BEHAVIOURAL AND MOLECULAR MECHANISMS OF LURASIDONE

INVESTIGATING THE BEHAVIOURAL AND MOLECULAR MECHANISMS OF LURASIDONE HYDROCHLORIDE IN A MK-801 MODEL OF SCHIZOPHRENIA

By

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ABSTRACT

Schizophrenia is a debilitating neuropsychiatric disorder that affects approximately one percent of the global population. Aberrant *N*-methyl-*D*-aspartate receptors and endoplasmic reticulum stress have been implicated in the pathogenesis of schizophrenia. Despite a century of extensive research, outcomes from best-practice treatments remain dismal. Lurasidone hydrochloride is a novel atypical antipsychotic drug with a unique receptor binding profile that can potentially treat the heterogeneous symptomology of schizophrenia. However, discrepancies in experimental design (i.e. animal models used, symptoms assessed etc.) have yielded conflicting results surrounding the procognitive and antidepressant properties of lurasidone. Furthermore, the limited aqueous solubility of lurasidone poses a considerable challenge for improving antipsychotic drug delivery to the brain and limiting the prevalence of adverse side effects. These obstacles coupled with the elusive pathophysiology of schizophrenia and its incurable nature, highlight the importance of investigating novel therapeutic targets and their underlying mechanisms to improve treatment and enhance the quality of life of patients with schizophrenia.

This thesis sought to accomplish three primary objectives: (1) validate the behavioural efficacy of lurasidone hydrochloride; (2) investigate the role of mesencephalic astrocyte-derived neurotrophic factor as a potential therapeutic target of lurasidone; and (3) evaluate the therapeutic potential of intranasal lurasidone administration as a novel method for antipsychotic drug delivery. The data presented within this thesis suggest that repeated lurasidone treatment may be effective at treating the positive, negative, and cognitive symptoms of schizophrenia, but not sensorimotor gating deficits. Furthermore, sub-chronic lurasidone treatment in rats significantly increased the relative expression of

mesencephalic astrocyte-derived neurotrophic factor in the rat prefrontal cortex, a primary site of impairment observed in schizophrenia. Lastly, we conclude that lurasidone administered via the nasal route using a novel poly(oligo ethylene glycol methacrylate)-based nanogel formulation required four times less drug to achieve a therapeutic response comparable to traditional intraperitoneal routes. The findings presented within this thesis suggest that lurasidone might be a favourable atypical antipsychotic drug that exerts its therapeutic effects through the modulation of neurotrophic factor expression in the brain regions affected by schizophrenia. This thesis offers new insight that can help guide future studies toward improving the prognosis of patients suffering from schizophrenia.

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ABBREVIATIONS

ΔΔCt	Delta Ct formula
5-HT	5-Hydroxytryptamine (Serotonin)
6-OHDA	6-hydroxydopamine
ACTB	Beta-actin
ANOVA	Analysis of variance
APD	Antipsychotic drug(s)
ARMET	Arginine-rich, mutated in early-stage tumors
ATF-6	Activating transcription factor 6
BAX	Bcl-2-associated X protein
BBB	Blood brain barrier
cDNA	Complementary DNA
CDNF	Cerebral dopamine neurotrophic factor
CHOP	C/EBP Homologous Protein
CNS	Central nervous system
COR	Cortex
Ct	Threshold cycle
DA	Dopamine
DEPC	Diethyl pyrocarbonate
DLPFC	Dorsolateral prefrontal cortex
DMT	N,N-Dimethyltryptamine
DSM-5	*Diagnostic and Statistical Manual of Mental Disorders*, Fifth Edition
DTNBP1	Dystrobrevin binding protein
ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum-associated protein degradation
ERSEII	Endoplasmic reticulum stress response element II
EPS	Extrapyramidal symptoms
FGA	First generation (typical) antipsychotic

GABA	Gamma-aminobutyric acid
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GDNF	Glial cell-derived neurotrophic factor
GLU	Glutamate
GRP78	78-kDA glucose-regulated protein
HIP	Hippocampus
HSD	Honest significant difference
i.n.	Intranasal administration
i.p.	Intraperitoneal administration
IRE1	Inositol-requiring enzyme
ISI	Inter-trial interval
KDEL	Lysine-aspartic acid-glutamic acid-leucine amino acid sequence
Ku70	Ku antigen p70 subunit
LSD	Lysergic acid diethylamide
MANF	Mesencephalic astrocyte-derived neurotrophic factor
MC	Methylcellulose
mGluR5	Metabotropic glutamate receptor 5
MK-801	Dizocilpine
mPFC	Medial prefrontal cortex
mRNA	Messenger RNA
NMDAR	*N*-methyl-*D*-aspartate receptor
NMR	Nuclear magnetic resonance spectroscopy
NO	Nitric oxide
NOR	Novel Object Recognition Task
NRG1	Neuregulin 1
NTF	Neurotrophic Factor(s)
NVHL	Neonatal ventral hippocampal lesion model of schizophrenia
PCP	Phencyclidine

PFC	Prefrontal Cortex
PERK	Pancreatic endoplasmic reticulum kinase
PET	Positron emission tomography
PGK1	Phosphoglycerate kinase 1
POEGMA	Poly(oligo ethylene glycol methacrylate)
PPI	Prepulse inhibition
RI	Recognition Index
ROS	Reactive oxygen species
RTDL	Arginine-threonine-aspartic acid-leucine amino acid sequence
RT-qPCR	Real-time quantitative reverse transcription PCR
SAP	SAF-A/B, Acinus, and PIAS
SAPLIP	Saposin-like protein
SEM	Standard error of the mean
SI	Social Interaction Paradigm
SN	Substantia nigra
SPECT	Single photon emission computed tomography
STR	Striatum
SZ	Schizophrenia
UPR	Unfolded protein response

CHAPTER 1: INTRODUCTION

1.1 A Brief History to Schizophrenia

The term “schizophrenia” is little more than a century old; yet, neuropsychiatric disorders have been affecting individuals since the dawn of human civilization. Specifically, the symptoms of schizophrenia (SZ) can be traced in written documents to Pharaonic Egypt, dating as far back as 2000 BC (Kyziridis, 2005). However, historical and contemporary understandings of psychiatric disorders were often believed to arise from supernatural origins, leading to little progress toward understanding and treating these conditions (Kyziridis, 2005). It was not until the middle of the 19th century that European psychiatrists began to describe mental disorders in a clinical context (Jablensky, 2010). Emil Kraepelin (1856-1926) was the first person to introduce the conceptual framework of SZ through his discovery of *dementia praecox* (early dementia) in 1878. Kraepelin hypothesized that specific combinations of fundamental symptoms could be integrated to comprise a single disease entity (Ebert & Bar, 2010). Originally, Kraepelin believed SZ was a neurodegenerative condition accompanied by a progressive decline in cognition based on longitudinal studies from his late-adolescent patients (Kyziridis, 2005). Later, Eugene Bleuler (1857-1939) criticized Kraepelin’s term citing a lack of global decline in cognition (Ashok, Baugh, & Yeragani, 2012; Kyziridis, 2005). In 1911, Bleuler coined the term “schizophrenia” (split-mind), redefining *dementia praecox* as a group of disorders characterized by dysfunction in different psychiatric domains that manifest in cycles of illness and remission (Falkai et al., 2015). Bleuler was also the first to categorize the disorder into *basic* (positive) and *accessory* (negative) clusters including delusions and hallucinations (positive) as well as deficits in social behaviour, speech

derailment, and affective incongruence (negative) (Ashok et al., 2012; Falkai et al., 2015; Kyziridis, 2005).

1.2 Schizophrenia in the 21st Century

Today, SZ is recognized as a chronic and debilitating neuropsychiatric disorder with developmental origins and point prevalence of 0.3% to 0.7% among the global population (Chong et al., 2016; van Os & Kapur, 2009). Charlson et al. (2018) estimate that approximately 21 million people are currently living with SZ worldwide, a majority of which live in low- and middle-income countries. Despite a relatively low lifetime prevalence (median 4.0 per 1,000 persons), SZ is ranked among the top 25 leading causes of disability worldwide, resulting in an enormous social and economic burden to patients, caregivers, and the broader society (Chong et al., 2016; Saha, Chant, Welham, & McGrath, 2005). The *World Health Organization* (WHO) estimates the direct costs associated with SZ to range between 1.6% and 2.6% of total healthcare expenditures in Western countries, comprising 0.02% - 5.46% of the gross domestic product (Chong et al., 2016). In Canada alone, the estimated cost of SZ to the Canadian economy surpassed 6.85 billion dollars in 2018 with most of the burden being attributed to productivity losses, hospitalizations, and premature mortality (Goeree et al., 2005; Schizophrenia Society of Canada, 2018). The overwhelming burden of SZ has been largely associated with its early onset and incurable nature (American Psychiatric Association, 2013; Chong et al., 2016). Thus, the disease burden attributable to SZ is expected to continue to rise due to a rapidly growing global population, increased life expectancies and poor treatment outcomes (Charlson et al., 2018).

As Bleuler hypothesized, SZ is a syndrome characterized by a complex group of symptoms – none of which appear to be pathogenic (Kyziridis, 2005; K. R. Patel, Cherian, Kunj, & Atkinson, 2014). The hallmark features of the disease include (but are not limited to) positive symptoms (i.e. hallucinations and delusions), negative symptoms (i.e. apathy, lack of emotion, and social withdrawal), and cognitive dysfunction (i.e. memory impairment, attention deficits, and poor executive functioning) (Fletcher & Frith, 2009; Liddle, 1987; Owen, Sawa, & Mortensen, 2016). These symptoms frequently hinder patients' capacity to think clearly, manage emotions, and make decisions, all of which may limit their ability contribute to society and foster meaningful relationships (Larquet, Coricelli, Opolczynski, & Thibaut, 2010; K. R. Patel et al., 2014). Schizophrenia is diagnosed through the assessment criteria outlined in the *Diagnostic and Statistical Manual of Mental Disorders*, Fifth Edition (*DSM-5*) (K. R. Patel et al., 2014). The *DSM-5* defines the diagnostic criteria for SZ as "the persistence of two (or more) of the following active-phase symptoms, each lasting for a significant portion of a least a one-month period: delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behaviour, and negative symptoms" with at least one of the qualifying symptoms being delusions, hallucinations or disorganized speech (American Psychiatric Association, 2013; K. R. Patel et al., 2014). Signs of SZ must also persist for at least a 6-month period, if left untreated (American Psychiatric Association, 2013).

Schizophrenia emerges during late adolescence or early adulthood with first-episode psychosis typically emerging between the ages of 18 to 25 in men and 25 to 35 in women (Ochoa, Usall, Cobo, Labad, & Kulkarni, 2012). A formal diagnosis and the onset of psychosis is often preceded by weeks, months, or even years of subtle

psychological and behavioural abnormalities such as mild disturbances in cognition, communication, motivation, and sleep (Larson, Walker, & Compton, 2010). This period of subclinical signs and symptoms is referred to as the prodromal period and is often overlooked as “normal” behaviour, particularly in adolescents. The SZ prodrome encompasses the period of time from the initiation of behavioural and cognitive abnormalities to the onset of psychosis (Duzyurek & Wiener, 1999). Moreover, a growing body of research suggests that earlier treatment intervention significantly improves the prognosis for patients with SZ (Cornblatt, 2001; Loebel et al., 1992). Thus, there has been recent interest in exploring the predictive validity of the prodromal period and investigating preventative pharmacological measures to delay or suppress the onset of psychosis and subsequent progression of SZ (Ruhrmann, Schultze-Lutter, & Klosterkotter, 2003).

1.3 Pathophysiology

1.3.1 Dysfunctional Dopaminergic Neurotransmission

Although the precise pathophysiological mechanisms of SZ remain elusive, the manifestation of symptoms has been hypothesized to arise from dysregulated dopaminergic neurotransmission (Featherstone, Kapur, & Fletcher, 2007; Howes & Kapur, 2009). This led to the development of the *Dopamine Hypothesis* which postulates that the overexpression of dopamine (DA) D_2 receptors coupled with hyperactive dopaminergic neurotransmission in the mesolimbic system contributes to the positive symptoms of SZ (Knable & Weinberger, 1997). This hypothesis was originally validated based on observations that pharmacological agonists of D_2 receptors (i.e. methamphetamine and cocaine) induce acute psychotic symptoms that are virtually indistinguishable from paranoia observed in SZ (Gründer & Cumming, 2016).

Consequently, these symptoms are rapidly ameliorated upon treatment with antipsychotic drugs (APDs), which are primarily D_2 antagonists (Angrist, Lee, & Gershon, 1974). More recently, advances in Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) studies have further substantiated the role of hyperactivated DA activity by demonstrating that drug-naïve patients with SZ exhibit increased levels of DA synthesis and release compared to age-matched controls (Abi-Dargham et al., 1998; Laruelle et al., 1996).

Moreover, DA deficiency in the prefrontal cortex (PFC) has also been implicated in the pathophysiology of SZ. In fact, reduced cerebral blood flow in the dorsolateral PFC (DLPFC) has been associated with poor cognitive functioning in patients with SZ (Daniel et al., 1991). This hypofunctional state is associated with reduced levels of the dopamine metabolite, homovanillic acid, in the DLPFC (Weinberger, Berman, & Illowsky, 1988). Therefore, the DA hypothesis also assumes that the negative and cognitive symptoms of SZ arise due to hypoactive subcortical DA projections that result from an under-stimulation of D_1 receptor-mediated neurotransmission in the PFC (Toda & Abi-Dargham, 2007). D_1 receptors are highly abundant in the PFC and play a prominent role in the regulation of working memory. PET studies involving drug naïve SZ patients have shown that these patients exhibit reduced expression of D_1 receptors in the PFC, which has also been linked to the negative and cognitive symptom subtypes (Okubo et al., 1997). This hypothesis has further validated based on observations that the negative and cognitive symptoms of SZ are primarily resistant to treatment with D_2 receptor antagonists, implying the involvement of separate mechanisms (Toda & Abi-Dargham, 2007).

Notably, hyper- and hypodopaminergic states in mesolimbic and mesocortical systems are not mutually exclusive. Animal studies modelling the DA hypothesis have demonstrated that hypodopaminergic activity in the PFC can co-occur and may even contribute to a hyperactive mesolimbic DA system (Featherstone et al., 2007). Therefore, these observations have led to a revised DA hypothesis that attributes the positive symptoms of SZ to hyperactive DA activity in the striatal and limbic systems and the negative and cognitive symptoms to hypoactive DA activity in subcortical regions. (Davis, Kahn, Ko, & Davidson, 1991; Howes & Kapur, 2009).

1.3.2 NMDA Receptors and Faulty DA-GLU-GABA Circuitry

Despite its empirical validation and clinical significance, the DA hypothesis fails to explain the cause of aberrant DA activity. Thus, the DA hypothesis has evolved into a multifactorial hypothesis that considers the interplay between other monoamines and neurotransmitters (i.e. dopamine, glutamate, and gamma aminobutyric acid) among complex neurocircuits (Carlsson et al., 2001). The *N*-methyl-*D*-aspartate receptor (NMDAR), a major glutamate (GLU) receptor subtype, has also been implicated in the development of SZ (Snyder & Gao, 2013). NMDAR's are heteromeric ion channels that are ubiquitous throughout the mammalian central nervous system (CNS) and play a critical role in the modulation of GLU activity (Schwartz, Sachdeva, & Stahl, 2012b). They have a well-defined three-dimensional structure comprised of NR1 and NR2 subunits that form heterotetramers resulting in a pore at the centre of the channel (Furukawa, Singh, Mancusso, & Gouaux, 2005). This pore allows for calcium influx, which contributes to neuronal depolarization and activation. NMDAR's high permeability to calcium ions plays a central role in the modulation of synaptic plasticity under normal physiological conditions

and inducing neuronal apoptosis under excitotoxic pathological conditions (Paoletti & Neyton, 2007). Recent studies have proposed that alterations in normal NMDAR functioning may disrupt a series of neural microcircuits that lead to downstream alterations in DA activity, previously described by the *DA hypothesis* (Schwartz et al., 2012b).

The *NMDAR Hypofunction Hypothesis* of SZ posits that a series of faulty NMDA glutamate receptors located at gamma aminobutyric acid (GABA) interneurons originating in the PFC indirectly contribute to the dysregulated DA activity described by the DA hypothesis (Schwartz et al., 2012b). This hypothesis was reverse engineered based on observations that healthy subjects treated with NMDAR antagonists (i.e. phencyclidine and ketamine) displayed schizophrenia-like phenotypes, whereas these drugs exacerbated symptoms in patients diagnosed with SZ (Javitt & Zukin, 1991; Lahti, Koffel, LaPorte, & Tamminga, 1995). Furthermore, NMDA-deficient mice exhibit behavioural phenotypes similar to those observed in other animal models of SZ, further implicating faulty NMDAR's in the pathophysiology of SZ (Mohn, Gainetdinov, Caron, & Koller, 1999). A particular consequence of hypofunctional NMDAR's is excessive GLU and acetylcholine release in the cerebral cortex (Adams & Moghaddam, 1998; Moghaddam, Adams, Verma, & Daly, 1997). Thus, it has been proposed that hypofunctional NMDARs lead to an overstimulation of postsynaptic GLU neurons that differentially alter mesolimbic and mesocortical DA activity, contributing to the heterogeneous symptomology of SZ (Figure 1) (Moghaddam et al., 1997; Olney, Newcomer, & Farber, 1999).

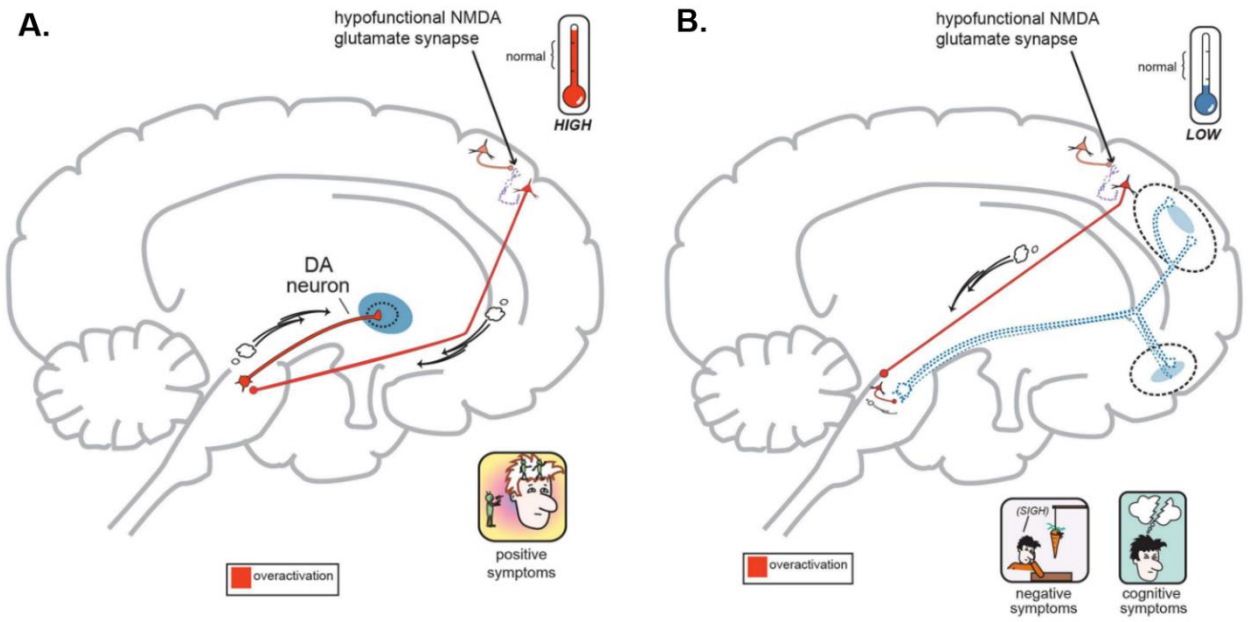


Figure 1. Proposed GABA-GLU-DA circuitry and the NMDAR Hypofunction Hypothesis of SZ. (A) Abnormal mesolimbic GLU-GABA-GLU-DA circuitry. A fully functional GLU neuron (orange) fires upon a GABA interneuron with hypofunctional NMDA receptors. This causes the GABA interneuron to become hypofunctional resulting in a loss of GABA tone. The reduced GABA tone causes the secondary GLU neuron to increase its firing rate (red). The hyperactive GLU neuron impinging on the mesolimbic system results in hyperactive mesolimbic DA activity which manifests as positive symptoms. **(B) Abnormal mesocortical GLU-GABA-GLU-GABA-DA circuitry.** Again, hypofunctional NMDA receptors in the PFC cause secondary GLU neurons to become hyperactive (red). In this instance, the hyperactive GLU neuron now impinges on a secondary GABA interneuron which causes an increase in GABA concentrations resulting in the subsequent inhibition of mesocortical DA activity (blue). *Images adapted from Schwartz, Sachdeva, & Stahl (2012).*

1.3.3 The Role of Serotonin

Abnormalities in 5-hydroxytryptamine (5-HT; serotonin) function have long been hypothesized to contribute to the pathophysiology of SZ. This evidence first emerged based on observations that psychedelic drugs such as lysergic acid diethylamide (LSD) and *N,N*-dimethyltryptamine (DMT) cause distortions in perception, paranoia, and cognition that closely resemble the positive symptoms of SZ (Geyer & Vollenweider,

2008). These drugs are structurally similar to serotonin and other indolamines that have a high affinity for 5-HT receptor subtypes. Specifically, the psychotomimetic properties of LSD and DMT have been linked to the hyperactivation of 5-HT_{2A} receptors (Aghajanian & Marek, 2000). These structural similarities led to several hypotheses that implicated serotonin alterations in the pathophysiology of SZ. However, several serotonergic hypotheses of SZ were abandoned throughout the 1970's and 1980's due to the realization that LSD-induced psychosis was clinically distinct from psychosis observed in patients with SZ and did not explain the negative symptoms of SZ (Abi-Dargham, Laruelle, Aghajanian, Charney, & Krystal, 1997; Roth, Lubin, Sodhi, & Kleinman, 2009).

Interest in the role of serotonergic mechanisms remerged in the 1990's with the introduction of atypical APDs (i.e. clozapine). These drugs were advertised as potent antagonists of 5-HT_{2A} receptors, a property that was believed to contribute to their superior efficacy and tolerability profiles compared to conventional antipsychotics (Meltzer, Li, Kaneda, & Ichikawa, 2003). Recent research into the mechanisms of atypical APD action has led to an accumulating body evidence that 5-HT_{2A} and 5-HT_{1A} receptor subtypes play a prominent role in the pathophysiology of SZ (Meltzer et al., 2003). 5-HT_{2A} and 5-HT_{1A} receptors are abundant on cortical and hippocampal pyramidal GLU neurons and GABA interneurons that modulate NMDAR activity in the PFC (Balu, 2016; Meltzer et al., 2003). Furthermore, many post-mortem studies and PET data suggest that patients with SZ display elevated expression of 5-HT_{1A} receptors and reduced expression of 5-HT_{2A} receptors in the PFC (Quednow, Geyer, & Halberstadt, 2010; Selvaraj, Arnone, Cappai, & Howes, 2014). Thus, it has been postulated that changes in serotonergic

signalling might contribute to the negative and cognitive symptoms of SZ by indirectly altering normal NMDAR functioning in the PFC (Balu, 2016).

1.3.4 Genetic Variables

Genetics play a prominent role in an individual's predisposition to particular diseases. Familial studies have illustrated that the likelihood of developing SZ is increased substantially based on an individual's degree of relatedness to other family members with SZ, suggesting a prominent genetic relationship (Tamminga & Holcomb, 2005). At present, there are no direct molecular markers for SZ and it is unlikely any particular gene is solely responsible for the development of the disease. However, several genes that code for proteins involved in glutamatergic and NMDAR processing are thought to play a role in one's susceptibility to SZ. Thus, it has been hypothesized that combinations of genetic abnormalities, coupled with several environmental insults during key periods of development, may lead to several pathophysiological irregularities that can potentially lead to the onset of SZ (Schwartz et al., 2012b).

An overwhelming body of evidence converges on the central theme that aberrant NMDAR's contribute to the final outcome of SZ. NMDAR's are comprised of small receptor subunits, any of which if altered by a mutation, could lead to a hypofunctional state and downstream irregularities in dopaminergic, serotonergic and/or glutamatergic activity (Schwartz et al., 2012b). NMDAR's are typically comprised of a single NR1 subunit, one of four subtypes of NR2 subunits (A, B, C, and D), and two variations of NR3 subunits (A and B), each encoded by a specific gene (Schwartz, Sachdeva, & Stahl, 2012a). The NR1 subunit is coded by the *GRIN1* gene whereas each of the NR2 subunits are encoded by *GRIN2A, GRIN2B, GRIN2C,* or *GRIN2D*, respectively (Schwartz et al.,

2012b). NR1 subunits are known to possess ion channel properties. Interestingly, NR1 knockdown mice expressing only five to 10 percent of normal NR1 subunit levels exhibit symptoms characteristic of SZ including increased motor activity, deficits in social behaviour and decreased prepulse inhibition (Duncan, Moy, Lieberman, & Koller, 2006; Halene et al., 2009; Mohn et al., 1999). Thus, it has been proposed that mutations in *GRIN1* that lead to downregulated NR1 subunit expression might cause a hypofunctional state of NMDAR's (Schwartz et al., 2012b). In contrast, the specificity and functional properties of NMDAR's arise from NR2 subunits, each with specific regional and temporal expression patterns (Qin et al., 2005). Of the four NR2 subtypes, the NR2A subunit has received the most attention for its potential role in SZ. NR2A subunits (coded by *GRIN2A*) are preferentially expressed in the PFC during adolescence. This is interesting since the spatial and temporal profile of NR2A subunits coincide with the age of onset of SZ (Mohrmann, Hatt, & Gottmann, 2000; Wenzel, Fritschy, Mohler, & Benke, 1997). Lastly, mutations in the neuregulin-1 gene (*NRG1*) on chromosome 8p, have been correlated with suboptimal NMDAR functioning and deficits in neurodevelopment (Hahn et al., 2006). *NRG1* is *a* gene that codes for a family of structurally-related proteins containing epidermal growth factor, which is required for neural and glial development (Schwartz et al., 2012a). Interestingly, mice lacking *NRG1* or its receptor, ErbB4, are significantly more likely to develop symptoms of SZ, an association that has also been supported by longitudinal studies in human Icelandic and Scottish Cohorts (Stefansson et al., 2003; Stefansson et al., 2002). These observations are further supported by evidence that the ErbB4 receptor influences neurobiological processes often altered in SZ such as NMDAR

expression and GABAergic functioning (Gu, Jiang, Fu, Ip, & Yan, 2005; Okada & Corfas, 2004).

Additionally, the NMDAR is not the only GLU receptor in the CNS. As previously described, aberrant in glutamatergic activity contributes to the downstream dysregulation of mesolimbic and/or mesocortical DA activity. Thus, it is possible for SZ to arise even if NMDA receptors are fully functional (Schwartz et al., 2012a). For example, alanine substitution mutations in the non-NMDA ionotropic glutamate receptor kainite-3 are associated with an increased risk of SZ (Bengi et al., 2002). Additionally, metabotropic glutamate receptor subtype 5 (mGluR5; encoded by *GRM5*) indirectly modulates the activity of glutamatergic NMDAR's (Matosin, Fernandez-Enright, Lum, & Newell, 2017). Several *GRM5* genetic variants have been negatively associated with cognition and hippocampal volume in patients with SZ compared to healthy controls (Devon et al., 2001; Matosin et al., 2018). However, more research is required to fully elucidate the potential genetic implications of non-NMDA receptors and their potential for predisposing people to SZ.

In addition, dystrobrevin binding protein 1 gene (*DTNBP1*) is a susceptibility gene that codes for dysbindin proteins which are involved in neurodevelopment and the modulation of dopaminergic and glutamatergic neurotransmission (Talbot et al., 2004). Interestingly, dysbindin proteins are reduced in the hippocampus and PFC of patients with SZ (Talbot et al., 2004; Weickert et al., 2004). Dysbindin is also believed to be involved in NMDAR clustering and net activity, which may contribute to synapse formation (Numakawa et al., 2004). Thus, alterations in *DTNBP1* that result in reduced dysbindin expression might also contribute to a dysfunctional glutamate system. Lastly, nitric oxide

(NO) modulates neurodevelopment, learning, memory, and neurotransmitter release in the CNS via the facilitation of neuron maturation and synaptogenesis (Oliveira, Zuardi, & Hallak, 2008). Under normal conditions, NO synthase-I (encoded by *NOS1*) generates NO gas which functions as a neurotransmitter to enhance the tone of GABA interneurons (Schwartz et al., 2012b). Functional imaging studies of patients with SZ that possess specific *NOS1* risk alleles exhibit reduced DLPFC functioning, consistent with the negative symptoms of SZ. Thus, genetic alterations to *NOS1* could also result in reduced cortical NO levels that lead to decreased GABA tone and a hypofunctional PFC (Figure 1B)(Reif et al., 2011).

1.5 Treatment

The therapeutic armamentarium for the treatment of SZ has expanded rapidly since the discovery of chlorpromazine over a half century ago (Miyamoto, Duncan, Marx, & Lieberman, 2005). Since then, APD therapy has become the hallmark of SZ treatment. First generation (typical) antipsychotics were originally developed in the 1950's in response to the positive symptoms of SZ due to their high affinity for D_2 receptors (Lieberman, Stroup, et al., 2005). They have been shown to successfully diminish the positive symptoms of SZ via postsynaptic blockades of mesolimbic D_2 receptors. Typical APDs fall into two broad categories: (1) phenothiazines (i.e. chlorpromazine, thioridazine, and trifluoperazine etc.) and (2) butyrophenones (i.e. haloperidol, bromperidol, and droperidol etc.) (Mathews & Muzina, 2007). Furthermore, *in vivo* PET and SPECT studies have demonstrated that the therapeutic efficacy of APDs are associated with limbic and striatal D_2 occupancies of 65 to 80%, whereas the risk of extrapyramidal symptoms (EPS) increases substantially with D_2 occupancies greater than 80% (Meltzer, Koenig, Nash, &

Gudelsky, 1989; Tarazi & Stahl, 2012). Given their high affinity for D2 receptors, most atypical APDs (i.e. haloperidol) tend to occupy greater than 80% of D_2 receptors, leading to a high incidence of neurological side effects such as EPS and tardive dyskinesia (Kusumi, Boku, & Takahashi, 2015). Despite their efficacy in treating the positive symptoms of SZ, these drugs have historically failed to address the negative and cognitive symptoms of the disease. This has largely been due to their poor affinity for serotonergic receptors, which have been associated with negative and cognitive improvement (Meltzer, Horiguchi, & Massey, 2011).

The invention of clozapine in 1975 led to the development of second generation (atypical) APDs, which were developed to treat the negative and cognitive symptoms of SZ while reducing the propensity of neurological side effects observed with typical APDs (Meltzer & Huang, 2008). Unfortunately, clozapine was discovered to cause agranulocytosis, which led to its early discontinuation. Two decades later, novel atypical APDs (i.e. olanzapine and lurasidone) were synthesized based on the chemical structure of clozapine. These drugs were observed to have comparably fewer side effects while maintaining a favourable therapeutic profile that also targets the negative and cognitive domains of SZ (Meltzer & Huang, 2008). Other atypical APDs include risperidone, quetiapine, aripiprazole, and sertindole (Kusumi et al., 2015).

The efficacy of atypical APDs can be explained via the *Serotonin-Dopamine Antagonism Hypothesis* proposed by Meltzer et al. (1989), which suggests that higher drug affinity ratios for serotonin 5-HT_{2A} receptors relative to dopamine D_2 receptors contribute to a therapeutic D_2 occupancy, while improving the negative and cognitive symptoms of SZ (Miyamoto et al., 2005). Atypical APDs are known as multi-receptor

antagonists given their potent affinity for both serotonergic and dopaminergic receptors. These drugs have been engineered with varying affinities for dopamine, serotonin, muscarinic, histamine and adrenergic receptor subtypes, all of which contribute to their unique therapeutic potential and specific side effects (Lally & MacCabe, 2015). Furthermore, electrophysiological studies have demonstrated that atypical APDs (but not typical APDs) enhance NMDAR-mediated neurotransmission, promote neurogenesis, and exhibit neuroprotective capabilities (Kurosawa et al., 2007; Yuen et al., 2012). More specifically atypical antipsychotics such as olanzapine have been reported to have neuroprotective effects against several brain insults such as cerebral ischemia and NMDAR antagonist-induced neuronal damage (Dickerson & Sharp, 2006). Additionally, olanzapine has also been shown to promote neurogenesis in adult rat brains, an important mechanism involved in preserving brain volume during the clinical course of disease. Interestingly, these additional therapeutic properties have not been observed following treatment with typical APDs (H. Y. Wang, Xu, Dyck, & Li, 2004). Given their broad therapeutic potential, atypical APDs have become the preferred course of treatment for SZ. However, many atypical antipsychotics are associated with increased metabolic side effects such as weight gain, diabetes, and hyperlipidemia, all of which contribute to an increased risk of cardiovascular disease and mortality among individuals treated with atypical APDs (K. R. Patel et al., 2014). Thus, recent research has shifted toward the development of novel APDs that address the heterogeneous symptomology of SZ, while minimizing the risk of metabolic and neurological side effects.

1.6 Lurasidone Hydrochloride

Lurasidone hydrochloride [(3a*R*,4*S*,7*R*,7a*S*)-2-[6,18]hexahydro-4,7-methano-2*H*-isoindole-1,3-dionehydrochloride] is a novel atypical APD that received regulatory approval by Health Canada as a monotherapy for the treatment of SZ and bipolar depression in June 2012 (Sunovion Pharmaceuticals Canada Inc., 2012). Lurasidone is a benzisothiazol derivative with a molecular mass of 529.14 g/mol and a molecular formula of $C_{28}H_{36}N_4O_2S{\cdot}HCl$ (Figure 2; Meyer, Loebel, & Schweizer, 2009). It is administered as a monotherapy with a recommended daily dose range of 40-160 mg/day, which should be taken with food to maximize absorption and bioavailability (Greenberg & Citrome, 2017). Lurasidone has garnered much attention as an effective therapy for the treatment of SZ due to its potential to treat the positive, negative, and cognitive symptoms of SZ, while maintaining a benign metabolic profile and a negligible risk of EPS (Ishibashi et al., 2010; Tarazi & Riva, 2013). *In vitro* functional analysis studies have shown that lurasidone acts as a potent antagonist at D_2 (K_i = 1.6 nM), serotonin 5-HT_{2A} (K_i = 2.0 nM) and 5-HT_7 (K_i = 0.5 nM) receptors and as a partial agonist at 5-HT_{1A} (K_i = 6.8 nM) receptors (Ishibashi et al., 2010). Lurasidone's unique receptor binding profile is believed to contribute to its ability to treat the heterogeneous symptomology of SZ (Table 1).

Lurasidone is moderately soluble in methanol, slightly soluble in ethanol, and virtually insoluble in water (0.224 mg/mL @ 20ºC) and most other nontoxic vehicular systems (Greenberg & Citrome, 2017). Due to its poor aqueous solubility, lurasidone is currently restricted to oral administration in humans, which limits its brain bioavailability to nine to 19 percent even when taken with a recommended meal of at least 350 kcal (Greenberg & Citrome, 2017; Preskorn, Ereshefsky, Chiu, Poola, & Loebel, 2013). In

healthy adult subjects, lurasidone reaches peak plasma concentrations within one to three hours and achieves steady state concentrations within seven days of treatment (Greenberg & Citrome, 2017; Lee, Chae, & Koo, 2011). The mean terminal half-life of lurasidone following oral administration is estimated to be 18 hours for a 40 mg dose, 22 hours for an 80 mg dose and 31 hours for a 120 mg dose in humans (Citrome, 2011; Cruz, 2011). Moreover, lurasidone is metabolized via the CYP3A4 pathway, producing two active metabolites (ID-14283, ID-14326) (Meyer et al., 2009). Lurasidone metabolites are primarily eliminated via the gastrointestinal tract with up to 80.1% of total metabolites being recovered in the feces, 9.2% in the urine and 10.7% left unrecovered (Dainippon Sumimoto Pharma America Inc., 2009).

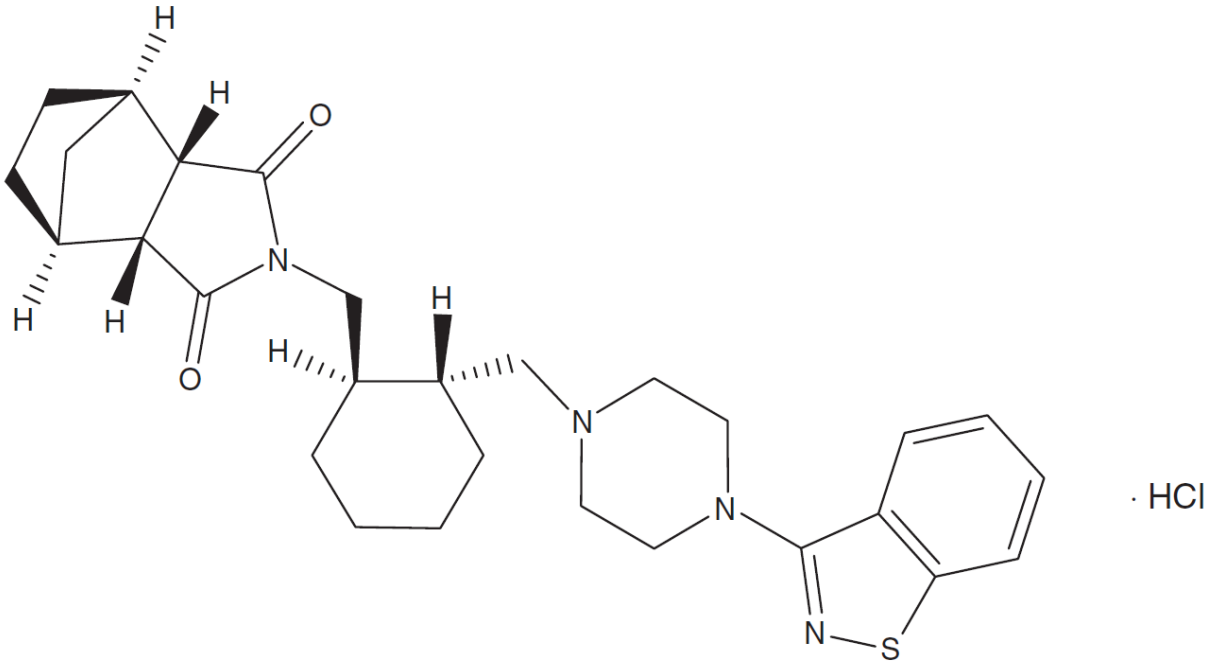


Figure 2. Molecular structure of lurasidone hydrochloride (Meyer et al., 2009).

Despite its promising therapeutic potential, the optimization of lurasidone treatment has been hindered by its poor aqueous solubility and limited bioavailability.

Likewise, much of lurasidone's action mechanism remains elusive, prompting the need for further clinical and preclinical research into its potential as a novel therapeutic drug for SZ. Overall, lurasidone appears to be a valuable addition to the rapidly growing list of antipsychotic medication for the treatment of SZ, but the above caveats must be addressed to fully appreciate its clinical significance.

Table 1. Relevant receptor binding affinities of lurasidone hydrochloride. All symbols represent the degree of receptor antagonism unless otherwise indicated. *Table adapted from Mayer et al. (2009).*

	D_1	D_2	D_3	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	5-HT_7	α_1	M_1	H_1
Lurasidone	+	++++	++	+++ (PA)	++++	+/-	++++	+	-	-
(-) = Clinically insignificant (K_i > 1000 nM); (+) = Low affinity (K_i < 275 nM); (++) = moderate affinity (K_i < 20 nM); (+++) = high affinity (K_i < 10 nM); (++++) = very high affinity (K_i < 5 nM); PA = Partial agonist										

1.7 Thesis Overview

This thesis investigated the behavioural, cognitive, and molecular implications of lurasidone treatment using a MK-801 model of SZ. To date, lurasidone has been shown to be effective in treating individual symptoms of SZ. However, many of these studies have yielded conflicting results, especially with respect to improving the negative and cognitive symptoms of SZ. This contradicting evidence can be attributed to the use of varying pharmacological (i.e. amphetamine, PCP, MK-801, etc.), genetic (i.e. *NRG1* and ErbB4 knockout models), and developmental models of SZ. This thesis sought to provide a comprehensive preclinical assessment of lurasidone hydrochloride. Chapter two attempted to validate the behavioural and cognitive efficacy of lurasidone using a consistent model that addressed the various symptomatic domains of SZ. This study

considered the preventative effects of lurasidone treatment with respect to its ability to attenuate and/or block MK-801-induced positive, negative, cognitive, and sensorimotor-gating deficits. Furthermore, the precise mechanism by which atypical APDs exhibit their therapeutic properties remains elusive. Chapter three examined the influence of lurasidone and MK-801 treatment on mesencephalic astrocyte-derived neurotrophic factor expression as a potential therapeutic target for the treatment of SZ. Lastly, the poor aqueous solubility of most APDs limits them to oral or injectable routes administration. This often requires excessive dosing regimens to achieve therapeutic concentrations in the brain. Conventional forms of APD delivery also expose non-target sites to unnecessary drug concentrations, which might contribute to the debilitating side effects accompanying most APDs. At present, there are no antipsychotic drugs that have received regulatory approval for intranasal administration. This thesis concludes with a preliminary assessment of intranasal lurasidone administration by utilizing a novel nanogel formulation to improve drug delivery. The goal of this thesis was to further elucidate the behavioural and molecular implications of lurasidone treatment with respect to the complex nature of SZ.

CHAPTER 2: VALIDATING THE PREVENTATIVE EFFECT OF LURASIDONE HYDROCHLORIDE WITH A MK-801 MODEL OF SCHIZOPHRENIA

2.1 Introduction

2.1.1 MK-801 Animal Model of Schizophrenia

NMDAR's are ionotropic, voltage-gated, GLU receptors that are primarily permeable to calcium (Paoletti & Neyton, 2007). Under normal conditions, the channel is blocked by a magnesium ion and requires the binding of excitatory amino acids (i.e. glutamate and glycine) to their respective agonist sites to allow the channel to open. The binding of GLU and glycine initiate the depolarization of the receptor, allowing for a voltage-dependent flow of sodium and calcium into the cell (Li et al., 2011). Under these conditions, NMDAR's have been shown to contribute to cognition and memory consolidation via their modulation of cellular maintenance and longevity, synaptic plasticity, and neurogenesis (Malhotra et al., 1996; Nacher & McEwen, 2006). In contrast, malfunctioning NMDAR's have been implicated in the pathophysiology of many diseases. GLU-induced neurotoxicity has been shown to contribute to many CNS disorders such as cerebral ischemia, neurodegenerative conditions, and epilepsy (Paoletti & Neyton, 2007). In theory, NMDAR antagonists are effective in treating GLU-induced excitotoxic conditions. However, these compounds have historically failed in clinical trials due to their unacceptable side effects that closely resemble SZ (Eyjolfsson, Brenner, Kondziella, & Sonnewald, 2006).

The activity of NMDAR's is affected by psychoactive compounds such as phencyclidine (PCP), alcohol, and dextromethorphan (Farber, Kim, Dikranian, Jiang, & Heinkel, 2002). Dizocilpine (MK-801) is a potent, non-competitive NMDAR antagonist that

is structurally and functionally similar to PCP. However, MK-801's potency and greater specificity for NMDAR's renders it a favourable pharmacological model of SZ in the context of the *NMDAR Hypofunction Hypothesis* (Seillier & Giuffrida, 2009). Unlike D_2 agonists (i.e. amphetamine) which only mimic the positive symptoms of SZ, treatment with MK-801 produces a comprehensive model of SZ by mimicking the positive, negative, cognitive, and sensorimotor-gating deficits (Rung, Carlsson, Ryden Markinhuhta, & Carlsson, 2005). MK-801 acts as an open channel blocker by binding to several PCP binding sites inside the NMDAR. The NMDAR blockade prevents the influx of calcium through the channel resulting in a hypofunctional state that causes downstream disruptions in GLU and DA transmission, which manifest as schizophrenia-like symptoms (Anis, Berry, Burton, & Lodge, 1983; Schwartz et al., 2012b).

2.2 Aims and Hypotheses

The objective of this study was to assess the preventative ability of the novel, atypical antipsychotic drug, lurasidone hydrochloride, to ameliorate the positive, negative, cognitive, and sensorimotor-gating symptoms of SZ, *in vivo*. To assess the efficacy of lurasidone, the NMDAR antagonist, MK-801, was used to induce a pharmacological rodent model that encompassed the heterogeneous symptomology of SZ. Rats were then subjected to a series of behavioural paradigms designed to assess a distinct class of symptom. This study also examined the implications of different treatment regimens (i.e. acute and sub-chronic treatment on rat behaviour.

Hypotheses:

1. Rats treated with MK-801 would elicit a significant increase in locomotor activity, a deficit in novel object recognition (NOR), diminished social behaviour, and a reduction in prepulse inhibition (PPI) compared to saline controls.
2. Pre-treatment with lurasidone would significantly attenuate MK-801-induced hyperactivity as well as deficits in NOR, social interaction (SI), and PPI.
3. Sub-chronic treatment with MK-801 would result in sensitization that would exacerbated behavioural deficits compared to acute treatment with MK-801.
4. Sub-chronic lurasidone treatment would be more effective at treating MK-801-induced behavioural deficits compared to acute lurasidone treatment.
5. Lurasidone would not have any significant effect on rat behaviour compared to saline controls.

2.3 Materials and Methods

2.3.1 Animals

Adult, male Wister Hans IGS (Catalogue #: 273; N = 30) rats weighing between 250-300 g (on arrival) were purchased from Charles River Laboratories (Montreal, QC) and housed in McMaster University's Central Animal Facility. Due to their nocturnal nature, all rats were housed under a 12:12 hour reverse light cycle (lights off at 7:00 am, lights on at 7:00 pm EST) to compensate for behavioural tests being performed during the day. Rats were housed individually in a room monitoring temperature (22ºC) and humidity (50 ± 5%) with access to food and water *ad libitum*. All rats were given seven days to acclimate to their new environment and then handled by experimenters for another five days prior to the start of the study. All animal procedures were approved by

McMaster University's *Animal Research Ethics Board* (AUP: 14-08-28; 18-06-27) in accordance with the ethical policies outlined by the *Canadian Council on Animal Care*.

2.3.2 Drug Treatment

Lurasidone was donated by Sunovion Pharmaceuticals, Inc. (Marlborough, MA) and prepared in an optimized vehicle solution of 0.5% (w/v) methylcellulose (MC) and 0.2% (v/v) Tween-20. MC and Tween-20 were obtained from Millipore Sigma (Oakville, ON). Lurasidone was suspended in the Tween/MC vehicle solution at a concentration of 1.5 mg/mL and administered at a final dose of 3.0 mg/kg. MK-801 was purchased from Millipore Sigma and dissolved in 0.9% sterile saline at a concentration of 0.35 mg/mL and administered at a dose of 0.35 mg/kg. All drug formulations were prepared on the day of administration and injected intraperitoneally (i.p.). All rats received acute (single injections) or sub-chronic (daily injections for seven days) injections prior to behavioural testing. Rats were divided into the following four treatment groups (N = 30):

1. Control (CNTL): Vehicle + Saline; n = 6
2. Lurasidone (LUR): Lurasidone + Saline; n = 6
3. MK-801 (MK): Vehicle + MK-801; n = 9
4. Combo (L + M): Lurasidone + MK-801; n = 9

For acute tests, drug naïve rats were pre-treated with a single dose of lurasidone (3.0 mg/kg; i.p.) or vehicle followed by MK-801 (0.35 mg/kg; i.p.) or saline respectively. On day one rat locomotor activity was monitored for two hours. Rats were given a 72-hour washout period to avoid any residual carryover effects and were then re-treated and subjected to NOR, SI, and PPI testing on day five (Figure 3A). For sub-chronic tests, rats received daily doses of lurasidone (3.0 mg/kg; i.p.) or vehicle followed by MK-801 (0.35

mg/kg; i.p.) or saline respectively for 10 days. Locomotor activity monitoring was conducted on day eight and NOR, SI, and PPI testing was conducted on day nine. All rats were sacrificed 30 minutes after the last injection on day 10 (Figure 3B).

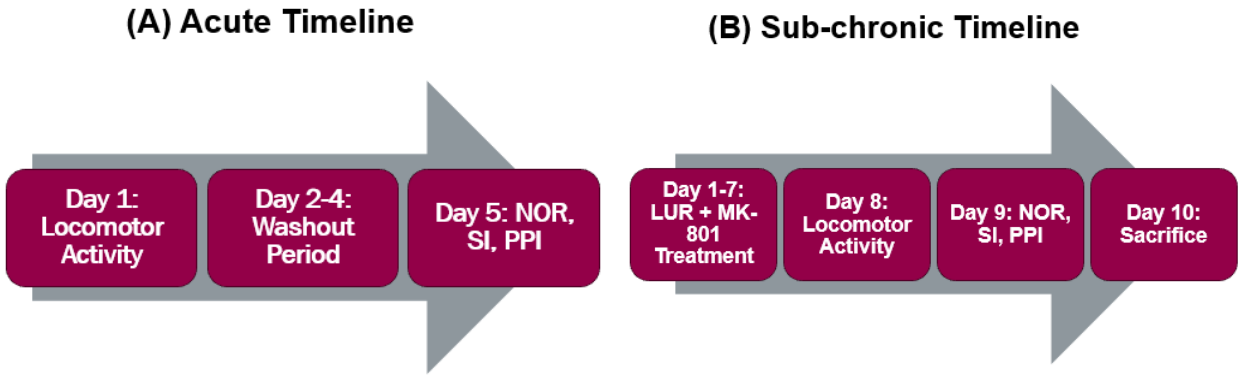


Figure 3. Summary of treatment regimens. (A) Acute study timeline. Locomotor activity monitoring was conducted on day one. Rats were given a 72-hour washout period and then re-treated and tested on NOR, SI, and PPI on day five. **(B) Sub-chronic study timeline.** Rats were pre-treated with daily doses of lurasidone (3.0 mg/kg; i.p.) followed by MK-801 (0.35 mg/kg; i.p.) for 10 days. Locomotor activity monitoring was conducted on day eight and NOR, SI, and PPI were conducted on day nine. All rats were sacrificed on day 10.

2.3.3 Behavioural Procedures

Rats were tested on a series of behavioural paradigms (described below) to assess a distinct class of symptom associated with the MK-801 model of SZ. Locomotor activity monitoring was used to assess the positive symptoms, NOR testing was used to evaluate the cognitive deficits, SI paradigm was used the monitor for negative symptoms, and PPI testing was used to assess sensorimotor-gating deficits. Figure 4AB describes the timeline of the behavioural tests relative to the lurasidone and MK-801 injections.

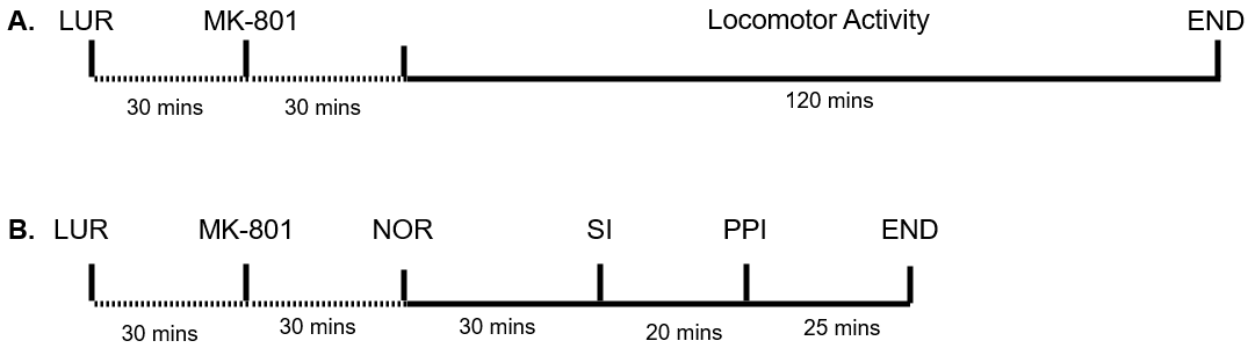


Figure 4. Timeline of behavioural testing relative to drug injections. (A) First day of testing. Locomotor activity was monitored for 120 minutes on the first day of behavioural testing. **(B) Second day of testing.** Due to the duration of locomotor activity monitoring, the remaining three behavioural tests were conducted separately. Pairs of unfamiliar rats were tested on NOR (15 mins each) beginning 30 minutes after the MK-801 injection. Following NOR, both rats were coloured with a water-based paint and given 10 minutes to settle in preparation for SI. Social behaviour was then monitored for the subsequent 10 minutes. Lastly, both rats were subjected to PPI testing for the final 25 minutes.

2.3.3.1 Locomotor Activity Monitoring

Increased locomotor activity in rodents has been established as a putative model of the positive symptoms of SZ using pharmacological challenges with D_2 agonists such as d-amphetamine or NMDAR antagonists such PCP or MK-801 (Sams-Dodd, 1998). Although hyperlocomotion is not a behavioural phenotype observed in humans with SZ, it has been linked to excessive dopaminergic neurotransmission in the mesolimbic and nigrostriatal systems of rats. Similarly, increased DA activity in the same systems is believed to manifest as hallucinations and delusions (positive symptoms) in humans (Cassidy et al., 2018). Despite its limited face validity, the locomotor activity test has become a useful tool for evaluating positive symptoms in preclinical models of SZ.

Locomotor activity was monitored using AccuScan computerized chambers (AccuScan Instruments, Columbus, OH) equipped with six infrared sensors that recorded

multidirectional movements via beam interruptions. Beam breaks were translated into several parameters including movement time (seconds) and total distance travelled (centimeters). A baseline assessment was conducted for 120 minutes one week prior to testing. Three days prior to baseline testing, rats were given a habituation period in which they were free to explore the chamber undisturbed for five minutes. On the day of testing, locomotor activity was monitored for 120 minutes and total distance travelled was calculated in 10-minute increments.

2.3.3.2 Novel Object Recognition

Patients with SZ also suffer from persistent cognitive dysfunction that often manifests prior to the onset of psychosis. Acute and chronic pharmacological challenges with NMDAR antagonists such as PCP, MK-801, or Ketamine have been shown to significantly impair recognition memory in healthy human and animal subjects (Meltzer et al., 2011; Meltzer & McGurk, 1999). Thus, the NOR task was developed to assess working and recognition memory in rodents, which allows for the quantification of cognitive symptoms in preclinical models of SZ (Silvers, Harrod, Mactutus, & Booze, 2007). This task operates on the assumption that rodents have an innate preference for novelty. Thus, when exposed to two objects, control rats should spend more time exploring a novel object compared to an object that they have already been familiarized with. This recognition preference is impaired in rodents that have been challenged with NMDAR antagonists (Grayson, Idris, & Neill, 2007).

The NOR test was carried out in two, five-minute sessions separated by a two-minute intersession interval (ISI) as described by Leger et al. (2013). An eight-arm radial maze was modified by blocking arms one, two, three, five, seven, and eight to form a V-

shaped configuration. Rats were given five minutes to habituate to the maze without any objects present, 24-hours prior to testing. On the day of testing, rats were given a five-minute familiarization trial in which they could explore two identical objects. After the familiarization trial, rats were returned to their home cages during the two-minute ISI. During this time, the maze and all the objects were cleaned with 75% (v/v) ethanol. Following the ISI, one of the identical objects was replaced with a novel object (exhibiting distinct differences in texture and colour). The position of the novel object was randomized for each test trial. Rats were then subjected to a five-minute test trial and allowed to explore the novel and familiar objects. Total time spent exploring each object during the familiarization and test trials was recorded. Object exploration was defined as sniffing, crawling, or touching the object when the rat's nose was within one centimeter of the object (Leger et al., 2013). NOR was quantified using *Recognition Index* (RI), which is a main index of memory retention (Antunes & Biala, 2012; Botton et al., 2010; Gaskin et al., 2010; Schindler, Li, & Chavkin, 2010). RI represents the percent of time exploring the novel object (T_N) relative to the total object exploration time (T_N + T_F) and was calculated according to the following equation $RI = \frac{T_N}{(T_N + T_F)}$. Rats were excluded from analysis if they failed to explore both objects for a minimum of three seconds in either the familiarization or retention trials.

2.3.3.3 Social Interaction Paradigm

Deficits in social behaviour (i.e. social withdrawal lack of motivation) represent the negative symptoms of SZ (Remington et al., 2016). These deficits can be modelled in animals using pharmacological challenges with NMDAR antagonists (Sams-Dodd, 1999). Since healthy rodents display a structured degree of innate social behaviour, deviations

from standard social activity can be easily assessed by monitoring interactions with other rats (Wilson & Koenig, 2014). Thus, the SI paradigm was developed to quantify the explorative and social behaviours of rodents in preclinical models of SZ (Sams-Dodd, 1999). Furthermore, interactions with other rats appear to be rewarding for rats living in social isolation (Wilson & Koenig, 2014). Therefore, control rats were expected to spend more time interacting with each other compared to rats manifesting the negative symptoms of SZ.

Rats were habituated to the social interaction arena for five minutes, 24 hours prior to testing. The SI arena consisted of a black polyvinyl open box (100 cm x 100 cm x 40 cm) with a ceiling mounted video camera located above the arena. On the day of testing, rats were marked with water-based paint for identification purposes, 10 minutes prior to the start of the test. Two unfamiliar rats (no prior interaction) were placed in the SI arena and social behaviour was recorded for 10 minutes. Video footage was manually analyzed by at least two observers blinded to the treatment groups. The total time each rat spent in “active interaction” was computed. Active interaction was defined based on the following behaviours: (1) sniffing the other rat; (2) following the other rat (i.e. within a tails distance); (3) crawling over/under the other rat, (4) grooming the other rat, and/or (5) aggressive behaviour (i.e. clawing/fighting with the other rat).

2.3.3.4 Prepulse Inhibition Test

Sensorimotor gating deficits are also characteristic of patients with SZ. Sensorimotor grating disturbances refer to an inability to differentiate extraneous stimuli from meaningful sensory inputs (Freedman et al., 1987). PPI of the acoustic startle reflex is a common technique used to assess sensorimotor gating deficits in patients with SZ

(Geyer et al. 1984). These deficits can also be applied to preclinical animal models of SZ using treatment with NMDAR antagonists. PPI refers to a response reduction to an auditory startle stimulus (pulse) when it is shortly preceded by a lower intensity, non-startling pre-stimulus (prepulse; Varty, Bakshi, & Geyer, 1999).

Animals were habituated to the PPI chambers for 10 minutes, one day prior to testing. PPI data was generated using a computerized SR-LAB Startle Response System (San Diego Instruments, San Diego, CA) consisting of Plexiglass cylinders and a piezoelectric accelerometer mounted to the bottom of the cylinder to measure the startle magnitude. Each testing session lasted approximately 25 minutes, beginning with a five-minute acclimatization period during which the rats were exposed to a 65-dB white noise present in the background. After the acclimatization period, rats were exposed to a series of five startle pulses (120 dB) without a prepulse stimulus to assess the initial response to the startle stimulus. Rats were then exposed to 65 randomized trials consisting of: (1) no pulse (65 dB background white noise only); (2) a startle-only pulse (120 dB; 40 ms); (3) one of three prepulse intensities (68 dB, 71 dB, 77 dB; 20 ms) presented 100 ms prior to the startle stimulus; or (4) one of the three prepulse intensities alone. The test concluded with five startle-only trials. The responses to the first five and last five startle pulses were excluded from analysis. Following the PPI session, the startle amplitude of each trial type was automatically averaged and used to compute %PPI using the following equation: $\%PPI = \frac{S-PS}{S} \times 100\%$, where S denotes the mean amplitude of the startle-only trials and PS denotes the mean amplitude of the startle response when preceded by a prepulse. All rats were tested in pairs and each completed locomotor activity, NOR and SI testing, prior to the PPI test.

2.3.4 Statistical Analyses

All statistical analyses were conducted using GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA). A two-way repeated measures analysis of variance (ANOVA) was performed to evaluate locomotor activity at each time point. An ordinary one-way ANOVA was conducted to assess the statistical significance of NOR and SI data. A regular two-way ANOVA was used to assess the statistical significance of the PPI data. The ANOVA's for locomotor activity, NOR, and PPI were followed-up with Tukey's honest significant difference (HSD) multiple comparisons test. Social interaction ANOVA's were followed-up with Fisher's least significant difference (LSD) multiple comparisons test. Lastly, a regular two-way ANOVA with Bonferroni's multiple comparisons test was used to compare the differences between acute and sub-chronic treatment regimens. Moreover, normality was confirmed using a Shapiro-Wilk test. Statistical outliers were removed via the ROUT outlier method using a default and conservative ROUT coefficient of one percent, as described by Motulsky and Brown, (2006). Statistical significance was determined at $p < 0.05$. All data was reported as mean ± standard error of mean (SEM).

2.4 Results

2.4.1 Experiment 1: Investigating the preventative effect of acute lurasidone treatment on MK-801-induced behavioural deficits

Acute administration of 0.35 mg/kg of MK-801 increased overall locomotor activity (21,213 cm ± 5,312) compared to control rats (8,107 cm ± 1,642; data not shown). However, locomotor activity in MK-801-treated rats was only significantly increased during the first 30 minutes of the test ($p < 0.05$; Figure 5). Furthermore, acute pre-treatment with lurasidone failed to attenuate MK-801-induced hyperactivity during the first

30 minutes ($p > 0.1105$; Figure 5). No significant changes in locomotor activity were observed across groups between 40 and 120 minutes (Figure 5). Treatment with only lurasidone did not have a significant effect on overall locomotor activity (3,585 cm ± 906.1) compared to control rats ($p = 0.6312$; data not shown).

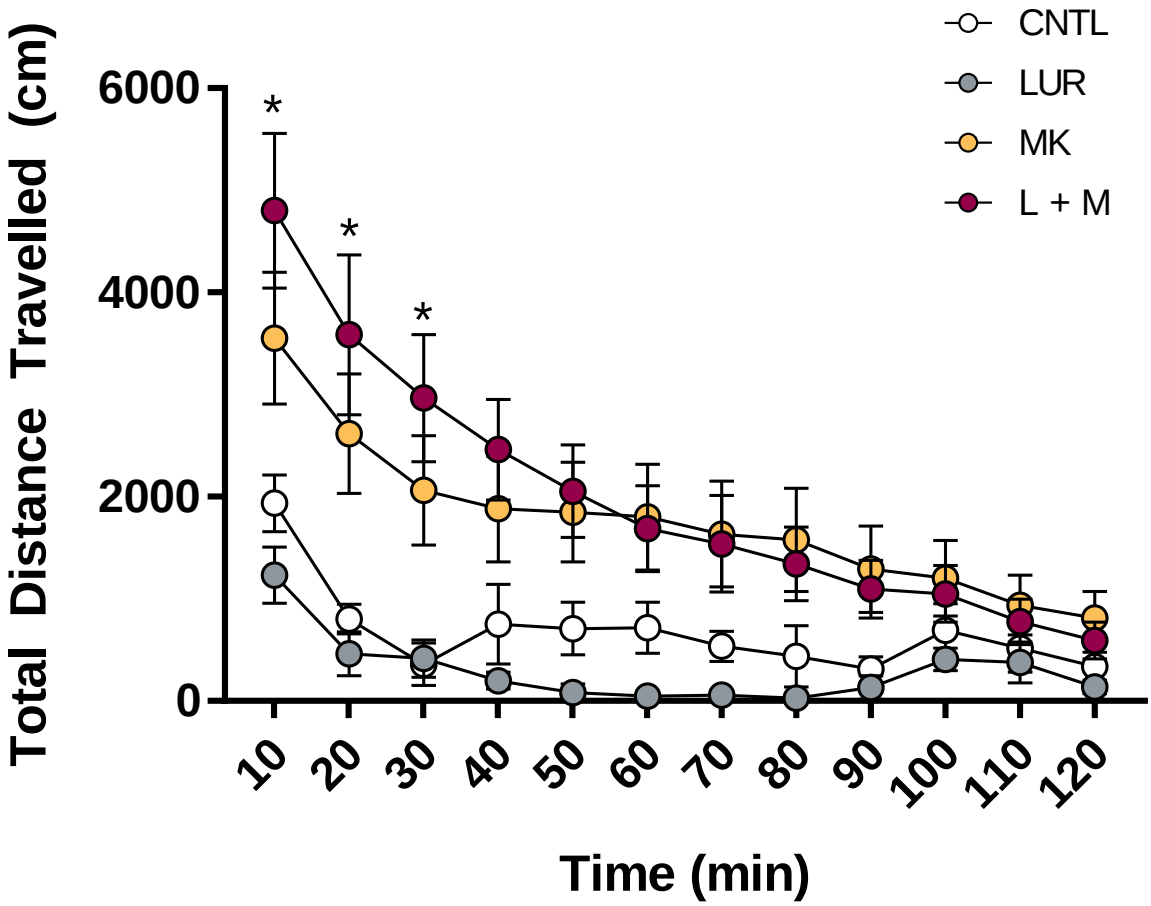


Figure 5. Summary of acute locomotor activity monitoring (CNTL, n = 6; LUR, n = 6; MK, n = 9; L + M, n = 9)**.** Data represent the total distance travelled (in centimeters) during each 10-minute interval. Two-way repeated measures ANOVA revealed a significant treatment effect ($F(3,26) = 5.146$, $p = 0.0063$). Tukey's HSD multiple comparisons test revealed that acute MK-801 treatment only induced hyperactive behaviour during the first 30 minutes of the challenge (*$p < 0.05$). Moreover, lurasidone failed to attenuate MK-801-induced hyperlocomotion during the first 30 minutes ($p > 0.1105$). Acute treatment with lurasidone alone had no significant effect on locomotor activity compared to the control group at any time point ($p > 0.7284$). Data represent mean ± SEM.

Furthermore, treatment with MK-801 caused a slight, but statistically insignificant reduction in RI (0.5068 ± 0.0427; Figure 6) compared to controls (0.6693 ± 0.0564; p = 0.1544). MK-801 rats pre-treated with lurasidone showed RI values comparable to controls (0.6460 ± 0.05315; Figure 6). However, the preventative effect of lurasidone could not be assessed due to the ineffectiveness of acute MK-801 treatment. Furthermore, treatment with lurasidone alone had no effect on recognition memory (0.6752 ± 0.0888; Figure 6) compared to control rats.

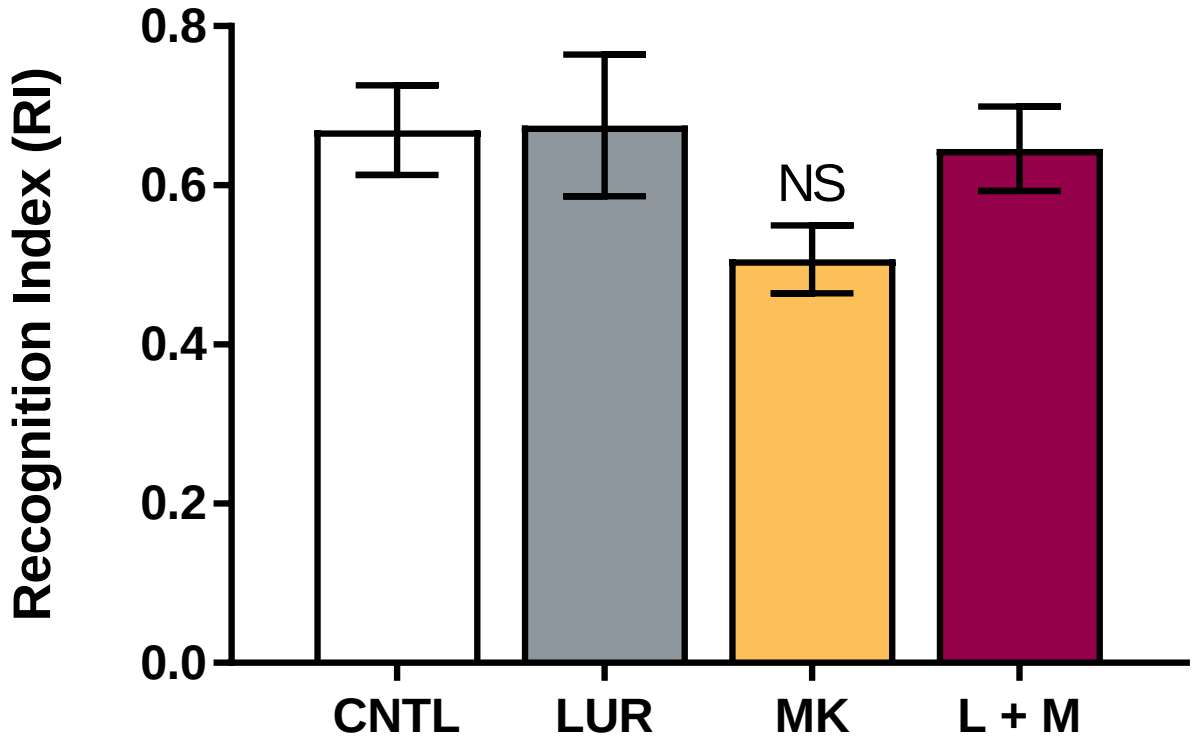


Figure 6. Acute novel object recognition summary (CNTL, n = 6; LUR, n = 3; MK, n = 9; L + M, n = 8). Data represent the mean recognition index per group. Ordinary one-way ANOVA did not reveal a significant difference between groups (F = 2.372, p = 0.0979). Tukey's HSD multiple comparisons test revealed that one-time administration of MK-801 caused a slight but insignificant reduction in RI (^{NS}p = 0.1544). Acute treatment with lurasidone alone had no effect on RI (p > 0.99). Data represent mean ± SEM.

Acute MK-801 treatment caused a significant reduction time spent in active interaction (approx. 75 sec ± 6) compared to control rats (approx. 144 sec ± 14; Figure 7). Furthermore, pre-treatment with lurasidone significantly increased the amount of time spent in active interaction (approx. 114 sec ± 10; Figure 7). Treatment with lurasidone alone caused a slight, but insignificant reduction in active interaction time (approx. 113 sec ± 15; Figure 7).

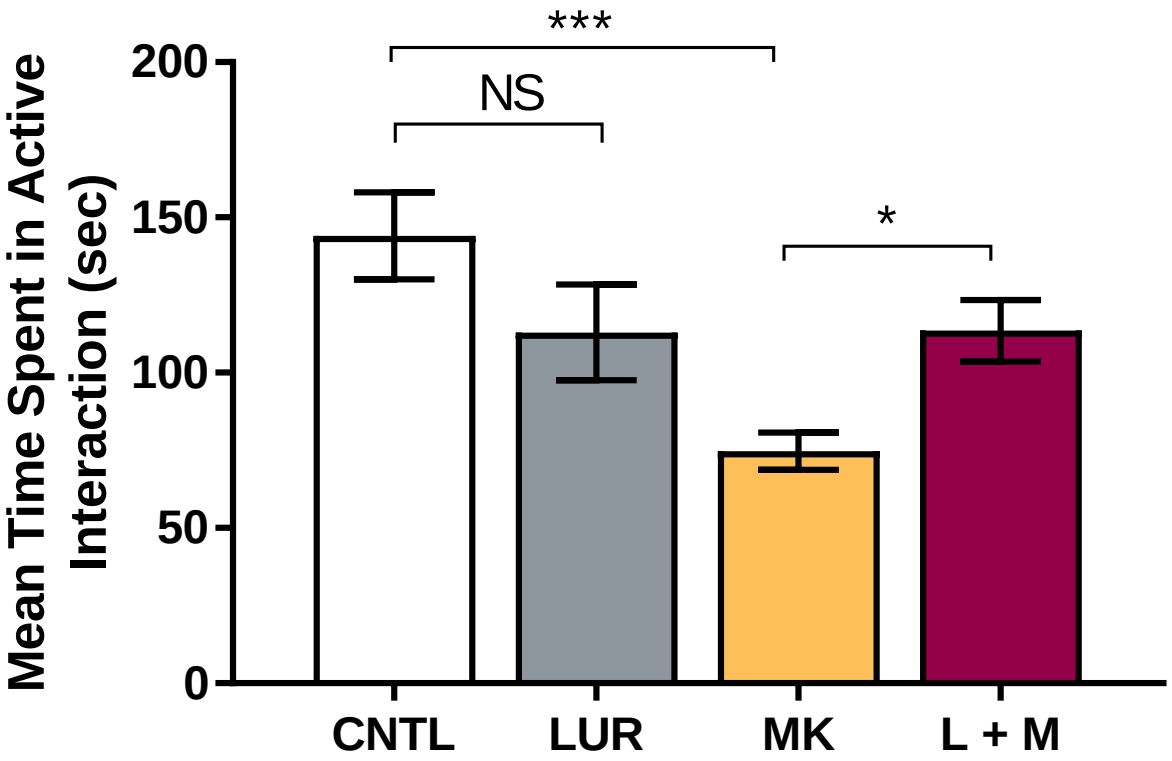


Figure 7. Acute social interaction summary (CNTL, n = 6; LUR, n = 6; MK, n = 8; L + M, n = 9). Data represent the average time spent in active interaction (in seconds). Active interaction was defined as aggressive behaviour, or sniffing, following, crawling over/under, and/or grooming the other rat. Ordinary one-way ANOVA identified a significant difference between groups ($F = 6.418$, $p = 0.0023$). Fisher's LSD multiple comparisons test revealed that acute MK-801 treatment caused a significant reduction in social interaction (***$p = 0.0002$). Acute lurasidone treatment slightly attenuated MK-801-induced social withdrawal (*$p = 0.0127$). Treatment with lurasidone alone had no significant effect on time spent in active interaction compared to controls ($^{NS}p = 0.0827$). Data represent mean ± SEM.

Acute MK-801 treatment caused a significant reduction in %PPI when the startle stimulus was preceded by a 71 dB prepulse (-2.42% ± 10.963) and a 77 dB prepulse (10.517% ± 10.284) but not a 68 dB prepulse (1.057% ± 10.321) compared to control rats (68 dB = 4.525% ± 14.909; 71 dB = 49.833% ± 12.284; 78 dB = 58.331% ± 11.424; Figure 8). Pre-treatment with lurasidone failed to attenuate MK-801 induced %PPI deficits at all three prepulse intensities (Figure 8). Furthermore, acute treatment with only lurasidone had no effect on %PPI at any prepulse intensity (68 dB = 22.865 ± 16.990; 71 dB = 44.002% ± 4.604; 78 dB = 55.017% ± 7.639) compared to controls (Figure 8).

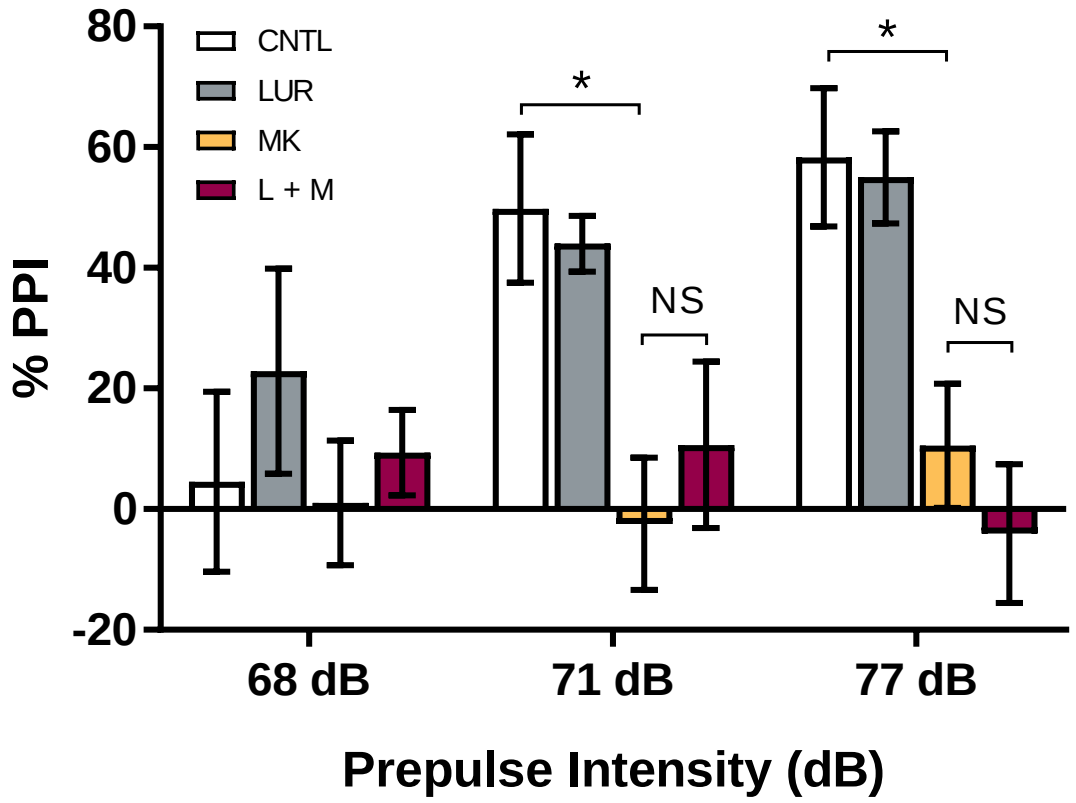


Figure 8. Acute prepulse inhibition summary by prepulse intensity (CNTL, n = 6; LUR, n = 6; MK, n = 8; L + M, n = 9). Data represent the mean %PPI when preceded by a 68 dB, 71 dB, or 77 dB prepulse. Regular two-way ANOVA identified a main effect of treatment ($p = 0.0058$). Tukey's HSD multiple comparisons test revealed that acute treatment with MK-801 caused a significant reduction in %PPI when the startle stimulus

was preceded by a 71 dB (*p = 0.0119) and 77 dB (*p = 0.0253) prepulse but not a 68 dB prepulse (p = 0.9967). LUR failed to attenuate MK-801-induced PPI deficits at a 71 dB prepulse (^{NS}p = 0.8138) and 77 dB prepulse (^{NS}p = 0.7606). Furthermore, treatment with LUR alone did cause any significant changes in %PPI compared to control animals at any prepulse intensity (p > 0.7433). Data represent mean ± SEM.

2.4.2 Experiment 2: Investigating the preventative effect of sub-chronic lurasidone treatment on MK-801-induced behavioural deficits

Repeated administration of 0.35 mg/kg of MK-801 for seven days significantly increased overall locomotor activity (51,240 cm ± 5,498) compared to control rats (5,060 cm ± 1,185; data not shown). Sub-chronic pre-treatment with lurasidone successfully attenuated MK-801-induced hyperactivity (21,835 cm ± 4,319) at 10, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 minutes (Figure 9; p < 0.05). Lastly, sub-chronic treatment with only lurasidone did not have a significant effect on overall locomotor activity (1356 cm ± 430.6) compared to control rats (p = 0.9463; data not shown).

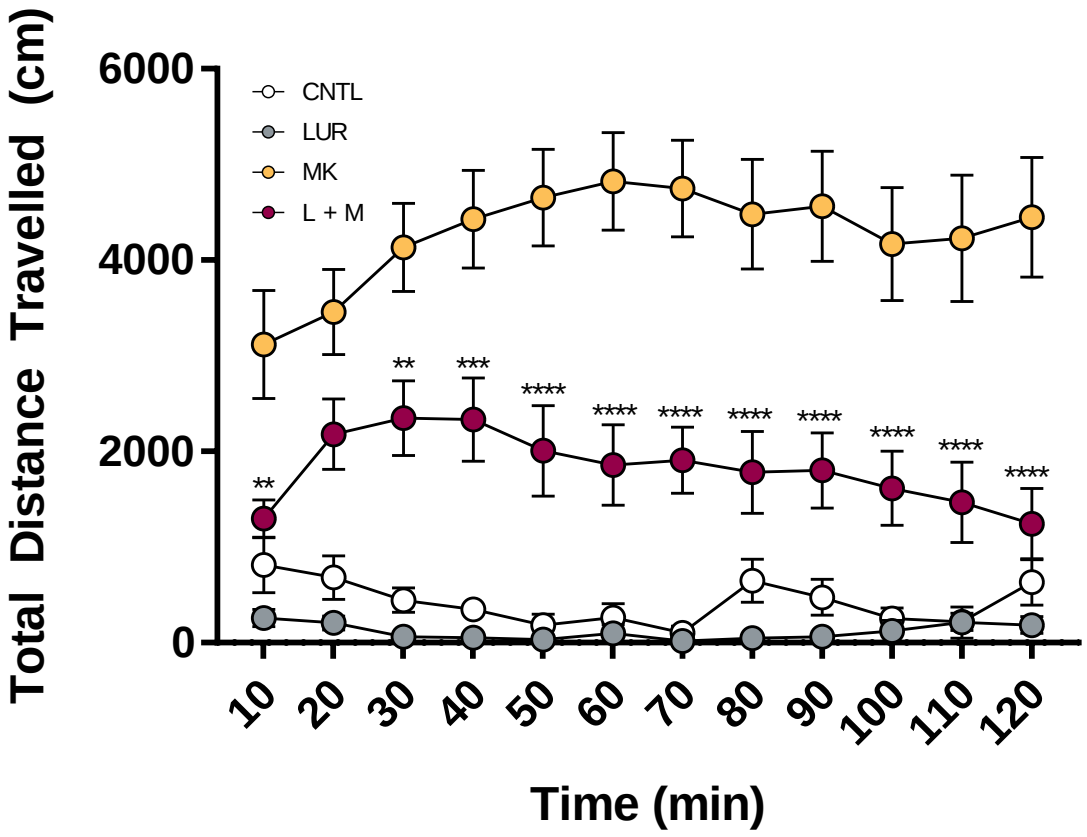


Figure 9. Sub-chronic locomotor activity monitoring summary (CNTL, n = 6; LUR, n = 6; MK, n = 9; L + M, n = 9). Data represent the total distance travelled (in centimeters) during each 10-minute interval. Two-way repeated measures ANOVA revealed a significant treatment effect ($F(3,26) = 28.99$, $p < 0.0001$). Tukey's HSD multiple comparisons test revealed that sub-chronic MK-801 treatment significantly increased locomotor activity at all time points ($p < 0.0008$). Furthermore, sub-chronic treatment with LUR significantly attenuated MK-801-induced hyperlocomotion at 10, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 minutes (**$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). Treatment with lurasidone alone had no effect on locomotor activity compared to healthy controls at any time point ($p > 0.8338$). Data represent mean ± SEM.

Sub-chronic MK-801 treatment significantly impaired recognition memory (RI = 0.4377 ± 0.0382) compared to control rats (RI = 0.6547 ± 0.0245; Figure 10). Sub-chronic pre-treatment with lurasidone successfully attenuated MK-801-induced deficits in RI (RI = 0.6881 ± 0.0474; Figure 10). Treatment with lurasidone alone had no effect on recognition memory (0.6735 ± 0.06484) compared to control rats (Figure 10).

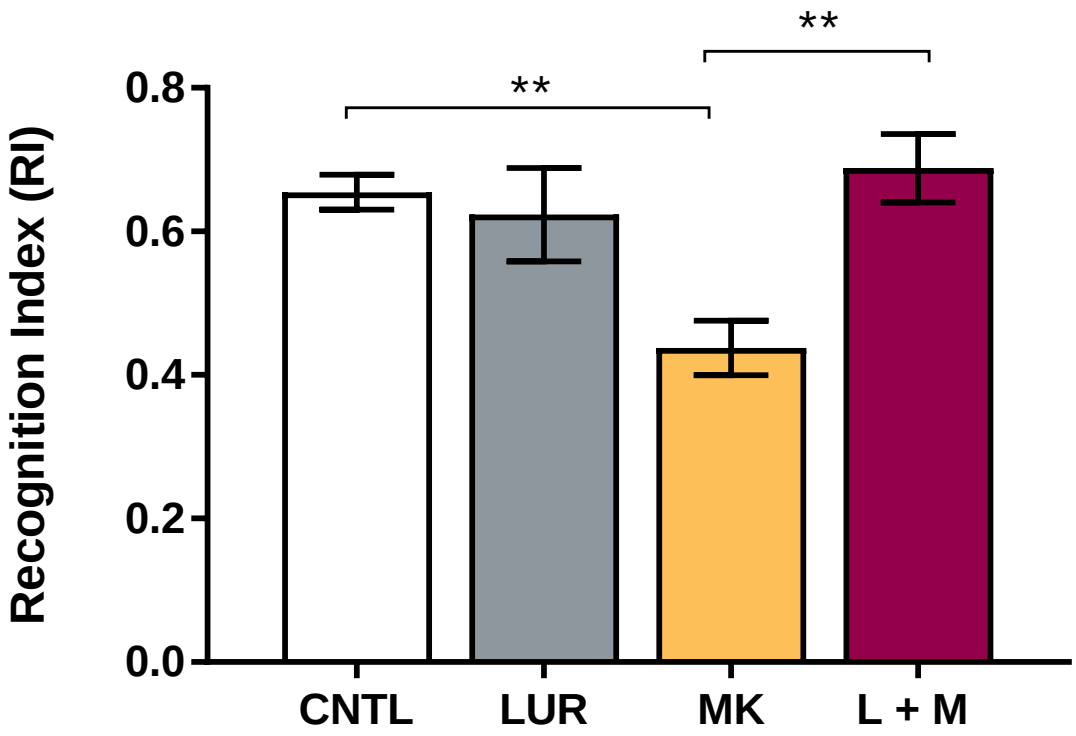


Figure 10. Sub-chronic novel object recognition summary (CNTL, n = 6; LUR, n = 6; MK, n = 9; L + M, n = 8). Data represent the mean recognition index per group. Ordinary one-way ANOVA revealed a significant difference between groups ($F = 7.596$, $p = 0.0010$). Tukey's HSD multiple comparisons test revealed that sub-chronic treatment with MK-801 caused a significant reduction in RI (**$p < 0.01$). Furthermore, sub-chronic lurasidone treatment successfully attenuated MK-801-induced RI deficits (**$p = 0.0011$). Treatment with lurasidone alone had no effect on RI compared to controls ($p = 0.9715$). Data represent mean ± SEM.

Sub-chronic MK-801 treatment caused a significant reduction in time spent in active interaction (approx. 50 sec ± 6) compared to control rats (approx. 147 sec ± 11; Figure 11). Furthermore, pre-treatment with lurasidone significantly increased time spent in active interaction (approx. 82 sec ± 10; Figure 11). Treatment with lurasidone alone did not significantly affect time spent in active interaction (approx. 134 sec ± 16) compared to controls (Figure 11).

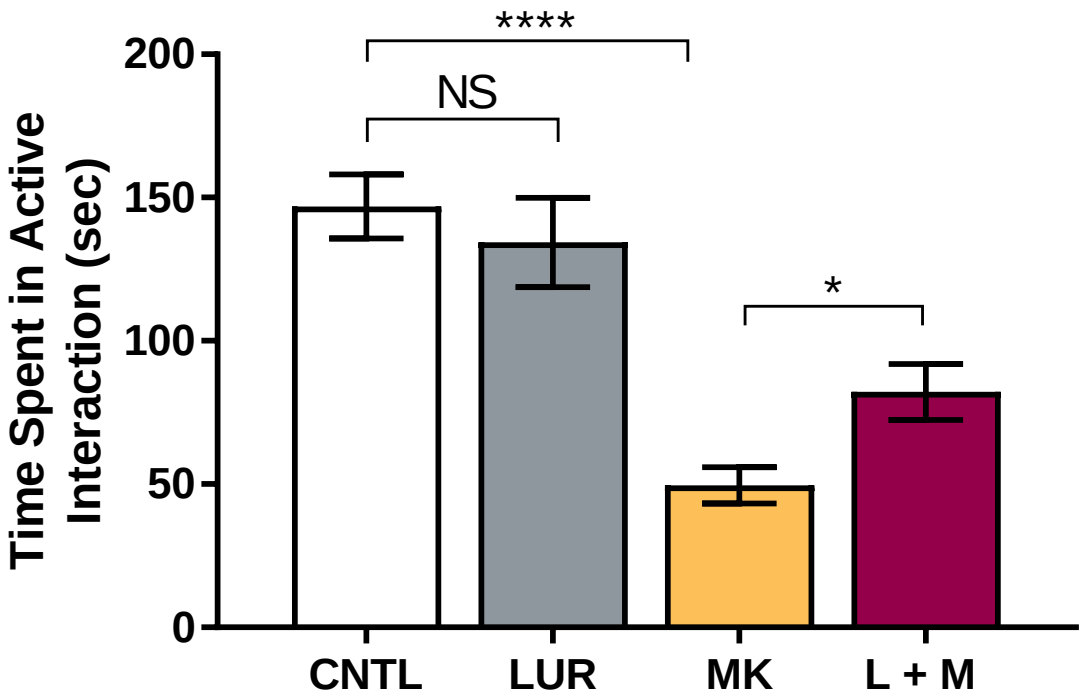


Figure 11. Sub-chronic social interaction summary (CNTL, n = 6; LUR, n = 6; MK, n = 9; L + M, n = 9). Data represent the average time spent in active interaction (in seconds). Active interaction was defined as aggressive behaviour, or sniffing, following, crawling over/under, and/or grooming the other rat. Ordinary one-way ANOVA identified a significant difference between groups ($F = 18.95$, $p < 0.0001$). Fisher's LSD multiple comparisons test revealed that sub-chronic MK-801 treatment caused a significant reduction in social interaction (****$p < 0.0001$). Acute lurasidone treatment slightly attenuated MK-801-induced social withdrawal (*$p = 0.0216$). Treatment with lurasidone alone had no significant effect on time spent in active interaction compared to controls ($^{NS}p = 0.4508$). Data represent mean ± SEM.

Lastly, sub-chronic MK-801 treatment caused a significant reduction in %PPI when the startle stimulus was preceded by a 71 dB prepulse (-3.910% ± 5.980) and a 77 dBb (-5.898% ± 3.392) prepulse but not a 68 dB prepulse (8.127% ± 6.715) compared to control rats (68 dB = 17.820% ± 20.317; 71 dB = 35.567% ± 13.795; 77 dB = 46.311% ± 12.762; Figure 12). Sub-chronic pre-treatment with lurasidone failed to attenuate MK-801 induced %PPI deficits at all three prepulse intensities (Figure 12). Furthermore, sub-

chronic treatment with lurasidone alone had no effect on %PPI at any prepulse intensity (68 dB = 34.472% ± 11.409; 71 dB = 54.636% ± 8.590; 77 dB = 57.397% ± 8.669) compared to control rats (Figure 12).

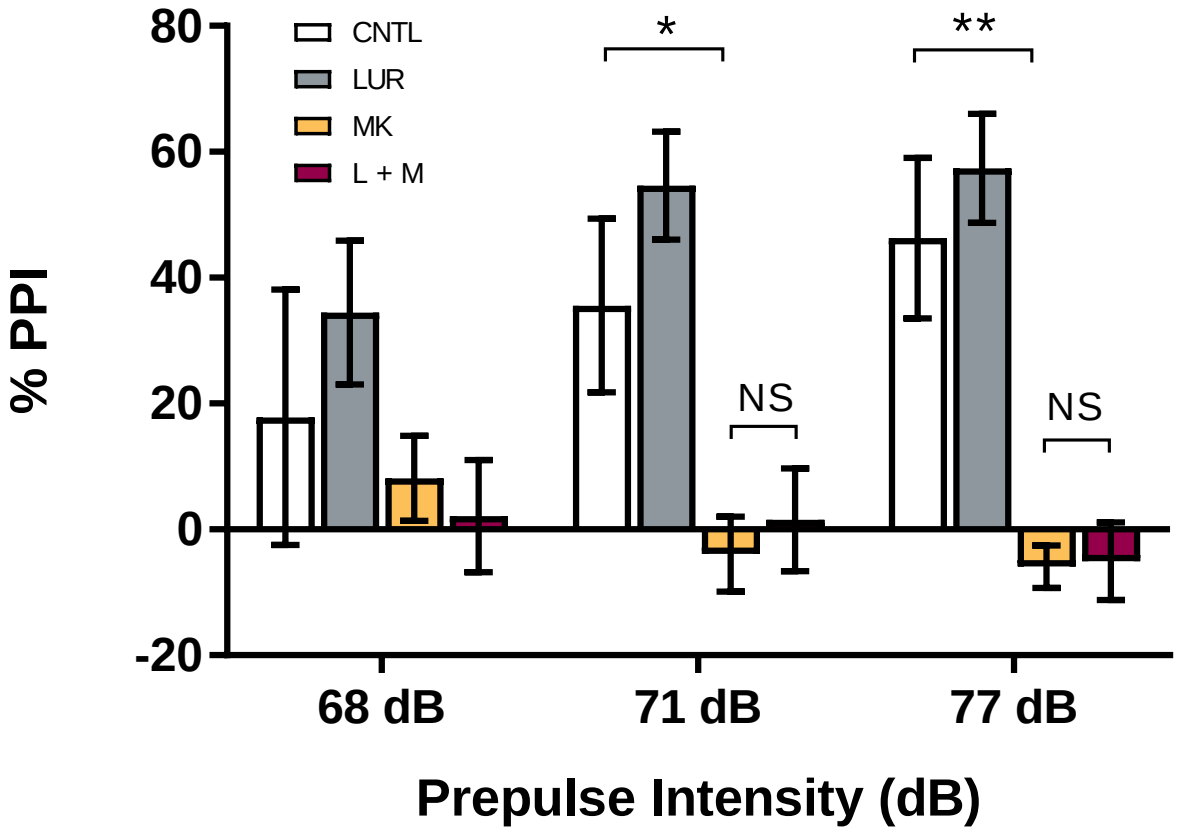


Figure 12. Sub-chronic prepulse inhibition summary by prepulse intensity (CNTL (All dB), n = 6; LUR (68 dB), n = 5; LUR (71 dB & 77 dB), n = 6; MK (77 dB), n = 8; MK (68 dB & 71 dB), n = 9; L + M (68 dB), n = 9; L + M (71 dB & 77 dB), n = 8). Data represent the mean %PPI when preceded by a 68 dB, 71 dB, or 77 dB prepulse. Regular two-way ANOVA identified a main effect of treatment ($p < 0.0001$). Tukey's HSD multiple comparisons test revealed that sub-chronic treatment with MK-801 caused a significant reduction in %PPI when the startle stimulus was preceded by a 71 dB (*$p = 0.0223$) and a 77 dB (**$p = 0.0017$) prepulse but not a 68dB prepulse ($p = 0.8882$). lurasidone failed to attenuate MK-801-induced PPI deficits at 71 dB prepulse ($^{NS}p = 0.9709$) and 77 dB prepulse ($^{NS}p = 0.9999$). Furthermore, treatment with lurasidone alone did not cause any significant changes in %PPI compared to control animals at any prepulse intensity ($p > 0.5685$). Data represent mean ± SEM.

2.4.3 Experiment 3: Comparison of acute and sub-chronic treatment regimens as models of schizophrenia

Repeated MK-801 administration (0.35 mg/kg; i.p.) significantly increased locomotor activity (51,240 cm ± 5,498) compared to acute administration (21,213cm ± 5,312; Figure 13). However, no significant differences in locomotor activity were observed between acute and sub-chronic treatment regimens for the CNTL, LUR, and L + M groups (Figure 13). Furthermore, no other significant behavioural differences were observed between acute and sub-chronic treatment for NOR (Figure 14), SI (Figure 15), or PPI (Figure 16) testing.

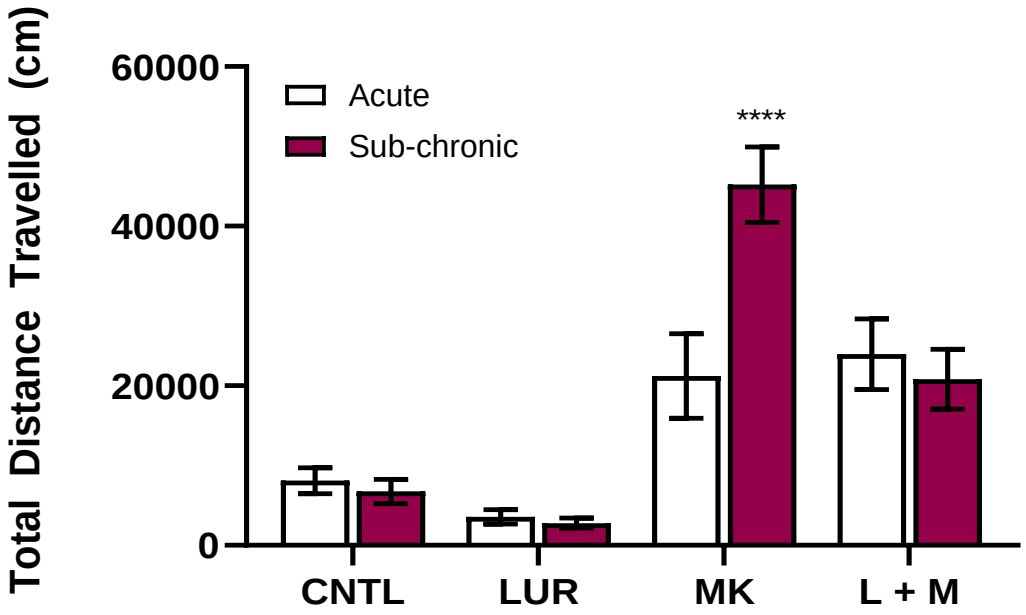


Figure 13. Effect of acute and sub-chronic treatment regimens on locomotor activity. Locomotor activity was monitored for 120 minutes following acute and sub-chronic treatment regimens. White bars indicate the mean total distance travelled following acute treatment and maroon bars represent the mean total distance travelled following sub-chronic treatment. Regular two-way ANOVA identified a significant interaction ($F(3, 52) = 5.906$, $p = 0.0015$). Bonferroni's multiple comparison's test revealed that locomotor activity was significantly greater following sub-chronic treatment with MK-801 compared to acute treatment (****$p < 0.0001$). There was no effect on locomotor activity between acute and sub-chronic treatment regimens for the CNTL, LUR, or L + M groups ($p > 0.9999$). Data represent mean ± SEM.

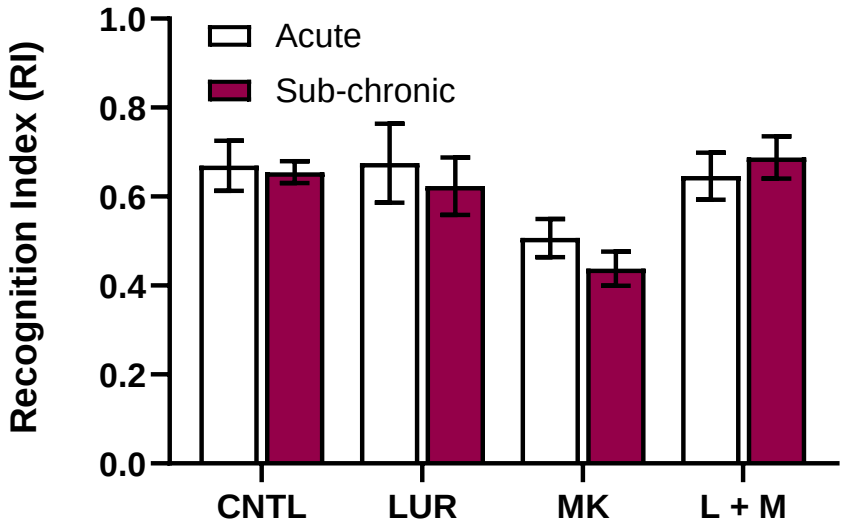


Figure 14. Effect of acute and sub-chronic treatment on recognition index during the test trial. Total time spent exploring the novel and familiar objects was recorded over a five-minute test trial and computed as an RI value. White bars represent the mean RI following acute treatment whereas maroon bars represent mean RI values following sub-chronic treatment. Regular two-way ANOVA did not identify a significant interaction between acute and sub-chronic treatment ($F(3, 46) = 0.5655$, $p = 0.6405$). Data represent mean ± SEM.

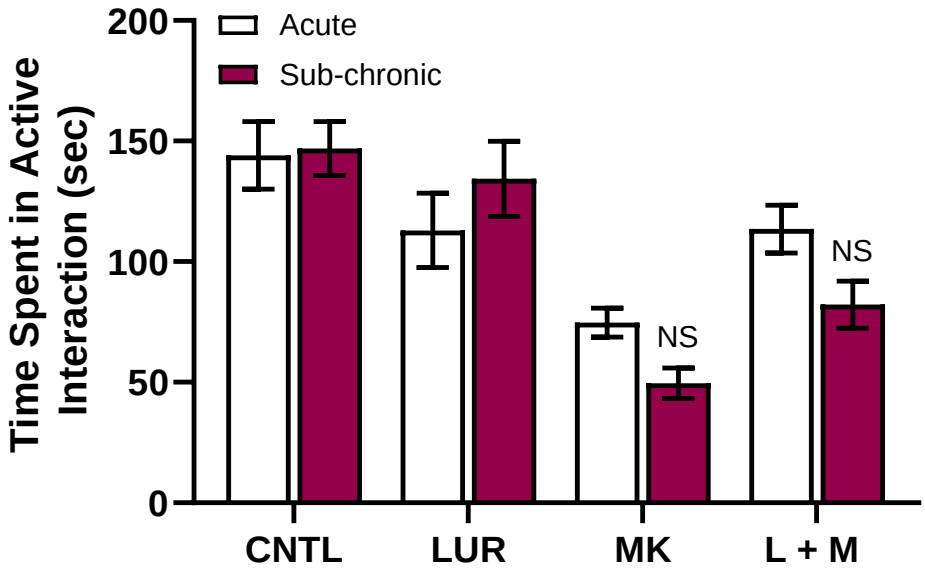


Figure 15. Effect of acute and sub-chronic treatment on social interaction. Total time spent in active interaction was calculated over a 10-minute test trial. White bars represent the mean time spent in active interaction following the acute treatment regimen. Maroon bars represent the mean time spent in active interaction following the sub-chronic treatment regimen. Regular two-way ANOVA did not identify a significant interaction between acute and sub-chronic treatment ($F(3, 51) = 2.543$, $p = 0.0664$). Data represent mean ± SEM.

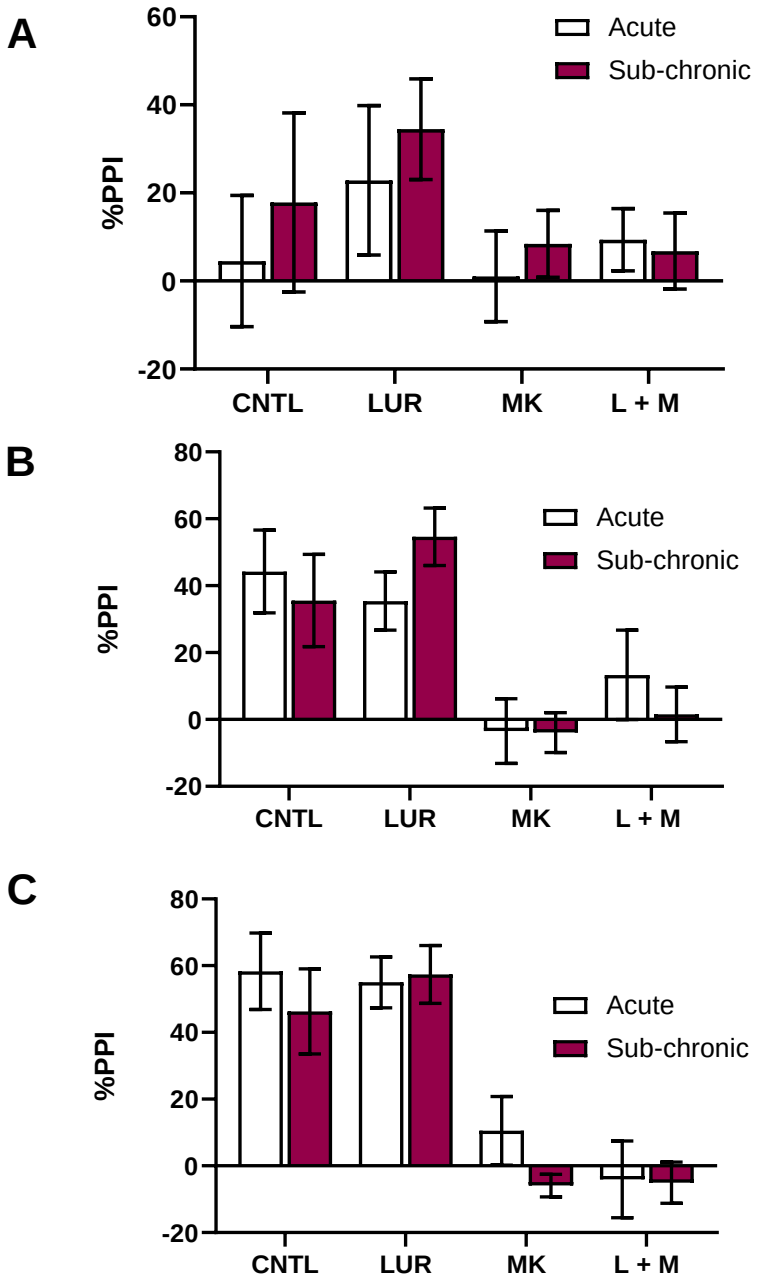


Figure 16. Effect of acute and sub-chronic treatment on %PPI. %PPI values for acute and sub-chronic treatment were computed for each pre-pulse intensity (A) 68dB; (B) 71 dB; (C) 77 dB. White bars represent the mean %PPI values following acute treatment whereas maroon bars represent mean %PPI values following sub-chronic treatment Regular two-way ANOVA did not identify a significant interaction between acute and sub-chronic treatment at any prepulse intensity. **(A)** 68 dB: ($F(3, 49) = 0.1876$, $p = 0.9043$). **(B)** 71 dB: ($F(3, 51) = 0.7909$, $p = 0.5046$). **(C)** 77 dB: ($F(3, 50) = 0.4415$, $p = 0.7243$). Data represent mean ± SEM.

2.5 Discussion

This study examined the preventative effect of acute and sub-chronic lurasidone treatment (3.0 mg/kg; i.p.) using a MK-801 (0.35 mg/kg; i.p.) model of SZ. Following each treatment regimen, rats were subjected to a series of behavioural paradigms designed to assess lurasidone's ability to block MK-801-induced deficits in the positive, negative, cognitive and sensorimotor gating domains of SZ. This study demonstrated that acute MK-801 treatment significantly induced observational deficits in locomotor activity (Figure 5), SI (Figure 7), and PPI (Figure 8), but not NOR (Figure 6). Likewise, pre-treatment with a one-time dose of lurasidone modestly attenuated MK-801-induced behavioural deficits in social interaction (Figure 7) but was ineffective at blocking MK-801-induced hyperactivity (Figure 5) and PPI deficits (Figure 8). Consequently, acute treatment with MK-801 caused a slight, but insignificant reduction in RI (Figure 6). Therefore, this test could not be used to assess the effect of lurasidone treatment on acute cognitive deficits. Interestingly, sub-chronic treatment with MK-801 resulted in significant deficits in all four behavioural paradigms. Moreover, sub-chronic lurasidone treatment was effective at blocking MK-801-induced deficits in locomotor activity (Figure 9), NOR (Figure 10), and SI (Figure 11), but not PPI (Figure 12). To the best of our knowledge this was the first preclinical study to evaluate the therapeutic potential of lurasidone using a comprehensive model that addressed the major symptomatic domains of SZ.

Sub-chronic treatment with MK-801 significantly increased the magnitude of its hyperlocomotor-producing effects compared to acute treatment (Figure 13). This is consistent with previous reports that repeated MK-801 injections result in behavioural sensitization to its own hyperlocomotor-producing effects (Lefevre, Eyles, & Burne, 2016).

Behavioural sensitization to MK-801 has been linked to altered DA release and turnover in the nucleus accumbens and PFC compared to non-sensitized rats (Cui et al., 2015). The temporary blockade of NMDA receptors reduces the firing rats of GABAergic interneurons in the PFC (Figure 1). This phenomenon results in an increase in extracellular DA, serotonin, GLU, and acetylcholine levels in the frontal area of rats and monkeys following acute treatment. The disinhibition of these neurotransmitter systems are hypothesized to contribute to increased locomotor activity following acute treatment (Bubenikova-Valesova, Horacek, Vrajova, & Hoschl, 2008). Additionally, Cui et al. (2015) showed that DA release in non-sensitized rats peaks within the first hour and returns to baseline within the second hour post challenge, whereas DA release tends to peak during the second hour in sensitized rats. These findings are consistent with the temporal profiles of locomotor activity observed our acute (Figure 5) and sub-chronic studies (Figure 9).

Contrary to acute administration, long-term exposure to NMDAR antagonists is associated with a decrease in frontal brain activity (Jentsch & Roth, 1999). In fact, long-term exposure to PCP in humans has been shown to reduce frontal lobe blood flow and glucose utilization (Jentsch & Roth, 1999). Furthermore, sub-chronic treatment with NMDAR antagonists is also followed by a decrease in the production and utilization of DA metabolites in the DLPFC, which corresponds with the hypofunctional state described by the *NMDAR Hypofunction Hypothesis* (Bubenikova-Valesova et al., 2008; Zuo et al., 2006). These molecular alterations could explain the differences in locomotor activity observed after acute and sub-chronic treatment with MK-801 (Figure 13). Furthermore, chronic, but not acute treatment with MK-801 resulted in altered expression of NR1 and NR2 subunits as well as a decrease in the relative number of parvalbumin-positive

interneurons in the rat hippocampus (Rujescu et al., 2006). These molecular observations are consistent with post-mortem findings involving human SZ brains (Jentsch & Roth, 1999). Thus, these observations suggest that repeated exposure to non-competitive NMDAR antagonists might provide a more accurate, qualitative representation of the behavioural and molecular symptoms of SZ compared to acute exposure (Jentsch & Roth, 1999).

Moreover, sub-chronic treatment with MK-801 did not significantly increase the magnitude of behavioural deficits observed in NOR, SI, or PPI (Figures 14, 15 and 16). However, differences in molecular responses to acute and sub-chronic treatment with NMDAR antagonists suggest that repeated administration may be necessary to induce neurochemical alterations that coincide with the behavioural phenotypes of SZ (Cui et al., 2015). Another important finding was that acute and sub-chronic treatment with only lurasidone did not differ significantly from control rats in any of the behavioural tests (Figures 13, 14, 15, and 16). These findings suggest that repeated exposure to lurasidone does not have any adverse effects on rodent behaviour.

Pre-treatment with lurasidone was effective in attenuating MK-801-induced hyperactivity following sub-chronic treatment (Figure 9) but not acute treatment (Figure 5). This is consistent with the typical course of action of APD treatment in humans, which typically takes six to eight weeks to reach maximum therapeutic efficacy (Kapur, Agid, Mizrahi, & Li, 2006; Lally & MacCabe, 2015). Lurasidone's high affinity for antagonizing dopamine D_2 receptors (Table 1) can be attributed to its attenuation of sub-chronic MK-801-induced hyperactivity. Studies have shown that hyperactive D_2 activity in the mesolimbic and nigrostriatal tracts contribute to increase locomotor activity in rodents,

whereas the same phenomenon emerges as positive symptoms (i.e. auditory hallucinations) in humans (Cassidy et al., 2018). These symptoms are consistently attenuated with repeated administration of both typical and atypical APD's. However, the delayed onset of antipsychotic action observed in this study does not correlate with the rapid time course of receptor blockades following the initiation of treatment in rats or humans (Grace, Bunney, Moore, & Todd, 1997). One proposed mechanism for this delayed antipsychotic effect is the *Depolarization Block Hypothesis*. The *Depolarization Block Hypothesis* suggests that repeated administration of APD's (i.e. D_2 antagonists) results in a time-dependent inactivation of DA neuron firing over the course of several weeks (Kapur et al., 2006). This has been further supported by observations by Valenti et al. (2011) that repeated administration of both haloperidol (typical APD) and sertindole (atypical APD) for one, three, give, seven, 15, and 21 days caused a gradual reduction in ventral tegmental area DA neuron population activity. In future studies, it would be interesting to test the locomotor activity of sub-chronically treated rats over a similar time course to determine if there is a progressive attenuation of symptoms that coincides with the depolarization block described by Valenti et al. (2011).

Furthermore, acute treatment with MK-801 resulted in a noticeable deficit in RI (Figure 6); however, this deficit was not statistically significant. In contrast, sub-chronic treatment with MK-801 did induce a significant reduction in RI compared to control animals (Figure 10). In addition, there was no significant difference in RI between acute and sub-chronic rats treated with lurasidone (Figure 14). The increase in the magnitude of the MK-801 deficit on RI is consistent with the sensitizing effects of NMDAR antagonists (Lefevre et al., 2016). Cognitive impairment is a fundamental feature of SZ and over 85

percent of patients with the disorder exhibit clinical impairment in cognitive domains including attention, working memory, declarative memory, and executive functioning (Palmer et al., 1997). Of the 14 known serotonin receptors, the 5-HT_{1A} receptor has been implicated in the procognitive properties of atypical APD's, including lurasidone (Huang, Horiguchi, Felix, & Meltzer, 2012; Meltzer & Sumiyoshi, 2008). Lurasidone is a partial agonist of 5-HT_{1A} receptors which has been shown to contribute to increased cortical DA activity, an effect that is diminished in 5-$HT_{1A}^{-/-}$ mice or upon treatment with 5-HT_{1A} antagonists (Ichikawa et al., 2001). These observations are consistent with the hypofunctional mesocortical pathways observed in SZ. Additionally, lurasidone has a strong affinity for antagonizing 5-HT_7 receptors (Huang et al., 2012). Findings from preclinical studies suggest that the blockade of 5-HT_7 receptors protect against schizophrenia-like cognitive deficits. More specifically, mice treated with the selective 5-HT_7 antagonist, SB-269970, show significant improvement in different variations of the NOR test, consistent with the rat studies in this manuscript (Figure 10; Freret et al., 2014) Thus, lurasidone's ability to partially agonize 5-HT_{1A} receptors and antagonize 5-HT_7 receptors, might contribute to the improved RI in MK-801 treated rats and its superior efficacy in attenuating cognitive impairment observed in patients with SZ.

Both acute (Figure 7) and sub-chronic (Figure 11) treatment with MK-801 caused a significant reduction in the average time spent in active interaction. Furthermore, sub-chronic treatment with MK-801 caused a greater reduction in time spent in active interaction (approx. 50 sec ± 6) compared to acute treatment (approx. 75 sec ± 6; Figure 15). Although this interaction was not statistically significant ($F(3, 51) = 2.543$, $p = 0.0664$), the trend was consistent with observations that repeated NMDAR antagonism

results in decreased frontal brain activity, which is also associated with the negative symptoms of SZ (Jentsch & Roth, 1999). Among the therapeutic properties of atypical APD's, their ability to treat the negative symptoms of SZ remains controversial. At present, the general consensus is that most atypical APD's are not therapeutically superior to their first-generation counterparts and any therapeutic improvement, in most instances, does not meet the threshold for clinically significant improvement (Fusar-Poli et al., 2015; Remington et al., 2016).

Further, acute and sub-chronic treatment with lurasidone resulted in a slight improvement in MK-801-induced social withdrawal (Figure 7; Figure 11). Interestingly, lurasidone has also been approved as a monotherapy or adjuvant therapy (with lithium or valproate) for the treatment of bipolar depression (Roberts, Lohano, & El-Mallakh, 2016). Lurasidone may improve negative symptoms by increasing PFC dopamine activity via its interactions with 5-HT_{1A} and 5-HT_7 receptors. Interestingly, 5-HT_{1A} partial agonists have also been shown to contribute to enhanced DA efflux in the PFC and hippocampus following a sub-chronic PCP challenge (Huang et al., 2012). These findings suggest that lurasidone's ability to improve the negative symptoms of SZ might be mediated via 5-HT_{1A}-related mechanisms. Additionally, lurasidone also has a strong affinity for 5-HT_7 receptors, which are known to improve mood (Bawa & Scarff, 2015). Cates et al. (2013) demonstrated that lurasidone improved the performance of normal mice during the tail suspension and forced swim tests. However, improvements in social withdrawal were abolished in 5-HT_7 receptor knockout mice (Cates et al., 2013). Despite its strong affinity for 5-HT_7, and 5-HT_{1A} receptors, lurasidone only modestly attenuated MK-801-induced deficits in social behaviour (Figure 7; Figure 11). Thus, lurasidone's full potential to treat

the negative symptoms of SZ may be hindered by its complex interactions with other receptors (Table 1).

Lastly, the acoustic startle response has been extensively used in both human and animal models as an assessment of sensorimotor-gating deficits (Wynn et al., 2004). In this study, both acute and sub-chronic MK-801 treatment caused a significant reduction in %PPI (Figure 8; Figure 12). However, in both instances, lurasidone failed to attenuate these deficits. These findings suggest that lurasidone is likely ineffective at treating sensorimotor gating deficits of SZ. To the best of our knowledge this is the first study to assess the effects of lurasidone on prepulse inhibition in any model of SZ. Other studies have observed discrepant results among other atypical APD's regarding their ability to treat sensorimotor gating deficits. However, the mechanism by which these drugs act to attenuate PPI deficits remains speculative. For example, some APD's such as clozapine and olanzapine significantly restore and/or block MK-801-induced PPI deficits, whereas treatment with other atypical APD's such as risperidone and zotepine failed to significantly improve these deficits (Bubenikova, Votava, Horacek, Palenicek, & Dockery, 2005; Varty et al., 1999). However, in both studies, even acute treatment with risperidone and zotepine appeared to reduce the effect of MK-801 on PPI despite being statistically insignificant, whereas lurasidone appeared to have no effect on PPI even after sub-chronic treatment (Figure 12). Attenuation of PPI deficits produced by NMDAR antagonists has been associated with each drugs affinity for 5-HT_{2A} and α_1-adrenergic receptors (Varty et al., 1999). Interestingly, lurasidone differs substantially from the aforementioned APD's in its affinity for α_1-adrenergic receptors. Lurasidone has a clinically insignificant affinity for α_1 receptors compared to risperidone, zotepine,

olanzapine, and clozapine, which all have moderate to high affinities for α_1 receptors (Mauri et al., 2014; Yasui-Furukori, 2012). Carasso et al. (1998) found that treatment with the selective α_1 agonist, cirazoline, significantly disrupts PPI. Furthermore, Bakshi and Geyer (1997) also demonstrated that the effects of PCP (structurally and functionally similar to MK-801) on PPI are significantly reduced following pre-treatment with the α_1 antagonist, prazosin. Thus, it is conceivable lurasidone's insignificant affinity for α_1 receptors might also contribute to its inability to block MK-801-induced PPI deficits following both acute and sub-chronic treatment.

2.6 Limitations and Future Directions

Pharmacological models of SZ are robust tools for initial assessments of APD efficacy. This study demonstrated that sub-chronic lurasidone treatment was effective in preventing MK-801-induced positive, negative, and cognitive deficits. However, these models have often been criticized as they lack etiological validity and do not support the neurodevelopmental origins of SZ. Thus, neurodevelopmental models have been developed to mimic the progressive onset of symptoms and more accurately represent the subtle changes in neural systems that underly the complex pathophysiology of the disease. The neonatal ventral hippocampal lesion (NVHL) model of SZ in rats is known to mimic the emergence of symptoms in humans (Tseng, Chambers, & Lipska, 2009). It would be interesting to determine if chronic lurasidone treatment is effective at preventing or attenuating NVHL-induced or other neurodevelopmentally-induced behavioural deficits. This may be a better indicator of the efficacy of early lurasidone intervention. Another noteworthy limitation of pharmacological disease models is that they can vary across sex and animal strains. This study only evaluated the preventative effects of

lurasidone treatment in male, Wistar rats. Thus, it is plausible that the findings presented in this thesis may be restricted to a specific niche. Future studies should explore the effects of both MK-801 and lurasidone across sex and species to obtain a better representation of the population.

Furthermore, both acute and sub-chronic social interaction studies revealed that lurasidone modestly attenuated MK-801-induced social deficits. To eliminate bias, the SI videos were manually analyzed by multiple different observers who were blinded to the treatment. Although efforts were made to concretely define different “active interactions”, the analysis was still prone to innate subjectivity and interpretation differences between observers. Thus, it is difficult to confidently conclude whether lurasidone’s modest attenuation of MK-801-induced social withdrawal was a legitimate therapeutic response to the drug or the result of inter-observer differences. Sams-Dodd et al. (1995) introduced an automated video-tracking system for objectively interpreting rat social behaviour using computerized parameters. It would be interesting to assess whether similar trends are observed in the SI paradigm using a video-tracking system.

Lastly, the NOR task has been criticized as an assessment for recognition memory (Young, Amitai, & Geyer, 2012). The test relies on the innate curiosity of rodents, which may be altered by pharmacological manipulations. Interestingly, treatment with MK-801 significantly increased the total exploration time compared to controls (data not shown). This is consistent with the hyperactive properties of MK-801. In some instances, rats that were not treated with MK-801 (CNTL and LUR groups) did not meet the inclusion criteria as they did not spend adequate time exploring both objects. This resulted in several exclusions and a very low sample size for the LUR group in the acute studies (n = 3;

Figure 6). It is also possible that other factors such as familiarity and olfactory cues may influence the exploratory behaviours of rats during the test. Thus, the NOR task is prone to a high rate of false positives that may skew the results (Young et al., 2012).

It was observed that acute lurasidone administration was ineffective at preventing MK-801-induced deficits in locomotor activity (Figure 5), social interaction (Figure 7), and PPI (Figure 8). It is worth mentioning that lurasidone was administered intraperitoneally 30 minutes prior to the MK-801 injection (Figure 4). This treatment timeline was consistent with other studies examining the acute effects of APD's (Arruda et al., 2008; Kolaczkowski, Mierzejewski, Bienkowski, Wesolowska, & Newman-Tancredi, 2014). However, previous reports have suggested that APD's can take up to an hour to reach their target receptors in the brain following acute administration in SZ patients (Valenti et al., 2011). Thus, it is presently unclear whether acute lurasidone treatment was ineffective, or if it required more time between injections to exert a therapeutic response. Future studies should look at the temporal profile of lurasidone relative to its acute effects on MK-801-induced behavioural deficits.

CHAPTER 3: INVESTIGATING THE ROLE OF MESENCEPHALIC ASTROCYTE-DERIVED NEUROTROPHIC FACTOR AS A POTENTIAL THERAPEUTIC TARGET FOR SCHIZOPHRENIA

3.1 Introduction

3.1.1 Neurotrophic Factors

Increasing evidence supports the notion that SZ is a subtle disorder of brain development and plasticity (Insel, 2010). Traditional, target-derived neurotrophic factors (NTFs) belong to one of three major families: (1) neurotrophins; (2) glial cell-line derived neurotrophic factor (GDNF); or (3) neuropoietic cytokines (Deister & Schmidt, 2006). These NTFs are naturally occurring endogenous secretory proteins that regulate the differentiation, localization, maintenance, and survival of CNS neurons. Different NTFs are known to influence specific subsets of neuronal subpopulations through the activation of transmembrane receptor tyrosine kinases or via kinases that interact with their receptors (Bespalov & Saarma, 2007; Lindholm & Saarma, 2010). A plethora of evidence has implicated NTFs in neuronal survival during critical periods of development. A key pillar of the neurotrophic theory posits that trophic molecules are only available in limited quantities during developmental periods of programmed cell death (Oppenheim, 1989). Only neurons that receive sufficient trophic support are capable of avoiding apoptosis and maintaining synaptic connections (Lindholm & Saarma, 2010). Therefore, it is not surprising that changes in NTF expression have been associated with various diseases such as SZ and Parkinson's disease (Chauhan, Siegel, & Lee, 2001; Nieto, Kukuljan, & Silva, 2013). Interestingly, only a handful of NTFs have been identified as potential

therapeutic targets for the treatment of neurogenerative and neurodevelopmental disorders (Lindholm & Saarma, 2010).

3.1.2 CDNF/MANF Family of Neurotrophic Factors

Cerebral dopamine neurotrophic factor (CDNF) and mesencephalic astrocyte-derived neurotrophic factor (MANF) belong to a fourth class of neurotrophic factors that are structurally and functionally distinct from traditional target-derived NTFs (Lindahl, Saarma, & Lindholm, 2017). Unlike the traditional classes of NTFs, which are primarily secretory proteins, MANF and CDNF are typically retained intracellularly in the endoplasmic reticulum (ER) (Apostolou, Shen, Liang, Luo, & Fang, 2008). Mammals are known to have both the MANF and CDNF proteins whereas invertebrates such as *Drosophila melanogaster* only inherit a single protein with a higher sequence homology to MANF (Palgi et al., 2009). Unlike their mature GDNF counterparts, which only have seven cystine residues, nuclear magnetic resonance (NMR) spectroscopy studies of CDNF and MANF have revealed a two-domain protein with eight cysteine residues that form four disulphide bridges (Parkash et al., 2009). These proteins also represent the first evolutionarily conserved family of NTFs from *Caenorhabditis elegans* to humans (Petrova et al., 2003). A growing body of evidence supports the role of the CDNF/MANF protein family in neuroprotection and the maintenance of ER homeostasis; however, due to the scope of this study, only the structural and functional properties of MANF were explored in detail.

3.1.3 MANF Structure

Mesencephalic astrocyte-derived neurotrophic factor (MANF; originally known as arginine-rich, mutated in early stage tumors; ARMET) was first described by Petrova et

al. (2003). Human MANF is an 18 kDa protein, comprised of a 179 amino acid sequence with a 21 amino acid signal peptide that is cleaved to produce a mature protein containing 158 amino acids, encoded by a 4.3 kb gene with four exons located on human chromosome 3 (Figure 17A). The secondary structure of MANF is primarily comprised of alpha helices (47%) and random coils (37%) with a virtual absence of β-sheets (Petrova et al., 2003). The crystal structure of MANF revealed a well-defined saposin-like protein (SAPLIP) N-terminal domain (residues 1-95) and an equally defined carboxy-terminal domain (residues 104-158) that is homologous to the SAF-A/B, Acinus, and PIAS (SAP) protein superfamily connected by a short, flexible linker sequence (residues 96-103) (Figure 17B; Hellman et al., 2011; Parkash et al., 2009). The unique structure of MANF is believed to contribute to its bifunctional role as a secreted neurotrophin and intracellular protein (Parkash et al., 2009).

3.1.3.1 Structural Significance of the N-terminal Domain

MANF's N-terminal domain has been proposed to contribute to its neurotrophic properties at dopaminergic neurons (Parkash et al., 2009). The N-terminal domain also contains a 21 amino acid signal sequence which is responsible for translocating MANF to the ER (Figure 17A; Mizobuchi et al., 2007). SAPLIP's are a diverse family of proteins that possess the ability to interact with lipids and/or membranes (Bruhn, 2005). The saposin-like fold at the N-terminal domain consists of five α-helices and a single 3_{10} helix that form a "closed leaf" conformation with a hydrophobic core stabilized by three disulphide bridges (Hellman et al., 2011). Additionally, the N-terminal domain contains positively charged lysine and arginine residues that are hypothesized to mediate lipid

interactions (Parkash et al., 2009). However, there is limited published data regarding potential lipid interactions with MANF (Lindahl et al., 2017).

3.1.3.2 Structural Significance of the C-terminal Domain

Moreover, the SAP-like C-terminal domain of MANF has been implicated in its antiapoptotic properties and the modulation of ER homeostasis (Hellman et al., 2011; Sawada, Hayes, & Matsuyama, 2003). The C-terminal domain of human MANF consists of three α-helices (α6, α7, and α8) with α7 and α8 forming a helix-loop-helix DNA binding motif (Hellman et al., 2011). The α7 and α8 helices are linked via a cystine bridge located in a CXXC motif^{127}CKGC^{130} in mature human MANF (Figure 17B). This cystine bridge has been implicated in MANF's intracellular survival-promoting properties and extracellular cytoprotective activities (Matlik et al., 2015). MANF's C-terminal domain is also homologous to Ku antigen p70 subunit (Ku70) proteins. Ku70 is known to prevent apoptosis by inhibiting the activity of proapoptotic factors such as BCL2-associated X (BAX) protein (Sawada et al., 2003). Given its structural homology, Hellman et al. (2011) hypothesized that MANF may exert its antiapoptotic actions via BAX-mediated interactions. Furthermore, MANF contains an arginine-threonine-aspartic acid-leucine (RTDL) amino acid sequence at the end of its C-terminal which is homologous to the lysine-aspartic acid-glutamic acid-leucine (KDEL) sequence involved in ER retention (Figure 17AB; Raykhel et al., 2007). KDEL receptors in the Golgi recognize the RTDL sequence and signal MANF back to the ER. Interestingly, cleavage of the RTDL sequence increases MANF secretion from primary neurons *in vitro* (Henderson, Richie, Airavaara, Wang, & Harvey, 2013). Thus, the C-terminal domain is also central to MANF's functional role in the ER.

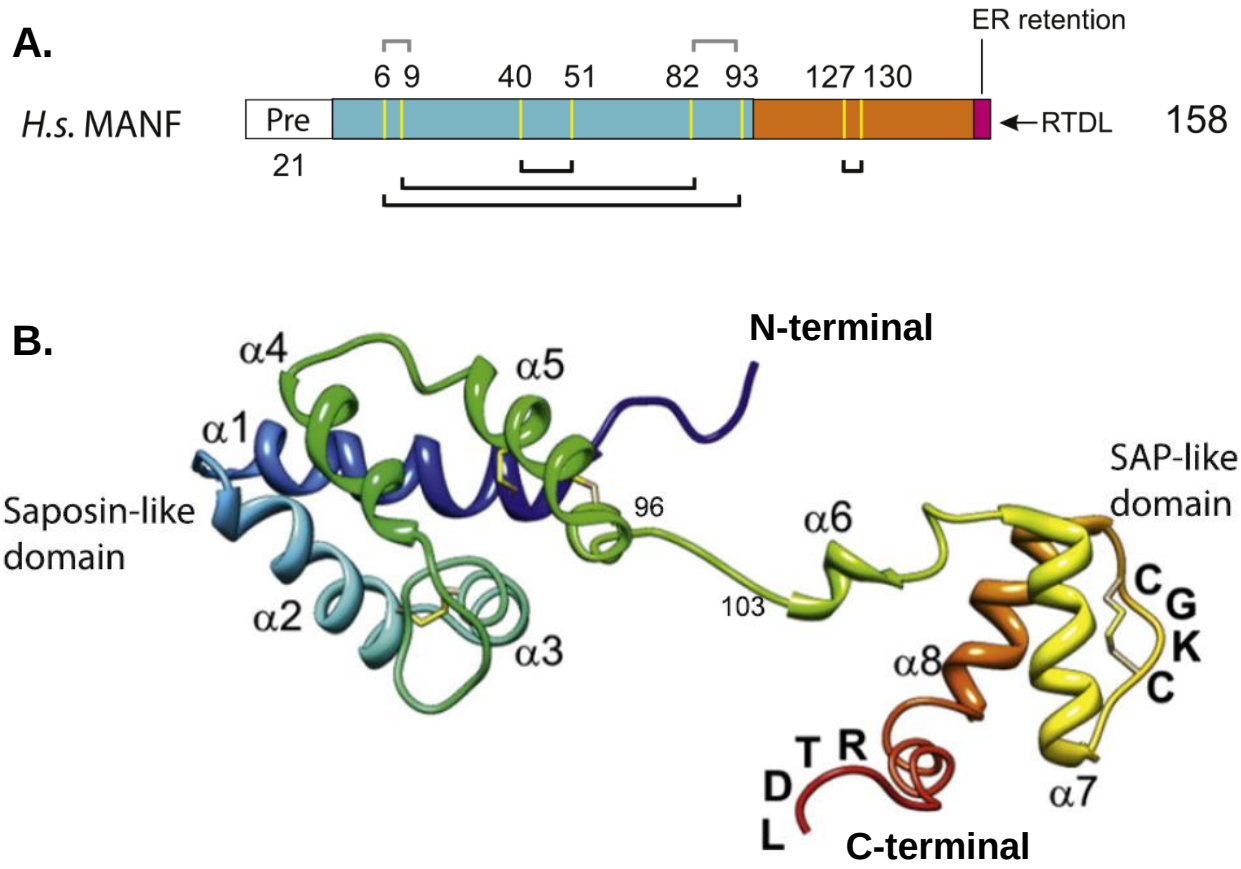


Figure 17. (A) Schematic representation of the primary structure of human MANF. The mature protein contains a unique 158 amino acid sequence with an RTDL endoplasmic reticulum retention sequence at the end of the C-terminal domain. The saposin-like domain is depicted in blue and the SAP-like domain is represented in orange. Cysteine residues are represented in yellow and numbered according to the mature protein sequence. Disulphide bridges are indicated by black connecting lines described by Parkash et al. (2009) and grey connecting lines represent suggested disulphide bridges described by Mizobuchi et al. (2007). **(B) NMR solution structure of human MANF.** The structure consists of an N-terminal saposin-like domain (residues 1-95) and a C-terminal SAP-like domain (residues 104-158) as described by Hellman et al. (2011). The linker region is defined as residues 96-103. The CXXC motif and ER retention signal (RTDL) are also depicted. *Images adapted from Lindahl et al. (2017).*

3.1.4 Physiological Function and Therapeutic Role of MANF

MANF was originally regarded for its neuroprotective and neurorestorative properties specific to midbrain dopaminergic neurons *in vitro* (Petrova et al., 2003).

Consistent with these findings MANF, has also been shown to potently protect and repair dopaminergic neurons in 6-hydroxydopamine (6-OHDA) models of Parkinson's disease and rescue cortical neurons in rat ischemic stroke models (Airavaara et al., 2009; Zhang et al., 2017). It is also known to protect against TATA box binding protein-mediated Purkinje cell degeneration (Yang, Huang, Gaertig, Li, & Li, 2014). In non-neuronal cells, $MANF^{-/-}$ mice develop severe insulin-dependent diabetes due to postnatal reductions in beta cell mass from increased apoptosis. Interestingly, recombinant MANF delivery significantly increased beta cell proliferation *in vivo* (Lindahl et al., 2014). MANF is also recognized as an intracellular protein that is upregulated via the unfolded protein response (UPR) during periods of ER stress. Upregulation of MANF has been shown to inhibit ER stress-induced cell death, which may contribute to its cytoprotective properties (Apostolou et al., 2008). Despite its therapeutic potential, the mechanisms underlying the cellular and neuroprotective functions of MANF and its surface receptors remain elusive.

3.1.5 Endoplasmic Reticulum (ER) Stress & MANF

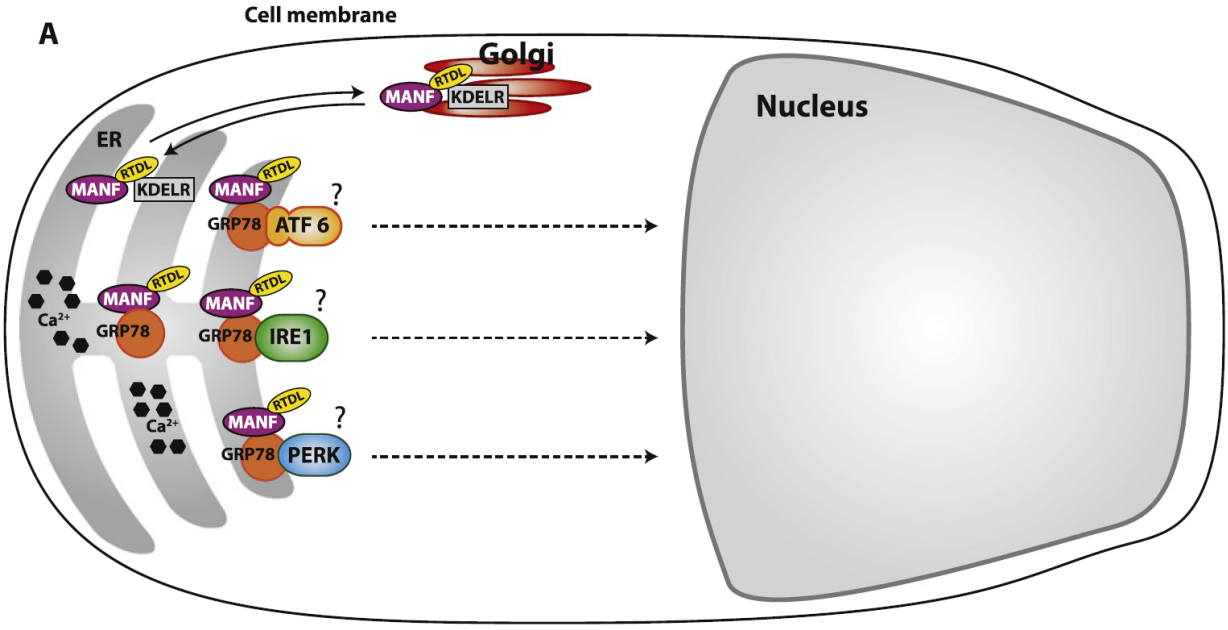
In eukaryotic cells, the ER is an organelle responsible for the synthesis, modification, and delivery of proteins to their target sites (Schroder & Kaufman, 2005). It is also an important site for calcium storage and lipid synthesis (Apostolou et al., 2008). However, these processes are susceptible to pathological perturbations such as pharmacological manipulations, genetic mutations, oxidative stress, and viral infections that can lead to the accumulation of misfolded or unfolded proteins, known as ER stress (Szegezdi, Logue, Gorman, & Samali, 2006). Cells utilize protein quality control systems such as ER-associated degeneration (ERAD) to counteract the effects of abnormal protein folding and prevent them from being secreted (McCracken & Brodsky, 2003).

However, during periods of prolonged ER stress, abnormal protein accumulation exceeds the functional capacity of ERAD. Thus, chronic ER stress has been implicated in a variety of pathological conditions including diabetes mellitus, cerebral ischemia, and degenerative disorders such as Alzheimer's disease and amyotrophic lateral sclerosis (Lindahl et al., 2017).

Excessive ER stress results in the activation of the UPR. The UPR is a temporary response mechanism that functions to restore ER homeostasis by inhibiting the translation of misfolded proteins, clearing unfolded proteins via ERAD, and upregulating molecular chaperones that specialize in normal protein folding (Lindahl et al., 2017; Schroder & Kaufman, 2005). Furthermore, ER stress is detected by three transmembrane UPR sensors: (1) pancreatic endoplasmic reticulum kinase (PERK); (2) activating transcription factor 6 (ATF6); and (3) inositol-requiring enzyme 1 (IRE1) (Lindahl et al., 2017). Under normal conditions, the glucose regulated protein 78 (GRP78) chaperone binds to the UPR sensors, rendering them inactive. Under ER-stress conditions, GRP78 is dissociated from the sensors to activate the UPR (Bertolotti, Zhang, Hendershot, Harding, & Ron, 2000). During periods of prolonged ER stress in which the UPR is unable to alleviate ER stress, higher order eukaryotes will initiate proapoptotic pathways in a final attempt to eliminate unhealthy cells and restore normal ER functioning (Szegezdi et al., 2006).

MANF mRNA and protein levels are upregulated during periods of ER stress *in vitro* and *in vivo* (Lindahl et al., 2017). For example, MANF expression is upregulated in *in vivo* mouse models of myocardial infarction and in culture medium of neonatal rat ventricular myocytes *in vitro* in the presence of ER stress (Tadimalla et al., 2008).

Likewise, MANF has an ER stress response element II (ERSEII) located on its promoter region that is recognized by UPR sensors (Oh-Hashi, Hirata, & Kiuchi, 2013). During ER homeostasis, MANF is usually located on the luminal side of the ER. However, during periods of ER stress, MANF secretion increases (Tadimalla et al., 2008). MANF retention in the ER is suggested to be the result of calcium-dependent interaction with GRP78 and KDEL receptors. Under normal conditions, MANF is retained in the ER by forming a calcium dependent complex with GRP78 and KDEL receptors. During periods of calcium-deficient ER stress, MANF dissociates from GRP78 and MANF secretion is increased (Glembotski et al., 2012). Interestingly, pancreatic beta cells of MANF$^{-/-}$ mice at E18.5 exhibit increased activation of UPR pathways prior to any signs of beta cell failure, suggesting that MANF is involved with keeping UPR sensors inactive (Lindahl et al., 2014). Thus, it has been speculated that the calcium-depended interaction between GRP78 and MANF is necessary for GRP78 to remain bound to UPR sensors (Lindahl et al., 2017). However, direct evidence supporting the interaction of MANF with GRP78 is still lacking.



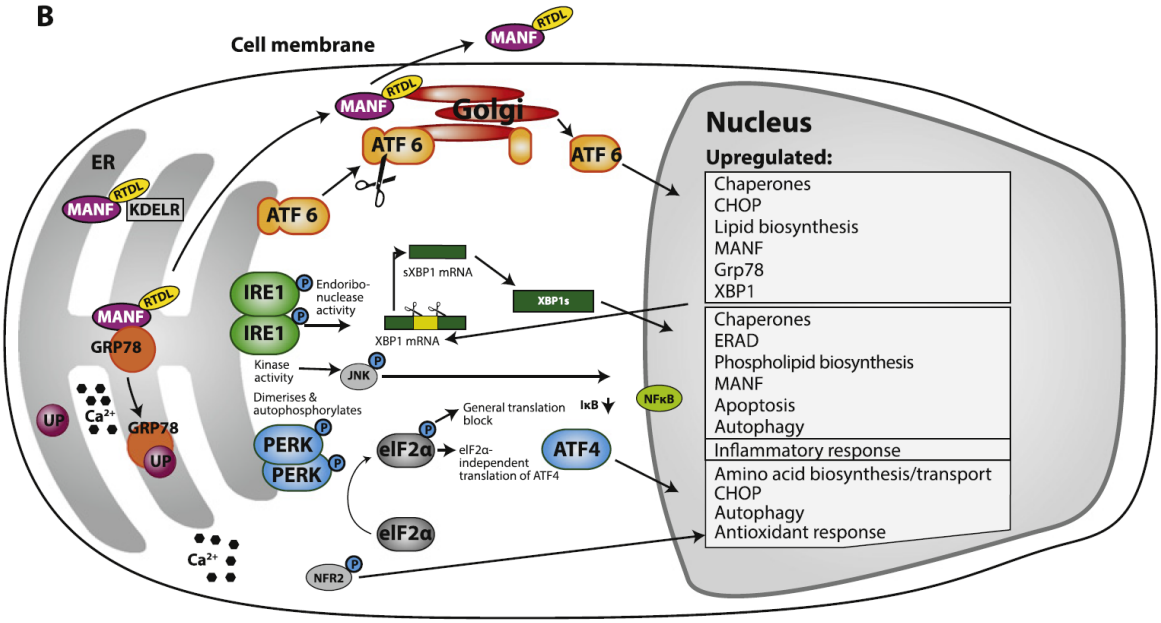


Figure 18. Hypothetical illustration of MANF's role in the ER. (A) Normal physiological conditions. In the absence of ER stress, MANF is retained in the ER via its RTDL sequence and interactions with GRP78 (Henderson et al., 2013). KDEL receptors (KDELR) in the Golgi recognize MANF's RTDL sequence and translocate MANF back to the ER. In the ER, MANF forms a calcium-dependent complex with GRP78 that remains bound to the luminal domain of UPR sensors (i.e. ATF6, IRE1, and PERK), rendering them inactive. **(B) Unfolded protein response during periods of ER Stress.** The presence of unfolded proteins (UP) initiates a state of ER stress. Periods of ER stress result in calcium depletion from the ER which may interfere with the calcium-dependent interaction between MANF and GRP78 (Glembotski et al., 2012). Thus, in the presence of ER stress, GRP78 binds to unfolded proteins and dissociates from MANF and UPR sensors causing them to become active. MANF may then be secreted to the extracellular space via its interactions with KDELR's. Active ATF6 translocates to the Golgi where it is cleaved by proteases and functions as a transcription factor to upregulate genes involved in protein folding and the expression of GRP78, MANF, CHOP, and other ER stress-related proteins. It also controls genes that encode for components of ERAD. Activated IRE1 leads to apoptosis of affected cells, reduces the translational workload and regulates autophagy genes in beta cells. Activated PERK inhibits general protein translation and increases the translation of ATF4. ATF4 upregulates the expression of pro-survival genes involved in the regulation of redox balance and apoptosis (Reviewed in Lindahl et al. 2017). *Images adapted from Lindahl et al. (2017).*

3.1.6 Schizophrenia: Degenerative or Developmental?

An abundance of evidence suggests that SZ is a neurodevelopmental disorder that is influenced by various genetic and environmental insults (Piper et al., 2012). It has been proposed that disruptive events during prenatal and perinatal development such as genetic mutations, nutritional abnormalities, infection, and pregnancy and/or birth complications may alter the trajectory of brain growth and involution over a lifespan (Piper et al., 2012). However, patients with SZ also show signs of degenerative processes. A plethora of neuroimaging studies have revealed a series of structural brain alterations in patients with SZ (Kochunov & Hong, 2014). One of the most consistent findings from these studies is that SZ patients have enlarged lateral ventricles and reduced regional brain volume in the frontal operculum and lateral temporal lobes (S. Patel, Sharma, Kalia, & Tiwari, 2017). Additional post-mortem studies have also revealed signs of cortical apoptosis and reduced hippocampal volume (Rund, 2018). Other studies have also revealed that the onset of psychosis is significantly associated with progressive reductions in cortical volume in the PFC (Dietsche, Kircher, & Falkenberg, 2017). Additionally, greater reductions in gray matter are more prevalent in poor-outcome patients (Sun et al., 2009). However, the topic of whether these morphological changes progress following the onset of illness is still subject to debate. Regardless, these findings highlight the importance of early treatment intervention to improve patient outcomes.

3.1.7 Schizophrenia and ER Stress

It has been documented that the interplay between reactive oxygen species (ROS) and ER stress may play a central role in the deterioration of untreated SZ patients (Kim, Kim, Rhie, & Yoon, 2015; S. Patel et al., 2017). The unique oxidative environment of the

ER requires tight regulatory control. Oxidative protein folding in the ER is dependent on homeostatic redox conditions which are disrupted during periods of ER stress (Kritsiligkou et al., 2018). Disruptions in the oxidative environment of the ER can lead to the production and accumulation of ROS. Furthermore, ER stress response proteins such as C/EBP homologous protein (CHOP) have also been shown to contribute to the generation of ROS and oxidative stress (Song, Scheuner, Ron, Pennathur, & Kaufman, 2008). Neuronal cells are particularly sensitive to oxidative stress due to their dependence on oxidative phosphorylation and their relatively poor antioxidant concentrations compared to other cell types. ROS toxicity results in apoptosis, gliosis, and further misfolding of proteins which are main culprits in the progression of neurodegenerative conditions (Chen, Guo, & Kong, 2012). Additionally, oxidative stress has also been shown to cause hypofunctional NMDAR's and to impair NMDA-dependent long-term potentiation (Koga, Serritella, Sawa, & Sedlak, 2016). Thus, it is plausible that prolonged periods of oxidative stress that arise from genetic or environmental insults during key developmental periods may also lead to a state of ER-stress and contribute to changes in brain morphology and the pathophysiological abnormalities observed in SZ patients.

3.1.8 Implications for Antipsychotic Drugs

A distinguishing feature among APDs is their potential to promote neuroprotection and neurogenesis (Kusumi et al., 2015). In general, typical antipsychotics such as haloperidol facilitate neuronal apoptosis and may even contribute to the abnormal brain morphology observed in SZ. For example, chronic haloperidol treatment has been associated with a greater reduction in gray matter volume in patients with SZ (Lieberman, Tollefson, et al., 2005) In contrast, the atypical APDs risperidone and quetiapine have

both been shown to protect against apoptosis in neuronal cell culture (Qing, Xu, Wei, Gibson, & Li, 2003). Furthermore, olanzapine has also been shown to protect against ischemia and NMDAR antagonist-induced neuronal damage (Dickerson & Sharp, 2006; Kurosawa et al., 2007). Previous studies have also indicated that haloperidol does not facilitate neurogenesis (Halim, Weickert, McClintock, Weinberger, & Lipska, 2004). However, olanzapine has been shown to stimulate neurogenesis in adult rat brains, which is an important mechanism in the prevention of brain volume reduction over the course of diseases (Lieberman, Tollefson, et al., 2005). Kurosawa et al. (2007) also demonstrated that olanzapine potentiates neuronal survival and neural stem cell differentiation through the regulation of ER stress response proteins, further implicating the ER stress response in the pathophysiology of SZ. Interestingly, upregulated MANF messenger RNA (mRNA) expression was observed in the PFC and COR of rats treated with lithium, a commonly used mood-stabilizing agent for the treatment of bipolar disorder (Prashar, 2017). Taken together, these findings suggest that atypical APDs may exert their neuroprotective and neurogenic effects through the modulation of ER-stress specific neurotrophic factors that may be potential therapeutic targets for SZ.

3.2 Aims and Hypotheses

The objective of this study was to determine if the behavioural changes observed in “Chapter 2” could be associated with fluctuations in MANF mRNA expression. This study aimed to determine whether lurasidone exerts its therapeutic actions through the modulation of MANF expression in different brain regions via an *in vivo* rat model of SZ. This study also sought to further implicate ER stress in the pathophysiology of SZ.

Hypotheses:

1. Sub-chronic treatment with only lurasidone would upregulate MANF mRNA expression in some or all of the brain regions analyzed.
2. Sub-chronic treatment with only MK-801 would downregulate MANF mRNA expression in some or all of the brain regions analyzed.
3. Sub-chronic pre-treatment with lurasidone would prevent MK-801-induced reductions in MANF mRNA expression.

3.3 Materials and Methods

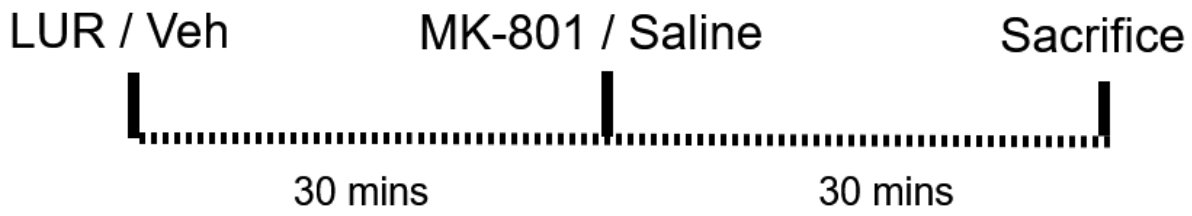
3.3.1 Animals

The sub-chronically treated rats from “Chapter 2” (Catalogue #273; N = 30) were used to evaluate changes in MANF mRNA expression. All rats were housed and handled according to the conditions outlined in “Chapter 2”. All animal procedures were approved by McMaster University’s *Animal Research Ethics Board* (AUP: 14-08-28; 18-06-27) in accordance with the ethical policies outlined by the *Canadian Council on Animal Care*.

3.3.2 Drug Treatment & Sacrifice

Rats received daily intraperitoneal injections of lurasidone (3.0 mg/kg) or vehicle (0.2% Tween-20 / 0.5% methylcellulose) followed by MK-801 (0.35 mg/kg) or saline. MK-801, saline, Tween-20, and methylcellulose were obtained from Millipore Sigma (Oakville, ON) and lurasidone was donated by Sunovion Pharmaceuticals Inc. (Marlborough, MA). All rats were sacrificed 30 minutes after the lurasidone injection on day 10 (Figure 19). On the day of sacrifice, rats were heavily anesthetized with isoflurane gas and monitored for loss of the foot withdrawal reflex in response to a toe pinch. Following the loss of the

withdrawal reflex, rats were rapidly decapitated. The brain was de-skulled, removed, and sectioned with the following brain regions from both hemispheres being harvested for analysis: (1) medial prefrontal cortex (mPFC); (2) PFC; (3) cortex (COR); hippocampus (HIP); striatum (STR); and substantia nigra (SN). All tissues were stored at -80ºC for



further analysis.

Figure 19. Relative injection timeline. Rats were pre-treated daily with LUR (3.0 mg/kg; i.p.) or Vehicle (Veh) 30 minutes prior to receiving MK-801 (0.35 mg/kg; i.p.) or saline for 10 days. Rats were sacrificed 30 minutes after the last injection on Day 10.

3.3.3 RNA Extraction from Rat Tissue

RNA was extracted using TRIzol reagent (Thermo Fisher Scientific; Waltham, MA). On day one, tissues were hand-homogenized in one millilitre of TRIzol reagent using autoclaved pestles until the sample was completely dissolved in TRIzol solution. Samples were then incubated at room temperature for five minutes. Following incubation, 200 uL of chloroform was added to each sample for every one millilitre of TRIzol reagent used. Each sample was shaken vigorously for 15 seconds and incubated for another 15 minutes at room temperature. All samples were then centrifuged at 12,000g for 15 minutes at 4ºC. Following centrifugation, the colourless RNA phase was removed and transferred to a new (autoclaved) 1.7 mL Eppendorf tube. Next, 500 uL of isopropanol was added to each sample for every one millilitre of TRIzol reagent used. The samples were shaken

vigorously for 15 seconds and allowed to incubate at room temperature for 10 minutes. All samples were then centrifuged again for 10 minutes at 12,000g at 4ºC. The remaining supernatant was discarded without disrupting the RNA pellet. Subsequently, one millilitre of 75% ethanol (prepared in diethyl pyrocarbonate (DEPC)-treated water) was added to each sample and all samples were stored at -80ºC for up to 24 hours.

On day two, all samples were thawed and vortexed thoroughly. The samples were centrifuged for five minutes at 7,500g at 4ºC. The supernatant was discarded without disrupting the RNA pellet and the samples were briefly centrifuged for 10 seconds. Residual ethanol was then removed with a P10 pipette and the RNA pellet was allowed to air dry for approximately 10 minutes. Each RNA pellet was then resuspended in 40 uL of DEPC-treated RNase-free water and incubated at 60ºC for 10 minutes in a preheated water bath. The samples were centrifuged again for 10 seconds and then vortexed thoroughly. RNA quantity and purity were then assessed using a NanoDrop One^{C} Microvolume UV-Vis spectrophotometer (Thermo Fisher Scientific; Waltham, MA), measuring absorption at 260 nm and 280 nm. The samples were then treated with a DNase I enzyme (Thermo Fisher Scientific; Waltham, MA) to enhance the purity by removing contaminating DNA. RNA quantity and purity were reassessed measuring absorbance values at 260 nm and 280 nm.

3.3.4 cDNA Synthesis from Rat RNA

Rat RNA was diluted to the lowest concentration among the samples using DEPC water. Any samples with an RNA concentration below 25 ng/uL were excluded from analysis. The isolated RNA was then converted to complementary DNA (cDNA) via reverse transcription using qScript cDNA SuperMix (Quanta Biosciences; Gaithersburg,

MD) at a ratio of four microlitres of qScript to 16 uL of RNA sample. Samples were then vortexed, quickly spun, and placed in a Mastercycler® Gradient (Eppendorf Canada Ltd; Mississauga, ON). All reactions occurred according to the following thermal profile: five minutes at 25ºC, 30 minutes at 42ºC, followed by five minutes at 85ºC, with a final hold at 4ºC.

3.3.5 Real-Time Quantitative Reverse Transcription PCR

Real-time quantitative reverse transcription PCR (RT-qPCR) was used to quantify MANF mRNA expression using the cDNA prepared from the rat brain tissue. RT-qPCR was performed using a QuantiFast SYBR Green Fluorescent dye PCR kit (Qiagen Inc; Toronto, ON). MANF and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers were synthesized at MOBIX (McMaster University; Hamilton, ON) and prepared at a concentration of five uM (Table 2). All samples were tested in triplicate with each reaction consisting of two microlitres of sample cDNA, 12.5 uL QuantiFast SYBR Green dye, five microlitres of forward primer, five microlitres of reverse primer, and 0.5 uL of nuclease-free water to a final volume of 25 uL per reaction. Absolute copy numbers were derived from a standard curve using 10-fold serial dilutions of cDNA ranging from one picogram to one attogram. The PCR was performed using a Stratagene MX3000P cycler (Qiagen Inc.) under the following thermal profile: (1) heat activation of 95ºC for five minutes; followed by 40 cycles of (2) denaturing at 95ºC for 10 seconds; and (3) combined annealing and extension step for 30 seconds at 60ºC. RT-qPCR conditions were optimized to ensure that amplification efficiencies remained constant over the course of the run. Relative MANF mRNA expression was determined using raw threshold cycle (Ct) values with GAPDH as the housekeeping gene.

Table 2. RT-qPCR Primer Sequences. List of specific primers and their respective sequence used for quantification of MANF mRNA expression.

Gene	Primer	Product Size	Primer Sequence (5' → 3')
Rat GAPDH	Forward	118 bp	5' – CAACTCCCTCAAGATTGTCAGCAA – 3'
Rat GAPDH	Reverse		5' – GGCATGGACTGTGGTCATGA – 3'
Rat MANF	Forward	129 bp	5' – CGGTTGTGCTACTACATTGGA – 3'
Rat MANF	Reverse		5' – CTGGCTGTCTTTCTTCTTCAGC – 3'

3.3.6 Statistical Analyses

Relative MANF mRNA expression was calculated using the delta Ct (ΔΔCt) method of analysis as described by Schmittgen and Livak (2008). Ct values were derived from the amplification plot generated by the MX3000P software. The Ct value represents the number of cycles necessary for the fluorescence of the PCR product to be detected above the background signal. The Ct value was defined by the intersection of the amplification plot with a predetermined fluorescence threshold value during the exponential phase of the reaction. The Ct values used for analysis represent the average of the triplicate Ct values for each sample. Any triplicates that varied by ± one cycle per sample were excluded from the analysis and the remaining duplicate values were averaged. The Ct values of MANF were normalized to GAPDH Ct values using the following equation:

$$2^{-\Delta\Delta Ct} = 2^{-\left((Ct_{MANF} - Ct_{GAPDH})sampleA\right) - \left((Ct_{MANF} - Ct_{GAPDH})sampleB\right)}$$

All statistical tests were performed using GraphPad Prism 8.0 software (GraphPad Software; San Diego, CA). Changes in relative mRNA expression and average GAPDH Ct values were analyzed using an ordinary one-way ANOVA with Tukey's HSD multiple

comparisons test to determine differences between groups. Moreover, normality was confirmed using a Shapiro-Wilk test. Statistical outliers were removed using Grubbs' Outlier test (Grubbs, 1969). Statistical significance was determined as $p < 0.05$. All data was reported as mean ± SEM.

3.4 Results

Ordinary one-way ANOVA did not reveal a significant difference in MANF mRNA expression between treatment groups in the mPFC ($F = 0.06944$, $p = 0.9757$; Figure 20), HIP ($F = 1.31$, $p = 0.2939$; Figure 21), or STR ($F = 2.351$, $p = 0.0955$; Figure 22). Moreover, ordinary one-way ANOVA revealed a significant difference between groups in the COR ($F = 3.034$; $p = 0.0487$; Figure 23) and PFC ($F = 11.61$; $p < 0.0001$; Figure 24). More specifically, Tukey's HSD multiple comparisons test revealed that treatment with both lurasidone and MK-801 significantly upregulated MANF mRNA expression by 142.6% ± 11.59 ($p = 0.0342$) in the COR relative to control rats, however, individual treatment with either compound had no effect on MANF mRNA expression (Figure 23). Furthermore, the greatest changes in MANF mRNA expression were observed in the PFC (Figure 24). Tukey's HSD multiple comparisons test revealed that treatment with only LUR upregulated MANF mRNA expression by 168.0% ± 7.57 ($p = 0.0290$) compared to controls whereas treatment with only MK-801 caused an 181.2% ± 15.79 increase in MANF mRNA expression ($p = 0.0030$). Likewise, treatment with both LUR and MK-801 upregulated MANF mRNA expression by 220.8% ± 15.99 ($p < 0.0001$; Figure 24). Furthermore, ordinary one-way ANOVA revealed a significant difference in GAPDH Ct values in the SN ($F = 16.3$, $p < 0.0001$; Figure 26E). However, GAPDH Ct values were stable across treatment groups for all other samples (Figure 26).

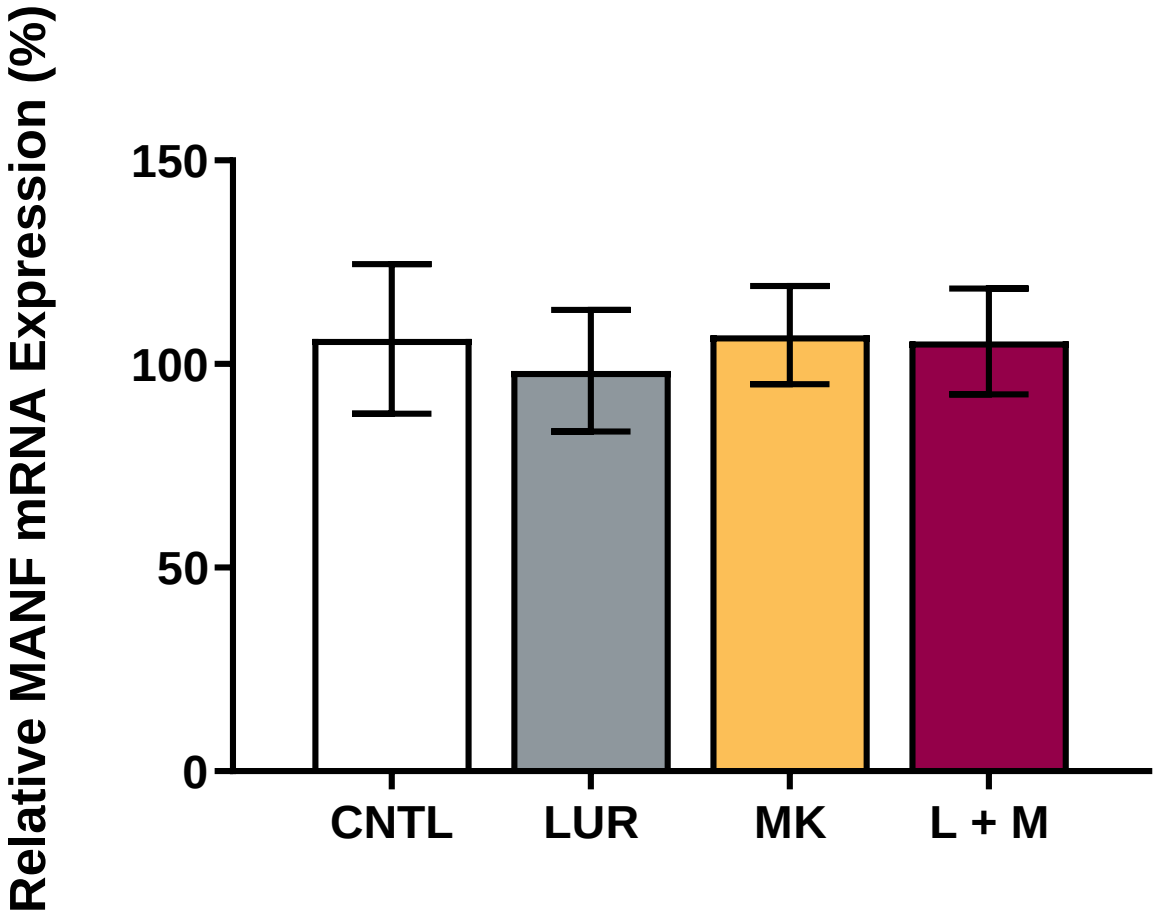


Figure 20. Relative MANF mRNA expression in the rat medial prefrontal cortex following treatment with LUR and MK-801 (CNTL, n = 6; LUR, n = 6; MK, n = 9; L + M, n = 9)**.** Data are presented as a percentage of the relative change in MANF mRNA expression normalized to the GAPDH housekeeping gene ($2^{-\Delta\Delta Ct}$ x 100%). Ordinary one-way ANOVA did not reveal a significant difference in MANF mRNA expression in the medial prefrontal cortex (F = 0.06944, p = 0.9757). Data represent mean ± SEM.

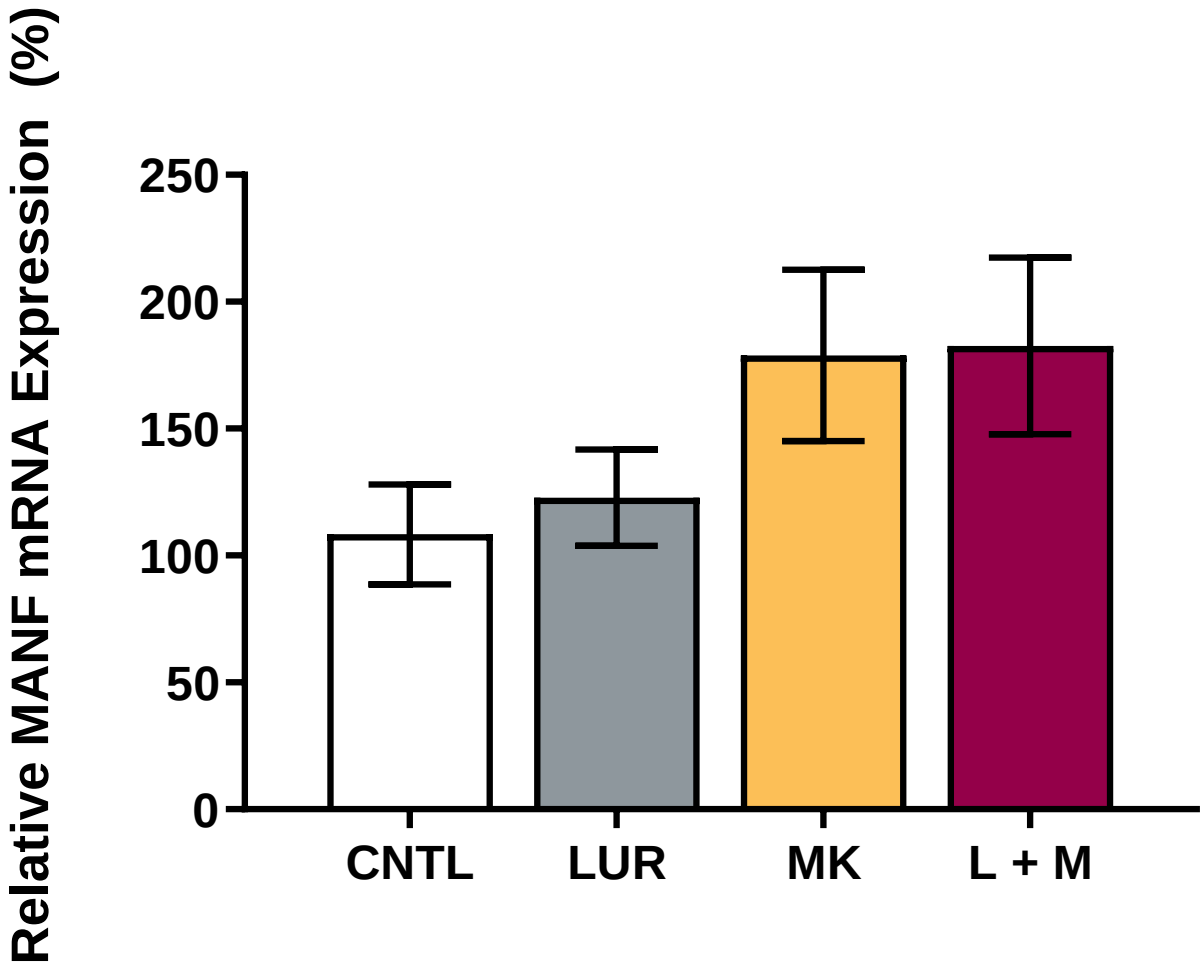


Figure 21. Relative changes in MANF mRNA expression in the rat hippocampus following treatment with LUR and MK-801 (CNTL, n = 6; LUR, n = 4; MK, n = 9; L + M, n = 8)**.** Data are presented as a percentage of the relative change in MANF mRNA expression normalized to the GAPDH housekeeping gene ($2^{-\Delta\Delta Ct}$ x 100%). Ordinary one-way ANOVA did not reveal a significant difference in MANF mRNA expression in the hippocampus (F = 1.314; p = 0.2939). Data represent mean ± SEM.

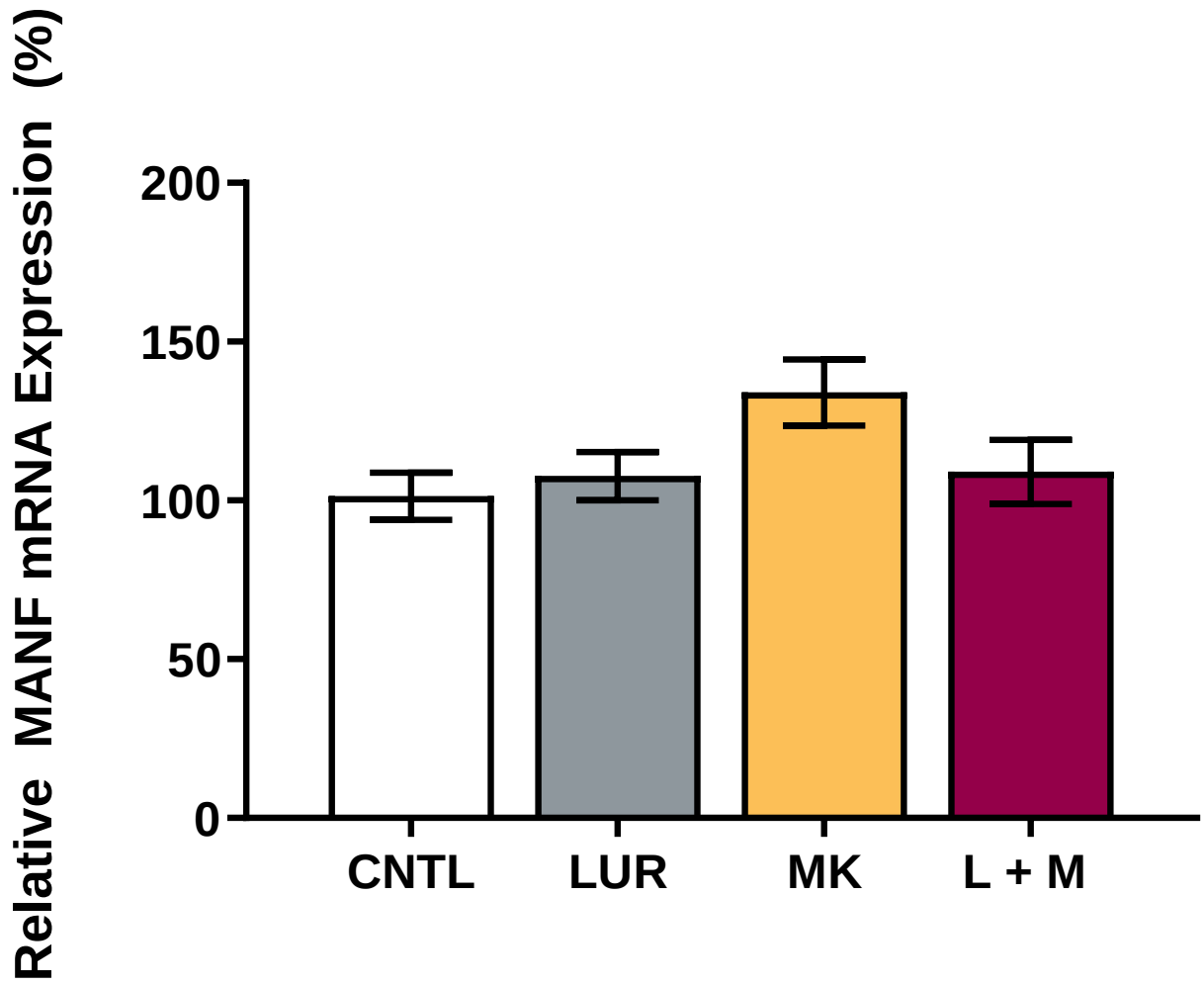


Figure 22. Relative changes in MANF mRNA expression in the rat striatum following treatment with LUR and MK-801 (CNTL, n = 6; LUR, n = 6; MK, n = 9; L + M, n = 9). Data are presented as a percentage of the relative change in MANF mRNA expression normalized to the GAPDH housekeeping gene ($2^{-\Delta\Delta Ct}$ x 100%). Ordinary one-way ANOVA did not reveal a significant difference in MANF mRNA expression in the striatum ($F = 2.351$; $p = 0.0955$). Data represent mean ± SEM.

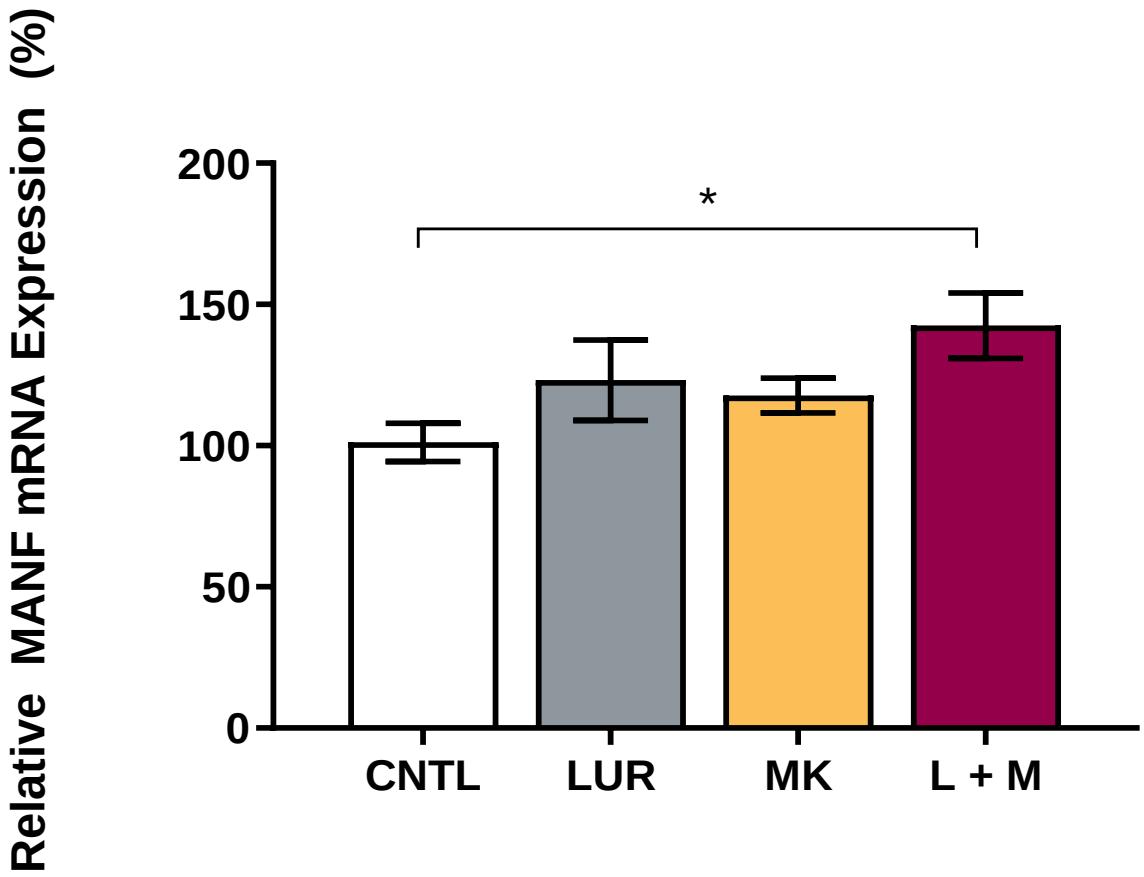


Figure 23. Relative changes in MANF mRNA expression in the rat cortex following treatment with LUR and MK-801 (CNTL, n = 6; LUR, n = 6; MK, n = 9; L + M, n = 9). Data are presented as a percentage of the relative change in MANF mRNA expression normalized to the GAPDH housekeeping gene ($2^{-\Delta\Delta Ct}$ x 100%). Ordinary one-way ANOVA revealed a significant difference in MANF mRNA expression in the cortex ($F = 3.034$; $p = 0.0487$). Tukey's HSD multiple comparisons test revealed that sub-chronic treatment with LUR and MK-801 together significantly increased MANF mRNA expression in the cortex compared to controls (*$p = 0.0342$). Treatment with LUR ($p = 0.5358$) or MK-801 ($p = 0.6647$) alone had no significant effect on MANF mRNA expression relative to control rats. No other significant differences were observed between groups. Data represent mean ± SEM.

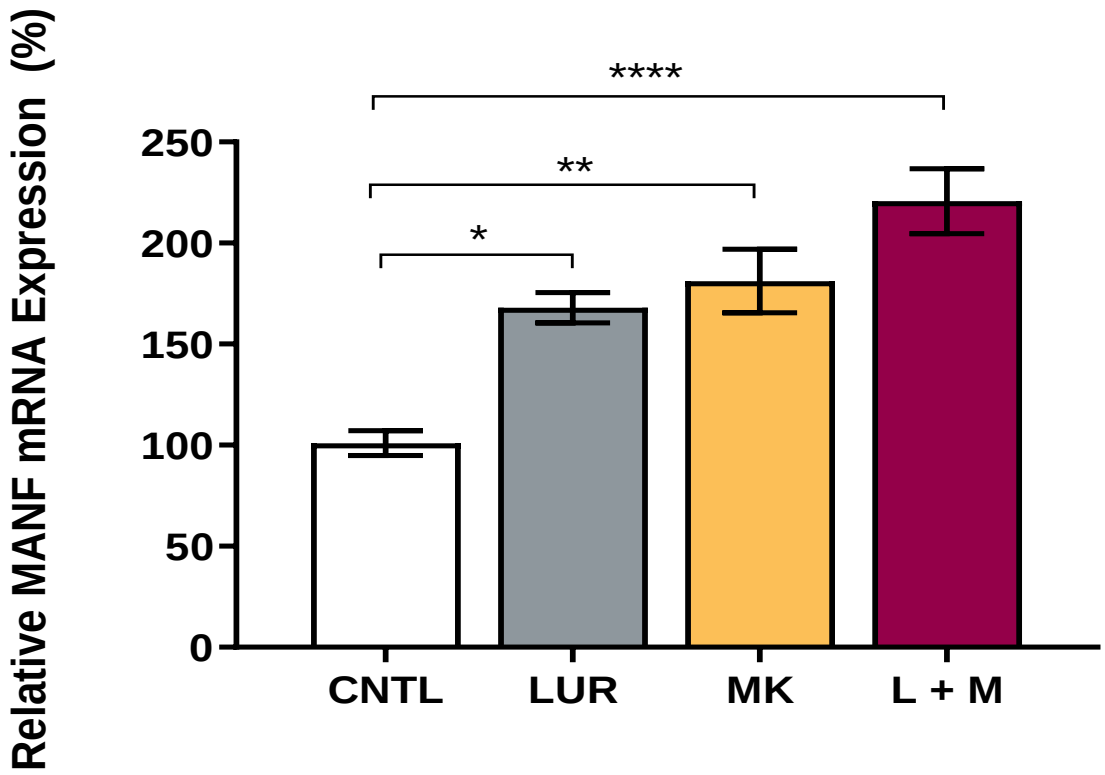


Figure 24. Relative changes in MANF mRNA expression in the rat prefrontal cortex following treatment with LUR and MK-801 (CNTL, n = 6; LUR, n = 6; MK, n = 9; L + M, n = 9). Data are presented as a percentage of the relative change in MANF mRNA expression normalized to the GAPDH housekeeping gene ($2^{-\Delta\Delta Ct}$ x 100%). Ordinary one-way ANOVA revealed a significant difference between groups ($F = 11.61$; $p < 0.0001$). Tukey's HSD multiple comparisons test revealed that sub-chronic treatment with LUR significantly increased MANF mRNA expression relative to control rats (*p = 0.0290). Interestingly, sub-chronic treatment with MK-801 also caused a significant increase in MANF mRNA expression relative to controls (**p = 0.0030). Lastly, treatment with both LUR and MK-801 caused the greatest increase in MANF mRNA expression relative to control rats (****p < 0.0001). No other significant differences were observed between groups. Data represent mean ± SEM.

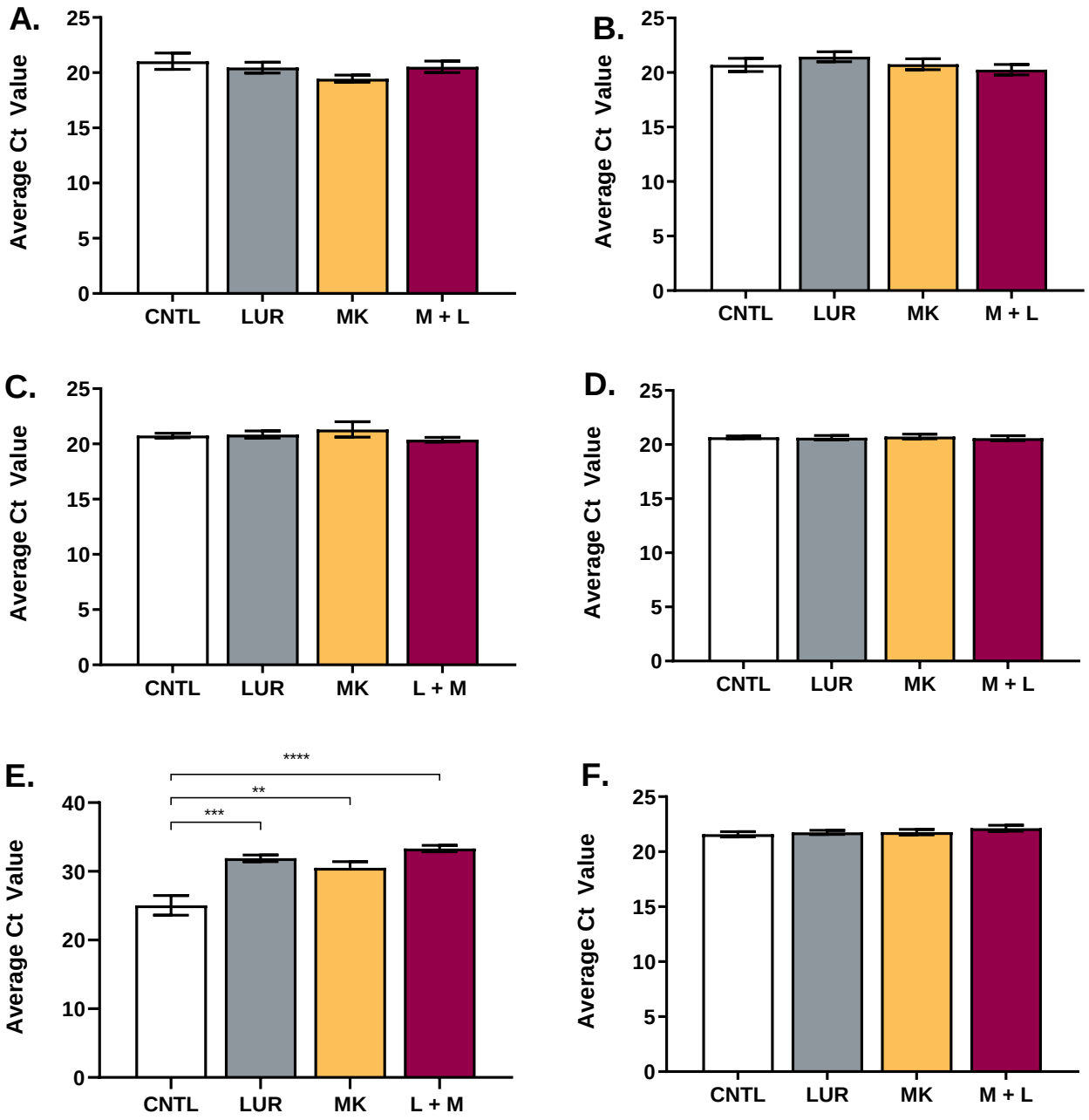


Figure 25. Summary of GAPDH housekeeping gene Ct values. Data are presented as an average of the individual GAPDH Ct triplicate values. Ordinary one-way ANOVA did not reveal a significant difference between treatment groups in the **(A)** mPFC ($F = 1.777$, $p = 0.1762$), **(B)** HIP ($F = 0.7558$, $p = 0.5298$), **(C)** STR ($F = 0.8424$, $p = 0.4831$), **(D)** COR ($F = 0.0993$, $p = 0.9596$), and **(F)** PFC ($F = 0.8497$, $p = 0.4794$). However, ordinary one-way ANOVA revealed a significant difference between groups in the **(E)** SN ($F = 16.30$, $p < 0.0001$). Tukey's HSD multiple comparisons test revealed that GAPDH Ct values were significantly increased following treatment with LUR (***$p = 0.0003$), MK (**$p = 0.001$), and L + M (****$p < 0.0001$). Data represent mean ± SEM.

3.5 Discussion

This study examined the effect sub-chronic lurasidone treatment (3.0 mg/kg; i.p) on MANF mRNA expression in a MK-801 (0.35 mg/kg; i.p.) model of SZ. Relative changes in MANF mRNA expression levels in each brain region were calculated via RT-qPCR and analyzed using the delta Ct method of analysis. This study demonstrated that sub-chronic treatment with LUR and/or MK-801 failed to alter MANF mRNA expression in the mPFC (Figure 20), HIP (Figure 21), and STR (Figure 22) relative to control rats. Additionally, only sub-chronic treatment with both lurasidone and MK-801 significantly upregulated MANF expression in the COR, whereas individual treatment with either compound had no effect on MANF expression (Figure 23). Interestingly, all 3 treatment groups significantly elevated MANF mRNA expression in the PFC compared to control rats (Figure 24). To the best of our knowledge this was the first study to investigate the relative changes in MANF mRNA expression in response to lurasidone treatment using an animal model of SZ. These results suggest a potential involvement of ER stress in the pathophysiology of SZ and implicate MANF as a potential therapeutic target of APDs for the treatment of SZ.

It was observed that sub-chronic treatment with only LUR significantly increased MANF mRNA expression in the PFC (Figure 24). This was an interesting observation given that most of the pathophysiological anomalies of SZ arise due to structural and molecular alterations in the PFC (Schwartz et al., 2012b; Sun et al., 2009). Additionally, the *NMDAR Hypofunction Hypothesis* posits that the symptoms of SZ arise due to aberrant NMDAR's at GABA interneurons in the frontal cortex (Figure 1). Interestingly, Zhou et al. (2006) showed that MANF is a potent enhancer of presynaptic GABAergic

synapses on nigral DA neurons. Furthermore, the sub-chronic studies from “Chapter 2” revealed that lurasidone significantly attenuated MK-801-induced deficits in the positive (Figure 9), negative (Figure 10), and cognitive domains of SZ (Figure 11). Thus, lurasidone may alleviate the symptoms of SZ via the modulation of MANF expression in the impaired PFC. Likewise, treatment with atypical APDs such as olanzapine and quetiapine have been shown to promote neuronal survival and stem cell differentiation *in vitro* via the modulation of ER chaperones such as GRP78 (Kurosawa et al., 2007). These findings suggest that the neuroprotective and neurogenic properties of atypical APDs, might be attributable to the cellular mechanisms involved in the attenuation of ER stress. Furthermore, it has been hypothesized that MANF fosters a calcium-dependent interaction with GRP78 under normal physiological conditions (Lindahl et al., 2017). During periods of ER stress, calcium depletion results in the dissociation of GRP78 from MANF leading to the activation of UPR sensors, which promote the downstream induction of genes that encode for molecular proteins that promote normal protein folding (Figure 18). Interestingly, the upregulation of MANF has also been associated with a reduction in ER stress-induced apoptosis following tunicamycin treatment *in vitro* (Apostolou et al., 2008). Thus, MANF may exert its cytoprotective effects in the ER through the modulation protein folding mechanisms. These findings are also consistent with previous reports that the relative expression of MANF mRNA in the PFC of Sprague Dawley rats was significantly upregulated approximately 1.5-fold following sub-chronic treatment with lithium, a commonly used mood stabilizer for the treatment of bipolar disorder, *in vivo* (Prashar, 2017). Therefore, it is plausible that lurasidone may act to alleviate the symptoms of SZ by inducing an overexpression of MANF in the impaired PFC. In the

future, it would be interesting to determine if the MANF mRNA expression patterns identified in the study follow similar trends *in vitro* and in humans.

Moreover, the average Ct values of the GAPDH housekeeping gene varied significantly between groups in the SN (Figure 25E). Thus, no legitimate conclusions could be made based on changes in MANF expression in the SN. This finding also suggests that treatment with LUR and/or MK-801 impacted GAPDH mRNA levels differently across brain regions. It is imperative for future studies to evaluate the effect of MANF mRNA expression in the SN using other rat housekeeping genes such as beta actin (ACTB) or phosphoglycerate kinase 1 (PGK1) (Svingen, Letting, Hadrup, Hass, & Vinggaard, 2015). These discrepant results may have also been due to the small size of the SN in the rat brain which made it difficult to obtain via manual sectioning techniques compared to larger brain regions. Thus, it is also possible that the changes in GAPDH Ct values in the SN were due to cross contamination with other brain regions during tissue sectioning.

MK-801 is believed to exert neurotoxic properties by interfering with intracellular calcium homeostasis, leading to ER stress (Chen et al., 2008). Furthermore, several preclinical studies have suggested that the blockade of NMDAR's leads to a paradoxical increase in glutamate activity in the PFC that can trigger excitotoxic neuronal injury in corticolimbic brain regions Figure 1; Ozyurt, Ozyurt, Akpolat, Erdogan, & Sarsilmaz, 2007; Plitman et al., 2014). Additionally, sub-chronic treatment with MK-801 displayed reversible neuropathological alterations specific to the posterior cingulate and retrosplenial cortices (Olney, Labruyere, & Price, 1989). Other NMDAR antagonists such as PCP have also been shown to decrease dendritic spine density in the PFC leading to

poor memory and cognitive performance (Hajszan, Leranth, & Roth, 2006). Therefore, the psychotomimetic and cognitive effects of MK-801 have been hypothesized to arise due to its neurotoxic effects in specific cortical regions (Olney et al., 1989). Periods of acute ER stress are known to upregulate MANF expression as the UPR attempts to restore ER homeostasis (Glembotski et al., 2012). In contrast, under severe and prolonged periods of ER stress, the UPR activates a series of proapoptotic pathways that lead to cell death (M. Wang, Wey, Zhang, Ye, & Lee, 2009). Thus, our original hypothesis proposed that the sub-chronic blockade of NMDAR's via MK-801-induced ER stress would disrupt MANF's calcium-dependent interaction with GRP78, resulting in MANF depletion and neuronal apoptosis. Interestingly, treatment with only MK-801 significantly upregulated MANF mRNA expression by approximately 1.75-fold in the PFC (Figure 24). Additionally, rats treated with only MK-801 also exhibited upregulated tendencies in MANF mRNA expression in the HIP (Figure 21) and STR (Figure 22), however these changes were not statistically significant.

One possible explanation for the upregulation of MANF mRNA expression in the PFC following MK-801 treatment is that sub-chronic treatment was not sufficient to result in substantial MANF depletion and the UPR was still active at the time of sacrifice. Furthermore, rats were sacrificed 30-minutes after the MK-801-injection to coincide with the behavioural timeline outlined in "Chapter 2" (Figure 4). Therefore, the increase in MANF expression following MK-801 treatment might be indicative of an acute ER-stress response. It would be interesting to analyze the temporal profile of MANF mRNA expression in the PFC following sub-chronic treatment with MK-801 by sacrificing at different time points relative to the MK-801 injection. Future studies should also evaluate

the effects of MK-801 on MANF mRNA expression using higher dosages and multiple daily injections to ensure a consistent and chronic blockade of NMDAR's. However, since the same rats were used for the behavioural experiments in "Chapter 2", it was not feasible to alter the treatment regimens without affecting rat performance on the behavioural paradigms.

Despite being neurotoxic to specific neuronal subpopulations under normal physiological conditions, MK-801 can also exert temporary neuroprotective effects against pre-existing NMDAR-mediated neurodegeneration (Olney et al., 1989). For example, treatment with MK-801 and/or PCP have been shown to protect CNS neurons against hypoxic-ischemia, hypoglycemic-, or epilepsy-related brain damage (all of which are believed to arise from hyperactive NMDAR activity; Farber et al., 2002). Under these conditions, it is believed that the temporary inhibition of NMDARs prevents degenerative processes when administered shortly after the insult (Kocaeli, Korfali, Ozturk, Kahveci, & Yilmazlar, 2005). Furthermore, coadministration of MK-801 with 6-OHDA partially attenuated 6-OHDA-induced neuronal damage by increasing cell viability in rat striatal, cerebrocortical, and hippocampal regions (Massari, Castro, Dal-Cim, Lanznaster, & Tasca, 2016). Interestingly, neuroprotective and neurorestorative effects of MANF have been demonstrated in 6-OHDA rat models of Parkinson's disease (Rangasamy, Soderstrom, Bakay, & Kordower, 2010). Thus, another plausible explanation for the upregulation of MANF mRNA expression in the PFC (Figure 24) is that MK-801 acts via a signalling pathways similar to lurasidone which exert their neuroprotective properties through the modulation of MANF expression. Future studies should confirm whether sub-chronic MK-801 treatment is adversely or therapeutically regulated by evaluating the

expression of ER-stress markers such as CHOP, GRP78, and/or BAX in response to LUR and MK-801 treatment.

3.6 Limitations and Future Directions

The first and most obvious limitation of this study was that it only examined the relative changes in MANF mRNA expression and not the total protein expression. Although, an upregulation in MANF mRNA expression might suggest a subsequent increase in protein expression, there are several post-transcriptional modifications that might interfere with protein translation. Thus, it is imperative for future studies to validate these results by examining the influence of sub-chronic LUR and MK-801 treatment on total MANF protein expression to determine if the upregulation of mRNA expression observed in this study correlates with relative changes in protein expression.

In vivo rat studies have demonstrated that MANF protein expression is developmentally regulated with peak expression levels observed during the first two weeks after birth and declining as the brain matures (Lindahl et al., 2017). This study examined the effects of lurasidone on MANF mRNA expression using a pharmacological model of SZ in adult rats. It might be more accurate to examine changes in MANF expression using a neurodevelopmental model of SZ or in adolescent rats, as this might more accurately correlate with MANF expression at the onset of psychosis.

Additionally, this was also the first report to examine relative changes in MANF mRNA expression in response to APD treatment in an animal model of SZ. Furthermore, this study was only conducted using a single rat cohort. Given the novelty of these findings, it would be beneficial to replicate these findings in a second cohort of rats. Likewise, future studies should also consider the effects of lurasidone on CDNF

expression in the rat brain. Unfortunately, due to complications with the CDNF primers in this study, it was not feasible to obtain accurate amplification values via RT-qPCR. Lastly, future studies should also aim to replicate the findings of this study in both cellular and human samples.

CHAPTER 4: INVESTIGATING THE EFFICACY OF INTRANASAL LURASIDONE ADMINISTRATION USING A POEGMA-BASED NANOGEL FORMULATION

4.1 Introduction

4.1.1 The Blood Brain Barrier

The blood brain barrier (BBB) is a highly selective, semipermeable barrier that separates the CNS from systemic circulation. The BBB consists of an intricate network of capillaries that vascularize the CNS and tightly regulate the movement of ions, molecules, and cells between the blood and the brain (Daneman & Prat, 2015). It is comprised of three elements of the brain microvasculature: (1) endothelial cells; (2) astrocyte end-feet; and (3) pericytes. Tight junctions between the endothelial cells form a barrier that selectively excludes blood borne substances from entering the brain (Ballabh, Braun, & Nedergaard, 2004). The specificity of the BBB prevents the passage of more than 98% of small molecules and nearly 100% of large molecules (> 500-Da) across the barrier (Pardridge, 2005). The precise regulatory control of the CNS protects neural tissue from harmful foreign substances such toxins and bacteria. Paradoxically, the BBB also poses a major challenge for delivering therapeutic molecules to the CNS in the case of neurological diseases (Lochhead & Thorne, 2012). Of the treatable CNS disorders, most are limited to “small molecule drug therapies”, which tend to be symptomatic treatments that require the systemic administration of excessive dosages to achieve a therapeutic response (Katare et al., 2015; Pardridge, 2005). Unfortunately, such large doses expose nontarget sites to unnecessarily high concentrations of drugs, which is often associated with adverse effects (Katare et al., 2015). Of more than 7000 small-molecule drugs, approximately five percent have been approved to treat only four CNS disorders including

depression, chronic pain, epilepsy, and schizophrenia (Pardridge, 2005). However, many of these molecules have limited aqueous solubility (i.e. lurasidone) and are restricted to oral administration. These drugs are often associated with poor neural bioavailability and metabolic side effects due to their exposure to hepatic and gastrointestinal metabolism (Lochhead & Thorne, 2012). Technically, neurotherapeutics can bypass the BBB via intracerebroventricular or intraparenchymal injections; however, due to the invasiveness and risk factors involved, these methods are not practical for instances where multiple dosing regimens are required (Dhuria, Hanson, & Frey, 2010).

4.1.1 Intranasal Delivery of Antipsychotic Drugs

Intranasal drug administration has historically been used to deliver therapeutic drugs to systemic circulation. However, a plethora of evidence now suggests that drug delivery to the CNS can be enhanced by exploiting innervating pathways in the nasal cavity (Katare et al., 2015). This provides a direct, non-invasive method to rapidly delivery therapeutic molecules from the nasal mucosa to the brain, while minimizing systemic exposure (Dhuria et al., 2010). Areas of the nasal cavity where the trigeminal and olfactory nerves terminate have been proposed as potential entry sites for drugs, molecules and/or peptides to directly bypass the BBB (Katare et al., 2017). From a pharmacokinetic perspective, direct nose-to-brain transport of drugs can also improve brain bioavailability by avoiding enzymatic degradation caused by exposure to the gastrointestinal tract and hepatic first pass effects (Katare et al., 2017). Thus, intranasal drug delivery offers several advantages compared to conventional oral and parenteral routes of administration; however, this method is also heavily restricted due to the volume of the nasal cavity (Table 3).

Most currently available APDs are limited to oral administration which requires entry into systemic circulation to reach the brain. Many atypical APDs are associated with severe metabolic and cardiovascular side effects (K. R. Patel et al., 2014). As a result, cardiovascular mortality rates among SZ patients are approximately 2.5 times greater than the general population (Saha et al., 2005). Scigliano and Ronchetti (2013) have proposed that the adverse effects of APDs are due to the blockade of peripheral dopamine receptors, which physiologically modulate sympathetic activity. Additionally, systemic circulation of antipsychotic drugs has also been shown to induce peripheral anticholinergic effects and increase the production of toxic hepatic metabolites (Igarashi, Kasuya, Fukui, Usuki, & Castagnoli, 1995; Lieberman, 2004). Thus, in bypassing systemic circulation via direct intranasal drug delivery, APDs may also improve the prognosis and quality of life of patients being chronically treated for SZ by reducing the risk of adverse effects. Interestingly, APDs have yet to receive regulatory approval for intranasal use (Katare et al., 2017).

Table 3. Summary of the advantages and challenges associated with intranasal drug administration. *Table adapted from Katare et al. (2017).*

Advantages	Challenges
→ Non-invasive technique	→ Limited deliverable volumes due to size of nasal cavity
→ Improved bioavailability due to bypass of hepatic and gastrointestinal metabolism	→ Limited aqueous solubility restricts drug formulation due to limited size of nasal cavity
→ Reduced effective dose due to direct transport	→ Prone to enzymatic degradation and rapid clearance in the nasal cavity
→ Rapid onset of pharmacological effects	→ Limited drug absorption due to rapid mucociliary clearance
→ Reduced side effects	→ Potential irritation to nasal mucosa

4.1.2 Mechanisms of Intranasal Drug Delivery

The precise mechanisms of intranasal drug delivery to the CNS are not fully understood. However, increasing evidence suggests that intranasal drug delivery occurs along the olfactory and trigeminal nerve pathways in the nasal epithelium, which provide direct access to the olfactory bulbs and brainstem, respectively (Dhuria et al., 2010). Thus, molecules must be transported across epithelial barriers and then migrate from the nasal mucosa to designated points of entry (Figure 26). From these initial entry points, the delivered molecules can then be distributed to other regions of the CNS. The proposed pathways of intranasal drug delivery are summarized in Figure 26.

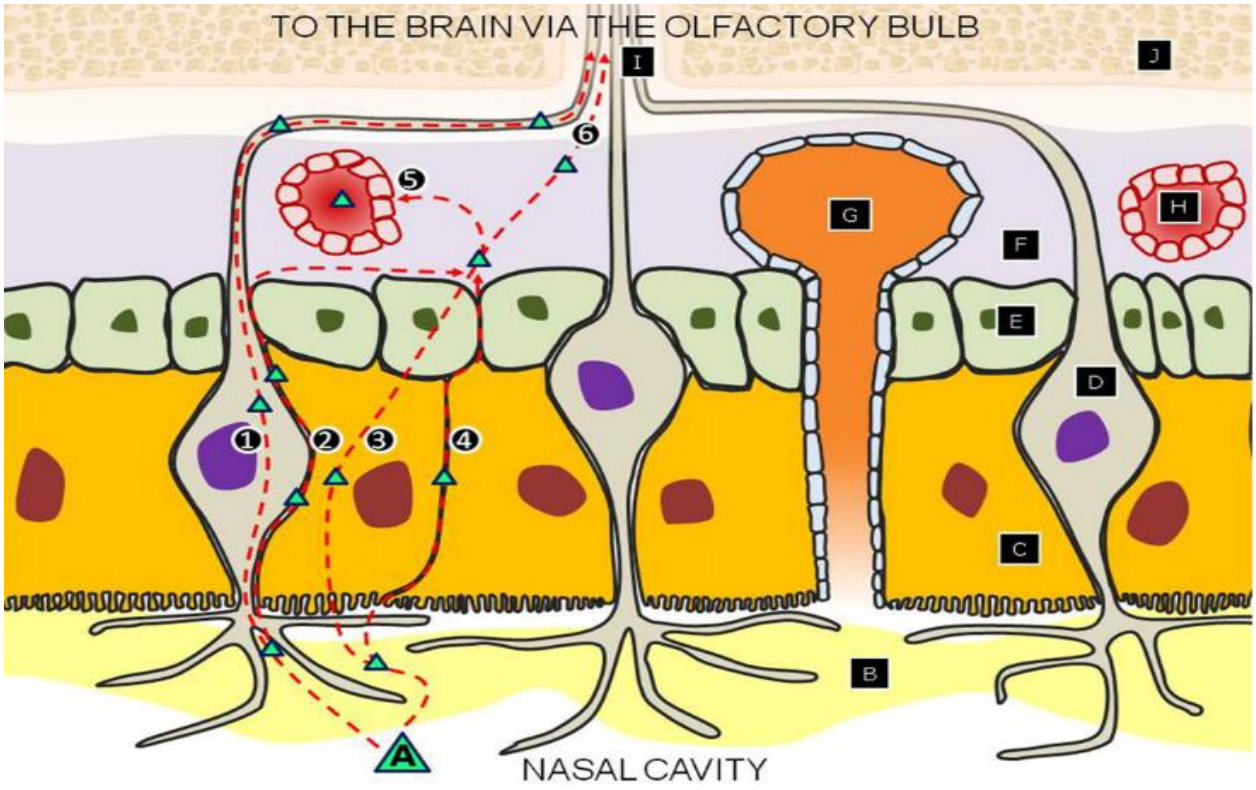


Figure 26. Schematic representation of the blood brain barrier and proposed pathways for direct nose-to-brain drug transport following intranasal administration. (A) Intranasally administered substance; (B) Nasal mucosa; (C) Olfactory epithelial cells; (D) Olfactory sensory neurons; (E) Basal cells; (F) Lamina propria; (G) Bowman's Gland; (H) Blood vessel; (I) Olfactory tract; (J) Cribriform plate;

(Pathway 1) Molecules can be transported transcellularly by travelling along the axon of an olfactory sensory neuron; (Pathway 2) Molecules can travel paracellularly between olfactory sensory neurons and olfactory epithelial cells; (Pathway 3) Molecules can be transported transcellularly across the epithelium via olfactory epithelial cells; (Pathway 4) Molecules can travel paracellularly between epithelial cells; (Pathway 5) Molecules can also enter systemic circulation via blood vessels; (Pathway 6) Once across the epithelium, molcules can travel via perineural or perivascular channels to reach the brain. *Image adapted from Katare et al. (2017); Pathways reviewed in Lochead and Thorne (2012).*

4.1.3 Nanocarriers

The development of suitable nasal formulations for the delivery of APDs (i.e. lurasidone) has been hindered by their poor aqueous solubility (Katare et al., 2017). Due to the limited volume of the nasal cavity, any formulation exceeding a single dose of 400 uL would likely be ineffective in a human population. Thus, most currently available APDs drugs would require substantial solubility enhancements to achieve a therapeutic dose range that would allow them to be incorporated into an suitable nasal formulation (Katare et al., 2017). These limitations have prompted investigations into the intranasal administration of novel drug delivery systems, such as nanocarriers, that act as protective mechanisms to improve drug targeting and brain bioavailability (Lombardo, Kiselev, & Caccamo, 2019). Furthermore, the effective dose would likely be less than conventional treatments dosages due to the protective nature of the nanocarrier and the bypass of gastrointestinal and hepatic metabolism (Katare et al., 2017).

Nanocarriers are colloidal drug carrier systems that are usually less than 500 nm in size (Lombardo et al., 2019). Their miniscule size allows for passage across BBB capillaries and diffusion into target tissue, allowing for controlled drug release directly at the target site (Mandal, Bisht, Pal, & Mitra, 2017). Nanocarriers are custom engineered transport modules that encapsulate a drug of interest and allow for precise drug targeting to specific tissues, while minimizing exposure to non-target tissue (Mandal et al., 2017).

Furthermore, their unique chemical structure allows them to be tailor-made to sense and respond to environmental differences (i.e. in the nasal mucosa) to ensure accurate, stimuli-controlled release *in vivo* (Raemdonck, Demeester, & De Smedt, 2009). Thus, nanocarriers represent a superior alternative to conventional pharmaceutical treatments as they allow for specific spatial and temporal distribution patterns, longer clearance periods, enhanced bioavailability, and improved solubility and stability (Lombardo et al., 2019). Moreover, Katare et al. (2015) showed that intranasal administration of the typical antipsychotic drug, haloperidol, loaded in a dendrimer-based nanoscopic carrier system required 6.7 times less drug to achieve a therapeutic response comparable to the same drug administered via intraperitoneal injections. Furthermore, the developed nano-formulation also increased the aqueous solubility of haloperidol by 100-fold (Katare et al., 2015). Therefore, intranasal drug delivery coupled with the use of appropriately engineered nanocarrier systems, could limit the adverse effects of APDs by enhancing brain bioavailability and reducing drug exposure to non-target sites. In doing so, this could also improve patient compliance and enhance the quality of life of patients treated with APDs.

4.1.4 Nanogels

Nanogels are three-dimensional, water-swollen networks of crosslinked hydrophilic or amphiphilic polymers ranging from 20 to 200 nm in size (Yadav, Halabi, & Alsalloum, 2017). Nanogels are primarily suited for hydrophilic drug transport, however, depending on the amphiphilicity of the polymer chains, they can also be optimized for hydrophobic drug delivery by engineering the polymer structure to allow for higher encapsulation efficiencies of poorly soluble drugs (Katare et al., 2017). Thus, poorly

soluble drugs (i.e. lurasidone) can be incorporated into nanogel formulations to improve their solubility, thereby enhancing the rates of cellular uptake compared to the free drug (Soni & Yadav, 2016). Furthermore, nanogels possess swelling properties in the presence of aqueous media which allow the formulation to absorb large quantities of water, resulting in a higher drug loading capacity (Yadav et al., 2017). The molecular composition, size, and morphology of nanogels can be optimized to respond to environmental changes to ensure spatial and stimuli-controlled release of the drug *in vivo* (Raemdonck et al., 2009). Thus, the versatile architecture allows for precise encapsulation and delivery of a wide variety of molecules without compromising their gel-like integrity. Therefore, intranasal delivery of nanogel formulations could be an effective way to enhance APD treatment, while reducing the propensity of adverse side effects.

4.2 Aims and Hypotheses

The objective of this study was to assess the efficacy of intranasal lurasidone administration using a novel poly(oligo ethylene glycol methacrylate) (POEGMA)-based nanogel system. This study also sought to compare intranasal lurasidone administration to intraperitoneal administration. To assess this, MK-801 was used to induce a pharmacological rodent model of SZ. Rats were then subjected to locomotor activity monitoring to assess lurasidone's ability to attenuate MK-801-induced hyperlocomotion.

Hypotheses:

1. Rats treated with MK-801 would elicit a significant increase in locomotor activity compared to control rats.
2. Substantially less drug would be required to elicit a therapeutic response via intranasal treatment compared to intraperitoneal treatment

3. Intranasal administration with a predetermined dose of lurasidone would significantly attenuate MK-801-induced hyperactivity.
4. Intraperitoneal administration of lurasidone with an equivalent dose as in hypothesis #3 would not significantly attenuate MK-801-induced hyperactivity (based on the observations from “Chapter 2”).

4.3 Materials and Methods

4.3.1 Animals

Adult, male Wister Hans IGS rats (Catalogue #: 273; N = 12) weighing between 250 and 300 g (on arrival) were purchased from Charles River Laboratories (Montreal, QC) and housed in McMaster University’s Central Animal Facility. All rats were housed and handled according to the conditions outlined in “Chapter 2”. All animal procedures were approved by McMaster University’s *Animal Research Ethics Board* (AUP: 18-06-27) in accordance with the ethical policies outlined by the *Canadian Council on Animal Care*.

4.3.2 Drug Treatment

Lurasidone was donated by Sunovion Pharmaceuticals, Inc. (Marlborough, MA) and loaded into a poly(oligo ethylene glycol methacrylate) (POEGMA)-based nanogel system prepared by Dr. Hoare’s lab. Nanogels were approximately 100 nm in size and were copolymerized with 10% butyl methacrylate to impart a hydrophobic domain and improve lurasidone uptake. MK-801 was purchase from Millipore Sigma and dissolved in 0.9% sterile saline at a concentration of 0.35 mg/mL and administered at a dose of 0.35 mg/kg. All drug formulations were prepared on the day of administration and injected via the intraperitoneal (i.p.) or intranasal (i.n.) routes. To determine an optimal dose, rats were pre-treated with intranasal doses of lurasidone (0.75 mg/kg or 1.0 mg/kg) or a blank

nanogel formulation followed by MK-801 (0.35 mg/kg; i.p.) or saline (i.p.). Due to the size of the rat nasal cavity, the total formulation volume administered via the intranasal route did not exceed 120 uL. Injections were administered 30 minutes and 60 minutes apart and locomotor activity was monitored 30 minutes following the MK-801-injection. Any rats receiving more than one treatment were given a 72-hour washout period to reduce the risk of residual carryover effects or behavioural sensitization. Rats received the following treatments (N = 12):

1. Control Group (CNTL): Saline + Saline; n = 6
2. MK-801 Group (MK): Blank Gel + MK-801; n = 6
3. 30-minute Intranasal Pre-treatment Group (30): LUR (i.n.) + MK-801 (i.p.); n =6
4. 60-minute Intranasal Pre-treatment Group (60): LUR (i.n.) + MK=801 (i.p.);n = 6
5. Intraperitoneal Group (IP): LUR (i.p) + MK-801 (i.p.); n = 6

4.3.3 Behavioural Testing

Locomotor activity was monitored using AccuScan computerized chambers (AccuScan Instruments, Columbus, OH) equipped with six infrared sensors that recorded multidirectional movements via beam interruptions. Beam breaks were translated into several parameters including movement time (seconds) and total distance travelled (centimeters). A baseline assessment was conducted for 120 minutes one week prior to testing. Three days prior to baseline testing, rats were given a habituation period in which they were free to explore the chamber undisturbed for five minutes. On the day of testing, locomotor activity was monitored for 90 minutes beginning 30 minutes after the MK-801 injection and total distance travelled was computed in 10-minute intervals.

4.3.4 Statistical Analyses

All statistical analyses were conducted using GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA). An ordinary one-way ANOVA with Dunnett's multiple comparisons test was used to evaluate the dose response of lurasidone relative to the MK-801 group. Furthermore, an ordinary one-way ANOVA with Tukey's HSD multiple comparison test was used to evaluate overall locomotor activity. Moreover, normality was confirmed using a Shapiro-Wilk test. Statistical outliers were removed via interquartile ranges. Statistical significance was determined at $p < 0.05$. All data was reported as mean ± SEM.

4.4 Results

4.4.1 Experiment 1: Determination of an effective dosing strategy for intranasal administration of lurasidone in Wistar rats

Ordinary one-way ANOVA revealed a significant difference between treatment groups when pre-treatment occurred 60 minutes prior to MK-801 administration ($F = 8.399$, $p = 0.0036$; Figure 28) but not 30 minutes ($F = 1.069$, $p = 0.3872$; Figure 27). Dunnett's multiple comparisons test revealed that intranasal administration of lurasidone at a does of 0.75 mg/kg failed to significantly reduce locomotor activity (24,323 cm ± 1,678) relative to the MK-801 group (34,787 cm ± 3,132) when it was administered 30 minutes prior to the MK-801-injection ($p = 0.0748$; Figure 27). Interestingly, intranasally administered lurasidone significantly blocked MK-801-induced hyperactivity at 0.75 mg/kg (16,258 cm ± 3,929; $p = 0.0029$) and 1.0 mg/kg (19,890 cm ± 3,032; $p = 0.0134$) when it was administered 60 minutes prior to the MK-801 injection (Figure 27).

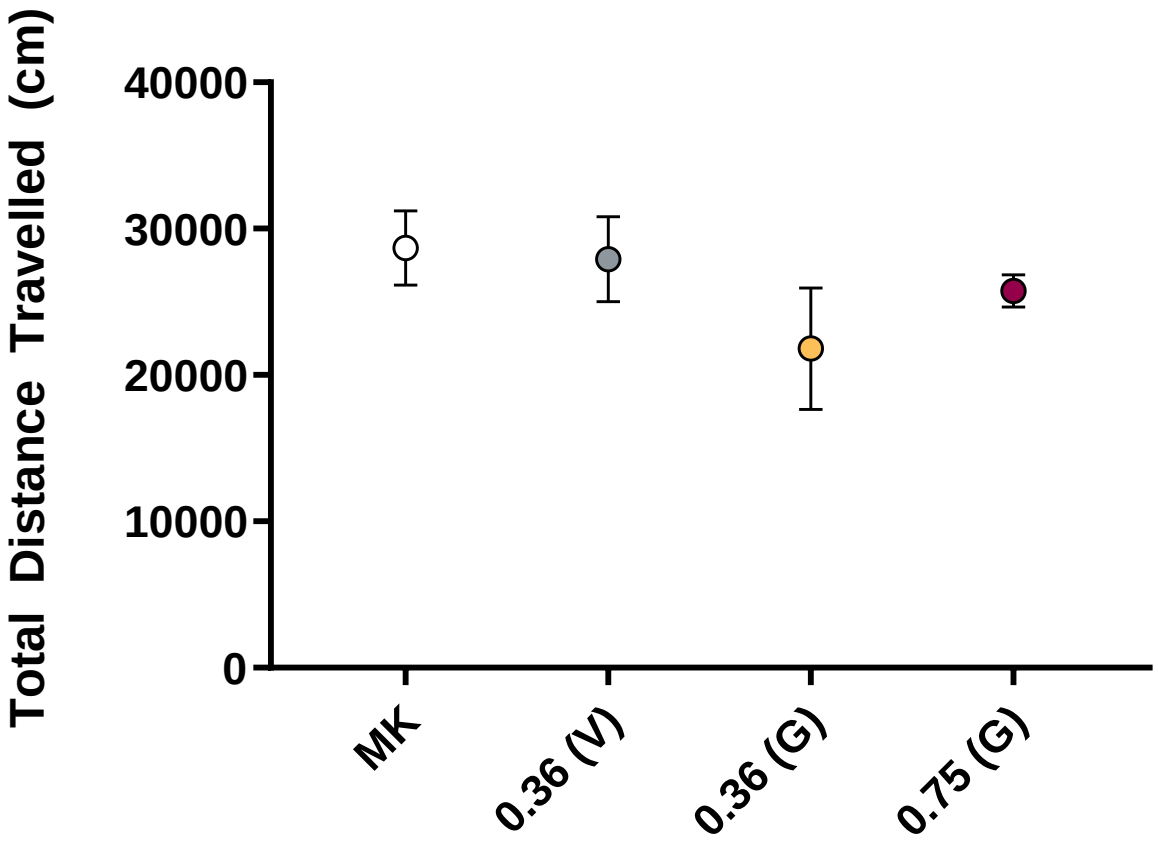


Figure 27. Summary of intranasal locomotor activity following 30-minute pre-treatment. Rats were pretreated with intranasal injections at doses of 0.36 mg/kg (vehicle; V), 0.36 mg/kg (nanogel; G), and 0.75 mg/kg (G). Ordinary one-way ANOVA did not reveal a significant difference between groups ($F = 1.069$, $p = 0.3872$).

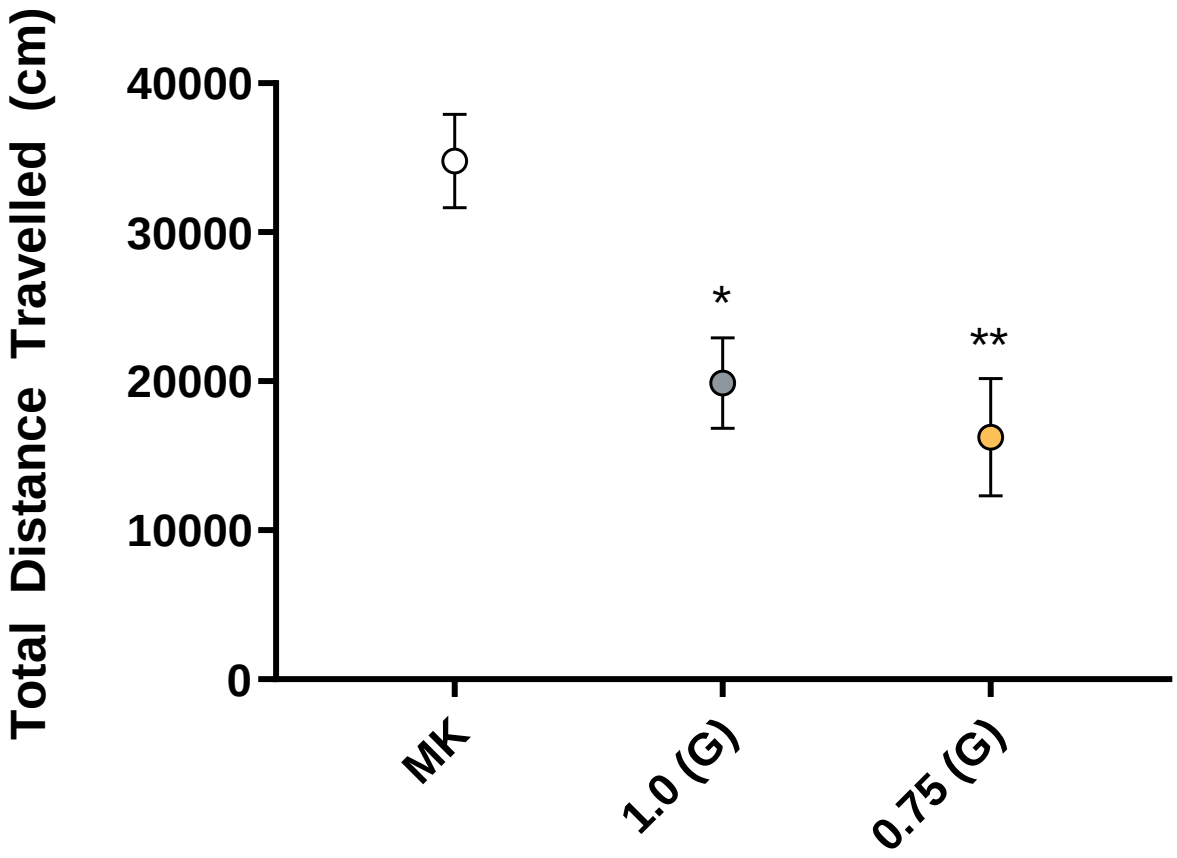


Figure 28. Summary intranasal locomotor activity following 60-minute pre-treatment. Rats were pretreated with intranasal injections of lurasidone using the nanogel formulation at doses of 0.75 mg/kg or 1.0 mg/kg followed by MK-801 (0.35 mg/kg; i.p.). Ordinary one-way ANOVA revealed a significant difference between groups ($F = 8.399$, $p = 0.0036$). Dunnett's multiple comparisons test revealed that intranasal administration of lurasidone at 0.75 mg/kg and 1.0 mg/kg significantly attenuated MK-801-induced hyperactivity (*$p = 0.0134$; **$p = 0.0029$).

4.4.2 Experiment 2: Comparing the efficacy of intranasal and intraperitoneal lurasidone administration using a MK-801 model of schizophrenia

Ordinary one-way ANOVA revealed a significant difference between groups ($F = 9.746$, $p < 0.0001$; Figure 29). Tukey's HSD multiple comparisons test revealed that MK-801 significantly increased locomotor activity (34,787 cm ± 3,132) relative to control rats (1,850 cm ± 451.3; $p < 0.0001$). Furthermore, intranasal administration of lurasidone at a dose of 0.75 mg/kg significantly attenuated MK-801-induced hyperlocomotion (16,258 ± 3,929; $p = 0.0109$). Interestingly, intraperitoneal administration of lurasidone at 0.75 mg/kg (gel) and 3.0 mg/kg (vehicle) failed to attenuate MK-801-induced hyperactivity. Lastly, intraperitoneal administration of 3.0 mg/kg (gel) of lurasidone significantly attenuated MK-801-induced hyperactivity.

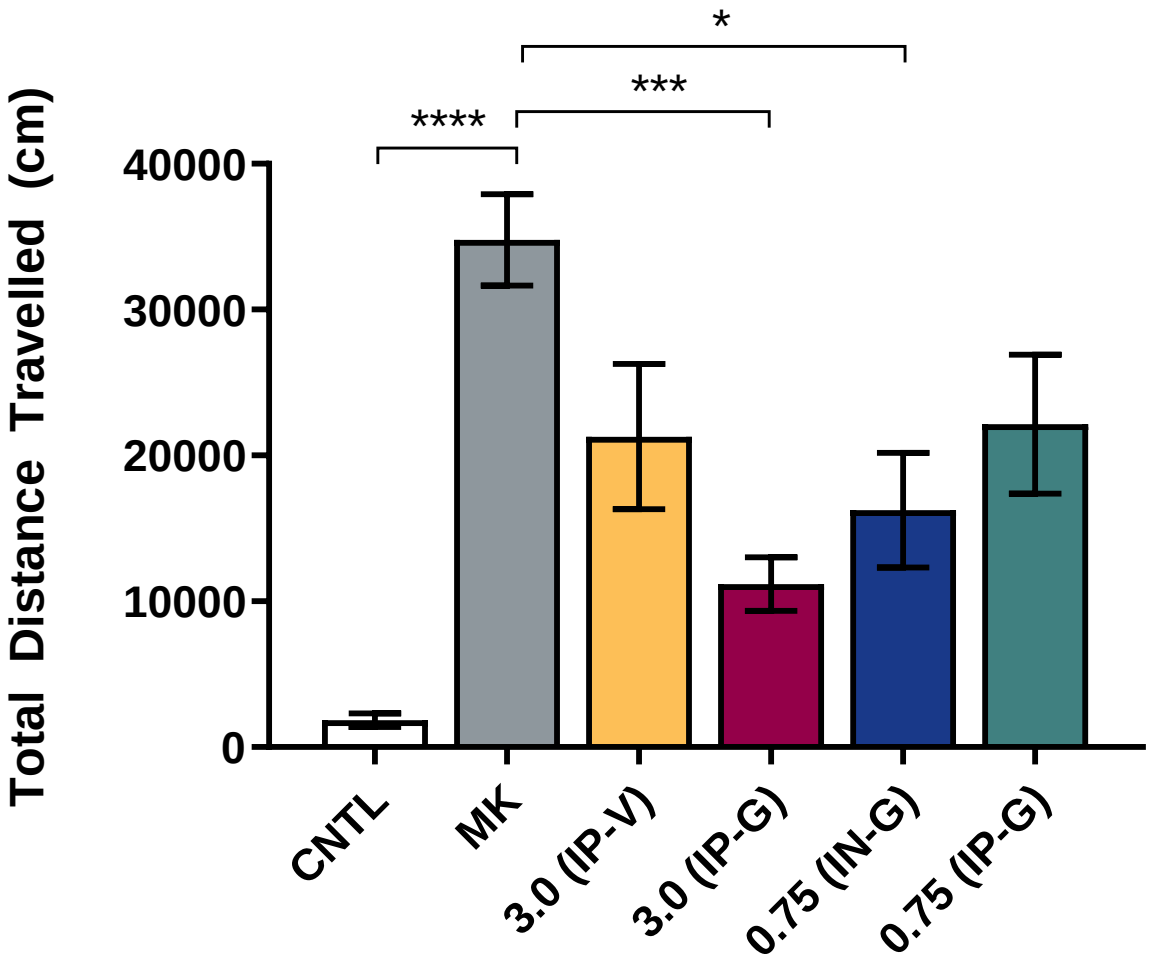


Figure 29. Comparison of intraperitoneal and intranasal lurasidone administration following 60-minute pre-treatment. Lurasidone was administered at a dose of 0.75 mg/kg or 3.0 mg/kg via the intranasal or intraperitoneal route 60 minutes prior to the MK-801 (0.35 mg/kg; i.p.) injection. Ordinary one-way ANOVA revealed a significant difference between groups ($F = 9.746$, $p < 0.0001$). Tukey's HSD multiple comparisons test revealed that MK-801 significantly increased locomotor activity relative to control rats (****$p < 0.0001$). Intraperitoneal treatment with 3.0 mg/kg of lurasidone in the tween/MC vehicle solution (IP-V) failed to attenuate MK-801-induced hyperactivity ($p = 0.1105$). However, intraperitoneal administration of lurasidone at the same dose with the gel formulation (IP-G) significantly attenuated hyperlocomotion (***$p = 0.0008$). Furthermore, intranasal administration of lurasidone at a dose of 0.75 mg/kg (0.75 IN-G) significantly attenuated MK-801-induced hyperlocomotion (*$p = 0.0109$) whereas intraperitoneal administration at an equivalent dose did not (0.75 (IP-G); $p = 0.1539$).

4.5 Discussion

This study sought to identify an effective dose for intranasal lurasidone delivery and evaluate the efficacy of intranasal and intraperitoneal lurasidone administration using a POEGMA-based nanoscopic carrier system. The locomotor activity monitoring test was used to assess the positive symptoms of schizophrenia using an acute MK-801 model of SZ. Intranasal administration of lurasidone at a dose of 0.75 mg/kg failed to attenuate MK-801-induced hyperactivity when pre-treatment occurred 30 minutes prior to the MK-801 injection (Figure 27). Interestingly when pre-treatment with lurasidone occurred 60 minutes before the MK-801 injection, lurasidone significantly blocked MK-801-induced hyperactivity at 0.75 mg/kg and 1.0 mg/kg, implying a temporal relationship (Figure 28). Furthermore, intraperitoneal administration with 0.75 mg/kg of lurasidone failed to significantly attenuate MK-801-induced hyperactivity, whereas intranasal administration with an equivalent dose resulted in significant attenuation (Figure 28). This study also demonstrated that acute treatment with lurasidone significantly attenuated MK-801-induced hyperactivity at dosages up to four times less than the original dose used for intraperitoneal administration in “Chapter 2” (Figure 29). These results suggest that intranasal administration of APDs could be a superior alternative to conventional oral and parenteral routes of APD delivery.

This study demonstrated that intranasal pre-treatment with doses of lurasidone as low as 0.75 mg/kg significantly attenuated MK-801-induced hyperactivity (Figure 28). This equates to a dose four times less than the median effective dose suggested for intraperitoneal lurasidone administration in rats (Kolaczkowski et al., 2014). These findings are also consistent with the report from Katare et al. (2015) who showed that

intranasal administration of haloperidol at doses 6.7 times less than that required for traditional intraperitoneal injections yielded a comparable therapeutic response. These observations highlight the effectiveness of nanogels in transporting drugs to the brain following intranasal administration. Furthermore, some atypical APDs produce severe cardiovascular, metabolic, and renal side effects which are partially due to the blockade of peripheral, non-target receptors (Scigliano & Ronchetti, 2013). Evidently, the high prevalence of adverse side effects of antipsychotic drugs has also been significantly correlated with patient noncompliance and increased risk of relapse (Haddad, Brain, & Scott, 2014). Therefore, by reducing the effective dose and exploiting direct nose-to-brain delivery pathways, intranasal delivery of antipsychotic drugs may reduce the rate of adverse effects and increase patient compliance.

Another interesting finding was that intranasal lurasidone treatment was ineffective when administered 30 minutes prior to MK-801 (Figure 27). However, lurasidone significantly attenuated MK-801-induced hyperactivity when pre-treatment was extended to 60 minutes prior to the MK-801 injection (Figure 28). This is an interesting finding because acute intraperitoneal injections of lurasidone (3.0 mg/kg) also failed to attenuate MK-801-induced hyperactivity in “Chapter 2” when administered 30 minutes prior to MK-801 (Figure 5). This could suggest that lurasidone requires at least 60 minutes to reach its target receptors in the rat brain and begin exerting its antipsychotic properties. Alternatively, this may have also been the result of delayed drug release from the nanogel formulation.

Moreover, when the lurasidone-loaded nanogel formulation was administered intraperitoneally at a dose of 0.75 mg/kg, it resulted in a noticeable but statistically

insignificant reduction in locomotor activity (Figure 29). In contrast, intranasal administration with an equivalent dose resulted in a significant attenuation of MK-801-induced locomotor activity. Interestingly, there was no significant difference between the intranasal and intraperitoneal groups (Figure 29). However, these findings support the notion that intranasal administration of therapeutic molecules results in a faster onset of pharmacological effects compared to conventional oral or parenteral routes (Lochhead & Thorne, 2012). Therefore, it is possible that intraperitoneal administration of lurasidone might be effective when pre-treated earlier relative to the MK-801 challenge. Thus, the acute antipsychotic effects of lurasidone should not be discounted. Future studies should assess the temporal relationship of antipsychotic drug efficacy following both intranasal and intraperitoneal injections relative to the MK-801 challenge.

4.6 Limitations and Future Directions

The first limitation of the present study was the lack of sample size (N = 12). This study served as a preliminary assessment of the effects of intranasal lurasidone administration using a MK-801 model of SZ. Due to the limited sample size, some rats received multiple MK-801 treatments. In “Chapter 2” we identified that repeated MK-801 treatment results in significant sensitization to its own hyperlocomotor-producing effects (Figure 13). To avoid MK-801 sensitization, all rats were given a minimum of 72-hour washout period between treatments. However, it is not presently clear whether intermittent exposure to MK-801 can result in behavioural sensitization. Additionally, repeated behavioural testing may have resulted in some rats becoming too familiar with the locomotor chambers, thereby affecting their performance. Thus, the rats tested more than once, may have had different behavioural responses compared to drug- naïve rats.

Therefore, this study lacked the appropriate sample size required to draw firm conclusions regarding the efficacy of intranasal lurasidone administration. However, it is worth noting that these findings are consistent with previous findings on the intranasal administration of antipsychotic drugs (Katare et al., 2015; Piazza et al., 2014). Future studies should attempt to validate these findings using drug naïve rats. Regardless, this pilot study has generated new insight regarding the effectiveness of intranasal lurasidone administration and the utilization of POEGMA-based nanogel systems for drug delivery. These data can guide future studies toward a more exhaustive investigation of the therapeutic potential of intranasally delivered antipsychotic drugs.

CHAPTER 5: CLOSING REMARKS

Available evidence has yielded conflicting results regarding the ability of atypical APDs to address the heterogeneity of SZ. For the first time, we used a comprehensive model of SZ to assess lurasidone's effect on the positive, negative, cognitive, and sensorimotor gating domains of SZ. We have successfully demonstrated that acute lurasidone treatment only attenuates the negative symptoms of SZ. In contrast, sub-chronic treatment resulted in significant attenuation of the positive, negative, and cognitive symptoms of SZ but not sensorimotor gating deficits. These findings suggest that sub-chronic treatment with MK-801 is a robust pharmacological model of SZ. Likewise, these behavioural results are consistent with the unique receptor binding profile of lurasidone given that sub-chronic lurasidone treatment demonstrated superior efficacy in attenuating deficits in three of the four major symptomatic domains of schizophrenia.

Furthermore, we have shown that sub-chronic treatment with lurasidone significantly upregulates MANF mRNA expression in the PFC. This was an important finding since the PFC represents the primary site of impairment (structurally and molecularly) observed in SZ. These findings suggest that lurasidone and possibly other atypical APDs might partially exert their therapeutic effect by modulating neurotrophic factor expression in the brain regions impaired by SZ. However, the novelty of these findings should be interpreted with caution. Future studies should attempt to replicate these findings in a separate rat cohort and determine if the relative changes in protein expression are consistent with the changes in mRNA expression described in this thesis.

Lastly, we provide support for the intranasal delivery of APDs. We demonstrated that acute intranasal administration of a lurasidone-loaded nanogel system with doses up

to four times less than the median effective dose described in “Chapter 2” significantly attenuated MK-801-induced hyperactivity. Interestingly, intraperitoneal administration of the lurasidone-loaded nanogel formulation with an equivalent dose failed to attenuate MK-801-induced hyperactivity. These findings are significant because APDs are currently only approved for oral and parenteral administration. Not only does intranasal drug delivery add to the therapeutic arsenal of available treatment options for patients with SZ, but we have also shown that it requires substantially lower drug concentrations to achieve a therapeutic response. This might reduce the prevalence of adverse side effects and increase patient compliance and quality of life. However, given our limited sample size, these results should be interpreted with caution.

In conclusion, lurasidone appears to be a promising drug candidate for the treatment of schizophrenia. Although these findings only provide a preclinical assessment, they do support the procognitive, antidepressant, and antipsychotic properties of lurasidone that can help guide future studies to unravel the significance of this drug within the context of a human population. Furthermore, while this manuscript does not provide a clear mechanism of action, it does identify a novel therapeutic target (MANF) that may help uncover the elusive mechanism of APDs. Moreover, the favourable tolerability and behavioural profiles of lurasidone coupled with the benefits of intranasal drug delivery should prompt further investigations into the predictive validity of the schizophrenia prodrome and the implications of earlier treatment intervention (i.e. before the onset of psychosis). Needless to say, more research is required to further comprehend the complex nature of SZ. However, this manuscript offers new insight to help guide future studies toward achieving this goal.

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