JNK 1 Regulates Dopamine Release in the Associative Striatum in MK801 Schizophrenia Model

Human Neuroscience

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Abstract

Various genes on the JNK1 pathway have been implicated in schizophrenia through genetic association studies. However, the effect of JNK1 on dopamine release has not been studied, the underlying mechanism remain poorly understood. Work from our group has previously using mass spectroscopy and bioinformatics analysis of GWAS data, provided evidence to suggest JNK1 may regulate brain functions that are disturbed in psychiatric disorders. We had identified that NMDA receptor subunits were downregulated at the cell surface in mice lacking Jnk1, suggestive of a "NMDA hypofunction" model of psychosis. This combined with a battery of behavioural anomalies representing a schizophrenia-like phenotype, led to the hypothesis to be tested, i.e. that hypofunction of NMDARs of interneurons would lead to hyperstimulation of the corticolimbic pathway, resulting in hyperdopaminergic release in the nucleus accumbens, otherwise referred to as the dorsal striatum.

This current study emphasises the role of JNK regulates dopamine release in associative striatum and in the control of schizophrenia-like behaviour in mice. By using *Jnk1* knock out mice and pharmacological inhibition of JNK pathway. This study aimed to investigate whether dopamine release was altered in the associative striatum in the MK801 schizophrenia mice model. We presently show that Jnk1 knockout mice exhibited mild but significant schizophrenia-like behaviour, as indicated by the range of behaviour test in an open-field and Y-maze shown showed that *Jnk1*-/- mice pronounced deficit in motor coordination and locomotion. Moreover, non-competitive NMDAr antagonists such as MK801 induced dopamine regulates in associative striatum which induced schizophrenia-like behaviour in mice. The intrinsic hyperactivity of *Jnk1*-/- mice, aligns with their reduced surface expression of NMDA receptor subunits, and a possible NMDAR hypofunction model. Furthermore, these findings highlight the JNK pathway as putative novel drug target against schizophrenia disorder. Further investigation would be needed to clarify the gene's mechanistic involvement and its potential therapeutic implications, especially in disorders characterized by NMDA receptor dysfunction. As we progress ahead in this line of research, understanding such intricate molecular-behavioural relationships becomes essential in developing targeted therapeutic strategies for neuropsychiatric conditions.

Keywords: JNK1, DLIGHT, associative striatum, MK801 schizophrenia model, NMDA hypofunction

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List of Abbreviations

AS	Associative striatum
DA	dopamine
FP	Fibre photometry
cpGFP	circular permuted green fluorescent protein
JNK1	c-Jun N-terminal kinase-1
MAP2K7	mitogen-activated protein kinase 7
NAcc	Nucleus accumbens
NMDA	N-Methyl-D-aspartate
OF	open field test
YM	Y maze
PPI	Prepulse inhibition
VTA	ventral tegmental area

Chapter 1: Review of the Literature

1.1 Schizophrenia

Schizophrenia (SCZ) is a complex and chronic neuropsychiatric disorder where the underlying causes remain unknow and in addition, the disease is challenging to treat effectively (Patel et al., 2014; McDonagh et al., 2017).

A debilitating lifelong illness such as schizophrenia affects 1 percent of the population worldwide; in men, symptoms usually appear in their late adolescents or early 20s, while women tend to show the first sign of symptoms in their 20s and early 30s (American Psychiatric Association [APA], 2013). Studies reviewed the prevalence and higher rates of mortality are associated with schizophrenia. For instance, in 2002, it was estimated at 5.1 per 1000 population in the United States and about 40 individually with conditions untreated (many people live with symptoms and without diagnosis or treatment) (Wu, Shi, Birnbaum, Hudson, & Kessler, 2006). In 2016, the global age-standardised point prevalence of schizophrenia was estimated at approximately 0.28% across 21 regions and 195 countries (Charlson et al., 2018). Moreover, Charlson and colleagues reported that the prevalence of schizophrenia rose from 13.1 million in 1990 to 20.9 million cases in 2016. Similarly, the World Health Organization stated that schizophrenia contributes 13.4 million lives with a disability to the burden of disease globally (Harrison et al., 2001; Laursen, Nordentoft, & Mortensen, 2014). Schizophrenia has become a global burden, chronic severe mental illness. Therefore, the cost-of-illness (COI) studies reported that schizophrenia is associated with a large economic burden driven by directed healthcare costs and productivity losses (Jin & Mosweu, 2017).

Schizophrenia has raised the number of cases from time to time with the significant financial burden that is demanded of society. Hence, it is essential to study the critical insight

into the aetiology, neuropathology, genetics, and treatment of schizophrenia, which can facilitate early identification, intervention and perhaps prevention of the illness.

1.1.1 Clinical Identification

Schizophrenia was defined as a chronic severe mental disorder that characterised by a disruption in the connection between thoughts, emotions, and behaviours. This disruption leads to distorted perception, inappropriate actions and emotions, withdrawal from reality and personal connections, and a feeling of mental disintegration.

Schizophrenia is mainly characterised by positive (i.e., hallucination, delusions, trouble concentrating, and motor symptoms), negative (i.e., apathy, emotional blunting, social dysfunction), and cognitive (disorganised thought, Impaired ability to focus and adhere to directions , problem completing task) symptoms (Association & Association, 2013). The diagnosis entails identifying a combination of signs and symptoms linked to reduced ability to perform work or engage in social activities. In order to diagnose Schizophrenia, it is necessary for there to be the presence of at least two symptoms from Criterion A for a significant amount of time, lasting a month or more (see Table1.).

Table 1. Diagnostic Criteria for Schizophrenia from Diagnostic and Statistical Manual of Mental Disorder (DSM-5)

Schizophrenia

Diagnostic Criteria **295.90** (F20.9)

A. Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated). At least one of these must be (1), (2), or (3):
   1. Delusions.
   2. Hallucinations.
   3. Disorganized speech (e.g., frequent derailment or incoherence).
   4. Grossly disorganized or catatonic behavior.
   5. Negative symptoms (i.e., diminished emotional expression or avolition).

B. For a significant portion of the time since the onset of the disturbance, level of functioning in one or more major areas, such as work, interpersonal relations, or self-care, is markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, there is failure to achieve expected level of interpersonal, academic, or occupational functioning).

C. Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or by two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).

D. Schizoaffective disorder and depressive or bipolar disorder with psychotic features have been ruled out because either 1) no major depressive or manic episodes have occurred concurrently with the active-phase symptoms, or 2) if mood episodes have occurred during active-phase symptoms, they have been present for a minority of the total duration of the active and residual periods of the illness.

E. The disturbance is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition.

F. If there is a history of autism spectrum disorder or a communication disorder of childhood onset, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations, in addition to the other required symptoms of schizophrenia, are also present for at least 1 month (or less if successfully treated).

1.1.2 Etiology of Schizophrenia

Schizophrenia represents a complex and multidimensional combination of hereditary, neurochemistry and environmental risk factors, and interaction underlines the development of schizophrenia (Misiak et al., 2018)

Although the etiologic of schizophrenia is still debatable, however, family, twin, and adoption studies offer compelling evidence that schizophrenia has a major genetic component. For instance, a comprehensive study of studies involving twins examined the role of genetics and the environment on susceptibility to schizophrenia. The findings revealed that around 80% of the liability to schizophrenia can be associated with genetic factors, while shared environmental impacts accounted for approximately 11% (Benson, 2012). The previous study suggested that the genetic architecture of schizophrenia may increase the risk of developing

diseases (Dennison, Legge, Pardinas, & Walters, 2020). Moreover, environmental factors that might play a role in the etiology of schizophrenia include obstetric complications (e.g., hypoxia, preeclampsia, low birth weight, and premature birth) and the seasonal of birth and prenatal or gestational exposure to infection. For instance, studies that investigated the environmental factor of schizophrenia discovered that the risk of schizophrenia is higher among people who were born in the spring-winter season (i.e., about 5% of people increased in risk associated with birth between December and May) (Michael-Titus, Revest, & Shortland, 2014), suggest that maternal influenza infection during pregnancy may compromise fetal brain development.

1.1.3 Pathophysiology of Schizophrenia

1.1.3.1 Hypothesis of Schizophrenia

It has long been discovered that alternation in brain chemistry in schizophrenia is associated with abnormal mental states. The most well-studied neurotransmitter implicated in schizophrenia is dopamine (DA). The dopamine hypothesis of schizophrenia has evolved over the decades and was derived from three critical observations. First, the pharmacological action of antipsychotic medications showed the improvement in patients by blocking dopamine receptor D2, which reduces dopamine levels in neurons (Creese, Burt, & Snyder, 1976; P. Seeman, Chau-Wong, Tedesco, & Wong, 1975). For instance, Schizophrenia is a result of the imbalance in neurotransmission within the brain's dopaminergic circuits, characterized by an increase in dopaminergic activity in the mesolimbic pathway and decreased dopamine signalling in the mesocortical pathway. (Davis, Kahn, Ko, & Davidson, 1991). Hence, the antagonism of the D2 receptor in the mesolimbic pathway will reduce dopamine activity and psychosis symptoms (Stahl, 2018; Seeman et al., 2006). The second evidence revealed amphetamine-induced psychosis (Young & Scoville, 1938). For instant, several studies found that abuse of amphetamine in non-schizophrenic people/ patients can result in a form of mental disorder characterized by paranoia and auditory hallucinations. (Bell, 1973; E. Ellinwood Jr,

1967; E. H. ELLINWOOD JR, Sudilovsky, & Nelson, 1973; Griffith, Oates, & Cavanaugh, 1968). Imaging studies have provided an additional source of data indicating that N-methyl-D-aspartate (NMDA) hypofunction in non-schizophrenic subjects can cause changes in DA levels that are comparable to those seen in schizophrenia. (Laruelle, Kegeles, & Abi-Dargham, 2003; Narendran et al., 2005). Together, several decades of research have shown that DA alteration has been associated with manifestations of schizophrenia.

1.1.3.2 NMDA hypofunction hypothesis of schizophrenia

Recently, the novel hypothesis centred on the hypoactivity of the NMDA transmitter system. Research has provided evidence supporting the idea that N-methyl-D-aspartate receptor (NMDAr) hypofunction plays a key role in functional dysconnectivity, which is an alternative model for symptoms (Zandi et al., 2011). The majority of evidence in humans suggests that NMDAr hypofunction helps clarify the aetiology and pathophysiology of schizophrenia. For instance, previous studies found that pharmacological approaches, such as systematic MK801, pCP or administering ketamine injections to rodents, which induce NMDA receptor hypofunction, is enough to impair both local and diffuse circuits as well as overall brain function. This also leads to psychosis-related behavioral symptoms (Eyjolfsson, Brenner, Kondziella, & Sonnewald, 2006). They also found *de novo* mutation of the NMDA receptor in a large proportion of schizophrenia patients. Furthermore, the NMDA receptor autoantibody has been found to induce schizophrenia in patients (Fromer et al., 2014). Moreover, The capacity of non-competitive NMDA receptor (NMDAr) antagonists such as phencyclidine, Ketamine, and MK801 to elicit a range of positive, negative, and cognitive symptoms resembling schizophrenia has given rise to the hypothesis that NMDAr hypofunction may play a role in the development of schizophrenia (Fromer et al., 2014; Krystal et al., 1994).

1.1.4 Treatment for schizophrenia

Schizophrenia required life-long treatment even when the symptoms subsided. Patients with schizophrenia typically undergo a combination of consistent pharmacological treatment and psychosocial therapy. Medications are widely used to treat schizophrenia; over decades, more than sixty antipsychotic medications have been developed; the ability to block dopamine D_2 receptors in the brain remained the same target among all antipsychotic drugs (M. V. Seeman & Seeman, 2014).

The antipsychotic medication can be classified into first-generation antipsychotics (FGAs) and second-generation antipsychotics (SGAs). The FGA drugs such as chlorpromazine and haloperidol were the first used to treat psychosis disorder worked as dopamine antagonists (i.e., blocking the action of dopamine, primarily by occupying the D_2 dopamine receptor).

FGAs have provided the patients with significant clinical improvement such as reduced hallucination and diminishing belief (Tandon, Nasrallah, & Keshavan, 2010). However, many patients on FGAs experienced extrapyramidal side effects (EPS) such as involuntary movement abnormality (muscle spasms, rigidity, shaking) and the other common side effect on these medications such as drowsiness, dry mouth, and weight gain (Tandon et al., 2010).

The SGA drugs such as clozapine are now widely used among clinicians in the United States and Europe as initially reserved for refractory treatment patients and causes fewer EPS symptoms than chlorpromazine or haloperidol and were all believed initially to be more efficacious and tolerable than FGAs (Keefe et al., 2007; Lieberman et al., 2005). Additionally, based on the NMDA hypothesis, Various preclinical investigations have shown that the effects of SGAs on responses to NMDAr antagonists raise the possibility that the therapeutic action of these agents may involve a correction of NMDAr hypofunction (Duncan, Miyamoto, Leipzig, & Lieberman, 2000). However, the results of large-scale studies such as the Clinical

Antipsychotics Trials of Intervention Effectiveness (CATIE), indicated that clozapine or other SGA medications may not be significantly more effective than FGAs medications. Additionally, there is no clear evidence to suggest that SGAs are associated with improved cognitive or social outcomes (Keefe et al., 2007; Lieberman et al., 2005), and the Clozapine does not appear to have superior efficacy compared to other drugs in the treatment of individuals who do not respond to standard treatments (Tandon et al., 2010). Furthermore, all antipsychotics currently on the market exhibit robust efficacy in treating positive symptoms, but their effectiveness in lowering negative symptoms is less consistent. The negative symptoms are largely attributed to a decrease in positive symptoms, while the negative symptoms linked with EPS worsen (Tandon et al., 2000). Together, the studies outline the approach of using antipsychotic drugs, leading to ongoing monitoring of the risks-benefit, as well as making decisions in collaboration with others. However, further studies of schizophrenia with the disciplined approach will enhance the better outcome for all schizophrenia patients later on.

1.1.5 Structural and Morphological Abnormalities in the Brain Associated with Schizophrenia

As briefly above, based on the hypothesis of schizophrenia, dopamine is the neurotransmitter that has been extensively researched and is associated with schizophrenia. . The four main dopamine pathways in the centra nerves systems (CNS) are 1) the mesolimbic pathway which projects from the ventral tegmental area (VTA) to D_1 and D_2 receptor rich in nucleus accumbens and the amygdaloid nucleus; 2) the mesocortical pathway, which projects VTA to temporal, frontal, and prefrontal cortices as those brain regions are rich in D_1, D_5 and D_2; 3) the nigrostriatal pathway which projects from the substantia nigra to the corpus striatum,

D_2, D_3, D_4 receptor are found to be concentrated in this brain region; 4) the tuberoinfundibular pathway which projects from the thalamus to median eminence of the D_2 receptor rich anterior pituitary (Kahn, Davis, Bloom, & Kupfer, 2000).

For instance, some studies have identified presynaptic abnormalities in dopamine functioning (i.e., too much dopamine is synthesised and released into the synapse) (O. Howes, McCutcheon, & Stone, 2015; O. D. Howes, McCutcheon, Owen, & Murray, 2017). Furthermore, some evidence shows that patients with schizophrenia have an increased number of D2 receptors (O. Howes et al., 2015). However, the increased D_2 receptor is small, not found in patients who have not taken antipsychotic medication. This suggests that the elevation in dopamine receptor density is more likely linked to treatment effects rather than being associated with schizophrenia itself (Fusar-Poli & Meyer-Lindenberg, 2013). Moreover, the mesolimbic pathway is also implicated in schizophrenia. For instance, abnormal activity of the mesolimbic pathway that carries dopamine from the VTA of the limbic striatum to the cerebral cortex is linked to the higher risk of schizophrenia (Laviolette, 2007). Likewise, significant dopaminergic innervation of the prefrontal cortex and other limbic areas linked to psychosis supports the idea that dopamine plays a role in schizophrenia (Stevens, 1979).

Moreover, positron emission tomography (PET) studies have shown strong evidence that schizophrenia patients have higher levels of dopamine synthesis and release capacity compared to control participants. This increase is observed in both the associative striatum and the midbrain origin of the neurons (O. D. Howes & Nour, 2016). Likewise, structural studies have reported the volumetric and morphological consistently reduced white matter volume in schizophrenia patients (Kuperberg et al., 2003; Pol et al., 2004; Rapoport, Giedd, & Gogtay, 2012; Van Horn et al., 2012). In addition, network analyses investigating the structure of brain networks using diffusion-weighted data have confirmed decreased levels of overall structural connectivity in patients. Specifically, the white matter connections between the frontal,

temporal, and parietal regions appear to be the most impacted (Van Den Heuvel & Fornito, 2014; Zhang et al., 2015).

1.1.6 Comorbidity of Schizophrenia

Psychiatric syndromes, including depression, anxiety, obsessive-compulsive disorder (OCD), substance use disorders, post-trauma stress disorder (PTSD), commonly co-occur with schizophrenia significantly. For example, depression and anxiety can cause secondary negative symptoms, panic attacks can lead to paranoid episodes, and cannabis abuse can worsen positive and cognitive symptoms (Buckley, Miller, Lehrer, & Castle, 2009). Moreover, the meta-analysis of 52 psychiatric comorbidities in schizophrenia studies reported a prevalence rate of about 12.4% for PTSD, 12.1% for OCD, 9.8% for panic disorder, and 14.9% for social phobia (Buckley & Hwang, 2015). However, the diagnostic and treatment issues of coexisting or overlapping psychiatric symptoms and schizophrenia disorder remain poorly understood (Abdullah, Azeb Shahul, Hwang, & Ferrando, 2020), suggested that the concept of comorbidity in schizophrenia may constitute distinct schizophrenia subtypes with a unique underlying biological pathogenesis (Insel, 2014). Besides that, animals' studies revealed that the psychosis-like phenotype in a rodent model for schizophrenia has developed in poor-coping stress, anxiety, and suspicious behaviour as an endophenotype (Millan et al., 2016). Suggested that animal models may address a clear splitting line between equivalent clinical traits and provide potentially crucial new insight into a range of brain mechanisms relevant to schizophrenia. In contrast, studying with a human would be complex due to technical and ethical points of view.

1.2 Animal Models in Schizophrenia

Animal models play a crucial role in examining the genetic, molecular, cellular, and environmental factors associated with many neuropsychiatric diseases. The use of animal

models leads to a better understanding of etiology, pathogenesis, brain pathologies, and behaviour abnormalities associated with schizophrenia in humans with better treatment outcomes with higher efficiency. Furthermore, animal models provide a rapid platform to examine the evolution of diseases compared to studying individuals. They also allow for invasive monitoring of the structural and molecular changes that are responsible for the development of diseases. Additionally, animal models enable the testing of new therapeutic approaches that cannot be performed on humans. Nonetheless, the presence of schizophrenia symptoms, such as paranoid delusions and auditory hallucinations, is limited to humans, which poses a challenge in interpreting findings derived from animal models (Canetta & Kellendonk, 2018).

Nevertheless, the animal models should have the following characteristics: face (symptomology parallel to that observed in humans), construct (replicate the theoretical neurobiological rationale and pathology), etiological (diseases that established by the same means such as environmental factors), and predictive (responding to treatment) validity to the clinical disorder being modelled (Geyer & Markou, 1995). For instance, schizophrenia should have a suitable pattern of behavioral and neurochemical abnormalities. These include the onset of symptoms after puberty, a decrease in connectivity and function in the hippocampus and cortex, dysregulation of dopamine in the limbic system, reduced activity of glutamate in the cortex, increased susceptibility to stress, abnormal response to rewards, social withdrawal, and cognitive impairment. Previous studies have reviewed the animal models of schizophrenia by comparing the potential applications of some standard models, which underline the predictive validity to evaluate novel compounds that could improve the cognitive and negative symptoms seen in schizophrenia (Floresco, Geyer, Gold, & Grace, 2005; Neill et al., 2010).

Animal models of schizophrenia have been established for over a decade, and various models share similar methodologies across distinct induction categories such as developmental,

drug-induced, lesion, or genetic manipulation (Carpenter & Koenig, 2008). Initially, animal models were developed based on dopamine hypothesised of schizophrenia (e.g., The pathogenesis of schizophrenia revolves around dopamine deficiency). However, with an increased understanding of genetics basis and potentials involvement of glutamate, animal models have been developed to explore the involvements in their disorder (Jones, Watson, & Fone, 2011). Nonetheless, NMDAr hypofunction models have proposed afterwards and have been wildly utilised to study the neurobiology of schizophrenia, as well as pharmacological (e.g., phencyclidine, ketamine, dizocilpine) and genetic approaches to induce NMDAr hypofunction (Collingridge et al., 2013; Lin, Lane, & Tsai, 2012) . The current study emphasises dizocilpine, also known as MK-801, to induce schizophrenia-like symptoms using rodents.

1.2.1 Drug-Induced Rodent Models

MK-801 is a non-competitive NMDAr antagonist which acts on GABAergic interneurons to reduce the inhibitory influence on excitatory pyramidal neurons leading to hyperexcitation in the prefrontal cortex neuronal circuit (Homayoun & Moghaddam, 2007). Moreover, MK-801 has substantially longer action of NMDAr blockade in rodents and higher specificity for NMDAr when compared to ketamine (Miyamoto, Leipzig, Lieberman, & Duncan, 2000). It has been suggested that MK-801 may produce a full range of schizophrenia-related behavioural phenotypes in both acute and chronic models due to its prolonged action, high potency, and specificity for NMDAr.

For instance, Administration of MK-801 in rodents causes hyper locomotor activity, a decrease in social engagement, and impairments in cognitive abilities (e.g., long-term spatial memory, working memory, and skill learning) (Abekawa, Ito, Nakagawa, & Koyama, 2007; Wiescholleck & Manahan-Vaughan, 2012). Moreover, the chronic MK-801 administration

leads to sensorimotor gating and social interaction deficits, increased anxiety-like behaviour and working memory deficits (Unal, Ates, & Aricioglu, 2018). Nonetheless, other studies found that the chronic MK-801 treatment reduced locomotor activity, decreased exploratory behaviour and increased anxiety-like behaviour, as well as learning impairments (de Leon & Diaz, 2003; Homayoun & Moghaddam, 2007). Prior research repeatedly demonstrated that prolonged administration of MK-801 results in heightened dopaminergic activity in the frontal cortex, nucleus accumbens, and striatum (Löscher, Annies, & Hönack, 1991). However, the use of pharmacology in rodent models to recapitulate the dysregulation of DA systems maybe need further consideration due to limitations as suggested continuous injection of MK-801 may result in heightened dopaminergic activity in the frontal cortex, which may not align with the hypodopaminergic state observed in patients with schizophrenia. .

The effect of MK-801 in rodents at the molecular, electrophysiological, and behavioural levels was assessed by various approaches. For instance, behaviours associated with positive symptoms of schizophrenia are examined as novelty-induced hyperlocomotion in the open field test. The forced swim test is commonly used to evaluate the negative symptom-like behaviour alteration that correlates with social withdrawal and anhedonia (Sams-Dodd, 1996), and the cognitive impairments are assessed by a variety of behavioural tests, such as the radial arm maze, Morris water maze, Y-maze, novel object recognised test. Moreover, the endophenotype corresponding to sensorimotor gating deficits in schizophrenia is detected as impaired prepulse inhibition (PPI) of the acoustic startle response (McKibben, Jenkins, Adams, Harte, & Reynolds, 2010). Hence, the positive symptom-like behaviour exhibited in MK-801 animal models showed increased stereotypic behaviour and ataxia; however, locomotor activity changes are inconsistently reported. The negative symptom-like behaviour is reduced in social interaction, while cognitive deficits range from impaired latent learning, attention, and cognitive flexibility deficits to decreased sensorimotor gating (Lee & Zhou, 2019a).

Moreover, studies on molecular and genetics have revealed several candidate genes that could be involved in the development of schizophrenia (Bunney et al., 2003). Recently, genetically modified mouse models played an important role in animal research in psychiatry and have become the method to understand better the role of genes in brain mechanisms in schizophrenia. For instance, applying molecular techniques, transgenic mice have been genetically modified to incorporate additional genes, resulting in an overexpression of the corresponding gene's products. Conversely, knockout mice have undergone mutations to eliminate or deactivate specific genes, leading to a deficiency in producing the products associated with those genes (Van Den Buuse, Garner, Gogos, & Kusljic, 2005). However, the previous studies have indicated that molecular biology, combinatorial chemistry, and computer modelling are unable to accurately anticipate the overall reaction in entire organisms or humans, especially when it comes to newly discovered genes (Alabaster & Group, 2002). This suggested that the implicated genes in schizophrenia have not been traditionally linked to other psychiatric illnesses. This can be challenged for *in vivo* neuroscientists to identify their role in animal models of schizophrenia, using the behavioural test relevant to the illness of interest. However, Understanding transgenic or knockout mouse models is important as their significant overexpression or deletion of gene activity does not accurately duplicate the subtle alterations in gene function observed in complex mental diseases like schizophrenia.

This current study emphasises the JNK family, which will state in the next section.

1.3 Mitogen-activated Protein Kinases

Mitogen-activated protein kinases (MAPK) are a specific type of protein kinase that catalyse the addition of phosphate groups to substrate proteins. They are specifically involved in directing cellular responses to various stimuli, such as mitogens, osmotic stress, heat shock,

and proinflammatory cytokines. MAPKs primarily target the amino acids serine and threonine. MAPKs regulate cellular function such as proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis (cell death). Regulation of the MAPK pathways have been widely studied and linked to the pathogenesis of several diseases particularly targeting for neurodegenerative disease (Harper & Wilkie, 2003). The MAPKs have a number of shared characteristic (e.g., activation dependent on two phosphorylation events) known as three-tiered; the signalling start with the top tier of kinases which called MAPK kinase kinases (MAP3Ks) activate MAPK kinases (MAP2Ks) by phosphorylation events then in turn activate the last and lower tier of MAPKs. This wide range of regulated by the MAPKs is mediated through phosphorylation of several substrates to prompt cellular response (Cargnello & Roux, 2012). The mammalian MAPK family of kinases comprise extracellular signal-regulated kinases (ERKs), p38 mitogen-activated protein kinases (p38s), and c-Jun N-terminal kinases (JNKs). The multiple of existed isoforms of each protein are shared among the cascades, hence enabling crosstalk and coordinated integration of external stimuli.

1.3.1 c-Jun N-terminal Kinases

The c-Jun N-terminal Kinases (JNKs) were initially recognized as stress-activated protein kinases (SAPKs) due to their exhibiting a response to various stressors (e.g., DNA damage, oxidative stress, cytoskeletal alterations, infection, or inflammation). The JNK pathway is one of the major signalling cascades of the mitogen-activated protein kinase (MAPK) signalling pathway, thus the studies of pathways regulated by JNKs have demonstrated their essential role to be indispensable for both cell proliferation and apoptosis. The outcome of JNK activation, whether it leads to cell proliferation or apoptosis, depends on the specific stimuli and cell type involved in the activation process. (Bogoyevitch, Ngoei, Zhao, Yeap, & Ng, 2010; Keshet & Seger, 2010). The JNK pathway has been originally found to be hyperactive in cancer, heart disease, axonal degeneration, neurodegenerative disease (e.g.,

Huntington's disease, Multiple sclerosis, Parkinson' s disease and Alzheimer's disease), obesity and insulin resistance (Hao et al., 2019; Pal, Febbraio, & Lancaster, 2016). Research has been actively progressing toward the generation of JNK inhibitors as a potential therapeutic target with less side effects (Tian et al., 2016).

JNK functions as the inflammatory signal, and protein synthesis, and can be activated by many stress events. JNK activation can be induced by upstream kinase activators or by altering the conformation of protein phosphatase enzymes that are sensitive to such changes. Normally, certain phosphatases block the activity of JNK itself and the activity of proteins associated with JNK activation (Zhan, Gurevich, & Gurevich, 2017). JNKs associated with scaffold proteins, including JNK interacting proteins (JIP), plenty of SH3s (POSH), β-arrestin, IκB kinase complex-associated protein (IKAP), JNK-binding protein 1 (JNKB1/MAPKBP1), and WDR62. Additionally, their activation is followed by the involvement of their upstream kinases JNKK1 and JNKK2 (Cohen-Katsenelson et al., 2013; Morrison, 2012). The process of phosphorylating c-Jun at the specific locations targeted by JNK (Ser-63 and Ser-73) leads to an enhancement in transcriptional activity. The phosphorylation site corresponds to Ser/Thr-Pro motifs situated in the activation domain of the transcription factor. JNK relies on a docking site to identify and attach to the substrate. Once activated, JNK can phosphorylate numerous substrates, including cytoskeletal proteins (e.g., microtubule-associated proteins, tau, and Neurofilament), proteins in the mitochondria, and proteins in the nucleus (such as ATF, c-Jun, and p53) (Bohush, Niewiadomska, & Filipek, 2018; Coffey, 2014; Zeke, Misheva, Reményi, & Bogoyevitch, 2016).

Three JNK genes, namely JNK1 (MKPK8), JNK2 (MAPK9), and JNK3 (MAPK10), have been found encoded in the mammalian genome: all three genes are expressed in the human and mouse brain and encode for ten different splice variants with molecular weights of 46 and 55 kDa (Zhan, Perez, Gimenez, Vishnivetskiy, & Gurevich, 2014). JNK1 and JNK2 have a

broad tissue distribution, whereas JNK3 is mainly localised in neurons and to a lesser extent in the heart and the testis.

1.3.2 c-Jun N-terminal Kinases in Schizophrenia

JNK has long been discovered more than 20 years ago. It has remained the subject of intense research interest with continued efforts to evaluate its biochemistry and regulation and contribute to cellular events under physiological and pathophysiological conditions (Yarza, Vela, Solas, & Ramirez, 2016). The importance of JNKs in the nervous system has been revealed using approaches of jnk-knockout mice and jnk inhibitor, more specific JNK signalling pathway – a multi-level kinase cascade involved in both physiological and pathological plasticity in neurons, and this may link with the evidence for abnormalities of signal transduction network in schizophrenia (Chen et al., 2005; McGuire et al., 2017).

A review of studies investigating the effects of JNKs on brain development and pathological states suggest that the JNK pathway is associated with a psychiatric disorder (Coffey, 2014). Other studies have also found that the JNK pathway is linked to intellectual disabilities, implicating an abnormality of synaptic structure (Marchisella, Coffey, & Hollos, 2016). Furthermore, recent evidence suggests that the JNK signalling pathway, specifically the kinase involved in JNK activation such as MKK7 (MAP2K7), poses a genetic risk in schizophrenia (Openshaw et al., 2019; Willis, Pratt, & Morris, 2020). For instance, Hong et al. (2021) found that JNK1 regulates the phosphorylation of more than one hundred schizophrenia polygene products, and JNK knockout mice show the molecular and behavioural phenotype of schizophrenia. Evidence from humans and rodents suggests that JNKs are important kinases in the brain, and the activities have been linked to neuropsychiatric disorders, particularly in schizophrenia-like behaviour. This would be a novel strategy that may improve treatment methods for schizophrenia.

1.4 Technological advances to study neurocircuitry of schizophrenia

1.4.1 Fibre photometry (FB)

The technique used in modern neuroscience to measure chemical signalling and neural activity of freely behaving animals is called fibre photometry. The early method has begun to provide insights into the function of specific brain circuits which is used to detect neural activity use electrophysiology which involves planting simple electrodes in the rodent brain, the electrodes allowed for recording the electrical activity produced by action potentials of neurons in the regions where they are implanted. Although this technique helps in understanding the function of different brain areas, however, electrophysiology still lacks specificity (Li, Liu, Guo, & Luo, 2019; Packer, Roska, & Häusser, 2013). Therefore, electrophysiology has been improved to the point that it currently allows measuring from specific cell population in specific brain areas. Traditionally, FP has been used to measure calcium signalling using genetically encoded fluorescent indicators such as GCaMP. More recently, a growing array of indicators has become available, allowing for measurements of many different kinds of chemical signals such as neurotransmitters, including serotonin and glutamate (Nguyen et al., 2010; Yamauchi et al., 2011) or dopamine.

1.4.2 dLight – Ultrafast Dopamine Indicator

Patriarchi et al. have developed dLight1, a genetically encoded fluorescent dopamine indicator that enables precise optical measurement of dopamine concentration in isolated target circuits. This indicator has exceptional spatiotemporal resolution and provides distinct benefits for investigating neuromodulatory mechanisms (Figure 1) (Cosme, Palissery, & Lerner, 2018; Patriarchi et al., 2018).

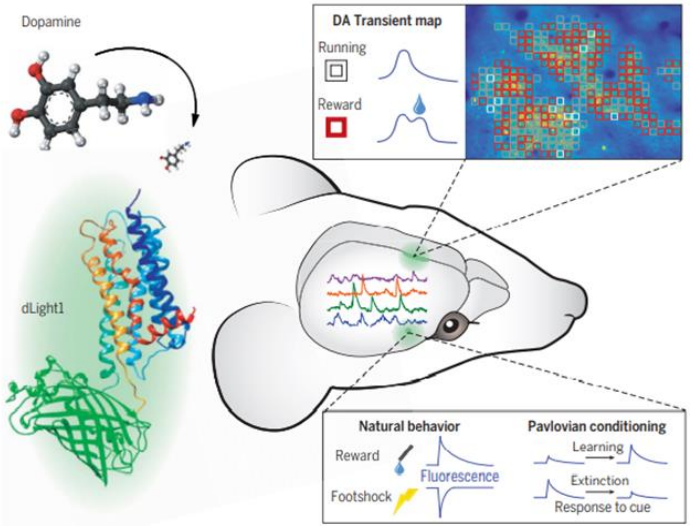

Figure 1. indicates the DLIGHT1 signal in vivo representing extracellular dopamine binding to the modified receptor (Patriarchi et al., 2018).

1.4.3 Fluorescence Protein

Fluorescence proteins (FP) are acquired by optimising the photophysical characteristics of blue to yellow variations derived from the green fluorescent protein (GFP) found in the Aequorea Victoria jellyfish. Additionally, FPs are also created from other animals to emit light across the entire visible spectrum (Shaner, Patterson, & Davidson, 2007). To date, the jellyfish variants have resulted in new monomeric BFP, CFP, GFP, and YFP variants, among others, and have yielded as such a powerful tool that provide great signal to noise contrast and allow visualization of cells for imaging applications (Chalfie, Tu, Euskirchen, Ward, & Prasher, 1994).

1.4.4 Fluorescence Microscopy

The absorption and subsequent re-radiation of light by these proteins results from a well-established physical phenomenon known as fluorescence (Hillenkamp & Peter-Katalinic, 2013). Fluorescence is a type of light emission that occurs with fluorophore molecules, where a fluorophore absorbs light of a certain wavelength and subsequently loses some energy

through heat generation and then emits the remaining energy as light. The emitted light is of lower energy level due to heat loss, and thus the emitted light is of a higher wavelength (lower energy) than the incoming excitation light. This is the excitation-emission process (Lichtman & Conchello, 2005). The microscope consists of the objective to collect the light, light sources containing different filters and a camera to capture the living cell. The sample is illuminated when the excitation light with a certain wavelength is directed towards the sample, and then the fluorescence is emitted, blocking the excitation light from arriving at the detectors (Murphy, 2001; Sanderson, Smith, Parker, & Bootman, 2014).

Chapter 2: Research Study

2.1 Objectives

This Master thesis work is part of a study in Coffey lab that examines the molecular action of JNK in models of affective and psychotic disorders. Results emanating from previous work using mass spectroscopy and bioinformatics analysis of GWAS data, provided evidence to suggest JNK1 may regulate brain functions that are disturbed in psychiatric disorders. The lab had identified that NMDA receptor subunits were downregulated at the cell surface in mice lacking *Jnk1*, suggestive of a "NMDA hypofunction" model of psychosis. This combined with a battery of behavioural anomalies representing a schizophrenia-like phenotype, led to the hypothesis to be tested, i.e. that hypofunction of NMDARs of interneurons would lead to hyperstimulation of the corticolimbic pathway, resulting in hyperdopaminergic release in the nucleus accumbens, otherwise referred to as the dorsal striatum. The nucleus accumbens' (NAcc) association with schizophrenia has been widely studied; hence it was important to explore whether JNK1 altered dopamine release in the dorsal and associative striatum (AS).

To this end, this study investigated the MK801 model of psychosis. It explored whether (1) the dopamine response in JNK knockout mice was elevated compared to wild-type mouse

and if injection with an NMDA receptor antagonist (MK801) would elicit a different response in of wild-type and knockout mice in terms of dopamine release. (2) The accompanying behavioural responses were also measured. MK801 is an NMDA receptor antagonist that induces a phenotype characterised by hyperactivity, stereotypies and cognitive underperformance. MK801 alters dopamine levels in the dorsal striatum (Lee & Zhou, 2019b; Powell & Miyakawa, 2006; Ranson et al., 2019). This study also aimed to measure the dopamine levels *in vivo* in the dorsal and associative striatum. The associative striatum dopamine irregularities have been described during psychosis but has not yet been explored in mouse models. The dLight1.1 sensor which fluoresces upon dopamine binding was used to measure dopamine transients in awake behaving mice using using fibre photometry. dLight1.1 is a genetically modified dopamine D1 receptor conjugated to cpGFP (circular permuted green fluorescence protein), allowing physiological and behavioural detecting of dopamine levels with green fluorescence (Patriarchi et al., 2018).

2.2 Materials and Methods

2.2.1 Animals

Seventeen male mice (4-month-old) – 9 wild type C57BL/6J and 8 *Jnk1-/-* mice – obtained from The Jackson Laboratory. The mice were housed under normal light conditions (12 hr of light dark cycle) and supplied with food and water *ad libitum*. All experimental methods were conducted following the guidelines outlined in the Finnish (62/2006 Act and 36/2006) and European (86/609/EEC, 2010/63/EU, 1986/ETS 123) directives, which were approved by the National Animal Experiment Board (ELLA).

2.2.2 Animal surgical preparation and virus injection

All the mice underwent stereotaxic surgery in order to be injected in the associative striatum (putamen) with an adenovirus (pAAV-CAG-dLight1.1; plasmid #111067) vector dLight1.1 dopamine sensor. The cannula, used to perform fibre photometry, was chronically implanted, and cemented using the stereotactic co-ordinates of the associative striatum (Anterior-posterior -0.6mm, medial lateral 1.5mm, -2.0mm to the dorsal ventral). Behavioral testing was carried out three weeks after surgeries.

2.2.3 Behavioural Testing

Measuring schizophrenia-related behaviours (i.e., stereotypy, ataxia and sensorimotor gating and cognitive tests) in rodents have provided means to understand the gene to behaviour relationship and examine the underlying pathophysiology of schizophrenia. Various behaviour tests such as OF and YM are done before and after IP injection (i.e., intraperitoneal injection). Mice are injected first with saline, to measure the baseline activity followed by MK801 after a week of rest (figure 2).

During behavioural testing, mice will be placed into their home cage in the open field for 10 minutes (for accommodation and Ethovision mice recognition) before being place in a YM field for 10 minutes followed by 30 minutes in an open field. Behaviour test was recorded using Ethovision video tracking system (Noldus Information Technology, Wageningen, Netherland). The dLight1.1 measure dopamine concentration in the associative striatum through the Fibre-Photometry (Patriarchi et al., 2018; Jessi et al., 2017; Gunaydin et al., 2014). All the mice are sacrificed at the end of the procedure for validation of dLight localization in the associative striatum. For all experimenters are blinded to the genotype or treatment.

2.2.4 Open Field

To investigate the change of behaviour, mice are released in the corner of the open field arena (30 x 30 cm) and stereotype behaviour was concomitantly measured for 30 minutes during the experiment. For the analysis, the stereotypy behaviour was rated for 5 minutes bins for 30 minutes before and after injection of MK801/saline (0 = normal explorative behaviour with no signs of repetitive movements; 1 = high rate of repeated sniffing and grooming compared to control; 2 = head weaving, circling movements and higher rate of grooming, as compared to 1; 3 = disorientation, rapid and continuous non-directed movements such as turning, back peddling or gagging) and ataxia (0 = normal baseline movements and no signs of uncoordinated motion; 1 = swaying, quivering movements but not falling over; 2 = rapid movements with falls or frequent leg slips; 3 = sedated, inactive and unable to move, especially restricted to the periphery of the open field). When the mice displayed multiple behaviours, the highest score will be addressed for 5 minutes interval.

2.2.5 Y-maze Spontaneous Alternation

The Y-shape maze, consisting of three white, opaque plastic arms positioned at a 120° angle from each other, was utilized to observe the schizophrenia-like behaviours. Mice are placed in the YM for 10 minutes right after the open field experiment. The arm entries and triads were recorded to determine the alternation percentage.

2.2.6 Tissue Processing and Immunofluorescence

Mice were sacrificed through intracardiac perfusion with PFA replacement of circulatory blood, and brains are extracted and fixated before the fluorescent immunostaining procedure. The cryo-protected brains were frozen in isopentane solution and cut in 40 μm sections in a microtome cryostat (reference of the instrument). The slices are placed in

phosphate-buffered saline (PBS) and stored at 4° before staining. Slices of the associated striatum were selected for every animal brain and stained with a procedure of fluorescent immunostaining. The staining procedure consists of washing the slices with 1x PFA, followed by incubation in blocking solution (1% BSA, 1xPFA, 0.4% Triton X-100), to finally fix primary antibodies (the following primary antibody dilutions will use at 1:2000 blocking solution with anti-GFP rabbit serum) for 72h, 4°C on gentle shaking and secondary antibodies conjugated to Alexa 488 in 1:500 dilution along with Hoechst-33342 (1:1000) solution for an hour at 4°. Lastly, the stained brain sections were imaged using ZEISS Axiozoom.V16 fluorescence microscope with PlannNeoFluar Z 1.0x objective (magnification 28).

2.2.7 Experiment Timeline

Figure 2 shows the timeline of the experiment starting from the surgery week. The behavioural test began after the rest week (approximately two weeks after surgery). Mice underwent various behaviour tests such as YM and OF, before and after saline injection and 10 minutes of rest on weeks 3 and 4. After 1 week of rest, mice underwent bahvaiours before and after the injection of MK801 The sacrifice, staining of tissue and validation (e.g., brain imaging and data analysis) were begun after the behavioural experiment (see method section)

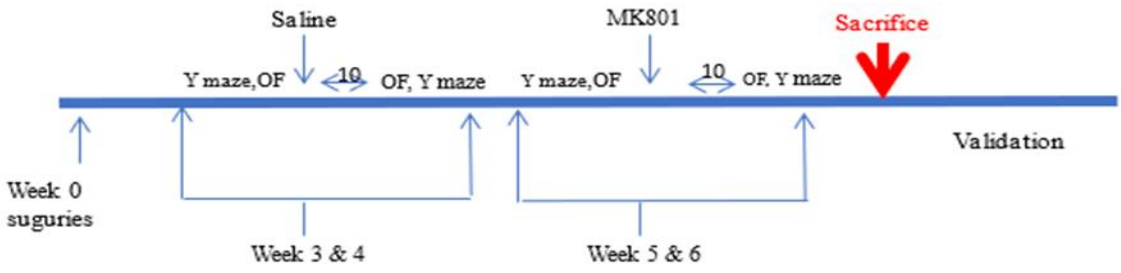

Figure 2. The timeline of *in vivo* schizophrenia mice model experiment

Chapter 3: Results and Discussion

3.1 Open-Field Test

The role of JNK1 in the regulation of dopamine release in the striatum, possibly in a schizophrenia mouse model induced by MK-801 can mimic certain schizophrenia-like symptoms in animals. Studies show that genetic risk for psychiatric disorders is associated with the JNK pathway and lacking JNK1 in mice produces the molecular and behavioural phenotype of schizophrenia (Coffey, 2014; Mohammad et al., 2018). To examine the effect of the associated striatum on activity response to MK-801 and saline before and after injection when compared to the time of locomotor activity as distance moved (in centimetres) in an open field between the WT and *Jnk1-/-* mice.

As shown in Figure 3, the *Jnk1* gene may play a role in the regulation of dopamine release in the striatum, a brain region crucial for motor function and reward processing. This study shows that *Jnk1*-/- mice moved significantly more than WT mice, both before and after the MK-801 injection. This increased locomotor response in the absence of JNK1 suggests that the gene may be part of pathways influenced by NMDA receptor activity which MK801 effects on these receptors (Figure 3b). The study also shows that both before and after saline injection, *Jnk1-/-* mice moved a significantly greater distance than WT mice, which could suggest that an inherent difference in baseline activity between the two groups emphasizes the potential inhibitory role of JNK1 in regulating movement under normal conditions (Figure 3a). Furthermore, this study observes the role of the Jnk1 gene in locomotor activity and behavioral response to MK-801 across 30-minute time bins showing that both WT and *Jnk1-/-* mice demonstrated a decrease in locomotor activity over a 30-minute period before any injection. This is consistent with typical habituation behavior observed in rodents placed in a new environment

(Rankin et al., 2009). Following the MK-801 administration, both groups showed a slight increase in locomotor activity. This finding reaffirms MK-801's expected impact on behavior.

Remarkably, during the first 15 minutes post-injection, WT mice exhibited a slightly more pronounced increase in activity compared to *Jnk1-/-* mice. This suggests a potential involvement of the Jnk1 gene in the early motor response to NMDA receptor blockade by MK-80. The Jnk1-/- mice generally displayed reduced locomotor activity compared to WT mice and did not respond to the MK-801 injection with as significant an increase in activity (Figure 3c).

Fig.3

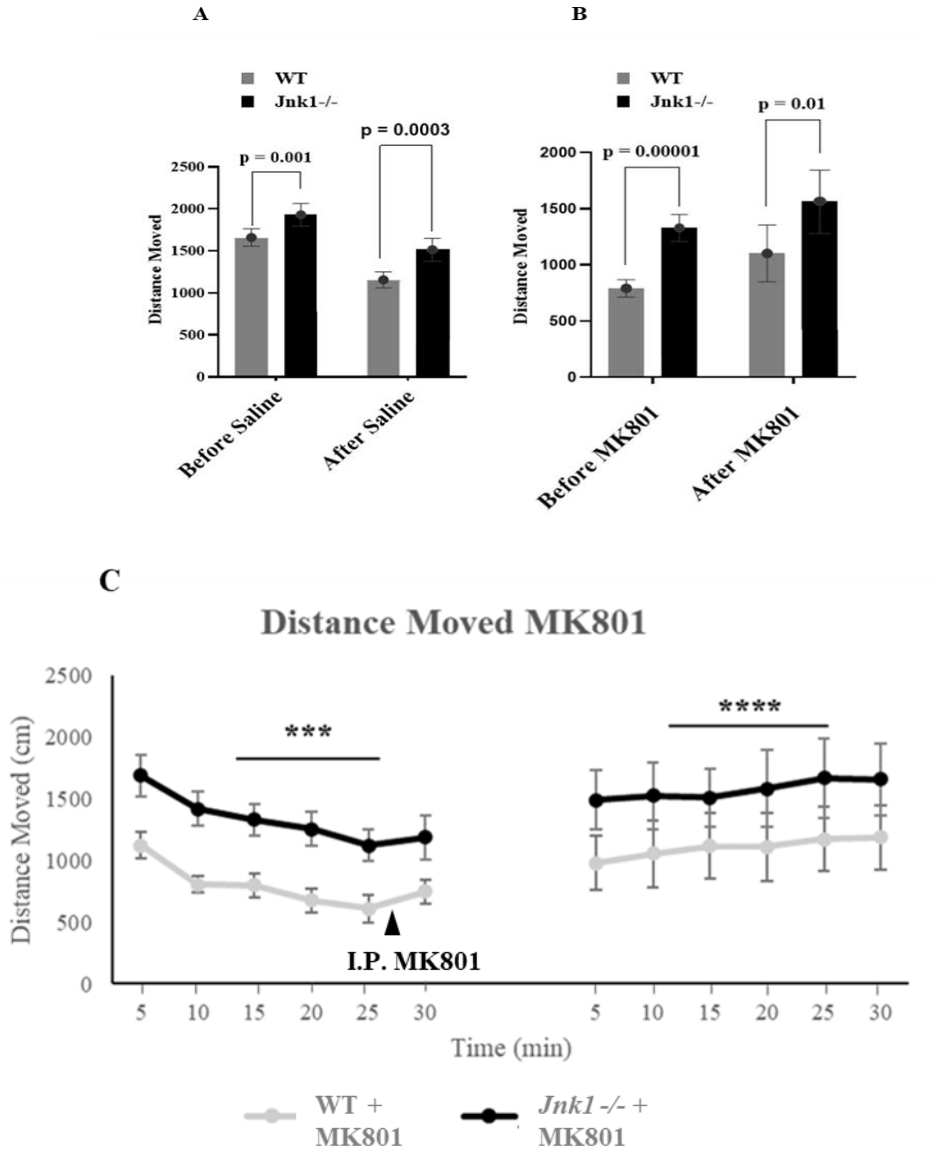

Figure 3. Examination of Locomotor Activity in WT and *Jnk1-/-* Mice Pre and Post Saline and MK-801 Administration. The graphs represent the mean distance moved by both WT (n=9) and Jnk1-/- mice (n=8), with error bars illustrating the standard error of the mean. (a) illustrates the distance moved pre- and post-saline injection, with significant differences observed in both pre (p=0.001) and post (p=0.0003) saline administration. (b) presents the distance covered pre- and post-MK-801 injection, displaying a highly significant difference pre-injection (p=0.00001) that remains significant post-injection (p=0.01). (c) provides a comparison of locomotor activity across 30-minute time bins for both mouse strains. A noticeable decrease in locomotor activity for both WT and Jnk1-/- mice was observed before MK-801 administration. Post MK-801 injection, a mild increase in locomotor activity was noted for both groups, with WT mice displaying a more marked increase in activity during the initial 15 minutes. It should be noted that for Figure 3c, asterisks denote levels of statistical significance as follows: * p = < .05, ** p = < .01, and *** p = < .001.

3.2 MK801-Induced Schizophrenia-like Behaviours in *Jnk1-/-* mice

MK-801 is used to model NMDAR hypofunction in rodents, providing a tool for studying this aspect of schizophrenia which is a complex mental disorder. Symptoms may induce stereotypy (repetitive behaviours) and ataxia (a lack of voluntary coordination of muscle movement), among others. This study aims to examine the effect of MK-801, and thereby NMDA receptor blockade, on the *Jnk1-/-* mice. The aim is to understand how the lack of *Jnk1* might interact with the schizophrenia-like behaviour induced by MK-801.

As it is shown in Figure 4, the effect of the MK-801 administration on stereotypy and ataxia behaviours was thoroughly studied in both WT and *Jnk1-/-* mice. Mk-801-induced behaviours were observed to be noticeably different when compared before and after the administration, with the drug-inducing hyperactivity in both mouse groups.

For the stereotypy-like behavior, there were significant differences observed between the pre-and post-administration time points in all mouse genotypes, which implies that the gene's influence is not solely related to the effect of MK-801 but, instead appears to play a

broader role in controlling stereotyped behaviours in general. Specifically, the stereotypy scores of *Jnk1-/-* mice for the post-MK-801-administered group show an increase in the stereotypy mean score after the administration of the drug when compared to WT mice, indicating that the *Jnk1* plays a role in regulating stereotype behavior, particularly in response to change in glutamatergic neurotransmission caused by MK-801 (Figure 4a).

In contrast to the stereotypy behaviours, the ataxia scores between the WT and *Jnk1-/-* mice showed significant differences both before and after the MK-801 administration. Following the injection, peak ataxia scores were observed within 10 to 20 minutes in the *Jnk1-/-* mice, indicating a profound loss of motor control (Figure 4b).

Taken together, our results show that the administration of MK-801 prompts notably stereotyped behavior and impairs motor function, inducing ataxia in both WT and *Jnk1-/-* mice. Notably, the absence of the Jnk1 gene significantly enhanced the ataxia, implying a potential role of JNK1 in the regulation of motor behaviours, which may be more prominent under the influence of drugs such as MK-801. Further research is necessary to delve deeper into the underlying mechanisms of these observations.

Fig. 4

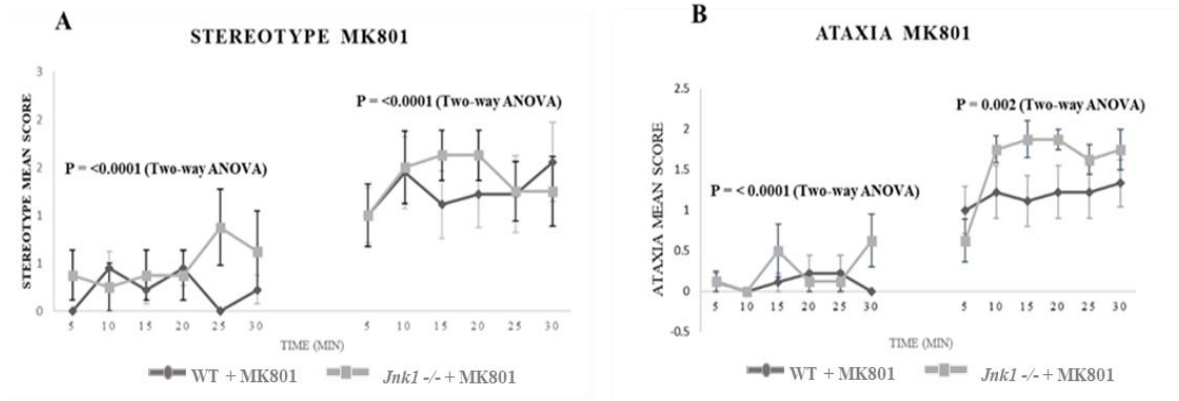

Figure 4. The administration of MK-801 induced stereotypy and ataxia, in both WT and *Jnk1*-/- mice. Stereotypy mean scores demonstrate a significant difference between WT and *Jnk1*-/- mice both before

($p < 0.0001$) and after ($p < 0.0001$) MK-801 injection (a). Ataxia mean score also reveals significant differences between the two groups, with $p < 0.0001$ before and $p = 0.002$ after MK-801 injection. These results highlight the pronounced impact of Jnk1 deletion on both stereotype and ataxia behaviours in response to MK-801.

3.3 Spatial Working Memory Using the Spontaneous Alternation Y-maze Test

Cognitive impairment in schizophrenia has drawn attention particularly important in morbidity in schizophrenia and impairment in working memory is a well-known aspect of cognitive impairments in schizophrenia (Elvevag & Goldberg, 2000). The present investigation was designed to evaluate the potential effects of the Jnk1 gene absence on spatial working memory in mice, as quantified by the spontaneous alternation task in a YM. Additionally, the study aimed to explore the impact of different treatments (saline and MK-801) and varying time points (before and after administration) on working memory.

Both WT and *Jnk1*-/- mice were subjected to the spontaneous alternation task in the YM following 10 minutes of exposure to the respective treatments. Functional abnormalities in the associative striatum, a crucial area for spatial working memory, were compared across genotypes and treatments.

As can be seen in Figure 5, our analysis revealed no significant difference in performance on the spontaneous alternation task between the two genotype groups, i.e., WT and Jnk1-/- mice. Similarly, neither MK-801 (Figure 5a) nor Saline (Figure 5b) treatment significantly influenced the task performance, irrespective of the administration time-point (before or after exposure). The finding suggests that the absence of the Jnk1 gene does not detrimentally impact spatial working memory in mice, at least under the conditions studied here. Moreover, the tested treatments and their timing do not seem to alter the performance of the YM task in a meaningful way. Further studies are needed to confirm these results and potentially investigate other cognitive domains that could be affected by the Jnk1 mutation.

Fig. 5

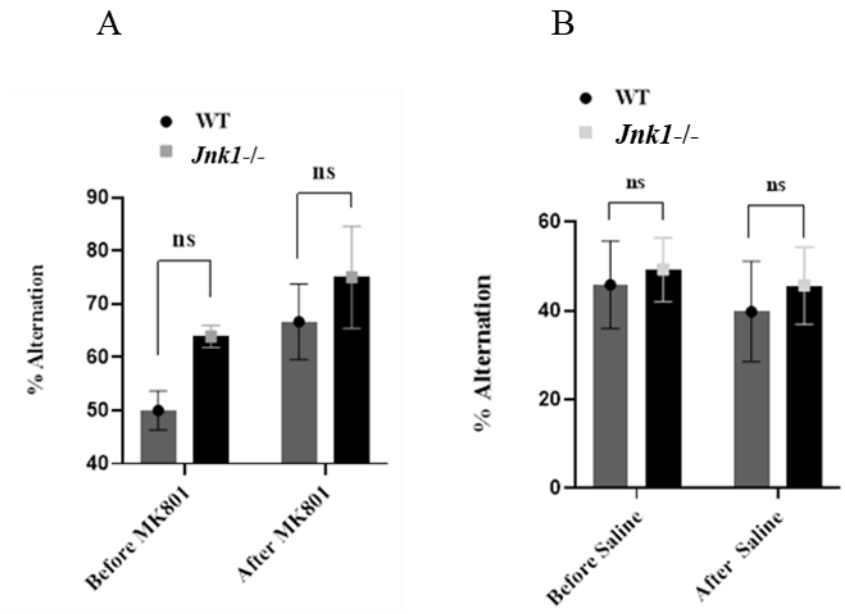

Figure 5. Impact of Genotype and Treatment on Spatial Working Memory. Performance of WT and *Jnk1*-/- mice on the spontaneous alternation task in the YM, following either saline or MK801 treatment. Data are presented for both pre-treatment (Before) and post-treatment (After) conditions. No significant differences were observed between genotypes or treatment groups, suggesting that the absence of the Jnk1 gene and the administered treatments do not significantly impact short-term spatial working memory under these conditions.

3.4 Immunohistochemistry staining

As it is shown in Figure 6, our study revealed that in both *Jnk1-/-* mice and WT (C57BL/6J) mice, there is an observable activity in the associative striatum (AS), which extends from Bregma 2.96 mm to Bregma -13 mm. This activity was successfully detected using an immunofluorescent staining method involving a primary anti-GFP rabbit serum and a secondary Alexa Fluor 568 donkey anti-rabbit IgG. The activation was visually apparent in both groups as indicated by the fluorescence signals. Significant differences in fluorescence labelling were observed when striatal activity was compared between WT and *Jnk1-/-* mice, indicating

changed neuronal activity or protein expression. Within the associative striatum regions of the WT mice, a baseline fluorescence pattern was seen, which was in line with anticipated neuronal activity. On the other hand, the *Jnk 1*-/-mice showed a significant increase in staining intensity in these areas, indicating increased activity or expression levels when the JNK1 gene is absent. These results suggest that JNK1 regulates the associative striatal neural pathways, which are important for cognitive processes and are known to be dysregulated in mental disorder like schizophrenia.

Fig.6

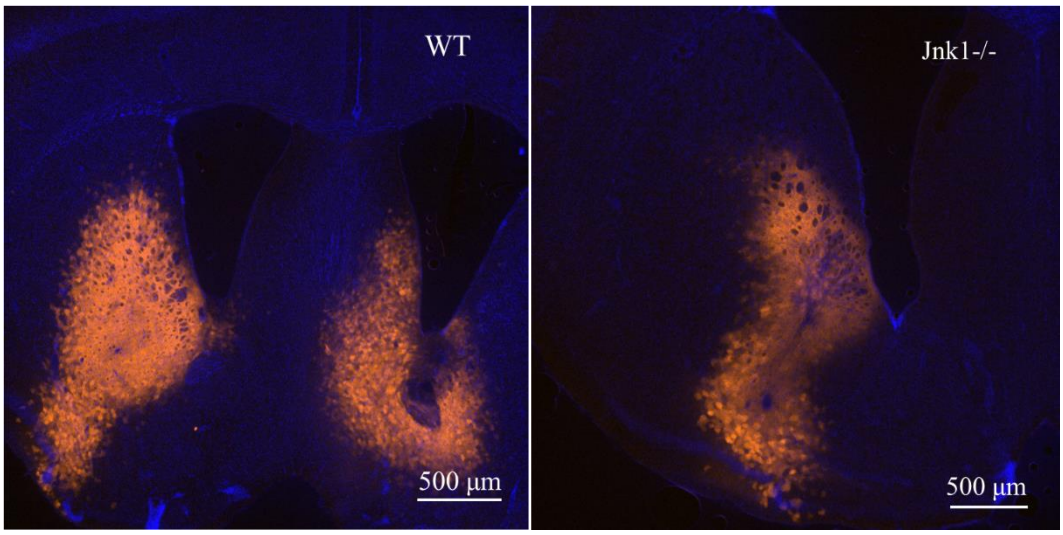

Figure 6. Immunofluorescence staining of the Associative Striatum (AS) in wild type (C57BL/6J) and *Jnk1-/-* male mice that had been injected with the dopamine reporter DLIGHT1. Tissue sections spanning from Bregma 2.96mm to Bregma -13mm were immunostained with anti-GFP rabbit serum as the primary antibody (to detect neuronal activity) and Alexa Fluor 568 donkey anti-rabbit IgG as the secondary antibody, scale bar of 500 µm were included in the images.

3.5 Discussion

Evidence from human studies have indicated that schizophrenia is polygenic (i.e., mutation in multiple different genes contribute to disease risk) and NMDA hypofunction

caused pathophysiology of schizophrenia has been extensively studied. JNK is a key signalling molecule of MAPK family and plays an important role in underlying symptoms of psychiatric disorder such as depression, anxiety, and schizophrenia (Alabaster & Group, 2002; Bunney et al., 2003; Eyjolfsson, Brenner, Kondziella, & Sonnewald, 2006; Coffey, 2014).

The aim of this study was to investigate whether dopamine levels in the dorsal and associative striatum were altered in *Jnk1-/-* mice using dLight1.1 dopamine sensor and the possible underlying mechanism of genetically modified mice model relevant to schizophrenia pathology. Across a range of behavioural paradigms, comparison behavioural tests in OF and YM and behavioural test score (stereotype and ataxia) between WT and *Jnk1-/-*mice, before and after treatments (Saline vs. MK-801) demonstrates support the hypothesis that (1) JNK1 knockout mice injected with an NMDA receptor antagonist (MK801) will show hyperactivity in the associative striatum and (2) would induce schizophrenia symptoms.

The results are partially consistent with previous studies. We find that in the open field test, *Jnk1-/-* mice exhibited significantly increase in distance moved between before and after saline injection, hence, between WT and *Jnk1-/-* mice showed significant different after MK-801 administration, indicating KO mice with MK801 injection were increased exploratory behaviour. This result suggests that *Jnk1-/-*mice exhibited schizophrenia-like behaviour compared to wild genotype mice hence, MK801-induced schizophrenia-like behaviour. Finding support previous research found that MK801 produces hyperlocomotion (i.e., it was analogized to positive symptoms based on increased activity of mesolimbic dopamine circuits), social flattening with low dose in rodents (Nilsson, Waters, Waters, Carlsson, & Carlsson, 2001). Constantly, other studies found repeated administration of NMDA antagonists induced behavioural alterations that mimic symptoms of psychosis as seen in schizophrenia, and JNK, its downstream target molecule plays important roles in regulating apoptosis in neural cell have been linked to the disease risk (Ahn et al., 2009). Moreover, the current finding indicated that

MK801 induced stereotyped behaviour and MK801-induced ataxia reflected in ordinal increases in impairment of motor behaviour in both WT and *Jnk1-/-* mice. Taken together, the heightened baseline activity, even under control conditions with saline, underlines a fundamental role for Jnk1 in maintaining regular locomotor behaviour suggest that the constant hyperactivity in *Jnk1-/-* mice may hint towards the involvement of neurotransmitter system, possibly the dopaminergic system which is linked to locomotor regulation. Similarly, to the previous research, which associated dopaminergic neurotransmission with various locomotor behaviours (Cangniard et al., 2006). Moreover, both groups of mice exhibited altered locomotor activity after MK801 injection, but the heightened responsiveness in *jnk1-/-* mice suggests a deeper linking of JNK1 pathways influenced by NMDA receptors activity. This particular interest considering the vital role that glutamatergic dysfunction plays in neuropsychiatric disorders like schizophrenia (Moghaddam & Javitt, 2012).

Consistently, the current findings are consistent with previous studies that found MK801-induced abnormality behaviour (stereotype and ataxia). For instance, the *jnk1-/-*mice displayed an increased response to the locomotor stimulating effecting of MK801 as previously shown for calcineurin knockout mice (Miyakawa et al., 2003). , hence in animal models of psychiatric disorders, research has indicated that the absence of JNK in rodents, following treatment with MK-801, leads to reduced dopaminergic activity in associative striatal brain regions, such as the caudate, which is crucial for associative learning and encoding action-specific value signals. This reduction in dopaminergic function results in impaired behavioral responses and motor coordination (de Leon & Diaz, 2003; Homayoun & Moghaddam, 2007; Löscher, Annies, & Hönack, 199; Sams-Dodd, 1996). Additionally, our findings from immunofluorescence analysis indicated that dopamine regulation was activated in the associative striatum brain area in both WT and *Jnk1*-/- mice which partially consistent with imaging studies found that amphetamine and MK801-induced dopamine release is

increased in the associative striatum of patients with schizophrenia, hence the amplitude of this increase is correlated with worsening of the psychotic symptoms of schizophrenia (see Review, (Grace, 2016)). Taken together, our results support the idea that JNK1 regulates the phosphorylation of schizophrenia polygene products and presented in behavioural phenotype of schizophrenia which suggest that lack of jnk1 gene in mice regulates dopaminergic dysfunction in associative striatum may contribute to the symptoms of schizophrenia. Moreover, the consistent hyperactivity of *Jnk1*-/- mice, strengthens the theory of an intrinsic genotype-driven hyperactivity. This hyperactivity may suggest a disruption in the balance between excitatory and inhibitory neurotransmission in the absence of Jnk1, thereby presenting a potential mechanism for the observed behaviour.

Our study further identified the validity of MK801-induced cognitive impairment (spatial working memory) in schizophrenia mice model. The results showed non-significant differences among genotypes; hence had no effect on MK801 induced memory impairment. Thus far, our observed that MK801 administered in mice failed to induced memory impairment is inconsistent with previous studies. For instance, in pharmacological and preclinical model for cognitive impairment associated with schizophrenia (CIAS) studies has been reported that NMDA receptor antagonists is known to elicit schizophrenia-like behaviour and cognitive impairments in healthy humans and worsen symptoms in schizophrenic patients (Krystal et al., 1994; Luby, Cohen, Rosenbaum, Gottlieb, & Kelley, 1959). Hence, review in rodents study reported MK801 produces behavioural disturbances which mimics schizophrenia including neurocognitive deficit (Large, 2007). Moreover, the study in adolescent rats found that an intraperitoneal injection of MK801 (0.05, 0.1, and 0.2 mg/kg) ones daily for 14 days showed that novel object recognition and spatial working memory in Morris Water Maze were significantly impaired by repeated MK801 treatment when animals were tested 24 hrs after drugs cessation (Li et al., 2011). Consistently, with clinical study reported that MK801

treatment is exacerbated psychosis in patients with schizophrenia and induced schizophrenia-like symptoms in control individuals when given repeatedly higher doses (Angrist, Sathananthan, Wilk, & Gershon, 1975; Janowsky, El-Yousef, Davis, & Sekerke, 1973).

However, similarly to the negative results, previous studies have reported that lower doses of MK801 administration or a single application of MK801 did not cause the deficit in spatial memory in rodents (Manahan-Vaughan, von Haebler, Winter, Juckel, & Heinemann, 2008; van der Staay, Rutten, Erb, & Blokland, 2011). The results suggested that chronic or repeated treatment with MK801 may induced cognitive deficits that resemble the symptoms of schizophrenia. There are, however, certain limitations that should be taken into consideration. The exact neural circuits and molecular pathways influenced by the absence of Jnk1 remain to be illuminated. Additionally, the broader translational implications, particularly concerning neuropsychiatric disorders need to be explored further with more specific experimental designs.

In conclusion, our results showed *Jnk1*-/-mice with a C57BL/6J background exhibited mild but significant schizophrenia-like behaviour, as indicated by the range of behaviour test in an OF and YM shown showed that *Jnk1*-/- mice pronounced deficit in motor coordination and locomotion. Moreover, non-competitive NMDAr antagonists such as MK801 induced dopamine regulates in associative striatum which induced schizophrenia-like behaviour in mice, but unaffected in cognitive function impairment (e.g., spatial working memory). Our study sheds light on the role of JNK1 in mouse behavior. The intrinsic hyperactivity of *Jnk1-/-* mice, aligns with their reduced surface expression of NMDA receptor subunits, and a possible NMDAR hypofunction model. Further investigation would be needed to elucidate the gene's mechanistic involvement and its potential therapeutic implications, especially in disorders characterized by NMDA receptor dysfunction. As we progress ahead in this line of research, understanding such intricate molecular-behavioural relationships becomes essential in developing targeted therapeutic strategies for neuropsychiatric conditions.

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