CHAPTER 2

LITERATURE REVIEW

2.1 Schizophrenia

Schizophrenia (SZ) is a complex neuropsychiatric disorder that affects about 0.32% of the world's population (WHO). Based on Malaysia's Second Report of the National Mental Health Registry on Schizophrenia, the prevalence of schizophrenia in Malaysia was stated as 5 cases/100,000 population each year, between 2003 to 2005, and has been estimated to increase up to 100 cases/100,000 population/year (Prakash et al., 2014). Patient with schizophrenia often experiences several symptoms known as positive, negative and cognitive symptoms. Due to the presentation of symptoms and impairments in schizophrenia, it has been ranked among the top 10 for mental and substance use disorders in global prevalence mental disorders (Marder and Cannon, 2019). To date, the aetiology of schizophrenia can be classified into several factors, including genetic, neurobiological, psychological, social, and environmental factors (Ang et al., 2012). Genetic factors have been contributing to most of the risk for schizophrenia as genetic variants and mutations such as deficits in genes associated with neuroplasticity including Fragile X Mental Retardation 1 (*FMR1*) and Cytoplasmic FMRP-Interacting Protein (*CYFIP1*) leads to presentation of symptoms in schizophrenia (Hall et al., 2014; Ghandi et al., 2014; Clifton et al., 2020). A study conducted by Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) has also identified multiple genes associated with schizophrenia that are aligned with the predominant aetiological hypotheses of the disorder. In addition, previous studies have shown associations between schizophrenia pathogenesis with several neurobiological mechanisms, including alterations in neurotransmitter (such as dopaminergic and glutamatergic) systems (Frohlich and Van Horn, 2014), alterations

in nitric oxide (NO) signalling (Miyamoto and Nitta, 2014), upregulation of inflammation status and oxidative stress (Onaolapo et al., 2017)

The current approaches to diagnosis and treatment of schizophrenia are focused on its psychotic or positive symptoms such as hallucination and delusions (Hany et al., 2024). In order to distinguish schizophrenia from other disorders that also involve psychotic symptoms, the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) states that the appearance of two or more symptoms that persist for a considerable amount of time is required for the diagnosis of schizophrenia (Patel et al., 2014). Recently, first-generation and second-generation antipsychotics have been known as common medications for treating psychotic symptoms in schizophrenia. According to the Malaysian Psychiatric Association, the first-generation or typical antipsychotics includes chlorpromazine, fluphenazine, haloperidol, loxapine, mesoridazine, perphenazine, thioridazine, thiothixene and trifluoperazine, while the second-generation or atypical antipsychotics includes clozapine, olanzapine, risperidone, ziprasidone and quetiapine. The main focuses of the medication are to alleviate symptoms, restore normal functioning and improve quality of life (Ceraso et al., 2020). Other than that, the current approach for schizophrenia symptoms includes management of treatment resistance therapy and psychosocial education (Hany et al., 2024). In contrast, there is no current medication available for treating the negative and cognitive symptoms in schizophrenia. Despites the ability of several antipsychotic drugs in reducing the severity of the negative symptoms in patient, the current treatment for negative and cognitive symptoms focuses on therapies to manage the symptoms as well as improving the life quality of the patient (Hany et al., 2024).

2.2 Positive, Negative and Cognitive Symptoms in Schizophrenia

As defined by the National Institute of Mental Health (NIMH), positive symptoms are often associated with the way the patient thinks, acts and experiences the world which includes hallucinations, disorganised thinking, delusions and experiences linked to mental state (Ang et al., 2021). A study on 90 patients with schizophrenia has illustrated that 57.7% of the patients have presented with positive symptoms. Among the positive symptoms, most of the patients measured at 64.1% experienced delusion (Mazumder et al., 2015). Positive symptom in schizophrenia is linked with hyperdopaminergic activity which leads to psychotic experiences observed in the patient (van den Buuse, 2010). Moreover, psychiatric disease including schizophrenia is also associated with psychomotor agitation that can cause excessive motor activities. According to a previous study, 50% of the cases that arrived at emergency with psychiatric agitation were reported to experience schizophrenia (San et al., 2016).

In contrast, negative symptoms are often related to impairment or deficit of normal functions or behaviours which can be presented with social withdrawal, avolition, alogia, blunted affect, and abnormalities in social interaction (Ellenbroek and Cools, 2000; Nestler and Hyman, 2010; Ang et al., 2021; Mosolov and Yaltonskaya, 2022). Records from the clinical practice show that at least one of the negative symptoms of moderate severity or worse was reported by 61% of schizophrenia outpatients who were still receiving antipsychotic medication. Furthermore, an assessment employing the five positive and negative syndrome scale (PANSS) Negative Subscale items (emotional withdrawal, verbal fluency, diminished affect, and poor rapport) revealed that, of the patients, 48% underwent social distancing, 42% suffered emotional distancing, and 39% experienced poor rapport; the

remaining patients exhibited all five negative symptoms (Correll and Schooler, 2020). Although the underlying mechanisms leading to the presentation of negative symptoms are complex, studies have suggested the hypofunction of NMDA activity which further leads to the reduction of dopaminergic activity as the main possible cause (Ang et al., 2021).

Cognitive symptoms have been among the main interest for scientists to do research in schizophrenia. This is due to the prevalence of the symptoms in patients with schizophrenia worldwide and the impacts on the quality of life of the patients. Among patients with early-stage schizophrenia 84.7% (144/170) has experienced cognitive symptoms which was much higher than that in the general population (Peng et al., 2021). In another study study, the appearance of cognitive deficits among patients with schizophrenia was 81.3% (Arunpongpaisal and Sangsirilak, 2013). Meanwhile, a study by Keefe et al (2005) has suggested that about 70% of patients with schizophrenia experienced moderate to severe cognitive impairments. The cognitive impairments in schizophrenia involved deficits in working memory, attention, and executive function deficiencies or impairments are typically associated with cognitive symptoms (Cirillo and Seidman, 2003; Simpson et al., 2010; Miyamoto and Nitta, 2014). Furthermore, as being suggested in previous study (Nuechterlein et al., 2004), seven domains for cognitive—processing speed, attention, working memory, verbal learning and memory, visual learning and memory, reasoning, and social cognition—have been linked to impairments associated with schizophrenia. Based on a study by Schaefer and co. patients with schizophrenia have scored significantly lower than controls across all cognitive tests and domains especially domains of processing speed (and episodic memory.

2.3 Neurobiology of Schizophrenia

Although the pathophysiology leading to the symptoms observed in schizophrenic patients is still unclear, few findings help in deducing the possible mechanism leading to the impairments. Abnormalities of neurotransmitter activities have been proposed as central to the pathophysiology of schizophrenia, which includes several main neurotransmitters including dopamine, serotonin, glutamate, and gamma-aminobutyric acid (GABA). However, the two most common hypotheses concluded by the researchers currently, are the dopamine and glutamate hypothesis. These two hypotheses have suggested that the neurobiological changes that occur in the brain are due to abnormal dopaminergic and glutamatergic activities, leading to disruption of other neurotransmission systems (Frohlich and van Horn, 2009; Shen et al., 2012; Javitt et al., 2012).

2.3.1 *Dopamine hypothesis*

Dopamine, a neurotransmitter that is produced and released in regions of the brain such as the substantia nigra and ventral tegmental is a catecholamine that acts as an inhibitory neurotransmitter (Wang et al., 2012; Brisch et al., 2014). The impairment of dopaminergic activity in schizophrenia is linked to positive and negative symptoms. The positive symptoms observed in schizophrenia can result from augmentation of D_2 receptor activity and increased subcortical release of dopamine, thus affecting the cortical pathway (Shen et al., 2012; Javitt et al., 2012). Hyperdopaminergic or over-activation of the dopaminergic system disturbs the cortical pathway which leads to psychotic episodes in schizophrenia such as hallucination and delusion (Shen et al, 2012; Brisch et al., 2014). In contrast to that, hypodopaminergic activity or reduced dopaminergic function is associated with the negative and cognitive symptoms in schizophrenia (Grunder and Cumming, 2016). Previous research has supported this

theory with their findings using radiotracer positron emission tomography (PET) which has associated the changes in D_1 receptor binding affinity to cognitive symptoms and decreased performance of working memory in animal models that were previously received D_1 receptor antagonists injection into the prefrontal cortex. (Karlsson et al., 2002; Abi-Dargham et al., 2002; Frohlich and Van Horn, 2013). However, the relevance of this hypothesis became a question due to the lack of mechanism explaining the differential in the dopamine dysfunction at different regions of the brain leading to the positive, negative and cognitive symptoms. Moreover, studies have proposed that the abnormal activity of dopamine in schizophrenia occurs in the dorsal involving D_1 and D_2 receptors rather than the D_3 receptors that are responsible for emotion, control reward and motivation in the limbic striatum (Leggio et al., 2013; McCutcheon et al., 2019).

2.3.2 *Glutamate Hypothesis*

Other than the dopamine hypothesis, a recent hypothesis known as glutamate hypothesis has been described as the underlying mechanism leading to the pathogenesis of schizophrenia. This hypothesis has proposed that the NMDA receptor as a culprit in which its hypofunction is responsible for the symptoms and pathology in schizophrenia (Frohlich and van Horn, 2009). NMDA receptor is a family of ionotropic glutamate receptors that relate to basic physiological functions such as memory and learning, and to pathophysiological conditions such as neurodegenerative disorders, stroke, and mental disorders when impaired (Ulbrich and Isacoff, 2009; Liu et al., 2019). The receptor is built up of hetero-tetrameric channels consisting of two compulsory GluN1 subunits and two other subunits, any of the other subfamily GluN2 (2A, 2B, 2C, 2D) or the subfamily GluN3 (3A or 3B) (Ulbrich and Isacoff, 2008). The

receptors for NMDA are neuronal excitatory receptors that play a crucial role in synaptic transmission and neuronal growth (Ulbrich and Isacoff, 2008). NMDA receptors are also important to brain plasticity mediators and are capable of translating complex neuronal activity patterns into long-term shifts in the structure and function of synapses that are thought to precede higher cognitive functions (Liu et al, 2019).

The glutamate hypothesis based on NMDA receptor hypofunction is based on findings where its antagonists lead to the presentation of schizophrenia-like symptoms in healthy individuals and worsening of the symptoms in schizophrenia patients (Allen and Young, 1978; Ellison, 1995; Coyle, 2009). Furthermore, a study using single photon emission computed tomography (SPECT) imaging on normal individuals shows a direct link between ketamine-induced alterations in the binding of a [123I]CNS-1261 radiotracer to NMDAR and negative symptoms of schizophrenia (Stone, 2008). On top of that, the glutamate hypothesis has been suggested as a more relevant hypothesis responsible for schizophrenia pathogenesis compared to the dopamine hypothesis as clinical observation indicates that glutamate neurotransmission blockade using antagonists at the NMDA receptor subtype produces a pathophysiological state that resembles the positive and negative symptoms of schizophrenia while in contrast, the dopamine model of amphetamine produces only positive symptoms (Domino et al., 2004; Coyle, 2012). In addition to that, it has been suggested that the dopaminergic dysfunction observed was a long-term consequence caused by NMDA receptor hypofunction (Roberts et al., 2010). Although the exact level of interactions is still unclear, it has been suggested that the interactions occurred between the glutamate and other neurotransmitter systems leading to the pathogenesis in schizophrenia (Tsai and Coyle, 2002). For example, studies have illustrated that NMDA hypofunction at cortical interneurons may lead to GABAergic interneuron

activations in the ventral tegmental area thus, increasing the interneuron activity and inhibiting mesocortical dopaminergic pathway stimulation, and reducing the dopamine release at the prefrontal cortex (Balu, 2016; Ellaithy et al., 2015).

2.3.3 Serotonin and GABA involvement in schizophrenia symptoms

Other than glutamate and dopamine hypothesis, the less popular neurotransmitter systems hypothesis that may possibly contribute to the pathogenesis of the symptoms in schizophrenia includes serotonin and GABAergic systems. However, compared to glutamate and dopamine hypothesis, these two neurotransmitter systems are often being linked with only certain symptoms.

Serotonergic neurons modulate a wide range of functions such as mood, perception, appetite, aggression, anxiety (Abela et al. 2020), and reward processing (Cohen et al. 2015). Due to its crucial roles in modulating other ligands, including several hormones and neurotransmitters, serotonergic system has been linked with wide range of biological functions (Pourhamzeh et al., 2021). Although its contribution towards onset of schizophrenia is still in question, serotonin has been found to be involved with certain symptoms in schizophrenia especially the negative symptoms. For example, serotonin receptor subtypes 5-HT7Rs has been found to serve as s therapeutic target of high-afnity binding to the several antipsychotics and antidepressants drugs in schizophrenia and could reverse the ketamine induced social withdrawal (Hołuj et al. 2015) Moreover, the role of serotonergic system in neurobiological mechanism of schizophrenia pathogenesis has often been linked with other main neurotransmitter systems including glutamatergic and dopaminergic systems. Past studies indicate that hyperactivation of serotonin receptor subtypes, 5-HT2ARs stimulate the released of glutamate in the VTA, thus activating the

mesolimbic pathway, which results in excess dopamine in the ventral striatum (Stahl, 2018).

The Gamma-aminobutyric acid (GABA) hypothesis started with the findings where glutamate decarboxylase (GAD) activity and GABA content were reportedly decreased in the nucleus accumbens and thalamus of schizophrenia patient (Bird et al., 1977). GABA is the major inhibitory neurotransmitter in the mammalian CNS (Olsen and Sieghart, 2009). Together with the main excitatory transmitter glutamate, the role of GABA is to maintain overall balance between neuronal excitation and inhibition in the CNS which is crucial for modulation of behaviour, emotion, cognitive impairment, neurodegeneration and genetic abnormalities (Uusi-Oukari and Korpi, 2010). In schizophrenia, the abnormal activities of GABA has been closely associated with the symptoms especially the cognitive symptoms. Previous studies indicate that reduction of parvalbumin (PV) neurons which are known as major GABAergic neurons in the schizophrenia patient (Uematsu et al., 2008; Gonzalez-Burgos et al., 2010). Furthermore, other evidence indicating possible role of GABA in schizophrenia neurobiology includes decrease in GABA concentrations in the cingulate gyrus in schizophrenia patient after first-episode psychosis (Nakahara et al., 2022) and reduction of GABA synthesis in postmortem brain studies of schizophrenia patient (Egerton et al., 2017). Other than cognitive symptom, the reduction of GABAergic function has also been associated with certain negative symptoms of schizophrenia. For example reduction of GAD67 which is crucial for GABA synthesis in the orbital frontal cortex (OFC) could lead to disturbances related to emotional and cognitive functioning which responsible for symptoms such as social withdrawal and apathetic behavior (Gur et al., 2000; de Jonge et al., 2017).

2.4 Animal model of schizophrenia

To help understand and identify the diversity of the symptoms of schizophrenia, the development of various animal models that represent the disease is essential. Animal models of diseases are crucial for rapid monitoring of disease progression. However, it is challenging to accurately replicate schizophrenia symptoms in animal models. The current models for schizophrenia that have been developed by the researchers including developmental, pharmacologically-induced, lesion or manipulation of genetics (Jones, Watson and Fone, 2011; Ang et al., 2021). Among the pharmacology model or drug-induced schizophrenia model, glutamatergic antagonists such as phencyclidine (PCP) and MK 801 have been reported to be able to cause a broad spectrum of behavioural responses that are similar to the symptoms in schizophrenia (Jones, Watson and Fone, 2011). Pharmacological, post-mortem, clinical, and animal researches have provided evidence suggesting that glutamatergic NMDA receptors play a major role in schizophrenia (Jones, Watson and Fone, 2011; Bondi, Matthews and Moghaddam, 2012; Ang et al., 2021). Therefore, it is thought that developing an animal model of schizophrenia based on glutamatergic receptor dysfunction will offer an experimental behavioural assay framework that can mimic particular symptoms seen in humans. This will be beneficial for studies to explore the neuropsychological foundations of the illness, the development of novel treatments for the illness, and the mechanisms of action of antipsychotic medications (Chatterjee et al., 2011). Both PCP and MK 801 act as an antagonist of NMDA receptors, which block the action of NMDA receptors leading to hypoglutamatergic activities and other pathways causing psychotic effects, making them suitable components for developing schizophrenia models. However, during the last few years, another PCP derivative

known as ketamine has emerged as another possible psychotic substance that can be used to develop schizophrenia models.

2.5 Ketamine

2-chlorphenyl-2-methylamino-cyclohexanone, or ketamine, is a PCP derivative that acts as a non-competitive NMDAR antagonist (Frohlich and Van Horn, 2013). It was first synthesised in 1962 by Calvin Stevens of the Parke-Davis pharmaceutical company (Rowland, 2005). Ketamine has been recognised as a dissociative anaesthetic with additional analgesic, amnesic, and at a lower dose, ketamine presented with possible fast-acting antidepressant properties (Rowland, 2005; Frohlich and Van Horn, 2013). Nowadays, ketamine is a widely used general anesthetic in both human and veterinary medicine across the globe (Rowland, 2005). It has also been acknowledged as a necessary medication for basic healthcare system (WHO, 2011). Due to its ability to cause several adverse reactions such as hallucinations, delirium, blurred vision and vivid dreams while at a lower level of neurotoxicity, ketamine is considered a possible or better pharmacological substance to develop schizophrenia model compared to other non-competitive antagonists of NMDA receptors (Frohlich and Van horn 2013). Although ketamine presents with adverse reactions that similar to those in schizophrenia, which can be observed after PCP administration, the emergence reactions of ketamine are lower in severity compared to PCP (Frohlich and Van Horn, 2013). In addition to that, the binding targets for ketamine are different compared to PCP and MK-801. Compared to MK-801 and PCP, ketamine also has a high binding affinity towards dopaminergic and serotonergic receptors which plays crucial roles in the symptoms progression and presentation in schizophrenia (Frohlich and Van Horn, 2013).

2.6 Ketamine-induced animal model of schizophrenia

Since ketamine is presented with the ability to induce psychotic behaviours similar to those observed in schizophrenia, ketamine has been used and studied to develop a psychosis model of schizophrenia. There are varieties of doses and protocols utilized by the researchers to develop a model that can mimic the schizophrenia presentations similar to those in humans. However, although psychotic behaviours could be induced in the animal model, due to the complex disease mechanisms, causing all three symptoms simultaneously is still hard. Moreover, some symptoms especially psychotic symptoms usually occur for an episode or a short duration of time and do not persist. Meanwhile, the lack of understanding of the brain circuitry involved in schizophrenia pathogenesis is one of the major limitations in ensuring the presentation of all the symptoms in the model.

Generally, the models can be categorised into two types according to the treatment regimes, which are acute and chronic administration of ketamine. Among the two types of regimes, there are different doses of ketamine used in previous studies. Most of the studies utilized sub-anaesthetic doses of ketamine which are usually used as an adjuvant to opioid for the treatment of pain and surgery to develop the psychotic model (Becker, 2003; Rushforth, Steckler and Shoaib, 2011; Chatterjee et al.,, 2011; Jeevakumar et al., 2015; Sheehy et al., 2017; Ebrahimi et al., 2023). However, most of the past studies focused on the acute psychotic effects of ketamine instead of the prolonged presentation of symptoms as those in schizophrenia patients (Canever et al., 2010; Mihara et al., 2017; Qin et al., 2021). Therefore, there is currently a lack of clarity regarding the most effective dose, withdrawal impact, and treatment plan required to mimic the typical symptoms in animal models.

Moreover, there are inconsistencies in the findings among the studies for example, a study using a single 10 mg/kg dose of ketamine showed a significant increase in distance travelled in an open field test (Canever et al., 2010) while another study using a single dose of 10 mg/kg ketamine showed no effect on distance travelled in comparison to control (Qin et al., 2021). Furthermore, differences have also been found between different treatment regimes, for example, a single treatment of 100 mg/kg of ketamine caused a significant effect on the tail suspension test (TST) but not in the forced swim test (FST), whereas repeated administration of 100 mg/kg ketamine for 10 days caused significant effect on FST but no effect on TST (Chatterjee et al., 2011). On top of that, the ability of the symptoms to persist for a certain amount of time is also different between the treatment regimes. For example, repeated administration of ketamine has caused significant effects on the tests which maintained for a long duration of time compared to the single administration of ketamine which caused only acute effects (Chatterjee et al., 2011; Rushforth et al., 2011).

Table 2.1 Some of the past studies using ketamine-induced psychotic animal model

Dose	**Behavioural Tests**	**Symptoms**	**Year**
Chronic: 25 mg/kg (7 days)	OFT Social interaction	1) Positive 2) Negative	Ebrahimi et al., 2023
Acute: (10, 50 and 100 mg/kg) **Chronic: (50 mg/kg (14 days)**	1) PPI 2) Y-maze	1) Positive 2) Cognitive	Qin et al., 2021
Acute: 20 mg/kg	1) PPI 2) OFT	1) Positive	Mihara et al., 2017
30 mg/kg daily after postnatal day (3 days)	1) Latent inhibition 2) NORT 3) Social interaction 4) EPM 5) OFT	1) Positive 2) Negative 3) Cognitive	Jeevakumar et al., 2015
Acute: 100 mg/kg **Chronic: 100 mg/kg (10 days)**	1) FST 2) TST	1) Negative	Chatterjee et al., 2012
Acute: 100 mg/kg **Chronic: 100 mg/kg (10 days)**	1) OFT 2) FST 3) Passive avoidance test (PAT)	1) Positive 2) Negative 3) Cognitive	Chatterjee et al., 2011
Chronic: 10 and 30 mg/kg (5 days)	1) Odor span task (OST)	1) Cognitive	Rushforth et al., 2011
Acute: 10, 25 and 50 mg/kg	1) OFT 2) Social interaction	1) Positive 2) Negative	Canever et al., 2010
Chronic: 30 mg/kg (5 days)	1) PPI 2) Latent inhibition	1) Positive	Becker et al., 2003

Although the exact actions of ketamine and other NMDA receptor antagonists used to induce schizophrenia are still unknown, there are previous findings indicating a possible association of neuronal systems such as glutamatergic, dopaminergic and serotonergic with the symptoms present in schizophrenia (Frohlich and Van Horn, 2013). For example, the psychotic presentation is associated with hypoglutamatergic activity due to antagonist effects that disrupt the activities of the dopaminergic and serotonergic systems. In schizophrenia, the positive symptom is closely related to hyperdopaminergic activity of D_1 and D_2 receptors at cortical pathway while the negative symptom is associated with hypodopaminergic of D_3 receptors at limbic regions (Frohlich and Van Horn, 2013; McCutcheon et al., 2019).

2.7 Behavioural tasks for symptom detection in an animal model of schizophrenia

Achieving the three primary requirements of face, construct, and predictive validity for the condition has been one of the most challenging aspects of creating an animal model (Jones et al., 2011). Predictive validity is the predicted reaction to both established and new therapeutics, construct validity is the alignment of pathophysiology and aetiology identified in the model with those experienced by humans, and face validity is the ability of the animal model to replicate the symptoms as in human patients (Hagan and Jones, 2005; Jones et al., 2011; Miyamoto and Nitta, 2014; Winship et al., 2019). Thus, developing the schizophrenia model while maintaining construct validity properties and confirming it through face and predictive values is crucial. In this case, the face and predictive validities can be confirmed by performing various behavioural tasks on the animal model. Thus, the primary objective of the behavioural activities is to identify the presentation of the three categories of symptoms that characterize schizophrenia: positive, negative, and cognitive symptoms.

2.7.1 *Behavioural task for detection of positive symptoms*

The positive symptom of schizophrenia is often linked with over-activation of the dopaminergic system. Over-activation of dopamine, which in the normal state is known to be involved in movement control, can lead to psychomotor agitation in schizophrenia (van den Buuse, 2010). Several neuropsychiatric disorders including schizophrenia often contribute to psychomotor agitation, causing hyperactivity or increased stereotypic movements (Larsson et al., 2013). Among the available behavioural tasks, the open field test (OFT) is one of the most suitable tests to measure

hyperactivity or hyperlocomotion activity. The animal will be kept in a restricted space, and during the course of the experiment, the distance it travels and the amount of time it spends traveling will be measured (Ang et al., 2021). Previous studies using different types of established models for schizophrenia such as developmental models (da Silveira et al., 2017; de Matos et al., 2018), genetically induced models (Karlsson et al., 2009; Lee et al., 2019) and pharmacological models (Bogale et al., 2016) have displayed an increased locomotor activity at the baseline level and in response to a novel environment. Other than OFT, another test that has been commonly used to study the positive symptom in the schizophrenia model is the pre-pulse inhibition (PPI) test. PPI can be represented by the level of startle reflex reduction when a non-startling (weak initial) acoustic stimulus is presented prior to a startling (strong) stimulus (Young et al 2009). Thus, it can be measured by recording the animal response in the presence or absence of a weaker, non-startling prepulse that precedes the startling pulse by a short delay (Ang et al., 2019). Studies have indicated that the schizophrenia patient has also experienced sensorimotor gating which leads to oversensitivity to sensory stimulation (Ang et al., 2021).

2.7.2 *Behavioural task for detection of negative symptoms*

Negative symptoms or defect symptoms of schizophrenia can be viewed as a reduction in normal functioning or disability thus reducing the quality of life (Ellenbroek and Cools, 2000). It involved the diminution of emotional expression and motivation related to human attributes at a certain level, which are not accessible readily in animals. However, the symptoms portrayed in patients with schizophrenia such as anhedonia, avolition, asociality, alogia, and blunted affect are often associated with socio-emotional deficits which can be measured with a different types of tests

such as elevated plus maze (EPM), forced swim test (FST), tail suspension test (TST) and three-chamber sociability test (Ang et al., 2021).

An elevated plus maze is a maze consisting of open and closed arms which is normally used to measure anxiety level. Due to the challenge in detecting the negative symptoms such as blunted effect, an alternative way of measuring the symptom has been studied by the researcher. In this case, the researcher has concluded that the measurement of anxiety is a suitable representation of the blunted effect in the animal model (O'Tuathaigh et al., 2010). To measure the anxiety level in the animal model, EPM is considered a useful tool which utilising the natural dislikes of the rodents towards open and high regions, and spontaneous exploratory behaviour when placed in new environments (Komada et al., 2008).

Other than that, FST and TST that were used to measure depression-like behaviours are known as suitable tools to measure the negative symptoms in schizophrenia model such as anhedonia and avolition (O'Tuathaigh et al., 2010; Ang et al., 2021). The principles behind FST and TST are based on the immobility or passive reactions after a period of struggle which reflects behavioural despair as a representation of depression level (Ang et al., 2021). Previous studies on developmental model (Babri et al., 2014), genetically induced models (Brielmaier et al., 2014; Fujikawa et al., 2014; Maksimovic et al., 2014) and pharmacologically induced models (Chatterjee et al., 2011; Chatterjee et al., 2012; Shin et al., 2011; Langen et al., 2012; Suarez-Santiago et al., 2017) have utilized FST and TST as tools to measure negative symptoms of schizophrenia.

Meanwhile, the three-chamber sociability test is usually performed to measure the level of social interaction in the animal model. One of the negative symptoms in schizophrenia known as asociality is characterised as a lower or lack of desire to