IMPAIRED PERFORMANCE ON TOUCHSCREEN OBJECT-LOCATION PAIRED ASSOCIATES LEARNING BY ACUTE SYSTEMIC MK-801 IS REVERSED BY L-GOVADINE BUT NOT D-GOVADINE OR CDPPB

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By Brittney R. Lins

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ABSTRACT

Schizophrenia is a serious psychiatric disorder that affects 1% of the population. Current theories implicate NMDA receptor hypofunction as a contributor to the symptomology and pathological alterations in schizophrenia. Cognitive impairments are increasingly recognized as not only fundamental to schizophrenia, but the strongest predictor of patient functional outcomes. Current antipsychotics do not improve the cognitive symptoms of the disorder; however, recent efforts have resulted in the identification of novel drug targets. One target is metabotropic glutamate receptors as they interact with and modulate NMDA receptors. Another approach focuses on dopamine, the neurotransmitter system targeted by traditional antipsychotics. Tetrahydroprotoberberines, such as D- and L-govadine, are synthetic compounds derived from traditional medicine that have demonstrated efficacy in treating schizophrenic symptoms. The present study assessed the effects of CDPPB (a metabotropic glutamate receptor 5 positive allosteric modulator), D- and L-govadine, and the typical antipsychotic haloperidol on the Paired Associates Learning (PAL) task in rats. The PAL task is impaired in patients with schizophrenia, has been adapted for use with rodents using touchscreen-equipped operant chambers, and has been promoted by MATRICS as a promising behavioural task with the potential to further translational health research in schizophrenia. The objectives of this study were: 1) examine the effects of acute NMDA receptor antagonism with MK-801 as a model for schizophrenia on performance of the PAL task; 2) test the effects of the putative antipsychotics, CDPPB and D- and L-govadine on reversing the effects of NMDA receptor antagonism on the task; and 3) to compare these novel therapeutics to a

classic antipsychotic. Two squads of male Long-Evans rats were trained to perform the PAL task in touchscreen-equipped operant chambers. After the rats reached criterion the following treatment schedules were divided between the two squads: 1) vehicle (10% cyclodextrin; i.p.), and CDPPB (1.0, 3.0, and 10.0 mg/kg, i.p.); or 2) vehicle (10% cyclodextrin; i.p.), CDPPB (3.0 mg/kg, i.p.), the NMDA receptor antagonist MK-801 (0.15 mg/kg, i.p.), and CDPPB with MK-801; or 3) vehicle (50% DMSO; s.c.), MK-801, D-govadine (1.0 mg/kg; s.c.), L-govadine (1.0 mg/kg; s.c.) and MK-801 with each isomer of govadine; or 4) vehicle (sodium acetate and acetic acid, pH 5.0, s.c.), and haloperidol (0.05 and 0.1 mg/kg, s.c.). Acute MK-801 significantly reduced the number of trials completed, impaired accuracy, and increased the number of errors in the PAL task. CDPPB had no effect on the PAL task and did not improve the MK-801 induced impairments. Administration of L-govadine, but not D-govadine, prior to MK-801 improved accuracy and reduced errors compared to MK-801 alone. L-govadine alone, but not D-govadine, reduced total responding compared to vehicle. Haloperidol caused a dose-dependent decrease in all activity in the task confounding interpretation of the results in regard to cognition. These data establish disruptive effects of acute MK-801 treatment on PAL task performance and demonstrate that L-govadine is capable of cognitive enhancement in a rodent model of schizophrenia.

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LIST OF ABBREVIATIONS

BVMT-R	Brief Visual Memory Test Revised
Ca^{2+}	Divalent Calcium
CANTAB	CAmbridge Neurological Test Automated Battery
CDPPB	3-Cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide
CNTRICS	Cognitive Neuroscience Treatment Research to Improve Cognition In Schizophrenia
D1	Dopamine Receptor Subtype 1
D2	Dopamine Receptor Subtype 2
DA	Dopamine
DISC1	Disrupted In Schizophrenia 1
DLS	Dorsolateral Striatum
DMS	Dorsomedial Striatum
DMSO	Dimethyl Sulfoxide
DTNBP1	Dystrobrevin Binding Protein 1
GABA	γ-amino-butyric acid
IP	Intraparitoneal Injection
LTD	Long-Term Depression
LTP	Long-Term Potentiation
LY354740	(1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate
LY379268	(1S,2R,5R,6R)-2-amino-4-oxabicyclo[3.1.0]hexane-2,6-dicarboxylic acid

LY404039	(-)-(1R,4S,5S,6S)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid
MATRICS	Measurement and Treatment Research to Improve Cognition in Schizophrenia
mGluR	Metabotropic Glutamate Receptor
mPFC	Medial Prefrontal Cortex
mRNA	Messenger Ribonucleic Acid
NA	Nucleus Accumbens
NMDAR	N-methyl-D-aspartate Receptor
NORT	Novel Object Recognition Task
NRG1	Neuregulin 1
PAL	Paired Associates Learning
PAM	Positive Allosteric Modulator
PCP	Phencyclidine
PFC	Prefrontal Cortex
PPI	Prepulse Inhibition
PRh	Perirhinal Cortex
PV	Parvalbumin
SC	Subcutaneous Injection
SOR	Spontaneous Object Recognition
VU29	*N*-(1,3-Diphenyl-1*H*-pyrazolo-5-yl)-4-nitrobenzamide

1. INTRODUCTION

1.1. Cognitive Impairment in Schizophrenia: MATRICS, CNTRICS, and PAL

Schizophrenia is a chronic psychiatric disorder which affects 1% of the general population. Recently characterised as a neurodevelopmental disorder, the etiology of schizophrenia is believed to depend on a complex interaction of genetic and environmental factors (Lipina et al., 2013). The onset of psychotic symptoms usually occurs in young adulthood and can lead to a lifetime of disability (Marshall and Rathbone, 2011). Although schizophrenia occurs in both men and women, men tend to experience earlier onset, more severe symptoms, and are affected more frequently (Champagne et al., 2014). Despite the introduction of neuroleptics in the late 1950s which effectively treat schizophrenia's most well-known symptoms, the hallucinations and delusions, patient outcomes are typically poor. It is estimated 80% of schizophrenic patients are unemployed and up to 70% are unable to live independently (Tregellas et al., 2014). The clinical manifestation of schizophrenia has three main categories of symptoms which are recognized as fundamental components of the disease. These are the positive symptoms which include hallucinations and delusions, the negative symptoms which include blunted affect and emotional flattening, and cognitive impairments such as memory and attentional deficits. While past research has been heavily driven by focus on the positive symptoms, it is becoming increasingly apparent that the negative and cognitive symptoms are highly detrimental to the patient's quality of life, even more so than the positive symptoms (Javitt, 2010;Nutt et al., 2013;Vinson and Conn, 2012;Nuechterlein et al., 2004). Importantly, the negative and cognitive symptoms are unresponsive to conventional antipsychotic treatment via D2 antagonism

(Vinson and Conn, 2012). While there have been claims of atypical antipsychotics enhancing cognition, these have been unsubstantiated (Vingerhoets et al., 2013). Cognitive impairment is of special clinical concern as deficits are present before the onset of psychosis, are stable throughout the course of illness, and are the strongest predictor of patient functional outcomes (Tregellas et al., 2014;Nuechterlein et al., 2004;Young et al., 2009;Green, 2006;Green, 1996). Furthermore, cognitive deficits persist even when psychosis is otherwise managed (Young et al., 2009). These data indicate a profound need for better understanding of cognitive processes as they apply to schizophrenia, as well as the development of treatments capable of improving the cognitive deficits.

The importance of cognitive symptoms to outcomes for those with schizophrenia and the dearth of treatment strategies prompted the National Institute of Mental Health to create the MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) initiative (Marder et al., 2004). The branch of MATRICS most concerned with preclinical research and standardization of rodent cognitive testing was given the title CNTRICS (Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia) (Young et al., 2009;Bussey et al., 2013). The main goals of MATRICS are to catalyse collaboration between academic, industrial, and government institutions to increase awareness of cognitive impairment as a core feature of schizophrenia, to characterise the nature of the deficits in schizophrenia, develop a standardized cognitive testing battery, and ultimately develop improved treatment (Young et al., 2009;Nuechterlein et al., 2004).

Cognition refers to a variety of conscious and unconscious mental processes. Cognitive processes are vital for normal, functional behavior (Keeler and Robbins, 2011). Through research catalysed by the MATRICS initiative, seven separable cognitive domains were identified as affected in schizophrenia. These seven domains are: processing speed, attention and vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving, verbal comprehension, and social cognition (Young et al., 2009). The MATRICS initiative continues to develop and scrutinize standardized methods of testing these seven domains in both humans and animals. Visual learning and memory is impaired in schizophrenia as well as in individuals at high risk of developing schizophrenia, and for this reason several behavioral tasks have been explored and chosen as promising methods to examine visual learning and memory. Such tasks include the Brief Visual Memory Task – Revised (BVMT-R) in which the participant is shown 6 geometric designs on a piece of paper for 10 seconds, and then must immediately reproduce the designs by drawing 3 times. After a 30 minute delay, the drawings are completed a fourth time, followed by the presentation of images where the participant answers 'yes' if they recognize the image, and 'no' if they do not recognize it (Young et al., 2009). Schizophrenic patients are impaired in all aspects of the task which are immediate recall; 30 minute delayed recall; and recognition (Schretlen et al., 2007). Another task used in humans is the visuo-spatial Paired Associates Learning (PAL) task from CANTAB (CAmbridge Neurological Test Automated Battery). The CANTAB PAL task, like all CANTAB tasks, is conducted on a touch screen computer. Participants are presented with 6-8 white rectangles arranged in a circle on a black screen and allowed

to see a geometric image located ‘behind’ each rectangle. After a variable delay, one of the geometric images is momentarily flashed in the center of the screen and the participant must select the white box in the location that the image was originally presented (Bussey et al., 2013;Talpos et al., 2009). Patients with schizophrenia, including first episode psychotics, are impaired at PAL (Donohoe et al., 2008;Wood et al., 2002;Chouinard et al., 2007;Aubin et al., 2009;Bussey et al., 2013), task performance correlates with symptom severity (Prouteau et al., 2005;Prouteau et al., 2004;Barnett et al., 2005;Bartok et al., 2005), and high risk individuals are impaired before the onset of psychosis (Wood et al., 2002;Bartok et al., 2005;Barnett et al., 2005). There are other conditions in which PAL is impaired, including neurodegenerative diseases such as Alzheimer’s disease and Huntington’s, however schizophrenia is the only psychiatric disorder to display a deficit in PAL. Even the very similar condition schizoaffective disorder does not cause impairment in PAL (Stip et al., 2005).

The touchscreen PAL task has several advantages that make it of particular relevance to translational health research. PAL is a visuo-spatial association task that is fully automated, non-verbal, and conducted on a touch screen computer. The automated computer platform makes it simple and time effective to administer PAL amidst a battery of tests in order to develop a comprehensive cognitive profile of each participant. The non-verbal component increases relevance to cognitive testing in animals where language communication is impossible. Finally, there are preclinical adaptations of the PAL task that assess visuo-spatial learning and memory in non-human primates and rodents. The rodent PAL task is sensitive to manipulation of the

hippocampus, by either lidocaine inactivation or local MK-801 infusion (Talpos et al., 2009). Systemic PCP and amphetamine impair task accuracy and decrease trials completed, and both systemic ketamine and PCP show a dose-dependent increase in response latencies (Talpos et al., 2014). LSD affects the number of trials completed and both response and reward collection latencies but not accuracy (Talpos et al., 2014). Furthermore, the CNTRICS initiative has stated that touchscreen visuo-spatial PAL task has sufficient evidence to support its recommendation as a promising task to assess long-term visual learning and memory in schizophrenia studies. This recommendation was driven by relevance to schizophrenia, potential for translation, and practicality considerations including amenability to drug testing, however further validation is needed (Bussey et al., 2013). The research described in sections 2 – 5 of this thesis utilizes the rodent PAL task and an established pharmacological model of schizophrenia to further validate the PAL task while also addressing the need for improved therapy by examining the effects of novel pharmaceutical compounds.

1.2. Dopamine, Glutamate, and GABA in Schizophrenia and Cognition

The traditional dopamine (DA) hypothesis of schizophrenia suggests over activity of the mesolimbic dopaminergic system as the underlying source of pathology (Vinson and Conn, 2012;Javitt, 2010;Lodge and Grace, 2008). This hypothesis is supported by the efficacy of typical and atypical antipsychotics in reducing or eliminating the positive symptoms of the disorder via antagonism of dopamine receptors, particularly D2 receptors which are present in high numbers in the striatum and nucleus accumbens (NA). Post-mortem studies of schizophrenic patients show increased D2 receptor

density and heightened sensitivity to amphetamine challenge indicated by increased DA release shown in imaging studies (Vinson and Conn, 2012;Seeman, 1987;Seeman, 2006). Another consideration is the occurrence of hallucinations and delusions as an adverse effect of administering L-Dopa, the precursor to DA, as treatment in Parkinson's disease (Schumacher-Schuh et al., 2013). These data support D2 hyperfunction in the striatum and nucleus accumbens as relevant in schizophrenia pathology. It was later shown that D1 hypofunction in the frontal cortex is also present and this prompted revision of the original D2 focused hypothesis (Laruelle, 2014). D1 hypofunction in the frontal cortex is associated with cognitive impairment and negative symptoms which aligns with a more complete depiction of the symptoms of schizophrenia, but also indicates the disturbances present are more complex than previously thought (Millan et al., 2012;Vinson and Conn, 2012).

The first indication of involvement of the glutamatergic system in schizophrenia came from observations that antagonism of N-methyl-D-aspartate receptors (NMDAR), an ionotropic glutamate receptor, by phencyclidine (PCP), ketamine, and MK-801 (dizocilpine) produced effects that closely resemble schizophrenia, including specific cognitive impairment, when administered to healthy individuals, as well as aggravate all categories of symptoms in schizophrenic patients (Vinson and Conn, 2012;Javitt, 2010;Ghoneim et al., 1985;Krystal et al., 1994). Another consideration is the presence of decreased NMDAR binding in unmedicated schizophrenic patients (Pilowsky et al., 2006). NMDAR activation requires binding of both glutamate and glycine, as well as membrane depolarization which then triggers calcium influx through the NMDAR channel (Vinson and Conn, 2012). Activation of these channels is necessary for long

term potentiation (LTP), a mechanism of synaptic plasticity required for the occurrence of learning and memory which implicates their involvement in cognition (Robbins and Murphy, 2006). NMDARs are distributed throughout the brain at excitatory synapses with a particularly high number in cortical and subcortical regions (Monaghan and Cotman, 1985;Monaghan et al., 1988;Ozawa et al., 1998). Particularly relevant to schizophrenia are NMDAR-containing glutamatergic projections from the mediodorsal thalamus to pyramidal cells in the prefrontal cortex, as well as inhibitory projections in the NA. In the thalamocortical circuit, glutamate binds NMDARs on GABAergic neurons which synapse onto glutamatergic thalamic neurons which then project to PFC pyramidal cells. NMDAR antagonism affects the GABAergic neurons and results in decreased inhibition of the thalamic neurons to which they project, and subsequent decreased inhibition of the PFC further downstream (Vinson and Conn, 2012;Moghaddam et al., 1997;Moghaddam and Adams, 1998). NMDAR antagonism via ketamine at sub anesthetic doses, but not anesthetic doses, increases extracellular glutamate in the PFC which could be related to decreased frontal inhibition. While NMDARs are diffusely distributed throughout the brain and present on both GABAergic and glutamatergic neurons, GABAergic interneurons have 10-fold greater sensitivity to NMDAR antagonists, coupled with elevated levels of the endogenous NMDAR antagonist kynurenic acid in schizophrenia. Together, these data provide a possible source of NMDAR hypofunction (Coyle, 2012).

A lack of brain inhibition as a central feature of schizophrenia is also supported by reports of altered GABAergic neurotransmission. GABA abnormalities are well documented in schizophrenia and include reductions in parvalbumin (PV)-containing

interneurons and reduced glutamic acid decarboxylase (GAD)-1 mRNA with resulting decreased GAD67 expression (Akbarian et al., 1995;Lewis et al., 2005;Volk et al., 2000;Hashimoto et al., 2003), an enzyme required in the synthesis of GABA, in the PFC and hippocampus (Lodge and Grace, 2008). This finding can be linked with NMDAR hypofunction due to the observation that mice treated repeatedly with ketamine have reduced PV and GAD67 due to superoxide generation which reduces inhibitory control of pyramidal cells (Zhang et al., 2008).

More recent theories have suggested that altered dopamine transmission may occur as a result of existing NMDAR alterations. When non-competitive NMDAR antagonists are persistently administered to animals, a deficit of DA in cortical regions and increased DA in subcortical regions arises (Laruelle, 2014). Furthermore, acute ketamine increases amphetamine induced DA efflux in healthy individuals as indicated by a reduction in D2 binding potential. Enhanced DA efflux in response to amphetamine is observed in human schizophrenic patients (Kegeles et al., 2000). In animal research, acute administration of ketamine and PCP increases forebrain DA transmission (Jentsch et al., 1997b;Doherty et al., 1980;Bowers, Jr. and Hoffman, Jr., 1986;Deutch et al., 1987;Hertel et al., 1995) while chronic PCP reduces frontal DA transmission (Jentsch et al., 1998;Jentsch et al., 1997c). PCP increases the firing rate of DA neurons (Grunze et al., 1996;French, 1994), and reduces cortical GABAergic function which results in enhanced glutamatergic transmission in the prefrontal cortex and ventral tegmental area to stimulate mesocorticolimbic DA transmission and enhance locomotive behaviour (Grunze et al., 1996;Yonezawa et al., 1998;Moghaddam et al., 1997;Mathe et al., 1998;Jentsch et al., 1997a). Non-competitive NMDAR antagonism is a well-

established animal model of schizophrenia due to its robust induction of symptoms isomorphic to the human condition. For this reason, rats treated with acute MK-801 injection was used as the animal model for the research described in sections 2 – 5 of this thesis.

1.3. Modelling Schizophrenia: Measurement and Validity

Schizophrenia is a complex disorder that is difficult to model in animals. When determining the value of any model in research, validity is an important consideration (Nestler and Hyman, 2010). Types of validity include face, etiological, predictive, and construct validity. If an animal model appears to replicate aspects of the human condition including specific symptoms, it is said to have face validity. Etiological validity depends upon the etiology of the human condition and the animal model being identical (Young et al., 2009). This presents a considerable challenge in schizophrenia as the etiology is largely unknown, although it generally believed to depend on an interaction between genetic and environmental factors which contribute to perturbed neurodevelopment (Lipina et al., 2013). Predictive validity is the ability of the model to make predictions about the human condition. For example, if a drug is able to improve cognition in the model and then also improves cognition in human schizophrenic patients, the model has predictive validity. Predictive validity can also be reversed to provide a positive control for the model. If a drug is known to have an effect in the human condition, it should have the same effect when applied to the model (Young et al., 2009). A current problem regarding predictive validity and cognitive enhancement in schizophrenia is a lack of positive controls as no drug treatments have been reliably

successful at improving cognition in humans (Floresco et al., 2005). Construct validity is of greater relevance in task design and choice than in determining the quality of an animal model as construct validity is maintained when a task measures what it is intended to measure, such as visual or working memory (Young et al., 2009).

Directly measuring many aspects of schizophrenia in rodents is challenging, particularly the characteristic hallucinations and delusions that accompany an episode of psychosis. Instead, researchers must rely on biochemical changes that correspond to hallucinations and delusions in humans as a sign of positive symptoms occurring in an animal model. One such biochemical abnormality is increased DA efflux in the ventral striatum. This also correlates with hyperlocomotion; therefore, hyperlocomotion has been used as a measure of positive symptoms in rodents (O'Tuathaigh et al., 2013;Moghaddam and Adams, 1998). The negative symptoms present an even greater challenge as emotional affect may not be present in rodents to the same extent as humans, if at all. Despite these species differences, behavioural tasks such as the social interaction task and sucrose preference are used to examine social withdrawal and anhedonia, respectively. Assessing the negative symptoms is disadvantaged by the lack of positive controls for predictive validity as no current treatment is known to affect these symptoms (Ellenbroek and Cools, 2000). As mentioned, the cognitive symptoms of schizophrenia also lack a positive control for determining the value of the model and task used however, the study of the cognitive symptoms has benefitted from the numerous behavioral tasks already in use for assessing animal cognition. A number of those tasks have been identified by MATRICS and CNTRICS as viable methods to assess the seven cognitive domains impaired in schizophrenia (Young et al., 2009).

Additionally, cognitive performance in animals and humans appear to depend on analogous circuitry and neural constructs (Jentsch and Roth, 1999).

Animal models of schizophrenia can be divided into four main categories which include pharmacological, genetic, lesion, and neurodevelopmental models (Ratajczak et al., 2013). Pharmacological manipulations include systemic injection of NMDAR antagonists such as MK-801, ketamine, and phencyclidine. Amphetamine, which stimulates DA release, and apomorphine, a DA agonist, are also used to model aspects of schizophrenia and are often used to induce hyperlocomotion and disrupt prepulse inhibition (PPI) in rodents (Geyer et al., 2001). Neurodevelopmental models of schizophrenia incorporate prenatal and neonatal insult as indicated relevant by epidemiological studies (Rees and Harding, 2004;Rees and Inder, 2005). Such models include maternal immune activation (MIA), prenatal methylazoxymethanol acetate (MAM) exposure, isolation rearing and maternal malnutrition. Genetic models include Neuregulin 1 (NRG1), dystrobrevin binding protein 1 (DTNBP1), and Disrupted in Schizophrenia 1 (DISC1) mutations (O'Tuathaigh et al., 2013). An example of a lesion model is Neonatal Ventral Hippocampal Lesion (NVHL) which involves a surgical intervention on postnatal day 7 (O'Tuathaigh et al., 2013).

1.4. Acute MK-801 Model of Schizophrenia

A widely used model of schizophrenia is achieved pharmacologically through NMDAR antagonism. As previously discussed, systemic administration of non-competitive NMDAR antagonists ketamine, phencyclidine (PCP), and MK-801 induce

symptoms specific to schizophrenia which include all three symptomatic domains in humans and heightens symptoms in schizophrenic patients (Krystal et al., 1994). In rodents, NMDAR antagonisms via these compounds induces hyperlocomotion (positive symptoms), disrupts sucrose preference (negative symptoms), and induces a variety of cognitive deficits including impaired spatial memory in an aversive learning task (Fowler et al., 2013) and impaired cognitive flexibility in a T maze set shifting task (Stefani and Moghaddam, 2010), as well as disrupts pre-pulse inhibition (thalamocortical loop dysfunction) (Stefani and Moghaddam, 2010;Fowler et al., 2013). Notably, the MK-801 model has never been examined in PAL. MK-801-induced hyperlocomotion can be reversed by clozapine, an atypical antipsychotic with high efficacy in treating the positive symptoms which indicates predictive validity, however there are no drugs currently able to affect cognition and thus no positive control for the cognitive symptoms (Floresco et al., 2005). NMDAR antagonism as a model of schizophrenia is benefitted by the relative ease of administering systemic injections when required, compared to models that require surgical intervention such as the neonatal ventral hippocampal lesion model. Furthermore, any observed effects can be attributed to NMDAR antagonism, as opposed to a broad effect of any number of abnormalities occurring as a result of perturbed neurodevelopment in other models. The MK-801 injection model also does not require the time investment required to produce other models, such as maternal immune activation (MIA). Finally, MK-801 injection allows the psychotic state to be induced only when desired, a luxury not available with neurodevelopmental and genetic models. This allows rats to be trained on behavioural tasks in a normal cognitive state, a valuable consideration given that many tasks require extensive training and it is

not known if rats with permanent cognitive deficits are capable of acquiring these tasks, and if so, the time it would require. A disadvantage of any pharmacological model lies in etiological validity. Schizophrenia is a neurodevelopmental disorder with evidence suggesting a role for prenatal and neonatal insult, as well as interplay with genetic susceptibility (Piontkewitz et al., 2012;Lipina et al., 2013). These factors are not considered in the pharmacological NMDA antagonism model.

Section 1.2. introduced the glutamate theory of schizophrenia and described the effects of pharmacologically induced NMDAR hypofunction. As previously mentioned, this hypothesis originated based on observation that the effects of NMDAR antagonists such as MK-801 bear a striking resemblance to schizophrenia (Coyle, 2012;Krystal et al., 1994;Ghoneim et al., 1985;Jentsch and Roth, 1999). Systemic administration of MK-801 to rodents is a well-established animal model of schizophrenia. The effects are robust and last several hours following systemic administration (Jentsch and Roth, 1999). One of the strengths of the MK-801 model is face validity as it produces several isomorphic symptoms to schizophrenia. The positive symptoms can be measured by proxy indices such as hyperlocomotion. NMDAR antagonism also induces cognitive deficits that fit the seven domains identified by MATRICS, particularly those that require the frontal lobe (Luby et al., 1959), and negative symptoms such as social withdrawal (Jentsch and Roth, 1999).

1.5. New Therapeutics for Schizophrenia

The recent theories implicating a role for NMDAR hypofunction in the pathology of schizophrenia have led to novel therapeutics targeting the glutamatergic system. While the obvious target for NMDAR hypofunction would appear to be an NMDAR agonist, stimulation of NR2B subunit-containing NMDARs is linked to apoptosis (Lai et al., 2011), therefore other approaches to modulate the activity of NMDARs have been considered. One group of novel targets are metabotropic glutamate receptors (mGluRs). mGluRs are G-protein coupled receptors (GPCR) (Niswender and Conn, 2010) which are categorized into three groups. Group I includes the subtypes $mGluR_1$ and $mGluR_5$. These are primarily located postsynaptically throughout the prefrontal cortex, striatum and hippocampus, making them of interest in cognition and schizophrenia (Stefani and Moghaddam, 2010). Group I mGluRs are coupled to G_q/G_{11} which, when activated, stimulate phospholipase C (PLC) to trigger hydrolysis of phosphotinositides (PI) and increase intracellular Ca^{2+}. Group II includes $mGuR_2$ and $mGluR_3$ which are found both pre- and postsynaptically throughout the forebrain where they are coupled to $G_{i/o}$ and their activation inhibits adenylyl cyclase to influence voltage-gated ion channels (Petralia et al., 1996;Gu et al., 2008). Finally, Group III include $mGluR_4$, $mGluR_6$, $mGluR_7$ and $mGluR_8$ which are found presynaptically and also couple to $G_{i/o}$. mGluRs have been identified for potential therapeutic benefit in schizophrenia, particularly Groups I and II which are present in the relevant circuitry. mGluRs provide an attractive option for drug design due to the presence of allosteric sites that are not highly conserved between subtypes which allows for allosteric modulation with a high degree of specificity (Conn et al., 2009).

Activation of Group II mGluRs stimulates $G_{i/o}$ to inhibit cAMP formation, inhibit voltage-gated Ca^{2+} channels, and activate K^+ channels to hyperpolarize the cell, prevent vesicle fusion to the cell membrane and overall decrease the levels of glutamate released (Fell et al., 2012). This results in reduced glutamatergic tone in brain regions of interest in schizophrenia such as the striatum, the PFC, and hippocampus (Yoshino et al., 1996;Battaglia et al., 1997;East et al., 1995;Lovinger and McCool, 1995). Psychotomimetics such as MK-801 increase glutamate activity in the PFC by disinhibiting the thalamocortical loop at the level of GABAergic neurons in the NA and Group II mGluR agonists reverse this effect (Moghaddam and Adams, 1998;Lorrain et al., 2003;Marek et al., 2000). Furthermore, a family of Group II mGluR agonists, best characterized by LY354740, have been successful at reversing psychotomimetic-induced impairments at the behavioral level, however there are variations between different behaviour paradigms, animal models, and animal strains (Conn et al., 2008;Cartmell et al., 1999;Imre et al., 2006b;Imre et al., 2006a;Monn et al., 1997;Profaci et al., 2011). In a neurodevelopmental animal model of schizophrenia in which social isolation induces hyperactivity, disrupts PPI, and impairs novel object recognition, LY404039 reversed hyperactivity (Fabricius et al., 2011), and LY379268 reversed hyperactivity, PPI impairment, and novel object recognition impairment (Jones et al., 2011). Currently, several Group II mGluR agonists have been involved in ongoing clinical trials with mixed results (Kinon et al., 2011;Patil et al., 2007).

$mGluR_5$, a Group I mGluR, has also been chosen as a promising novel therapeutic target for schizophrenia as its activation increases excitation of midbrain GABAergic neurons to restore inhibitory regulation to the thalamocortical pathway and

PFC and correct the effects of NMDAR hypofunction (Vinson and Conn, 2012). $mGluR_5$ are involved in regulating the activity of NMDARs. One mechanisms of this regulation is NMDAR positive modulation through PKC phosphorylation and tyrosine kinase phosphorylation (Lu et al., 1999;Collett and Collingridge, 2004;Kotecha et al., 2003). Likewise, NMDAR activity activates calcineurin which dephosphorylates $mGluR_5$ as a PKC phosphorylation site (Alagarsamy et al., 1999). Furthermore, NMDAR and $mGluR_5$ are physically linked via binding and scaffolding proteins (Ehlers, 1999). More specifically, $mGluR_5$ bind Homer proteins, and Homer proteins can cluster with PSD-95 through the postsynaptic density Shank protein. Finally, PSD-95 interacts with NMDAR (Fagni et al., 2004;Naisbitt et al., 1999;Tu et al., 1999). As previously stated, NMDAR are critical for LTP, a process necessary for normal memory function which occurs through up-regulation of membrane proteins. $mGluR_5$ knockout mice show less NMDAR-mediated hippocampal LTP and are impaired in NMDAR-dependent learning tasks (Jia et al., 1998;Lu et al., 1997). These data provide evidence for a role of $mGluR_5$ in cognition, particularly learning and memory. Drug design has focused on the development of allosteric modulation at these receptors as stimulating the receptor directly via agonists induces long term depression (LTD) and seizure activity in hippocampal slices and in vivo (Wong et al., 2005).

1.6. CDPPB

CDPPB, 3-Cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide, is an $mGLuR_5$ positive allosteric modulator (PAM), the first of such compounds to allow for in vivo administration due to improved solubility, increased potency, and improved ability to

enter the central nervous system compared to earlier compounds (Lindsley et al., 2004;Kinney et al., 2005;Chen et al., 2007). CDPPB interacts with the MPEP site on $mGluR_5$, is $mGluR_5$ selective, and does not interact with any of 175 proteins that commonly interact with drugs (Chen et al., 2007). CDPPB reduces amphetamine-induced hyperlocomotion and amphetamine-induced PPI disruption in rats (Kinney et al., 2005). Hyperlocomotion is due to increased DA in the ventral striatum and is associated with the positive symptoms of schizophrenia, while disrupted PPI indicates abnormalities within the thalamocortical pathway (Alsene et al., 2011). CDPPB also reverses MK-801 induced disruption of sucrose preference in rats, a trait relevant to the negative symptoms of schizophrenia (Vardigan et al., 2010). These data indicate promise for CDPPB, and $mGluR_5$ activity enhancement in general, in treating symptoms of schizophrenia.

LTP and LTD are both critical for the proper formation of memories. In order for CDPPB to have potential as a cognition enhancer, it must not disproportionately influence LTP or LTD, as that could have negative effects on cognition (Vinson and Conn, 2012). An analogue of CDPPB known as VU29 has been examined for such effects at the Schaffer collateral-CA1 synapse following afferent-stimulation induced LTP and LTD. VU29 enhanced both LTP and LTD without altering the presynaptic activity necessary for each type of synaptic plasticity to occur. Furthermore, CDPPB and ADX47273, a functionally distinct $mGluR_5$ PAM, improved performance in the Morris water maze task in mice, suggesting efficacy as a cognitive enhancer (Vales et al., 2010;Liu et al., 2008;Chan et al., 2008;Darrah et al., 2008;Uslaner et al., 2009). These data provide another indication of the potential for $mGluR_5$ receptor enhancement via

such compounds as CDPPB in the treatment for schizophrenia as cognitive impairment remains unmanaged with current treatment options (Vinson and Conn, 2012).

1.7. Govadine

Govadine is a synthetic compound from the family of tetrahydroprototerberines which exists in two isomers, D- and L-govadine. This putative antipsychotic provides a dramatic contrast to CDPPB as its effects are predominately within the DA system opposed to the glutamatergic system. While relatively little is known about govadine, recent studies have indicated efficacy in improving all three symptomatic domains in schizophrenia (Lapish et al., 2014;Lapish et al., 2012). Both isomers have been shown to have a higher binding affinity for DA-D_1/D_5 receptors than DA-D_{2L} receptors, and have modest affinity for adrenergic receptors and low affinity for serotonin receptors. The D2 binding affinity of L-govadine is greater than that of D-govadine, and L-govadine is an antagonist at D2 receptors. Behavioral studies show L-govadine improves abnormalities representative of positive symptoms such as amphetamine induced hyperlocomotion as well as causes catalepsy and impairs conditioned avoidance responding, therefore acting like an atypical antipsychotic. D-govadine improves cognition in untreated rats by reducing errors in the spatial win-shift task. Both isomers improve measures associated with negative symptoms such as amphetamine-disrupted latent inhibition and restore impaired social interaction in the NVHL model. At an electrophysiological level, when DA neuron firing is suppressed by quinprole, a D2 agonist, L-govadine, but not D-, restores firing by suppressing G-coupled inward rectifying K^+ channels. Microdialysis studies show L-govadine increases DA efflux

throughout the PFC and nucleus accumbens while D-govadine only increases DA efflux in the mPFC (Lapish et al., 2014;Lapish et al., 2012).

1.8. Hypothesis

This thesis seeks to explore cognitive impairment in schizophrenia, specifically visual learning and memory using the touchscreen visuo-spatial PAL task, acute MK-801, and novel putative antipsychotics, CDPPB and govadine. Due to the well documented effects of systemic NMDAR blockade causing cognitive impairment (Moghaddam and Adams, 1998;Lecourtier et al., 2007;Kegeles et al., 2000;Darrah et al., 2008;Stefani and Moghaddam, 2010;Moghaddam et al., 1997), and demonstration of intra-hippocampal MK-801 disrupting PAL (Talpos et al., 2009), I hypothesize acute, systemic MK-801 will reduce accuracy and increase errors in PAL. Similarly, CDPPB has successfully restored performance to control levels in multiple spatial cognitive tasks when faced with pharmacologically induced NMDAR hypofunction (Moghaddam and Adams, 1998;Lecourtier et al., 2007;Kegeles et al., 2000;Darrah et al., 2008;Stefani and Moghaddam, 2010;Moghaddam et al., 1997), therefore I hypothesize that PAL, as a hippocampal dependent cognitive task, will utilize overlapping circuitry as the tasks previously explored and CDPPB will attenuate MK-801 induced deficits. Finally, D-govadine has also displayed efficacy as a cognitive enhancer, although it has never been examined alongside an NMDAR antagonist such as MK-801. As discussed earlier, D1 hypofunction in the PFC is associated with cognitive impairment and D-govadine increases DA efflux in the mPFC, presumably increasing potential for D1 binding. Given these past results, I hypothesize D-govadine will improve PAL performance when faced

with acute MK-801. In contrast, L-govadine acts as an atypical antipsychotic, a class of drugs not associated with cognitive enhancement. Despite increasing DA efflux in the PFC with potential to combat frontal D1 hypofunction, previous research with L-govadine has not demonstrated cognitive enhancing abilities (Lapish et al., 2014), thus I do not expect L-govadine to improve PAL performance.

2. METHODS

2.1. Subjects

Two squads of 16 male Long-Evans rats were used (Charles River Laboratories, Quebec, Canada). Rats were single housed in clear plastic cages in a temperature controlled environment. Lighting was controlled automatically on a 12:12 hour cycle, lights on at 7:00 am and all handling and experimentation occurred within the light phase. Rats were food restricted to 85% of their free feeding body weight and maintained on a food restricted diet with sufficient intake to support normal growth throughout the experiment. All experiments were performed in accordance with the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Care and Use Program and the Animal Research Ethics Board.

2.2. Training Apparatus (refer to Figure 1)

All training and testing was conducted using 8 touchscreen equipped operant chambers from Lafayette Instruments. The apparatus consists of a wooden box that can

be latched shut with two clips and contains a fan to circulate air and create background noise. The operant chamber is located on a sliding shelf at the base of the wooden box. An upper sliding shelf holds the sugar pellet dispenser and a video camera. The video camera outputs to a monitor for a live feed of the rat's activity within the chamber. The operant chambers are trapezoidal in shape with the wider end consisting of a touch screen monitor. The monitor is covered with a black polycarbonate mask. Masks are interchangeable depending on the task being conducted. In this case, the mask has three rectangular windows which allow the rats to contact the touchscreen monitor only in the areas where the stimuli are presented. Directly below the three windows is a spring loaded 'response shelf'. This forces the rats to intentionally stand and press the shelf down in order to contact the touch monitor and make a selection.

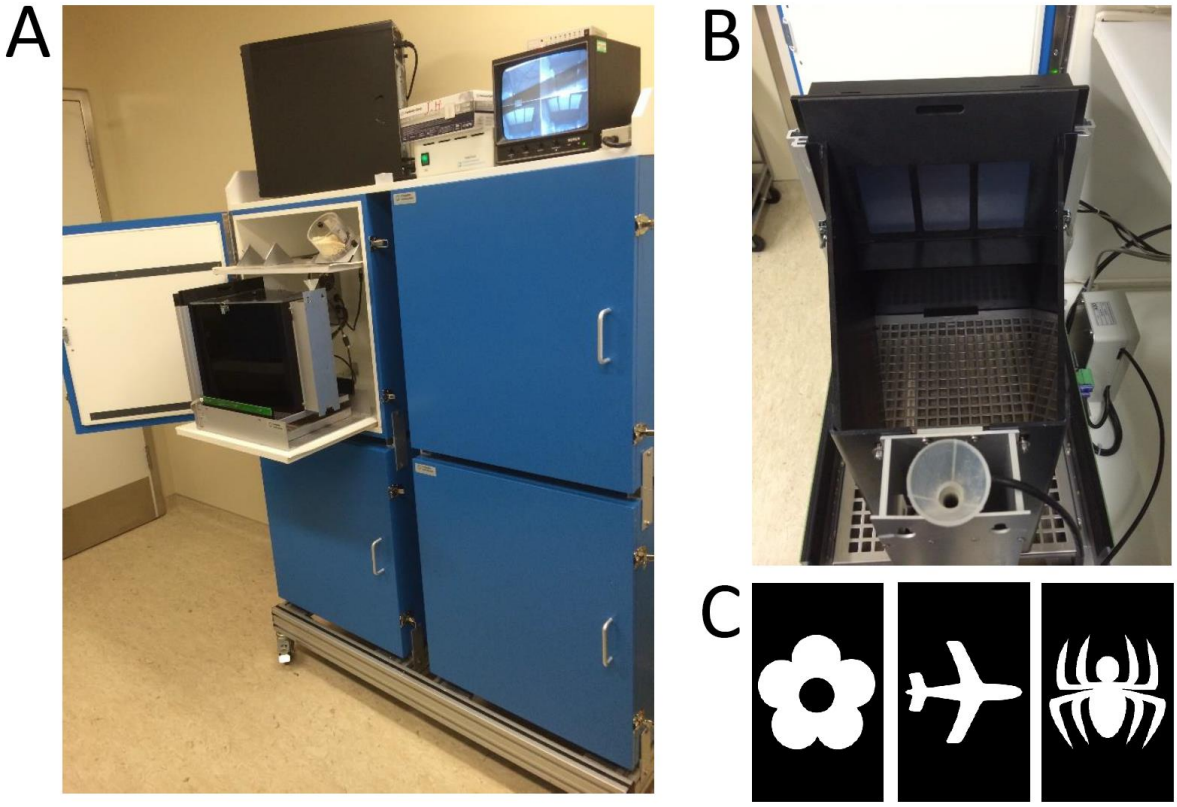

Figure 1: Touchscreen Apparatus. [A] The full apparatus including the blue wooden box which houses the touchscreen-equipped operant chamber on the lower sliding shelf, and the food magazine and camera on the upper sliding shelf. **[B]** The interior of the chamber as it is set up during the PAL task. Note the mask with three windows open to the touch monitor and the spring-loaded response shelf. The funnel shaped opening opposite the touchscreen guides the sugar pellet reward to the food port. **[C]** The three images displayed on the monitor during PAL. The images are ordered in respect to their correct window in the task.

2.3. Touchscreen Habituation and Pretraining (refer to Table 1)

All rats were left undisturbed for a minimum 5 days following transport and arrival to the animal holding facilities. Prior to training, rats were extensively handled over the course of 5 days. On day one of habituation, rats were introduced to transportation to the testing room and the testing room itself. Rats were brought from the vivarium to the testing room via a push cart and elevator and left undisturbed for 1 hour with all equipment running (8 chambers, 2 computers), lights dim, and 5 reward pellets placed in each cage. For all other days of training, rats were left undisturbed for 15-20 minutes following transport to the testing room before being introduced to the chambers.

Pretraining protocols were conducted as per the instructions and software that accompanies the Lafayette touchscreen chambers with the exception of eliminating the optional dPAL Acquisition stage. Pretraining began with two days of habituation in which the rats were placed in the chambers with 5 reward pellets in the food port and left undisturbed for 30 minutes. Criterion was reached if all pellets were located and consumed in that time frame. For all pretraining, each phase was repeated daily until criterion was reached. Initial Touch Training consisted of one of three windows on the screen being illuminated. The location of the illuminated window was pseudorandom in that the same window would not be illuminated for three consecutive trials. If the rat touched the illuminated screen, 3 pellets were rewarded. If the illuminated screen was not touched, 1 pellet was rewarded. The stimulus remained illuminated for 30 seconds, or until the rat touched the screen. Following each trial is a 20 second inter-trial interval which began when the rat entered the food port to collect the reward. Criterion was 100 trials completed in 1 hour. Must Touch Training also involved the illumination of one of

three windows, however the rat must touch the illuminated window to receive 1 reward pellet. There was no reward if the rat touched a blank window. Criterion was 100 trials completed in 1 hour. This was followed by Must Initiate Training where the illumination of the window had to be triggered by the rat nose poking into the illuminated food port, followed by touching the illuminated window, to receive a reward pellet. Criterion was 100 trials in 1 hour. The final stage of pretraining was Punish Incorrect Training in which the rat had to touch the illuminated window to receive a reward. An incorrect touch was followed by a 5 second time out and then a correction trial in which the same window was illuminated. Correction trials continued until the correct selection was made, followed by delivery of a food reward. The 20 second inter-trial interval began when the reward was collected. Criterion was 100 trials completed in 1 hour with 80% correct for 2 days in a row. Following Punish Incorrect, training on the full version of the task could begin.

Table 1: Timeline of training and treatment events.

Squad 1 Week	Stage	Squad 2 Week	Stage
1	Handling and habituation	1	Handling and habituation
2-4	Pretraining	2-4	Pretraining
3-8	Task acquisition	3-7	Task acquisition
9	CDPPB (1.0, 3.0, 10.0 mg/kg)	8-12	MK-801 (0.15 mg/kg) and CDPPB (3.0 mg/kg)
10	Washout	13	Washout
11	MK-801 Pilot	14	Re-baseline
12	Washout and re-baseline	15	Haloperidol (0.05, 0.1 mg/kg)
13	MK-801 and CDPPB Pilot		
14	Washout and re-baseline		
15	MK-801 (0.15 mg/kg) and Govadine (1mg/kg)		

2.4. dPAL Full Task (refer to Figure 2)

Rats were presented with two of three images on each trial in a pseudorandom order. Each image could appear in any one of the three windows on the touchscreen monitor. Of these three windows, each image had a correct location and two incorrect locations. The images were flower (f), airplane (a), spider (s), plus a blank window (b), and could be presented as $f^{+}/s^{-}/b^{-}$, $f^{+}/b^{-}/a^{-}$, $b^{-}/a^{+}/f^{-}$, $s^{-}/a^{+}/b^{-}$, $b^{-}/f^{-}/s^{+}$, $a^{-}/b^{-}/s^{+}$. The flower was always correct when presented in the left window, the airplane was always correct in the middle window, and the spider was always correct in the right window. Selections were made by nose poke directly onto the screen. Each correct selection was rewarded with a sugar pellet, incorrect selections were punished with a 5 second delay. Following an incorrect selection, the rat was given a correction trial in which the same pair of stimuli were presented repeatedly until the correct selection was made. Correction trials were not included in the number of trials completed or task accuracy which were based only on the first presentation of each stimulus pair. Rats were trained on this task until performance was stable for a minimum of 3 consecutive days at 90 trials completed in 1 hour with a minimum of 80% correct.

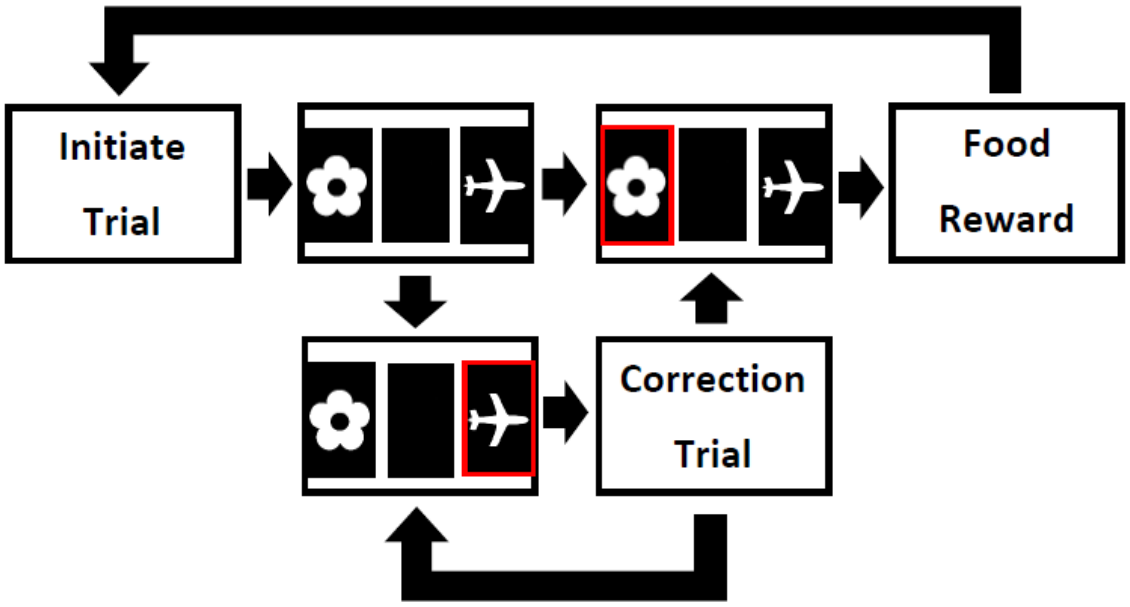

Figure 2: dPAL Full Task Schematic. A flow chart of the events that occur in the PAL task. The trial begins with a free food delivery into the illuminated food port located opposite the screen. The rat nose pokes into the food port to initiate the trial and a pair of stimuli appear on the screen. If the rat makes a correct section (the flower in this example), the choice is coded as correct and a food delivery is made. Following an inter-trial interval of 20 s, the food port illuminates and the rat may initiate the next trial. If the rat makes an incorrect selection, the choice is coded as incorrect the rat is punished with a 5 s delay before the same stimuli are presented again. The trials with repeated stimuli are known as correction trials, and will repeat until the correct choice is made. Note that incorrect selections made in the correction trials are not included in the accuracy measurement and the number of correction trials are not included in trials completed which is instead based on the first presentation of the stimuli only. Also, a trial is considered complete once the correct choice is made, either following the first presentation or a correction trial. Total trials represents the number of trials plus correction trials.

2.5. Drug Administration (refer to Table 1)

Drug treatments were administered following training in a counterbalanced order following a within-subjects design with the exception of pilot trials. Squad 1 rats received vehicle (10% cyclodextrin), 1.0 mg/kg CDPPB, 3.0 mg/kg, 10.0 mg/kg CDPPB. Following this, a subset of rats from squad 1 (n = 7) were tested in a pilot experiment to determine optimal doses of MK-801 and CDPPB (data not included in this document). Rats were treated with MK-801 a maximum of 2 times at doses of 0.1, 0.15, or 0.2 mg/kg. Finally, squad 1 rats were treated with vehicle (50% dimethyl sulfoxide), 0.15 mg/kg MK-801, 1.0 mg/kg D-govadine, 1.0 mg/kg L-govadine, 0.15 mg/kg MK-801 with 1.0 mg/kg D-govadine, and 0.15 mg/kg MK-801 with 1.0 mg/kg L-govadine. Vehicle, D-, and L-govadine were given via subcutaneous injection, MK-801 via intraperitoneal injection.

Squad 2 rats were treated with vehicle (10% cyclodextrin), 0.15 mg/kg MK-801, 3.0 mg/kg CDPPB, and 0.15 mg/kg MK-801 plus 3.0 mg/kg CDPPB. In a subsequent series of treatments, we examined the effects of haloperidol on the dPAL task. Haloperidol was dissolved in 1M acetic acid and diluted with 0.5M sodium acetate and the final pH was adjusted to 5.0. A subset of 8 rats received vehicle, 0.05 mg/kg haloperidol, and 0.1 mg/kg haloperidol. All rats were re-baselined following washout periods to ensure adequate proficiency in the dPAL task (performance at criterion for 3 days in row) before subsequent series of testing began. All drugs were injected at a volume of 1 mL/kg except CDPPB which was 2 mL/kg.

2.6. Data Analysis

All data are presented as group means plus or minus the standard error of the mean. Dependent measures analysed were accuracy (% correct selections), number of trials completed, number of correction trials completed, total trials completed (trials plus correction trials), mean correct response latency, mean incorrect response latency, and mean reward collection latency. The PAL task is fully automated which eliminates observer bias and no data scoring is required. Data analyses were conducted using SPSS Version 21. All pharmaceutical manipulations were treated as within-subjects factors. Two-way repeated measures ANOVAs were used to analyze the MK-801 with CDPPB and MK-801 with govadine data (D- and L-govadine were analyzed separately). One-way repeated measures ANOVAs were used to analyze the CDPPB alone and haloperidol data. Tukey's HSD was used for post hoc analysis.

Two rats from squad 1 were removed due to failure to learn the PAL task and did not participate in any drug treatments, reducing the final number of rats in the CDPPB alone treatment from 16 to 14. Three more rats failed to reach baseline criterion near the end of the govadine treatment schedule despite several attempts to re-baseline and did not complete the final treatments. One rat failed to complete any trials when treated with L-govadine and was removed from analysis. The final number of rats included in the govadine analysis is 12 per drug treatment, however it is not the same 12 rats in each treatment group which necessitated analyzing the D- and L-govadine treatments separately in order to maintain a true within-subjects design. Finally, the haloperidol treatment contained outliers however none were removed from analysis because removal would not affect significance, and the overall increased variability and the high

standard error of the mean (SEM) reflects the presence of the outliers (refer to Figure 7).

3. RESULTS

3.1. The effects of CDPPB on PAL

CDPPB was administered to Squad 1 rats via IP injection in three doses (1 mg/kg, 3 mg/kg, 10 mg/kg) and a vehicle control (10% cyclodextrin). Task accuracy was unaffected by CDPPB (Figure 3A; $F(3,39)=0.86$, $p>0.05$). There was a significant effect of CDPPB on the number of trials completed (Figure 3B; $F(3,39)=3.97$, $p<0.05$), and post hoc analysis indicates rats treated with the 10 mg/kg dose completed fewer trials. Despite this, there was no effect on the number of correction trials (Figure 3C; $F(3,39)=0.37$, $p>0.05$), or the total number of trials (trials plus correction trials) (Figure 3D; $F(3,39)=2.50$, $p>0.05$). Several latency measures were also included in analysis, revealing a significant effect on latency to make a correct decision following initiating the trial by nose poke into the food port (Figure 3E; $F(3,39)=3.42$, $p<0.05$), with the 10 mg/kg treated rats taking longer to make a correct response as indicated by post hoc breakdown. There was no effect on latency to make an incorrect response (Figure 3E; $F(3,39)=0.36$, $p>0.05$), and latency to nose poke into the food port to collect the sugar pellet following a correct response (Figure 3E; $F(3,39)=0.89$, $p>0.05$).

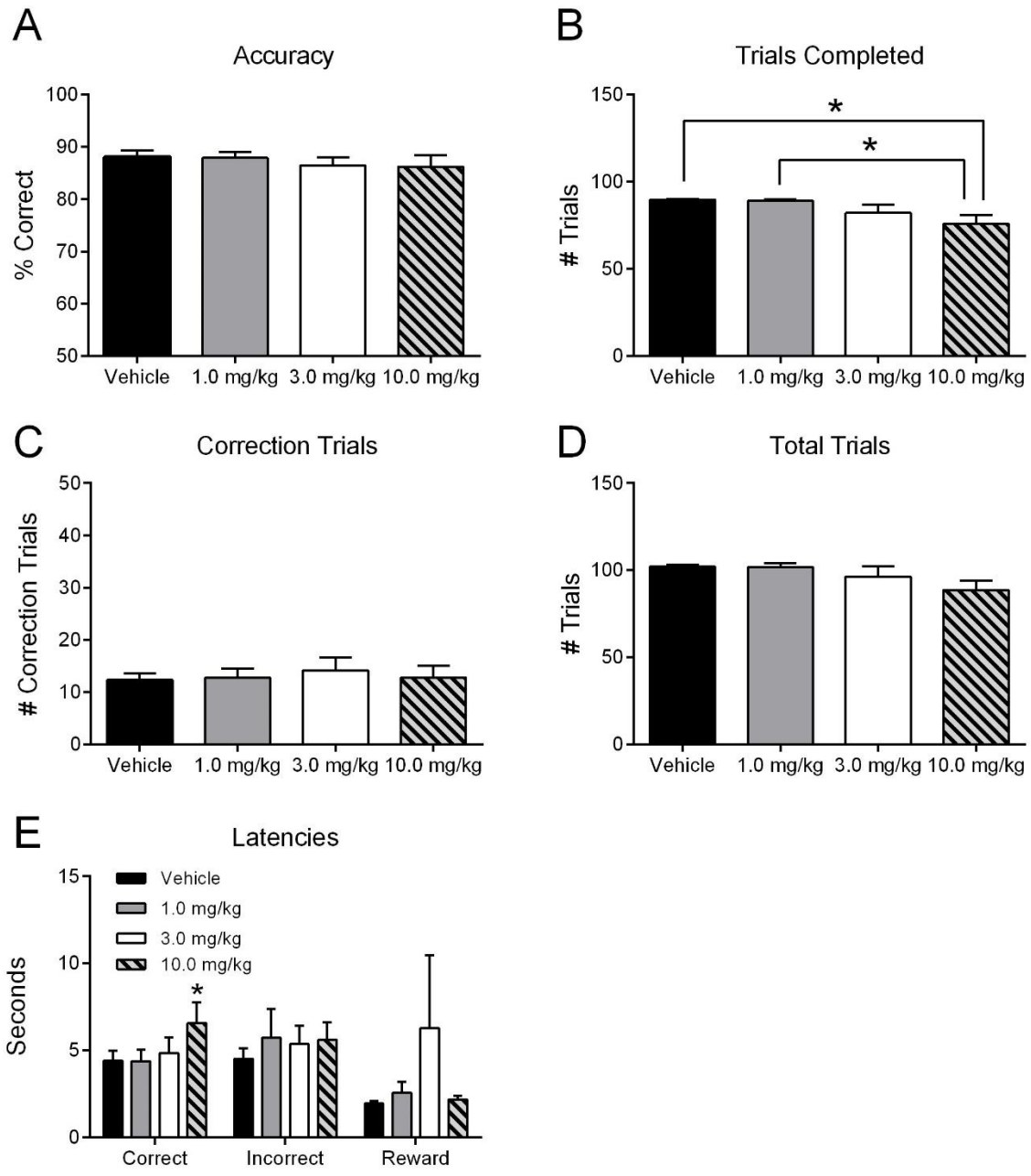

Figure 3: The effects of CDPPB on PAL. [A] CDPPB did not affect accuracy (% correct). **[B]** CDPPB significantly reduced the number of trials completed at a dose of 10 mg/kg. **[C]** CDPPB did not affect the number of correction trials performed. **[D]** CDPPB did not affect total trials. **[E]** The 10 mg/kg dose of CDPPB significantly increased correct response latency, but did not affect other latency measures.

3.2. The effects of MK-801 and CDPPB on PAL

Acute injection (i.p.) of CDPPB (3 mg/kg), MK-801 (0.15 mg/kg), CDPPB alongside MK-801, and a vehicle control were administered to Squad 2 rats following a within-subjects design. The 3.0 mg/kg dose was chosen as it was the highest dose in previously examined that did not reduce the trials completed or affect latency. The MK-801 and MK-801 with CDPPB groups had significantly lower accuracy than the vehicle control and CDPPB alone groups due to a significant main effect of MK-801 treatment (Figure 4A; $F(1,15)=13.21$, $p<0.01$). No main effect of CDPPB (Figure 4A; $F(1,15)=1.15$, $p>0.05$) and no interaction (Figure 4A; $F(1,15)=0.36$, $p>0.05$) were observed. The number of trials completed were also significantly reduced in the MK-801 and CDPPB with MK-801 groups as shown by a significant main effect of MK-801 treatment (Figure 4B; $F(1,15)=8.84$, $p<0.01$). No main effect of CDPPB (Figure 4B; $F(1,15)=3.94$, $p>0.05$) and no interaction (Figure 4B; $F(1,15)=1.74$, $p>0.05$) were observed. The MK-801 and CDPPB treated rats made more errors than when treated with vehicle or CDPPB alone, as indicated by significantly increased correction trials. There was a significant main effect of MK-801 on the number of correction trials completed (Figure 4C; $F(1,15)=21.48$, $p<0.001$), but no main effect of CDPPB (Figure 4C; $F(1,15)=0.23$, $p>0.05$), and no interaction (Figure 4C; $F(1,15)=0.29$, $p>0.05$). When trials and correction trials were summed to determine the total number of trials completed regardless of accuracy there was a significant main effect of MK-801 treatment (Figure 4D; $F(1,15)=19.84$, $p<0.001$) indicating the rats carried out more trials when treated with MK-801 and MK-801 with CDPPB compared to vehicle and CDPPB alone. No main effect of CDPPB treatment (Figure 4D; $F(1,15)=1.27$, $p>0.05$), and no interaction

(Figure 4D; $F(1,15)=1.31$, $p>0.05$) were observed. Latency to make a correct response was differentially affected by MK-801 and CDPPB. There was a significant main effect of MK-801 treatment reducing latency (Figure 4E; $F(1,15)=9.56$, $p<0.01$), and a significant main effect of CDPPB increasing latency (Figure 4E; $F(1,15)=8.96$, $p<0.01$). No interaction was observed (Figure 4E; $F(1,15)=0.03$, $p>0.05$). Incorrect response latency decreased due to a significant main effect of MK-801 treatment (Figure 4E; $F(1,15)=5.70$, $p<0.05$), but there was no main effect of CDPPB treatment (Figure 4E; $F(1,15)=2.02$, $p>0.05$), and no interaction (Figure 4E; $F(1,15)=0.63$, $p>0.05$). Mean reward latency was significantly reduced by MK-801 (Figure 4E; $F(1,15)=13.53$, $p<0.01$), and increased by CDPPB (Figure 4E; $F(1,15)=8.44$, $p<0.05$), but there was no interaction (Figure 4E; $F(1,15)=3.06$, $p>0.05$). Over parameters such as accuracy and correction trials, MK-801 impaired cognitive performance in a manner consistent with what would be expected in a model of schizophrenia and these deficits were not corrected by administration of CDPPB. Some effects of CDPPB on latencies are contrary to what was seen with Squad 1, however other measures are consistent with the previous experiment.

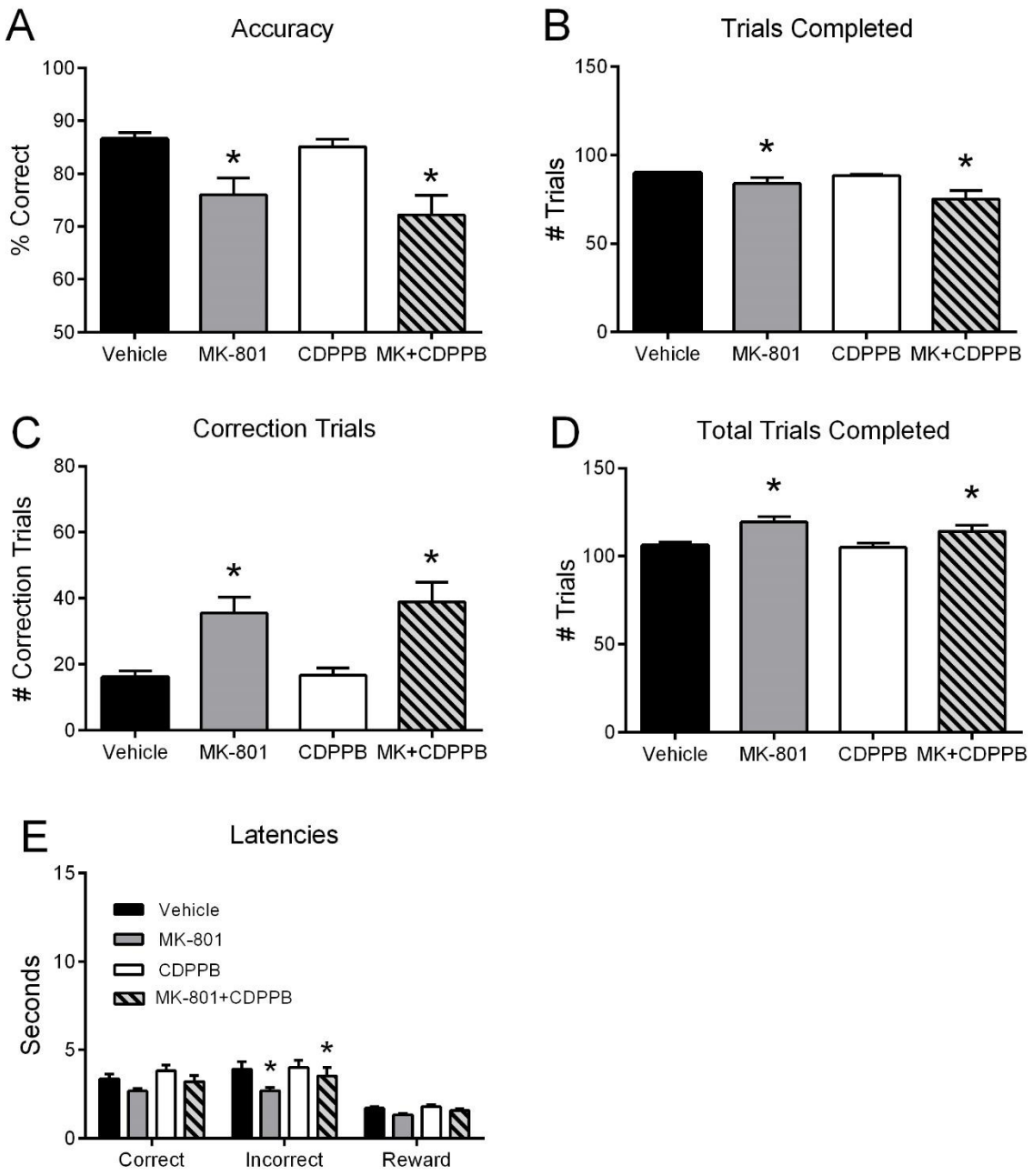

Figure 4: The effects of CDPPB and MK-801 on PAL. [A] MK-801 significantly reduced accuracy. CDPPB alone had no effect and did not reverse the deficit. **[B]** MK-801 significantly reduced trials completed. CDPPB alone had no effect and did not reverse the deficit. **[C]** MK-801 significantly increased correction trials. CDPPB alone had no effect and did not improve the impairment. **[D]** MK-801 increased the number of total trials (total responses). CDPPB had no effect. **[E]** MK-801 reduced all latency measures and CDPPB increased correct response latency. Asterisks were omitted for clarity for correct and reward latency.

3.3. The effects of MK-801 and D-govadine on PAL

Acute injection of MK-801 (0.15 mg/kg, i.p.) and D- and L-govadine (1.0 mg/kg (s.c.) were administered alone and in combination (with both govadine isomers kept separate), along with a vehicle (50% DMSO). MK-801 treatment significantly reduced task accuracy (Figure 5A; $F(1,11)=18.30$, $p<0.01$) while there is no effect of D-govadine (Figure 5A; $F(1,11)=0.44$, $p>0.05$), and no interaction (Figure 5A; $F(1,11)=2.14$, $p>0.05$). There was a nearly significant reduction of the number of trials completed due to MK-801 treatment (Figure 5B; $F(1,11)=4.79$, $p=0.051$), with no effect of D-govadine (Figure 5B; $F(1,11)=0.00$, $p>0.05$) and no interaction (Figure 5B; $F(1,11)=3.63$, $p>0.05$). Correction trials were significantly increased by MK-801 treatment (Figure 5C; $F(1,11)=33.86$, $p<0.001$), but not D-govadine (Figure 5C; $F(1,11)=0.56$, $p>0.05$), and no interaction was observed (Figure 5C; $F(1,11)=1.02$, $p>0.05$). Likewise, MK-801 significantly increased total trials completed (Figure 5D; $F(1,11)=43.79$, $p<0.001$), with no effect of D-govadine (Figure 5D; $F(1,11)=1.82$, $p>0.05$), and no interaction (Figure 5D; $F(1,11)=0.25$, $p>0.05$). There was no significant effect of either MK-801 (Figure 5E; $F(1,11)=2.45$, $p>0.05$) or D-govadine (Figure 5E; $F(1,11)=0.06$, $p>0.05$) and no interaction (Figure 5E; $F(1,11)=1.23$, $p>0.05$) regarding correct response latency. Similarly, incorrect response latency was unaffected by MK-801 (Figure 5E; $F(1,11)=3.88$, $p>0.05$) and D-govadine (Figure 5E; $F(1,11)=2.32$, $p>0.05$), with no interaction (Figure 5E; $F(1,11)=2.43$, $p>0.05$). As previously observed, reward latency was reduced by MK-801 (Figure 5E; $F(1,11)=34.53$, $p<0.001$), but there was no effect of D-govadine (Figure 5E; $F(1,11)=1.41$, $p>0.05$) and no interaction (Figure 5E; $F(1,11)=0.01$, $p>0.05$). Overall, D-govadine alone had no effects on PAL performance at

any examined parameter, but MK-801 reduced task accuracy, increased correction trials and total trials despite a near significant reduction in trials, as well as reduced reward latency. D-govadine did not demonstrate efficacy in restoring the MK-801 induced PAL impairments.

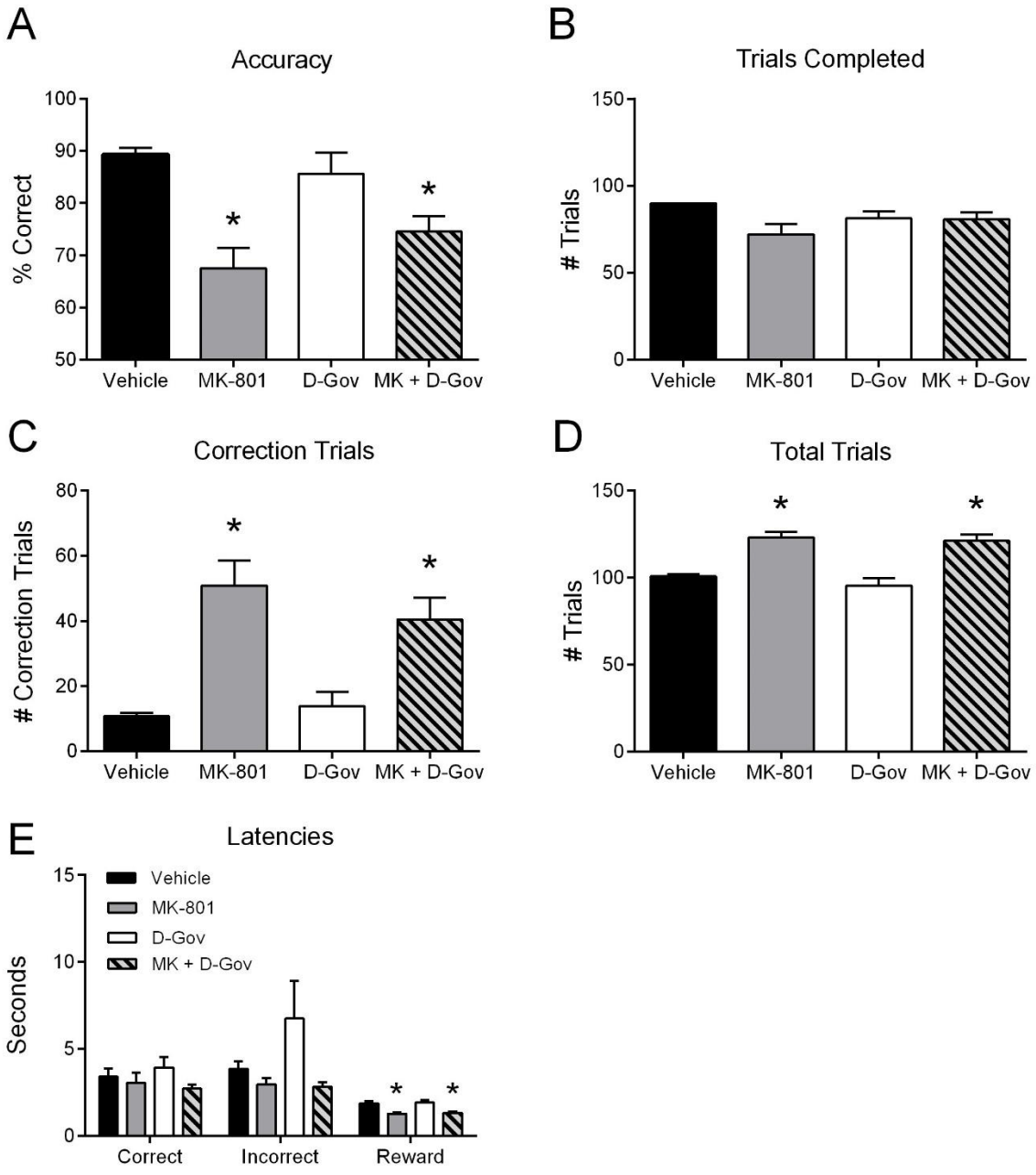

Figure 5: The effects of D-govadine and MK-801 on PAL. [A] MK-801 significantly reduced PAL accuracy. D-govadine alone had no effect and did not reverse the deficit. **[B]** MK-801 caused a near significant (p=0.51) reduction in trials completed. D-govadine alone had no effect and did not reverse the deficit. **[C]** MK-801 significantly increased correction trials. D-govadine alone had no effect and did not improve the impairment. **[D]** MK-801 increased the number of total trials (total responses). D-govadine had no effect. **[E]** MK-801 reduced reward latency, D-govadine had no effect, and no effects were observed on the other latency measures.

3.4 The effects of MK-801 and L-govadine on PAL

Acute injection of MK-801 (0.15 mg/kg) and D- and L-govadine (1.0 mg/kg) were administered alone and in combination (with both govadine isomers kept separate). PAL task accuracy was significantly reduced by MK-801 (Figure 6A; $F(1,11)=13.33$, $p<0.01$). Furthermore, there was a significant main effect of L-govadine (Figure 6A; $F(1,11)=13.44$, $p<0.01$), and a significant interaction (Figure 6A; $F(1,11)=12.23$, $p<0.01$). Post hoc analysis indicated the MK-801 treated group had significantly reduced accuracy compared to vehicle, L-govadine, and L-govadine with MK-801. L-govadine alone reduced the number of trials completed (Figure 6B; $F(1,11)=5.73$, $p<0.05$), while there was no effect of MK-801 (Figure 6B; $F(1,11)=0.10$, $p>0.05$). A significant interaction (Figure 6B; $F(1,11)=6.89$, $p<0.05$) was observed for the number of trials completed and post hoc analysis revealed the L-govadine treatment causes completion of fewer trials than the other treatment groups. There was a significant main effect of MK-801 (Figure 6C; $F(1,11)=20.94$, $p<0.01$), a significant main effect of L-govadine (Figure 6C; $F(1,11)=30.49$, $p<0.001$), and a significant interaction (Figure 6C; $F(1,11)=19.38$, $p<0.01$) regarding the number of correction trials completed. Post hoc analysis indicated MK-801 treatment increased correction trials, but L-govadine and MK-801 with L-govadine were not different from the vehicle treatment. Regarding the total trials completed, MK-801 caused a significant increase (Figure 6D; $F(1,11)=14.35$, $p<0.01$), but L-govadine caused a significant reduction (Figure 6D; $F(1,11)=20.91$, $p<0.01$). There was no interaction observed for the total number of trials completed (Figure 6D; $F(1,11)=0.00$, $p>0.05$). There was no significant effect of L-govadine (Figure 6E; $F(1,11)=3.83$, $p>0.05$), or MK-801 (Figure 6E; $F(1,11)=1.36$, $p>0.05$) on correct

response latency and no interaction was observed (Figure 6E; $F(1,11)=1.14$, $p>0.05$). Likewise, there was no significant effect of L-govadine (Figure 6E; $F(1,11)=3.78$, $p>0.05$), or MK-801 (Figure 6E; $F(1,11)=0.28$, $p>0.05$), and no interaction (Figure 6E; $F(1,11)=0.22$, $p>0.05$) for incorrect response latency. In contrast to the other latency measures, L-govadine significantly increased reward latency (Figure 6E; $F(1,11)=40.16$, $p<0.001$) while MK-801 caused a significant reduction (Figure 6E; $F(1,11)=25.99$, $p<0.001$). No significant interaction was observed (Figure 6E; $F(1,11)=1.23$, $p>0.05$). Note the large group mean and SEM for incorrect response latency following treatment with L-govadine and L-govadine with MK-801. These increases were driven by a small subset of rats with exceptionally high latency, and as it was a minor portion of the sample population, their exclusion would not alter the results of the one-way repeated measures ANOVA (n.s). This may illustrate heightened sensitivity of some individuals to adverse effects of L-govadine. The effect of MK-801 on reward latency was consistent with that from Squad 1, however other latency effects were not replicated. Overall, MK-801 consistently impaired accuracy and increased correction trials across two independent samples, as well as increased the total number of trials completed while other task measures have more variable results. L-govadine demonstrated efficacy in reversing the MK-801 induced impairments in accuracy and correction trials however it also reduced the total trials completed.

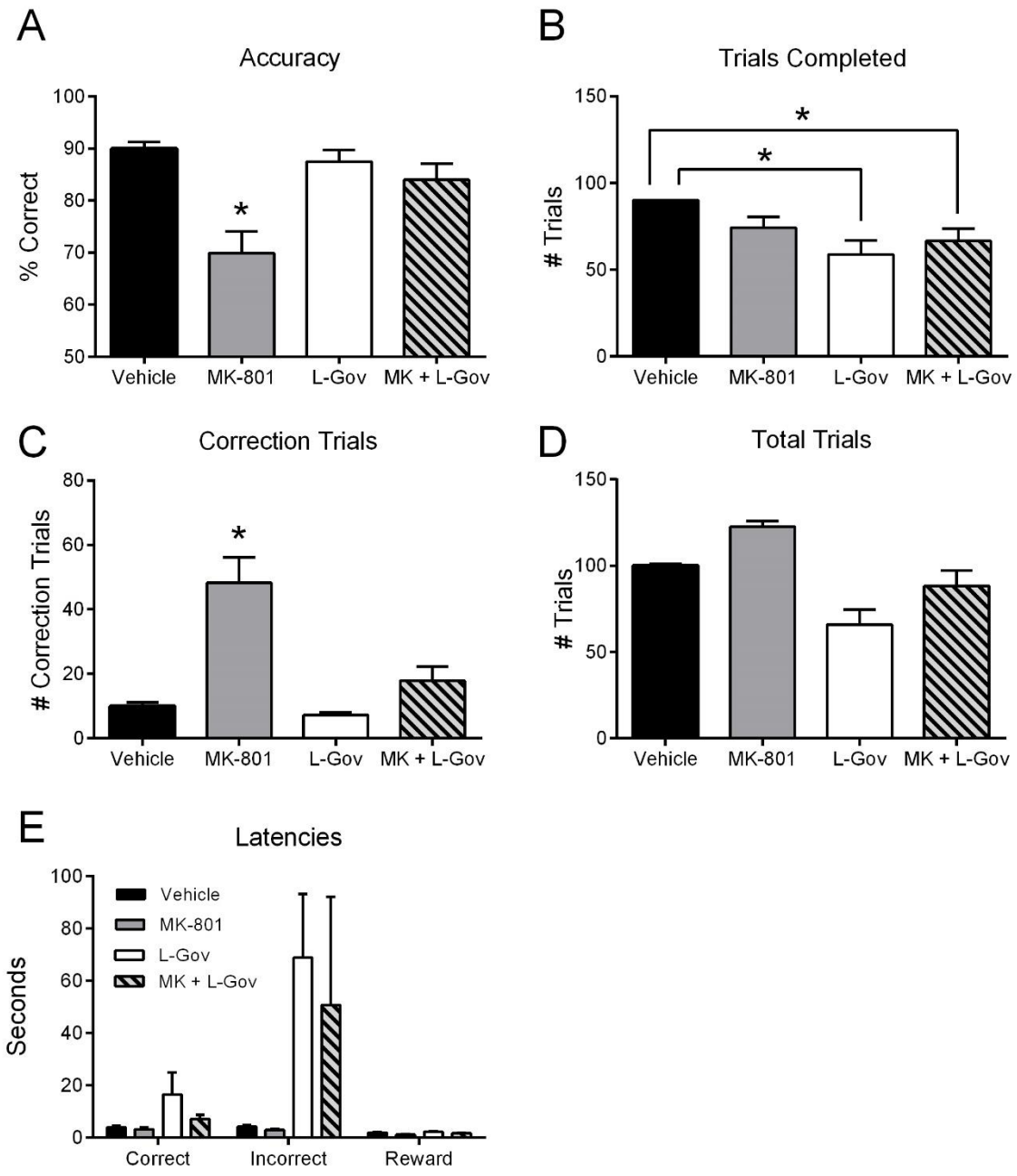

Figure 6: The effects of L-govadine and MK-801 on PAL. [A] MK-801 significantly reduced accuracy. L-govadine reversed the deficit but had no effect alone. **[B]** MK-801 had no effect on trials completed but L-govadine caused a significant reduction. **[C]** MK-801 significantly increased correction trials. L-govadine restored correction trials to control levels, but had no effect alone. **[D]** MK-801 increased the number of total trials (total responses) and L-govadine decreased total trials. Asterisks were omitted for clarity. **[E]** MK-801 reduced reward latency and L-govadine increased reward latency with no effect on other latency measures. Asterisks were omitted for clarity for reward latency.

3.5. The effects of Haloperidol on PAL

Haloperidol was administered subcutaneously in 2 doses, 0.05 mg/kg and 0.1 mg/kg, as well as a vehicle control (acetic acid and sodium acetate, pH adjusted to 5.0). Haloperidol did not impair task accuracy compared to the vehicle treatment (Figure 7A; $F(2,14)=2.74$, $p>0.05$), however a significant effect was observed on trials completed (Figure 7B; $F(2,14)=29.02$, $p<0.001$). Post hoc analysis indicated that treatment with both doses reduced trials completed than the vehicle treatment and the two doses differ from each other as well, decreasing in a stepwise dose-dependent manner. There was also a significant effect of haloperidol on correction trials (Figure 7C; $F(2,14)=8.01$, $p<0.01$) with post hoc analysis indicating the high dose of 0.1 mg/kg resulted in fewer correction trials than the 0.05 mg/kg dose and vehicle, which did not differ. When examining the total trials completed, there was a significant effect of haloperidol treatment (Figure 7D; $F(2,14)=28.63$, $p<0.001$). Post hoc analysis indicated both doses of haloperidol treatment reduced total trials compared to vehicle and the two doses were significantly different from each other. The latency to make a correct choice was not affected by haloperidol (Figure 7E; $F(2,14)=3.99$, $p>0.05$), however a significant effect of haloperidol was found to increase incorrect response latency (Figure 7E; $F(2,14)=6.83$, $p<0.05$). Post hoc testing showed rats took significantly longer to make an incorrect selection when they received the high dose of 0.1 mg/kg. No effect of haloperidol was observed on reward latency (Figure 7E; $F(2,14)=1.12$, $p>0.05$). Note the high mean and SEM shown in Figure 7E for correct response and reward latency. A small subset of rats are responsible for increasing the mean, and this is represented in the large SEM. These rats were considered for exclusion from analysis but were

ultimately included as their exclusion would not have changed the overall outcome of the one-way repeated measures ANOVA (n.s.), as well as to demonstrate the variability in sensitivity of individual subjects to the haloperidol treatment.

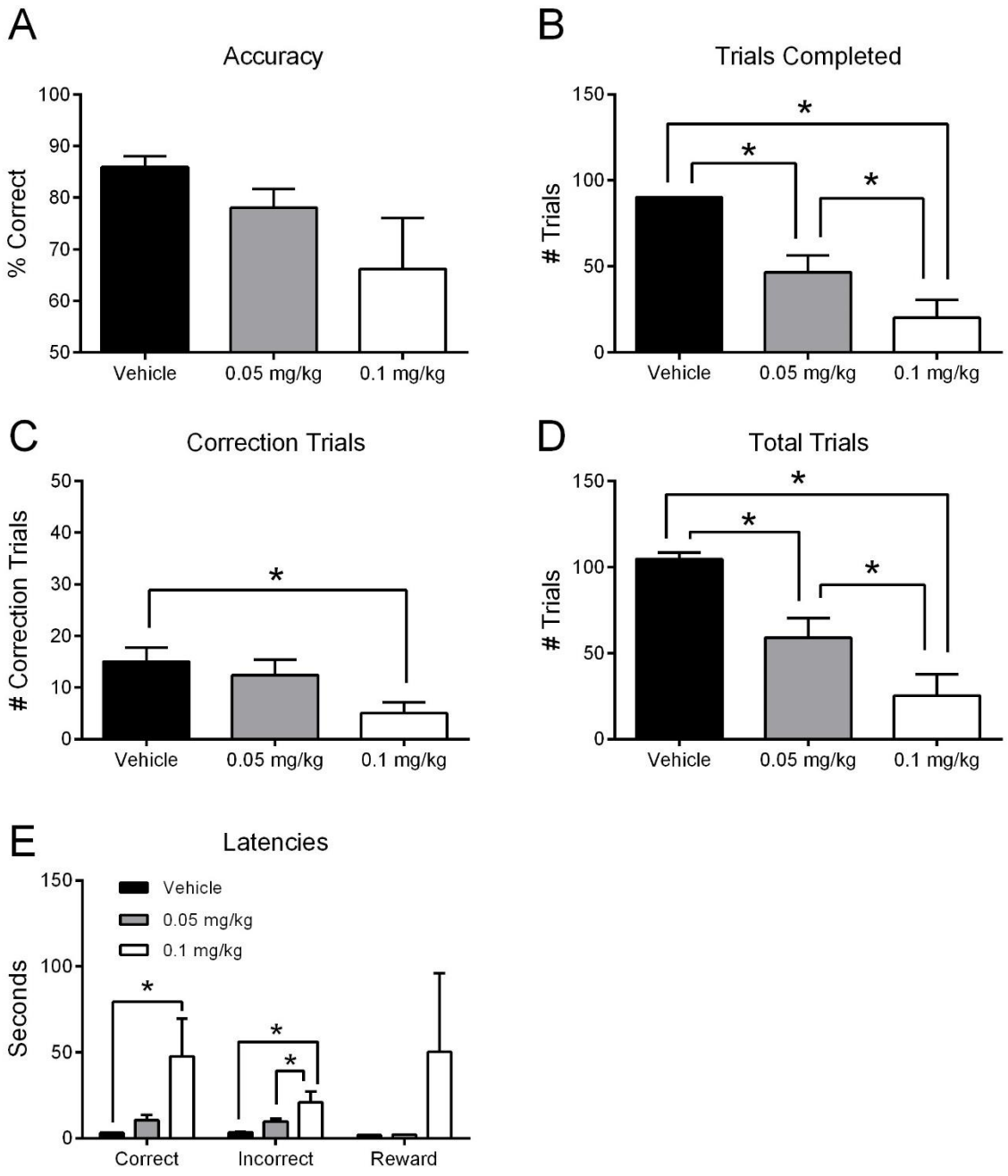

Figure 7: The effects of haloperidol on PAL. [A] Haloperidol did not significantly affect accuracy. **[B]** Haloperidol caused a dose-dependent decrease in the number of trials completed. **[C]** Haloperidol significantly reduced the number of correction trials at 0.1 mg/kg. **[D]** Haloperidol caused a dose-dependent decrease in the number of trials completed. **[E]** Haloperidol significantly increased correct and incorrect response latency at the 0.1 mg/kg dose with no significant effect on reward latency.

4. DISCUSSION

The present experiments resulted in novel data regarding the MK-801 animal model of schizophrenia in the touchscreen visuo-spatial PAL task and the putative antipsychotics CDPPB and govadine. No effects of CDPPB were found, either when administered alone (Figure 3) or alongside MK-801 (Figure 4), except for a significant reduction in the number of trials completed at the 10 mg/kg dose of CDPPB (Figure 3B). MK-801 at a dose of 0.15 mg/kg reliably reduced accuracy, increased correction trials, and increased total trials across two independent samples (Figure 4ABCD, Figure 5ABCD, Figure 6ABCD). More variable were the effects of MK-801 on the latency measures (Figure 4E, Figure 5E, Figure 6E). D-govadine alone had no effect on any of the measured parameters and did not affect the MK-801 induced deficits (Figure 5). L-govadine alone decreased the total number of trials completed and increased reward latency, while also attenuating the MK-801 induced deficits to control levels (Figure 6). Haloperidol did not affect PAL accuracy at either examined dose but caused a dose dependent decrease in trials completed, correction trials and total trials while increasing incorrect response latency at the 0.1 mg/kg dose. Overall, these results indicate that the MK-801 model of schizophrenia induces deficits in visuo-spatial PAL, a task that measures one of the seven cognitive domains affected in schizophrenia. These data further indicate that positive allosteric modulation of $mGluR_5$ and systemic administration of D-govadine do not enhance PAL in either untreated rats or those faced with a pharmacological challenge via systemic MK-801, however systemic L-govadine reversed the MK-801 induced deficits.

4.1. Acute MK-801 injection impairs cognitive performance in PAL

Systemically administered MK-801 impairs cognitive performance in a variety of tasks including recognition memory, set-shifting, and spatial learning and memory (Vales et al., 2010) however its effects on performance of the PAL task had never been examined until the present study. The dose used in the present experiment is 0.15 mg/kg is within the typical range for rats (0.1 to 0.3 mg/kg) (Darrah et al., 2008;Stefani and Moghaddam, 2010). MK-801 was administered to two independent squads of rats and significantly reduced accuracy (% correct), increased correction trials (errors), and increased total trials in both squads, but trial number was only significantly reduced in squad 2 (Figure 4B). Correction trials follow incorrect selections in which the same pair of stimuli are repeatedly presented until the correct choice is made (Figure 2), thus an increase in correction trials indicates an increase in perseverative errors. Perseveration is usually assessed in set-shifting tasks to refer to the number of times a participant reverts to an old strategy despite requiring a new strategy to complete the task or receive a reward. However, the definition of perseveration is repetitive maladaptive errors, such as repeatedly selecting the same image despite indication that it is incorrect or inappropriate as is the case with the visuo-spatial PAL task correction trials. Perseveration is a hallmark feature of cognitive impairment in schizophrenia and is associated with prefrontal cortex dysfunction (Pantelis et al., 1999) and NMDAR hypofunction (Darrah et al., 2008;Stefani and Moghaddam, 2010). The increase in correction trials following MK-801 treatment indicates increased relevance of PAL to the human condition of schizophrenia, as well as provides another way to assess perseveration in a task not requiring cognitive flexibility.

Total Trials is a novel measure that has not been included in previous PAL publications. This is the sum of trials completed, which is a count of how many times a rat responded to the first presentation of a stimulus pair (not including correction trials), plus correction trials. The total trials measure provides more information as to how the rats are spending their time in the touchscreen chamber. Previous work reports a decrease in trial number to indicate decreased responding (Talpos et al., 2014), but makes no reference to correction trials despite each correction trial also indicating a response. As mentioned in section 2, criterion on the PAL task is 90 trials completed in 60 minutes. The session automatically ends after 60 minutes or 90 trials are completed, whichever occurs first. As correction trials are not included in the response count for trials completed, when correction trials increase, first-presentation trials will decrease as long as the 60 minute time limit is enforced simply due correction trials taking up an increased portion of the allotted 60 minutes. Therefore, trials completed alone does not fully account for the number of responses made. In this experiment, MK-801 treatment increased total trials signifying that MK-801 does not reduce responding (Figure 4D, 5D, 6D). This does not necessarily indicate that MK-801 increases responding because the vehicle treated rats make less errors allowing them to move through the 90 trials more quickly, causing the session to end and preventing further responding. Therefore, the total trials measure can only indicate if responding is decreased but not if it is increased, as whether or not the 90 trials are completed affects the time frame in which responding can occur. The knowledge that MK-801 does not reduce the ability of a rat to make a response strengthens the argument that the reduction in accuracy and increase in

errors is due to cognitive impairment, not other factors such as impaired motor function or motivation which could also reduce responding and effort in the task.

When assessing a visual based task, it is important to consider the possibility that the drug administered may affect visual perception. The effects of various pharmaceuticals have been assessed in a touchscreen visual discrimination task and found no compelling evidence that either MK-801 or PCP induce selective impairments in perception, indicating NMDAR antagonists are appropriate for use in researching cognition in visual based tasks (Talpos et al., 2012).

Overall, these data indicate that NMDAR hypofunction via systemic MK-801 impairs performance in the PAL task. The cognitive impairment is consistent with what is observed in human schizophrenic patients as an increase in perseverative errors robustly occurred in both independent squads tested without reducing responding.

4.2. CDPPB alone affects trials completed but not cognitive performance in PAL

The results displayed for the vehicle treated rats in Figure 3 are comparable to previous studies that examined PAL with the mean accuracy achieved near 90% (Talpos et al., 2014;Talpos et al., 2009). The only significant effect of CDPPB alone was a reduction in trials completed at the highest administered dose, 10 mg/kg. Previous studies have examined motor effects of CDPPB using the rotorod test and open field locomotion and no significant locomotor effects were found using doses of up to 30 mg/kg (Fowler et al., 2013;Stefani and Moghaddam, 2010). Despite no significant effects, previous authors have noted that the time required to complete maze-based

tasks takes 20% longer when rats receive 10 mg/kg and 30 mg/kg CDPPB than when they are vehicle treated (Fowler et al., 2013;Vales et al., 2010). This indicates potential for doses of CDPPB 10 mg/kg and higher to affect locomotor ability in some capacity that may not be directly measured in an open field locomotor assessment, such as reaction time. It is worth noting the highest dose assessed using a rotorod was 5 mg/kg (Vales et al., 2010), half of the dose that caused a reduction in trials completed in the present experiment. Furthermore, there was no effect on task accuracy or correction trials, indicating no negative cognitive effects of CDPPB that could have influenced trials completed.

CDPPB did not affect accuracy or correction trials which is consistent with previous research as there are currently no reports of CDPPB alone improving cognition in conditions where performance is already high (further specified in section 4.3.). It is possible that CDPPB alone could enhance performance in a task with more freedom to manipulate the difficulty, such as the touchscreen TUNL task, a non-match to sample task in which both the delay between the sample and test phases and the locations of the stimuli can be modified to increase difficulty (Oomen et al., 2013). In PAL the vehicle treated rats are already performing at a mean of approximately 90% correct which may indicate a ceiling effect.

4.3. CDPPB does not reverse MK-801 induced impairments in PAL

CDPPB was administered alongside MK-801 at the moderate dose of 3 mg/kg to determine if the $mGLuR_5$ PAM would attenuate the MK-801 induced deficits. The only

effect of CDPPB was increased correct response latency with no effect on any of the measures directly related to cognition, namely accuracy and correction trials. As previously mentioned, CDPPB has improved cognition in spatial based tasks. Specifically, CDPPB reduced perseverative errors during reversal learning in the Barne's maze (Fowler et al., 2013), enhanced performance in a delayed-alternation T-maze task at increased delays (Fowler et al., 2013), and reduced MK-801 induced impairments in spatial aversive learning with no effect on aversive learning in controls (Fowler et al., 2011). CDPPB also restores MK-801 induced deficits in a set-shifting task using a T-maze by restoring trials to criterion and perseverative errors to control levels, once again with no effect in control animals (Stefani and Moghaddam, 2010). Given that CDPPB has repeatedly demonstrated efficacy in improving perseveration due to cognitive inflexibility following MK-801 administration but not in the current study suggests fundamental differences between two types of perseverative behaviors that can both be caused by NMDAR hypofunction. The PAL task is rigid in structure in that it requires learning three distinct visuo-spatial associations over a relatively long-term training phase. These visuo-spatial associations do not change over the course of experimentation which makes PAL different from tasks that assess cognitive flexibility. Cognitive flexibility depends on dopaminergic and glutamatergic transmission in the medial prefrontal cortex, the major neurotransmitter systems involved in current theories of schizophrenia (Javitt, 2010;Coyle, 2012). CDPPB attenuates MK-801-induced abnormalities in the mPFC such as increased firing and decreased burst frequency (Lecourtier et al., 2007) thus enhanced $mGLuR_5$ activity measurably affects mPFC dysfunction due to NMDAR antagonism. This is presumed to underlie the CDPPB

related improvements observed in cognitive flexibility tasks. The data collected in the current experiment indicate that this same effect is not sufficient when persistent error making occurs in a more rigid task setting. A possible explanation of the impairment could be that the disturbance observed in the present study is the disturbance of a learned habit. PAL is an operant task, the like of which tend to be goal-oriented, but can become driven by habit following extended practice (O'Tousa and Grahame, 2014). Habitual action relies on the dorsolateral striatum, distinct from the reward-driven behaviors dependent on the dorsomedial striatum, however these regions interact in reward-driven habit forming (Burton et al., 2014), which could describe the rigid touchscreen PAL task. One potential caveat is habitual learning is not impaired in schizophrenic patients (Weickert et al., 2002), although no data could be found regarding maintenance of an already acquired habit during an episode of psychosis, which would be much more relevant to the present study. If PAL does involve habit formation, these data may indicate a disruption of habitual behavior in acute psychosis. One effect of NMDAR antagonism is increased DA release in the striatum (Jentsch and Roth, 1999), providing a potential mechanism of striatal modulation. However, $mGluR_5$ are also present in the striatum which raises the question as to why there was no effect of CDPPB in correcting these deficits. These data necessitate a closer look at PAL, habit development and maintenance, and NMDAR antagonism in the striatum and surrounding circuitry.

4.4. L-govadine, but not D-govadine, reverses MK-801 induced impairments in PAL

Govadine differs greatly from CDPPB as it affects the DA system. When combined with acute MK-801 the two govadine isomers produced opposite results with L-govadine attenuating the MK-801 deficits (Figure 6) and no effect of D-govadine (Figure 5). More specifically, L-govadine restored accuracy (Figure 6A) and correction trials (Figure 6C) to control levels when administered with MK-801 while D-govadine and MK-801 treated rats did not differ from MK-801 alone for accuracy (Figure 5A) or correction trials (Figure 5C). Previous research shows that D- and L-govadine have different but complementary effects: L-govadine acts like a typical antipsychotic capable of improving positive symptoms, generally attributed to greater efficacy as a D2 antagonist, while D-govadine is a cognitive enhancer (Lapish et al., 2014), contrary to the precognitive effects of L-govadine observed in the present study. The main differences between the two isomers are L-govadine's greater affinity for D1 receptors and greater D2 antagonist effects than D-govadine, and L-govadine increases DA efflux in the NA (ventral striatum), and throughout the PFC whereas D-govadine is limited to the mPFC (Lapish et al., 2014). As previously mentioned, cognitive impairment is partially attributed to D1 hypofunction in the PFC (Coyle, 2012). The cognitive enhancing effects of L-govadine may be due to increasing DA effux in regions of the PFC beyond the mPFC.

The prospect of PAL involving habitual action was introduced in section 4.3. and goal-directed habitual action depends on striatal structures (Burton et al., 2014), therefore implicating the involvement of circuitry under greater influence of L-govadine due to its unique ability to increase DA efflux in the ventral striatum. For example, the

orbitofrontal cortex and ventral striatum feed into circuits involved in habitual behavior (Balleine and O'Doherty, 2010). Furthermore, an fMRI study in humans reports activity in the OFC and ventral striatum in response to visual stimuli predictive of a food reward, which may be of relevance in the visuo-spatial PAL task. Seemingly contradictory however, is the ability of NMDAR antagonism to increase DA efflux in the ventral striatum (Jentsch and Roth, 1999). Taken together, L-govadine's action as a D2 antagonist working in unison with increased DA efflux in the ventral striatum, as a result of both its own effect and NMDAR hypofunction, combined with a general increase of DA efflux throughout the PFC may create conditions suitable for normal performance in the PAL task, restoring accuracy and correction trials to control levels. While the systemic injections utilized in this study cannot implicate specific brain regions, these data, when considered in light of the discussed work on habitual behavior appear to support visuo-spatial PAL containing elements of habit.

Trials completed and total trials were also differentially affected by D- and L-govadine, where D-govadine had no effect (Figure 5B, 5D) and L-govadine significantly reduced both (Figure 6B, 6D). As described in section 4.2. total trials is a measure of total responding, indicating less activity overall following L-govadine treatment. Given that L-govadine has effects in the striatum, locomotor impairment due to catalepsy could be responsible but this is not likely considering response latencies were not severely affected (although there was a slight increase in reward latency). It is worth noting that a small number of rats showed exceptionally high response latency when treated with L-govadine which may indicate individual sensitivity to the drug treatment. While three latency measures are calculated by the software, a fourth latency measure is not

quantified, and this is the latency to initiate a new trial following reward collection. By order of elimination, if the other three latency measures are not abnormally altered, latency to initiate a trial must be increased to account for the allotted 90 minute time frame. It is possible that L-govadine's ability to affect the striatum as previously mentioned may also have an effect on motivational and goal-directed behavior, as this is a function of the dorsomedial striatum (Burton et al., 2014;Balleine and O'Doherty, 2010), therefore decreasing the total number of responses.

4.5. Haloperidol lowers task activity in a dose-dependent manner.

Haloperidol is a typical antipsychotic and potent D2 antagonist. Despite the reports of conventional antipsychotics being ineffective in improving cognitive deficits (Javitt, 2010;Young et al., 2009;Millan et al., 2012) the success of L-govadine, a drug that acts as a D2 antagonist among other previously discussed effects, in improving cognitive parameters in PAL prompted further investigation of D2 receptors. While PAL accuracy decreased with each increasing dose of haloperidol, no significant effect was observed (Figure 7A). Trials, correction trials, and total trials were all reduced in a dose dependent manner with the 0.1 mg/kg treatment group completing less than half of the total responses as the vehicle group. A significant reduction in correction trials would generally be taken as a reduction in errors and therefore cognitive enhancement, however in this case the reduced correction trials are proportional to the reduced total trials. This severe effect on responding is likely due to the well documented extrapyramidal effects of classic antipsychotics which include muscle rigidity known as catalepsy (Marrocco et al., 2013). These motor effects confound the ability of the PAL

task to provide insights into cognitive effects which may be directly related to D2 antagonism.

4.6. Limitations and future directions

Limitations of the research presented in this thesis include the use of only one behavioural task. While the extensive training regimen would have made the addition of another task difficult in the given time frame, the PAL task is not able to provide direct information on attention or motivation which would have given greater insights into the nature of the deficits produced by MK-801 and could have helped explain the decrease in total trials seen following L-govadine administration. Future research directions include using local infusions to elucidate the brain regions involved in the PAL task. Such targets include inactivation of the mPFC and dmSt, followed by more specific manipulations such as D1 antagonism in the mPFC in attempt to mimic, and reverse, the deficits from MK-801. These studies will help explain why a pro-cognitive effect of L-govadine, but not D-, was observed. Another future direction is to address the aforementioned limitation and repeat the pharmaceutical treatments used in this study with other behavioural tasks, such as TUNL and the 5-choice serial reaction time task.

5. CONCLUSION

This thesis presents novel findings regarding cognitive deficits in a pharmacological rodent model of schizophrenia. These data demonstrate the efficacy of acute MK-801 in inducing deficits relevant to schizophrenia in a visuo-spatial association task which has

been promoted by MATRICS and CNTRICS as a promising technique with translational potential. These data further indicate that the $mGluR_5$ PAM CDPPB is not effective at restoring the MK-801 induced deficits in PAL. This thesis also contains the first data indicating cognitive enhancement due to L-govadine, an effect which may be due to the PAL task containing elements of habit. Finally, these data demonstrate the adverse effects of haloperidol which confound interpretation of cognitive performance. These findings constitute a valuable contribution to the study of cognition and the collaborative effort to improve treatment in schizophrenia.

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