MK-801-INDUCED IMPAIRMENTS ON THE TRIAL-UNIQUE, DELAYED NONMATCHING-TO-LOCATION TASK IN RATS: EFFECTS OF ACUTE SODIUM NITROPRUSSIDE

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ABSTRACT

The cognitive symptoms observed in schizophrenia are highly prevalent and predictive of patient functional outcome but are not usually alleviated by conventional antipsychotics. In a recent pilot study, sodium nitroprusside (SNP), a nitric oxide donor, was identified as a promising adjunct treatment to reduce the working memory impairments experienced by schizophrenia patients. Adjunctive SNP has also been reported to decrease the positive and negative symptoms experienced by patients for weeks following a single administration. The mechanisms underlying these changes and the areas of cognition affected remain largely unknown. Therefore, it is of interest to examine the effects of SNP using a rodent model of schizophrenia that has demonstrated predictive validity. The aim of the present experiment was to explore the effects of SNP on the acute MK-801 rodent model of schizophrenia using a highly translatable task in order to establish its validity. Working memory and pattern separation were measured using the trial-unique, delayed nonmatching-to-location (TUNL) task in touchscreen-equipped operant conditioning chambers. Acute MK-801 administration 25 minutes prior to task initiation impaired both areas of cognition. When SNP and MK-801 were administered within 5 minutes of each other, no interaction was observed. Interestingly, SNP improved performance on trials with difficult to discriminate patterns (p=0.058). Previous rodent studies using the ketamine model of schizophrenia and the novel object preference task observed a preventative effect of SNP administration. When we administered SNP nearly 4 hours prior to MK-801, no cognitive improvements were observed. Our results suggest that SNP may have intrinsic cognitive enhancing properties but is not capable of reducing MK-801-induced working memory and pattern separation impairments in the TUNL task. This study failed to mirror the results of the human pilot study that observed improved working memory following SNP administration.

Further, it did not replicate previous animal studies using ketamine. Ultimately, the findings suggest that the effects of MK-801 in the TUNL task may not hold the predictive validity needed for its use in the study of SNP. In order to advance the understanding of SNP, future studies should investigate other translatable paradigms to establish validity.

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LIST OF ABBREVIATIONS

AMPA	Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid
cGMP	Cyclic Guanosine Monophosphate
Cn^-	Cyanide ion
D1	Dopamine Receptor Subtype 1
D2	Dopamine Receptor Subtype 2
dlPFC	Dorsolateral Prefrontal Cortex
GABA	γ-Aminobutyric Acid
GABAergic	γ-Aminobutyric Acid releasing
GAD67	67-kDa isoform of Glutamate Acid Decarboxylase
GC	Guanylate Cyclase
IP	Intraparitoneal Injection
MATRICS	Measurement and Treatment Research to Improve Cognition in Schizophrenia
MK-801	Dizocilpine
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
nNOS	Neuronal Nitric Oxide Synthase
mPFC	Medial Prefrontal Cortex
mRNA	Messenger Ribonucleic Acid
NMDA	*N*-Methyl-D-Aspartate
NMDAR	*N*-Methyl-D-aspartate Receptor
PCP	Phencyclidine

PFC	Prefrontal Cortex
PV	Parvalbumin
PV+	Producing Parvalbumin
TUNL	Trial-Unique, Delayed Nonmatching-to-Location

1.0 INTRODUCTION

1.1 Schizophrenia: Cognition, Working Memory and Pattern Separation

Schizophrenia is a chronic psychiatric condition that affects nearly 1% of the population and is ranked among the top ten illnesses contributing to the global health burden of disease (Murray and Lopez 1996; McGrath et al. 2008). This neurodevelopmental disorder is the consequence of an interaction between genetic susceptibility and environmental factors (Davis et al. 2016). Symptom presentation, severity, and response to treatment is highly heterogeneous in this patient population (O'Connor and O'Shea 2015). The symptoms of schizophrenia are categorized into three domains: positive, negative, and cognitive (Bozikas et al. 2004; Carbon and Correll 2014). The positive symptoms are distortions in perceptual and thought-processes including hallucinations and delusions. These symptoms are typically managed by conventional antipsychotics. In contrast, negative symptoms include social withdrawal and anhedonia, while cognitive symptoms involve deficits in attention, working memory, and impulse control (Lewis et al. 2005). To date, no available treatment or combination of treatments reduces symptom presentation across all three domains (Vingerhoets et al. 2013). Importantly, cognitive symptoms are highly indicative of patient functional outcome, including community functioning and interpersonal interactions, implicating them in patient quality of life (Bhagyavathi et al. 2015). The cognitive symptoms are experienced by 80% of patients and remain stable throughout illness progression. These impairments appear prior to diagnosis and may be a vulnerability marker of psychosis (Bora et al. 2014; Carbon and Correll 2014). Further, cognitive symptoms persist after positive symptoms are managed (Nuechterlein et al. 2004; Young et al. 2009). Therefore, it is imperative that treatments become available to alleviate these symptoms.

The Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative has identified 7 cognitive domains that are typically affected in the disorder (Green et al. 2004). These domain 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).

Working memory is the ability to temporarily store and manipulate information. Impaired working memory reduces the capacity of schizophrenia patients to maintain information across a delay and protect it from interference (Fleming et al. 1995; Kim et al. 2004; Fatouros-Bergman et al. 2014). Due to their high prevalence, robustness, and severity, working memory deficits have been proposed as a core component to the diagnosis (Mesholam-Gately et al. 2009). Further, these impairments critically contribute to other symptoms and neuropsychological deficits in schizophrenia (Silver et al. 2003; Lewis and Moghaddam 2006). For example, disorganized speech patterns common to patients may reflect the inability to mentally maintain a linguistic schema (Ragland et al. 2007). Working memory performance depends upon the dorsolateral prefrontal cortex (dlPFC, Ragland et al. 2007) and impairments may be the result of abnormal neural activation, anatomy, or integration within this region. Functional imaging studies have shown reduced dlPFC activation in schizophrenia patients is accompanied by increased activation of surrounding neural regions, however this compensation is insufficient to return performance to control levels (Manoach et al. 1999). Reduced dlPFC activation appears specific to schizophrenia, as individuals with non-schizophrenia psychosis (MacDonald et al. 2005), bipolar disorder (Hamilton et al. 2009), or depression (Barch et al. 2003) do not present with a comparable functional abnormality. Reduced dlPFC activation occurs in treatment-naïve or antipsychotic-treated patients, suggesting it is not a consequence of

medication (Dreher et al. 2012; Kyriakopoulos et al. 2012; Koike et al. 2013). Further, the subset of schizophrenia patients who do not exhibit working memory deficits present with increased dlPFC activation compared to controls, implying reduced dlPFC efficiency across patients (Callicott et al. 2003). Patients with greater dlPFC changes perform worse on working memory tasks, suggesting dlPFC dysfunction correlates with symptom severity (Perlstein et al. 2001). In addition, anatomical studies show a positive correlation between the dlPFC grey matter volume in schizophrenia patients and their working memory ability (Sui et al. 2015). Other studies have suggested that working memory symptoms may be a consequence of functional and anatomical disconnections of the PFC from the rest of the brain (Zhou et al. 2015).

Pattern separation is the ability to keep two similar but distinct patterns as separate representations within the mind and evidence suggests that this cognitive function is impaired in schizophrenia. Although the research exploring pattern separation impairments is limited, patients appear to perform worse than controls on pattern separating tasks (Das et al. 2014). This impairment may be attributed to more basic visual discrimination and recognition deficits (Martinelli and Shergill 2015). Pattern separation is dependent upon the dentate gyrus, a hippocampus region with altered functioning and reduced volume in schizophrenia (Haukvik et al. 2015; Faghihi and Moustafa 2015; Stan et al. 2015). Taken together, working memory and pattern separation are cognitive domains impaired in schizophrenia that may reduce patient quality of life.

In order to efficiently develop treatment strategies to alleviate these impairments, an understanding of the pathophysiological changes in schizophrenia that lead to these cognitive symptoms is required. One predominant hypothesis in the literature suggests that alterations in the neurotransmitter glutamate and its receptors underlie the cognitive symptoms of this disease.

Therefore, focusing on this dysfunctional system may accelerate antipsychotic development (Moghaddam and Javitt 2012).

1.2 Glutamate and NMDA Receptor Dysfunction in Schizophrenia

The dopamine hypothesis of schizophrenia postulates that patient symptoms result from dopamine dysfunction (Lieberman 2004; Lodge and Grace 2008; Javitt 2010). Within the striatum and nucleus accumbens, D_2 receptors are abundant and patients with schizophrenia have increased D_2 receptor densities as well as heightened amphetamine sensitivity (Seeman 1987; Seeman 2006; Vinson and Conn 2012). Increased dopamine resulting from hyperactivity of the mesolimbic system is thought to underlie the positive symptoms (e.g. hallucinations, delusions) of schizophrenia. Lending support to this theory, typical and atypical antipsychotics antagonise D_2 receptors and alleviate the positive symptoms (Seeman 2006; Takahashi et al. 2008). Similarly, increasing dopamine levels by administering a dopamine precursor, levodopa, to Parkinson's patients can produce psychosis-like adverse effects including hallucinations and delusions (Schumacher-Schuh et al. 2013). However, the negative and cognitive symptoms of schizophrenia are unresponsive to D_2 receptor antagonists (Vinson and Conn 2012). The dopamine hypothesis was later extended to include dysfunction of the mesocortical pathway as the mechanism underlying the negative and cognitive symptoms (Laruelle 2014). Specifically, reduced D_1 receptor binding within the PFC is implicated in the working memory impairments of schizophrenia (Lieberman 2004). It has also been proposed that dopaminergic changes in patients may be secondary to glutamate dysfunction.

Glutamate is the primary excitatory neurotransmitter within the central nervous system and is found within 40% of all human synapses (Tsai and Coyle 2002). Glutamate receptors are classed as either metabotropic or ionotropic (Ozawa et al. 1998). Metabotropic glutamate

(mGlu) receptors are G-protein coupled and utilize secondary messenger cascades. These receptors modulate glutamate release and receptor function (Schoepp and Conn 1993). The second class of glutamate receptors are ionotropic. These receptors are ligand-gated ion channels, permeable to cations, and responsible for fast, excitatory post-synaptic potentials. There are three identified ionotropic receptors: alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), *N*-methyl-D-aspartate (NMDA), and kainate receptors (Lewis and Moghaddam 2006). All three types of ionotropic receptors have been implicated in schizophrenia (Kerwin et al. 1990; Breese et al. 1995) and are believed to contribute to the disease but the majority of evidence emphasizes the critical role of NMDA receptors in schizophrenia.

NMDA receptor activation requires ligand-binding and a voltage change in the neuronal membrane. More specifically, their activation requires glutamate and glycine co-binding to their respective sites. In addition, a partial membrane depolarization is required to remove noncompetitively bound magnesium ions within the receptor pore (Dingledine et al. 1999). NMDA receptors are located at excitatory synapses throughout the brain, with particularly high densities in cortical and subcortical regions (Monaghan and Cotman 1985; Monaghan et al. 1988). Activation of these receptors is required for long-term potentiation, a mechanism of synaptic plasticity, and blocking NMDA receptor function impairs learning and memory (Robbins and Murphy 2006). Several lines of evidence support the role of NMDA hypofunction in schizophrenia. Initial support followed the observation that behaviors similar to schizophrenia were produced following treatment with an NMDA receptor antagonist, such as ketamine, phencyclidine (PCP), or Dizocilpine (MK-801). The experiences of non-schizophrenic subjects administered ketamine replicated the positive, negative and cognitive symptom domains of

schizophrenia to a level indistinguishable from patients (Krystal et al. 1994; Adler et al. 1999). Further, when ketamine was administered to schizophrenia patients, symptoms were exacerbated (Malhotra, 1997). Patients with schizophrenia have a reduced level of NMDA receptor subunits in the PFC, including the dlPFC (Errico et al. 2013; Dean et al. 2016). Further, levels of endogenous NMDA receptor agonists are also reduced in patients with schizophrenia and non-medicated patients exhibit reduced NMDA receptor binding compared to healthy controls (Pilowsky et al. 2006; Errico et al. 2013).

NMDA receptors are implicated in the clinical manifestation of schizophrenia leading to alterations in glutamatergic as well as other neurotransmitter systems. Within the thalamocortical circuit, glutamate binds to NMDA receptors on inhibitory interneurons within the mediodorsal thalamus. These GABAergic interneurons synapse onto glutamatergic thalamic neurons, which project to pyramidal cells with PFC. When NMDA receptor function is reduced, depolarization of interneurons is impeded, resulting in impaired GABAergic inhibition. This disinhibition increases the glutamatergic excitatory firing in the PFC (Moghaddam et al. 1997; Vinson and Conn 2012). NMDA receptor dysfunction may also have secondary effects on the dopamine system. Chronic administration of NMDA receptor antagonists increasing dopamine levels in subcortical regions (Laruelle 2014). Further, NMDA receptor antagonist treatment increases glutamate function in the ventral tegmental area, stimulating mesolimbic dopamine transmission (Mathé et al. 1998; Jentsch et al. 1998). Therefore, reducing NMDA receptor function in rodents may enable the pathophysiological changes in schizophrenia to be greater understood.

1.3 NMDA Receptor Hypofunction as a Model of Schizophrenia

Animal models of disease bypass the ethical restraints that restrict clinical experimentation and provide a better understanding of human conditions (Markou et al. 2009). One way to induce a disease-like state in rodents is using pharmacological manipulations. By administering specific receptor agonists or antagonists, animal models can be produced that mimic precise pathophysiological changes within a disease. A key advantage of pharmacological models is that they provide a researcher with temporal and spatial control (Nestler and Hyman 2010). As mentioned, patients with schizophrenia have reduced functioning of NMDA receptors and administration of non-competitive NMDA receptor antagonists in controls induces a schizophrenia-like phenotype (Krystal et al. 1994; Adler et al. 1999; Errico et al. 2013). Similarly, non-competitive NMDA receptor antagonism in rodents produces behaviors analogous to the positive, negative, and cognitive symptoms of schizophrenia (Moghaddam and Krystal 2012). For example, locomotor hyperactivity (Manahan-Vaughan et al. 2008; Howland et al. 2012; Zemanova et al. 2013; Mahmood et al. 2016), reduced sociability (Morales and Spear 2014), and performance deficits on visual learning and working memory tasks (Brown et al. 2013; Zemanova et al. 2013; Kumar et al. 2015b; Kumar et al. 2015a; Lins and Howland 2016) are observed following MK-801 administration, mirroring the positive, negative, and cognitive symptoms respectively. Beyond its direct effect on NMDA receptors, MK-801 administration indirectly mimics other pathophysiological features of the disease (Lewis et al. 2005).

Noncompetitive NMDA receptor antagonists preferentially bind to NMDA receptors located on γ-Aminobutyric acid (GABA)-releasing interneurons (Coyle 2012). At sub-anesthetic doses, ketamine increased extracellular glutamate in the PFC (Coyle 2012). This is because the effect of NMDA receptor antagonists on GABAergic interneurons impairs the inhibition of these

neurons on thalamic glutamatergic neurons. Glutamatergic neurons then project to PFC pyramidal neurons and increase the excitatory firing in the PFC (Moghaddam et al. 1997; Vinson and Conn 2012). More specifically, sub-anesthetic doses of MK-801 bind to interneurons containing parvalbumin (PV), a calcium-binding protein. Interneurons containing parvalbumin (PV+) are fast spiking and produce non-adaptive firing patterns. PV+ cells innervate pyramidal neurons, at their soma (basket cells) or their axon (chandelier cells), and other interneurons that target proximal dendrites of projection neurons (Lewis et al. 2005). These PV+ interneurons may be particularly vulnerable to glutamate dysfunction for several reasons (Lewis and Moghaddam 2006). First, these cells receive more excitatory inputs from pyramidal neurons than other GABA subtypes in the prefrontal cortex (PFC) and hippocampus (Gulyás et al. 1999; Melchitzky and Lewis 2003). Second, these PV+ interneurons express more glutamate receptor subunits within the temporal cortex and PFC. For example, the R1 subunit of NMDA receptors is expressed in more PV+ than other interneurons (Huntley et al. 1997; González-Albo et al. 2001). Lastly, the activation of PV+ interneurons may be highly dependent upon glutamate receptor activation, resulting in their high sensitivity and vulnerability to NMDA receptor antagonism (Lewis and Moghaddam 2006). NMDA receptor antagonism reduces PV mRNA expression levels by up to 25% in the PFC in rodents without changing neuron density, suggesting these neurons have the capacity to alter PV+ interneuron translational processes (Cochran et al. 2003). The preferential binding of non-competitive NMDA receptor antagonists to GABAergic, specifically PV+ interneurons, indirectly models altered GABA function in schizophrenia and may be related to working memory symptoms.

1.4 Validity of the Acute MK-801 Model

Animal models are critical to the advancement of novel therapeutic drug production but no animal model perfectly replicates human disorders (Markou et al. 2009). In the case of schizophrenia, modeling the disease is particularly tricky. The diagnosis of schizophrenia, similar to other psychiatric disorders, is based on clinical observations and occurs without objective physiological irregularities such as changes in synapses, cells, or neural circuits. Further, schizophrenia is marked by aspects of the disease that appear to be uniquely human, such as psychosis. Even in the case of more concrete disease characteristics, including cognitive impairments, animal behavioral changes are only approximated to human resemblance. Accounting for these short-comings, animal models of schizophrenia provide irreplaceable insight into the condition (Nestler and Hyman 2010). Therefore, the strengths and limitations of an applied model must be appreciated in order to draw appropriate conclusions from data (Markou et al. 2009).

Three types of validity have been proposed to the assess an animal models clinical utility: etiological, face, and predictive (Nestler and Hyman 2010). The acute MK-801 model of schizophrenia can be evaluated for each type. Etiological validity measures how consistent the development of the modeled condition is with the human disorder. The development of schizophrenia is due to an interplay between genetic and environmental factors (Davis et al. 2016). Therefore, the acute administration and short-term behavioral changes that follow a single MK-801 injection are indicative of limited etiological validity. In contrast to pharmacological models, models rooted in genetic or environmental manipulations produce chronic behavioral changes and more closely replicate cause of the schizophrenia. However, high etiological validity can come at a cost. When measuring cognition, testing may be

confounded by differences in learning (Vorhees et al. 2012; Vorhees et al. 2015). In addition, these models often produce a variety of nonspecific and diverse neurological changes, thus preventing the study of isolated systems (Hadar et al. 2015). The second type of validity used in the assessment of animal models is face validity. Face validity is the degree to which a model presents with the pathophysiological manifestations and behavioral changes seen in a disease state (Nestler and Hyman 2010). An acute injection of MK-801 produces neural and behavioral changes similar to those seen in schizophrenia (see *1.2 MK-801 Model of Schizophrenia*). As mentioned, schizophrenia is characterized by NMDA receptor dysfunction, reduced PV+ interneuron GABA transmission, and three symptom domains. The acute MK-801 model of schizophrenia demonstrates high face validity because rodents administered the drug display similar changes (Lewis et al. 2005; Moghaddam and Krystal 2012). Predictive validity is the final measure of clinical utility and is directly related to the development of novel drugs. Sometimes termed pharmacological validity, predictive validity is the capacity of a model to foreshadow how a patient population will respond to a treatment. In experiments assessing predictive validity, negative controls are drugs known to be ineffective in patients. At best, haloperidol, clozapine, and other conventional antipsychotics inconsistently and moderately reduce the cognitive symptoms of schizophrenia (Feinstein and Kritzer 2013). In contrast, a positive control is the "gold standard" drug used in comparison with the new treatment. This drug is believed to be of the highest available quality and tends to be the most frequently prescribed for the condition. Unfortunately, no available drugs alleviate the cognitive symptoms of schizophrenia and the lack of a positive control prevents the thorough evaluation of an animal model's predictive validity (Markou et al. 2009). Predictive validity of the acute MK-801 model of schizophrenia is inconsistent (Adell et al. 2012). However, this may be a consequence of the

task translatability in assessing novel treatments in the MK-801 model, rather than the model itself.

1.5 Trial-Unique, Delayed Nonmatching-to-Location Task

Within the last decade, several reports have highlighted the suboptimal progress in novel pharmaceutical development, particularly emphasising the halt in psychiatric disorders (Markou et al. 2009; Nestler and Hyman 2010). In the case of schizophrenia, the tasks used to measure cognitive function in animals may be a key hindrance (Nestler and Hyman 2010). By developing assessments with high cross-species applicability, failed clinical drug trials will be reduced and the understanding and treating of human conditions using novel drugs will become more rapid (Markou et al. 2009).

One rodent task used to assess working memory is the delayed nonmatching-to-position paradigm. During this task, a rat is presented with a lever in one of two positions. After pushing the lever and a subsequent delay, two levers are presented in each location. To be rewarded, the non-matched lever must be pressed (Sloan et al. 2006). The usefulness of this task was questioned following the observation that trained rats developed mediating strategies to improve performance, thereby reducing reliance on memory (Chudasama and Muir 1997; Panlilio et al. 2011). The Trial-Unique, Delayed Nonmatching-to-Location (TUNL) task was developed to address the weaknesses of the delayed nonmatching-to-position task. The TUNL task is conducted in touchscreen-equipped operant conditioning chambers (Oomen et al. 2013). By using an automated touchscreen system, variability amongst trials is reduced and researcher bias during data collection is eliminated (Talpos et al. 2010; Oomen et al. 2013). Further, this system makes the administration of tasks simple, time-efficient, and consistent. The touchscreen is covered with a mask composed of 14 open squares and each trial is composed of three phases

(Figure 1). During the sample phase, 1 of the 14 squares becomes lit and a rat nose-pokes the square. This is followed immediately by a delay phase lasting for a predetermined period of time. After the delay, the rat pokes its nose into a port at the opposite end of the operant chamber to queue the choice phase. During the choice phase, the previously-lit sample square and a novel square become lit and the rat must correctly non-match by selecting the novel square to obtain a food reward. The location of the correct square is random and cannot be predicted by the rat, reducing the likelihood of mediating strategies (Talpos et al. 2010). By manipulating the length of delay and distance between the sample and choice square, the demand on working memory and pattern separation can be respectively altered. For example, longer delays require a greater demand on working memory and closer together squares are more difficult to maintain as separate patterns (Oomen et al. 2013). As mentioned, one way to improve the predictive validity is to focus on the preclinical-clinical translation of cognitive tasks. Reduced disparity between preclinical and clinical measures is likely to enhance predictive validity in animal models of disease. Similar to animal models, it is important to critically evaluate the validity of a task to ensure it is appropriate for the cognitive domain being studied. Construct validity indicates whether the domain measured by an animal task is similar to the human cognitive domain it is being compared to (Markou et al. 2009). One way to measure this is by comparing the neural correlates underlying the domain in both species.

In humans, working memory is dependent upon the dlPFC and functional alterations in this region are observed in patients with schizophrenia (Lewis and Moghaddam 2006). Therefore, if TUNL were to show construct validity for working memory then lesions to the medial PFC (mPFC), the rodent cortical region equivalent to the human dlPFC, should impair performance. Indeed, when the mPFC is lesioned, rats perform significantly worse than shams

following longer delays, regardless of the pattern separation difficulty. Further supporting the mPFC in the working memory component of the TUNL task, the removal of inter-trial intervals produces performance interference following PFC lesions (McAllister et al. 2013). Pattern separation is heavily reliant on the dentate gyrus in humans (Gaffan 1985). Rodents are sensitive to the effects of pattern separation on the TUNL task, as smaller separations between sample and choice stimuli reduces accuracy. However, this reduction is exaggerated in rats with hippocampal lesions. This reflects the hippocampal dependence in pattern separation (Talpos et al. 2010). Taken together, these two findings suggest that the TUNL task has high construct validity and may be appropriate to measure working memory in rodent models of schizophrenia.

1.6 Alleviation of the Cognitive symptoms

Current antipsychotics alleviate the positive symptoms of schizophrenia, including hallucinations and delusions, that are core to the DSM diagnostic criteria and a hallmark of the disease. However, negative and cognitive symptoms are severe and associated with long-term disability yet are untreated by conventional antipsychotics (Hyman and Fenton 2003).

The hypothesis that NMDA receptor hypofunction underlies the cognitive symptoms of schizophrenia has created a new model and target in novel pharmaceutical development focused on improving this disruption (Matsui et al. 1995). NMDA receptor activation requires the co-binding of glutamate and glycine (Dingledine et al. 1999), thus administering agonists to these binding sites may recover NMDA receptor function. Exogenous glutamate leads to nervous system damage, therefore research primarily focused on targeting the glycine site (Olney 1990; Tsai et al. 1999; Tsai and Coyle 2002; Tsai et al. 2006). Although co-administration of glycine with ongoing antipsychotic treatment reduced symptoms in patients even after its

discontinuation, these improvements were inconsistent (Evins et al. 2000; Javitt et al. 2001; Heresco-Levy et al. 2004; Buchanan et al. 2007). A meta-analysis of 18 short-term trials found that D-serine or glycine, NMDA receptor agonists to the glycine site, reduced the negative symptoms of schizophrenia but the results were inconsistent and the findings were inconclusive. In addition, D-cycloserine, a partial agonist of the NMDA receptor glycine site, was ineffective at reducing any patient symptoms (Tuominen et al. 2006). Pharmacological shortcomings in clinical trials are not limited to glutamatergic targets. Putative antipsychotics that alter dopaminergic, serotoninergic and cholinergic mechanisms have also reached clinical trials but have not produced desired results (Carbon and Correll 2014).

Most commonly, drug discovery is unidirectional, with information flowing from preclinical to clinical studies (Markou et al. 2009). It often begins with the identification of a particular target. *In vitro* studies give insight into the practical aspects of novel pharmaceutical development such as cellular toxicity, metabolism as well as other pharmacodynamics and pharmacokinetic properties. However, clinical efficacy is initially studied using *in vivo* animal models and eventually progresses into human clinical trials (Markou et al. 2009). Unfortunately, this prevents the pragmatic and rational modification of animal experiments. Sodium nitroprusside (SNP) is a nitric oxide donor with a developed clinical profile that is traditionally used to treat hypertensive crisis in clinical settings (World Health Organization 2014). Recently, SNP has shown promise as an adjunct treatment to alleviate the cognitive symptoms of schizophrenia, presenting a unique situation whereby human and rodent study can happen simultaneously (Maia-de-Oliveira et al. 2015a). SNP is a water soluble salt with the chemical formula $Na_2[Fe(CN)_5NO]$. In hypertensive crisis (World Health Organization 2015), SNP reduces mean arterial blood pressure and vascular resistance, while increasing cardiac output.

This potent molecule induces rapid venous and arterial vasodilation. The onset of vascular effects is virtually immediate and begins within 30 s of administration. Once infusion is completed, the effects rapidly dissipate and the half-life of SNP is < 2 minutes. Patients with schizophrenia were administered SNP adjunct to their antipsychotic treatment and, eight hours following SNP administration, patients had improved performance on the Stroop and n-back task. This is indicative of reduced selective attention and working memory impairments respectively (Maia-de-Oliveira et al. 2015a). Further, positive and negative symptoms were reduced following a single treatment for up to four weeks (Hallak et al. 2013). Taken together, these studies suggest that SNP in conjunction with antipsychotics may lead to reduced symptom presentation in all three symptom domains long-term.

1.7 Rationale and Hypotheses

Nitric oxide function is altered in schizophrenia, implicating it as a potential mechanism for pharmaceutical treatment (Beninger et al. 2009). Recently, human clinical trials have reduce positive, negative and some cognitive symptoms in schizophrenia following the administration of SNP (Hallak et al. 2013; Maia-de-Oliveira et al. 2015a). Importantly, one of the assessed cognitive domains was working memory (Maia-de-Oliveira et al. 2015a). Although the precise mechanisms underlying this effect are unknown, SNP has revealed promise in the treatment of all three symptom domains of schizophrenia.

The overarching aim of this thesis is to determine whether MK-801-induced impairments in the TUNL task are an appropriate model of cognitive impairment in schizophrenia to study the effects of SNP. Although this model has demonstrated face validity in other tasks, its predictive validity is inconsistent. Thus, the first objective of this research is to demonstrate the face

validity of the MK-801 model of schizophrenia in the TUNL task. Only one previous study has investigated performance changes following acute MK-801 administration in the TUNL task (Kumar et al. 2015a). The results of this experiment showed 0.05 mg/kg of MK-801 impaired task accuracy with 1 s and 20 s delays. In the current experiments, we will expand on the previous findings by using less dramatic differences in delay. It is hypothesized that acute MK-801 administration will disrupt TUNL task performance and reduce accuracy on trials with 2 s and 6 s delays and face validity of MK-801 in the TUNL task will be demonstrated. Further, previous studies have identified perseverative errors following MK-801 administration (Lins et al. 2015), and we expect MK-801 will increase the number of correction trials completed by a rat during the TUNL task.

The second objective is to explore the therapeutic potential of SNP using the MK-801 model in the TUNL task. Human studies have suggested SNP as an adjunct treatment to reduce working memory impairments in schizophrenia patients (Maia-de-Oliveira et al. 2015a). Further, SNP has the potential to prevent and rescue ketamine-induced impairments in cognitive tasks (Kandratavicius et al. 2015; Maia-de-Oliveira et al. 2015b). Therefore, it is hypothesized that, similar to ketamine studies, acute MK-801-induced impairments on the TUNL task will be reversed when rats are pre- or co- treated with SNP. If SNP reduces MK-801-induced impairments, then the MK-801 model in the TUNL task will demonstrate predictive validity and the paradigm may be used in future animal studies for SNP in schizophrenia.

2.0 MANUSCRIPT

2.1 Title Page

MK-801-induced impairments on the Trial-Unique, Delayed Nonmatching-to-Location task in rats: effects of acute sodium nitroprusside

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***** The following sections (*2.0 Introduction* to *8.0 Conclusion*) are the text of a manuscript to be submitted to the journal Psychopharmacology. The manuscript contains all the experiments described and completed for this master's thesis.*****

2.2 Abstract

Rationale: The cognitive symptoms observed in schizophrenia are not alleviated by conventional antipsychotics. Following a recent pilot study, Sodium Nitroprusside (SNP) has been identified as a promising adjunct treatment to reduce the working memory impairments experienced by schizophrenia patients.

Objective: The present experiments aimed to explore the effects of SNP on the acute MK-801 rodent model of schizophrenia using the highly translatable trial-unique, delayed nonmatching-to-location (TUNL) task to examine the face and predictive validity of this model.

Methods: SNP (0.5, 1.0, 2.0, 4.0 mg/kg) and MK-801 (0.05, 0.075, 0.1 mg/kg) were acutely administered to rats trained on the TUNL task.

Results: Acute MK-801 treatment impaired TUNL task accuracy. Administration of SNP (2.0 mg/kg) with MK-801 (0.1 mg/kg) failed to rescue performance on TUNL. SNP (5.0 mg/kg) administration nearly 4 hours prior to MK-801 (0.05 mg/kg) treatment had no preventative effect on performance impairments. SNP (2.0 mg/kg) improved performance on a subset of trials.

Conclusion: These results suggest that SNP may possess intrinsic cognitive-enhancing properties but is unable to block the effects of MK-801 treatment on the TUNL task. This experiment does not mirror the alleviating effects of SNP in schizophrenia patients, suggesting that MK-801 in the TUNL task may not hold the predictive validity necessary for its use in the study of SNP as a treatment in schizophrenia.

Key words: schizophrenia, NMDA receptor, nitric oxide donor, working memory, pattern separation

2.3 Introduction

Schizophrenia affects 0.7% of the population (McGrath et al. 2008) and is characterized by positive, negative, and cognitive symptoms (Carbon and Correll 2014). The cognitive symptoms are distinct and core characteristics associated with the disease (Bozikas et al. 2004; Aquila and Citrome 2015) and their severity influences patient functional outcome (Bhagyavathi et al. 2015). Although these symptoms are experienced by the majority of patients, they remain unresolved by available antipsychotic treatments (Palmer et al. 1997). Working memory deficits have been emphasized as key impairments in schizophrenia by the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative (Marder and Fenton 2004). Working memory impairments are robust and highly prevalent among patients (Mesholam-Gately et al. 2009). The severity of impairment is highlighted by the collection of working memory vulnerabilities, including the inability to maintain information across a delay or protect it from interference (Fleming et al. 1995; Kim et al. 2004; Fatouros-Bergman et al. 2014). Pattern separation, the ability to keep separate patterns as distinct internal representations, is a less frequently studied domain of cognition impaired in schizophrenia. This impairment is observed in reduced accuracy on tasks that require face matching, matching-to-sample, or the spatial discrimination of sound (Perrin et al. 2010; Soria Bauser et al. 2012). The trial-unique, delayed nonmatching-to-location (TUNL) task simultaneously measures working memory and pattern separation in rodents. The high cross-species translatability of this task makes it a promising and unique platform to study potential treatment of cognitive impairment in schizophrenia (Bussey et al. 2012). To date, only one experiment has examined task performance in a rodent model of schizophrenia (Kumar et al. 2015a) and none have attempted to reverse any observed impairments.

Sodium Nitroprusside (SNP) is a nitric oxide donor that is traditionally used to treat hypertensive crisis by rapidly inducing arterial and venous vasodilation (Hottinger et al. 2014). Recently, SNP has been investigated as an adjunct treatment to reduce symptom severity in schizophrenia. Intravenous administration of SNP significantly reduced the positive, negative and cognitive symptoms experienced by schizophrenia patients (Hallak et al. 2013; Maia-de-Oliveira et al. 2015a). Of particular importance, patient performance improved 8 hours after SNP administration on working memory and selective attention tasks (Maia-de-Oliveira et al. 2015a). Although cognitive changes were not examined in a subsequent follow-up, positive and negative symptoms were reduced four weeks following the single SNP infusion (Hallak et al. 2013). This suggests that SNP may alleviate all three symptom domains of schizophrenia over long-term periods. Rodent studies using acute NMDA receptor antagonist models of schizophrenia have mirrored the human findings. In these studies, the administration of SNP in conjunction with an NMDA receptor antagonist attenuated locomotor behavior, social behavior, and novel object recognition abnormalities. These measurements are believed to be rodent analogues to aspects of the positive, negative and cognitive symptoms of schizophrenia (Maia-de-Oliveira et al. 2015b; Trevlopoulou et al. 2016). Rodent effects were observed up to one week after administration, further supporting the long-term efficacy of SNP (Maia-de-Oliveira et al. 2015b). Although the mechanism underlying these changes are not fully understood, this evidence supports SNP as a viable treatment to alleviate the symptoms of schizophrenia and improve patient quality of life. SNP is different from putative antipsychotics, whose unidirectional study begins in animal models and proceeds to humans, because its medical application (Friederich and Butterworth 1995) allows SNP to be concurrently studied in clinical

populations and animal models. The present study aims to exploit the bidirectional study of SNP to explore the predictive validity of an animal model of schizophrenia.

NMDA receptor dysfunction is a pathophysiological change implicated in the symptom manifestation of schizophrenia. Human controls who received a non-competitive NMDA receptor antagonist, such as ketamine or phencyclidine, present with a schizophrenia-like phenotype that is indistinguishable from symptoms exhibited by schizophrenia patients (Krystal et al. 1994; Adler et al. 1999). Further, these drugs exacerbate symptoms in diagnosed patients (Malhotra 1997). Non-competitive NMDA receptor antagonism in rats has demonstrated face validity as model of schizophrenia (Moghaddam and Krystal 2012). Specifically, impaired working memory performance has been observed as MK-801 administration in rodents reduces performance on the TUNL task (Kumar et al. 2015a). However, the predictive validity of this model is difficult to interpret as there are currently no positive controls that reduce cognitive symptom presentation in schizophrenia patients (Markou et al. 2009). Therefore, the primary aim of the proposed experiment is to explore the effects of SNP on MK-801-induced working memory impairments in the TUNL task in order further characterize its pharmacological validity as a rodent model of schizophrenia. As mentioned, the TUNL task possesses high translational capacity, making it an ideal platform to study predictive validity (Bussey et al. 2012). The secondary aim of this research is to explore the effects of SNP on pattern separation following MK-801 administration.

2.4 Materials and Methods

2.4.1 Subjects

Forty-four male Long-Evans rats were trained on the TUNL task in two separate cohorts, referred to as Squad 1 (n=22; Charles River Laboratories, Quebec, Canada) and Squad 2 (n=20; Charles River Laboratories, New York, USA). All subjects were housed in clear, ventilated plastic cages and contained within a temperature-controlled vivarium. Animals were given in-cage enrichment consisting of a plastic tube. Rats were maintained on a 12h:12h light-dark cycle and all experimental procedures were completed during the light phase. Subjects were food restricted in order to maintain 85% of their free-feeding weight with normal growth accounted for. Water was available *ad libitum*, except during testing. All experiments were approved by the University of Saskatchewan Research Ethics Board and were conducted in accordance with the standards of the Canadian Council on Animal Care.

2.4.2 Training Apparatus

All experimental training and testing occurred within eight touchscreen-equipped operant conditioning chambers (Lafayette Instruments, Lafayette, IN, USA). Each individual chamber was trapezoidal in shape, with the face of the wide edge comprised of a touchscreen (Fig 1.). The touchscreen was covered by a black polycarbonate mask with 14 small squares arranged in a 7x2 pattern. The lower part of the mask was covered by a spring-loaded "response shelf" that rats must intentionally use in order to touch the exposed touchscreen. On the opposite end of the chamber was a food magazine where odorless reward pellets (Dustless Precision Pellets, 45 mg, Rodent Purified Diet; BioServ, Frenchtown, NJ) were dispensed. This food magazine also contained a reward light and an infra-red nose-poke detector. The floor of the chamber was

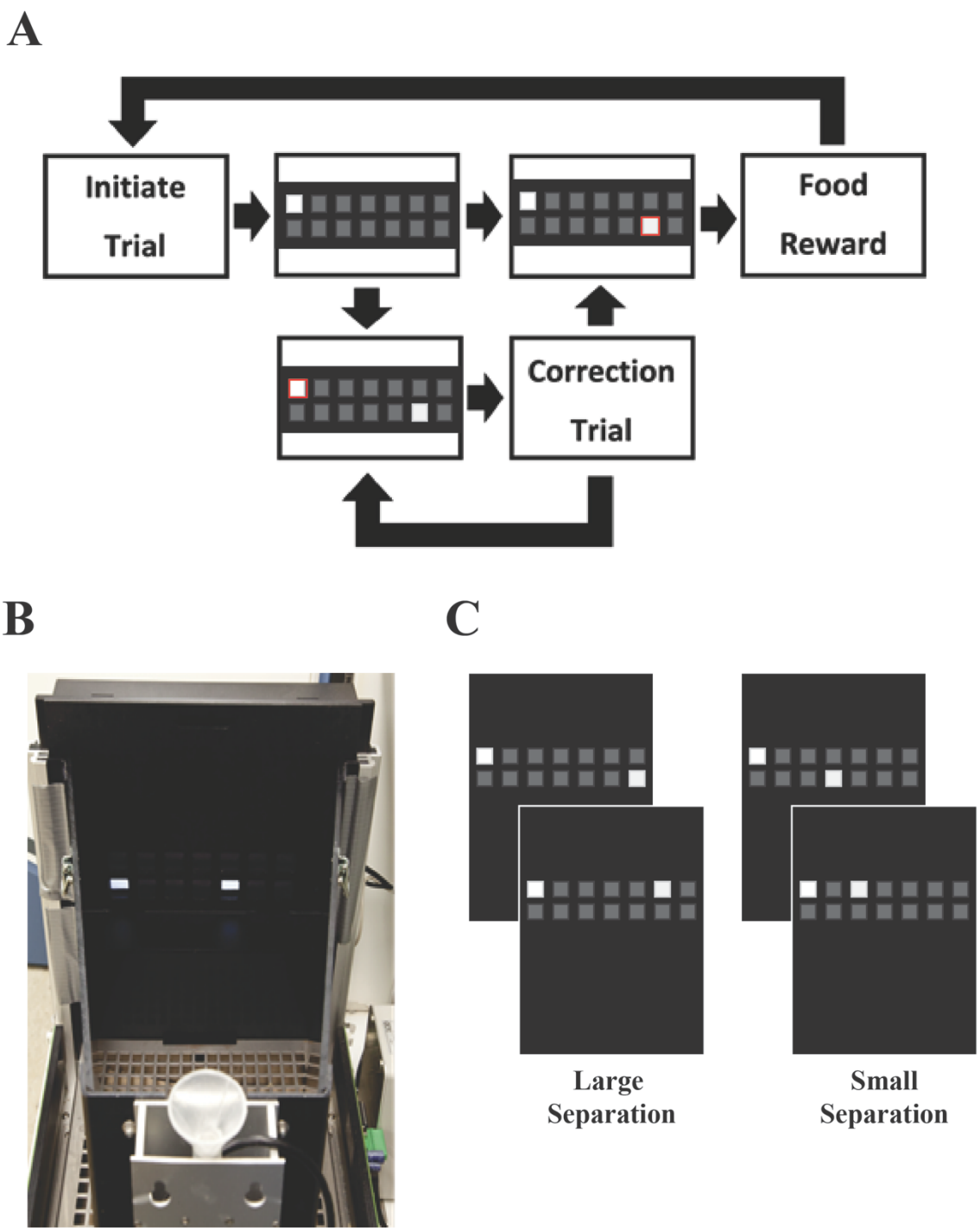

Fig 1 Touchscreen-equipped operant chamber and task schematic. **A.** Flow chart depicting TUNL trial procedure and outcome. See Methods for procedural details. **B.** Photograph of the touchscreen apparatus and stimuli presentation corresponding to the choice phase of the task. **C**. Samples of the two categories of pattern separations (large and small).

composed of metal mesh and the roof was a plastic lid. Each operant conditioning chamber was located on a sliding shelf at the base of a sound-attenuating large wooden box. In addition to the operant conditioning chamber, boxes contained a pellet dispenser, video camera, small ventilation fan, and a house light that was activated following incorrect responses.

2.4.3 Handling and Habituation

Rats were undisturbed in the vivarium for a minimum of five days following their arrival and then individually handled for three consecutive days. Throughout handling, rats were placed on carts and travelled from the vivarium, up an elevator, to the touchscreen room in order to familiarize them with the route of transportation. Following handling, rats were habituated. On the first day of habituation, rats were left in the touchscreen room for one hour with all technology turned on (2 computers and 8 chambers). Ten reward pellets were placed in each home cage to familiarize them with the food reward. Rats were left undisturbed in the touchscreen room for 15 minutes prior to being placed in the operant conditioning chambers on all proceeding training and testing days. Due to the limited number of chambers, rats were run in groups of 8 and an effort was made to consistently put rats into the same chamber throughout training and testing. During the second and third days of habituation, rats were placed into the operant conditioning chambers for 30 minutes with 10 pellets in the food reward port. The chambers were turned on but no stimuli was present on the screen. All behavior was recorded within the touchscreens and an external monitor presented a live video feed of each rat's activity.

2.4.4 TUNL Pretraining

The pretraining protocol followed a modified version of the instructions and software provided by Lafayette that accompanied the touchscreen chambers and each phase was repeated until criterion was reached. TUNL pretraining was composed of four training stages: initial

touch, must touch, must initiate, and punish incorrect. Initial Touch Training introduced the relationship between touchscreen stimuli and a food reward. During each trial, 1 of 14 squares was illuminated. If a rat touched the illuminated square, 3 reward pellets were immediately dispensed but if the square was not touched after 30 seconds, the stimulus was removed and a single reward pellet was dispensed. Each trial was proceeded by a 20s inter-trial interval (ITI). Rats must have completed 100 trials in 60 minutes to reach criterion. The Must Touch Training was similar to Initial Touch Training but the square remained illuminated until it was touched by a rat. Touching the square led to a single reward pellet being dispensed and criterion was 100 trials in 60 minutes. The proceeding Must Initiate Training required a rat to poke its nose in the food magazine to initiate trials identical to those in Must Touch Training. Criterion was 100 trials in 1 hour. The final pretraining stage was Punish Incorrect Training; each trial began with a rat poking its nose into the food reward port, leading to the presentation of a stimulus. If a rat touched the stimulus, a reward pellet was dispensed and a new trial initiated following an ITI. However, if the rat touched an unilluminated square, a timeout began. During a timeout, no reward pellet was dispensed and the house light turned on for 5 s followed by an ITI. The previous trial was then repeated until the rat correctly selected the stimulus; these repeated trials were termed as 'correction trials'. Criterion was the completion of 100 trials within 60 minutes with >80% accuracy on two consecutive days.

2.4.5 TUNL Task Acquisition

Once a rat completed pretraining, it immediately began learning the standard TUNL task (Fig. 1). Each trial began by a rat poking its nose into the reward magazine and initiating the sample phase where 1 of 14 squares was lit. Once the lit square was touched, the stimulus was removed from the screen and a 2 s delay began. In 33% of trials a reward pellet was dispensed

in order to maintain motivation. Following the delay, the rat was required to poke its nose into the reward magazine to start the choice phase. During this phase, the sample square and a novel square were illuminated simultaneously. A correct response was made when a rat non-matched to the sample square and touched the novel square. Correct responses were rewarded with reward pellet and followed by a 20s ITI. A new 'selection trial' began at the end of the ITI, with different stimuli from the previous trial. If an incorrect response was made, with the rat selecting the sample square, the house light turned on for 5 s and no reward was dispensed. This time out was followed by a 20 s ITI and the proceeding trial was identical to the previous one. Trials that repeat the most recent selection trial are termed 'correction trials' and are repeated consecutively until the correct response is made. Accuracy was a measure of the percent of correct responses made during selection trials. Rats were initially trained to complete 40 trials with >75% in 35 minutes. Following this, the second criterion required was 70 trials with >75% correct in 1 h. Rats treated with SNP and MK-801 received additional training that included 2 and 6 s delays with criterion set at 75% accuracy on a session over 2 consecutive days. Once the last criterion was met, rats were left undisturbed in the vivarium while the remaining rats were trained to criterion. Rats were given reminder training sessions once a week to maintain performance until testing began (Oomen et al. 2013). Once all rats were trained to the second criterion, they were reintroduced to daily testing for at least three days to collect baseline measurements prior to drug treatment. Animals that failed to reach criterion were not used for subsequent testing.

2.4.6 Drug Treatments

The order of treatment administration was counterbalanced using a within-subjects design. Rats receiving MK-801 and SNP was quasi-randomly assigned such that MK-801 was not administered on two consecutive treatment days. Dose received for dose-response curve data

was given in ascending order with the initial treatment counterbalanced across rats. MK-801 (Abcam, Cambridge, MA) and SNP (Sigma-Aldrich, St. Louis, MO) were dissolved in saline and drugs were protected from light exposure to prevent photodecomposition (Bisset et al. 1981). All injection volumes were 1.0 mL/kg body weight and saline was used as the vehicle treatment. The doses of MK-801 and SNP used in the TUNL task were determined from existing literature and previously published touchscreen data collected within our lab (Gourgiotis et al. 2012; Kandratavicius et al. 2015; Maia-de-Oliveira et al. 2015b; Kumar et al. 2015a; Lins et al. 2015; Lins and Howland 2016). Dose-response curve data were collected following intraperitoneal administration of MK-801, SNP, or saline 25 minutes prior to starting the TUNL task. Treatment-free days were allowed between testing and baseline measurements were recollected prior to each treatment to ensure a sufficient washout period. A total of 5 rats (1 from squad 1 and 4 from squad 2) did not learn the task and were not treated. Rats trained in squad 1 were used for the MK-801 dose-response curve (n=10; 0.05, 0.075, 0.1 mg/kg), SNP dose-response curve (n=10; 0.5, 1.0, 2.0, and 4.0 mg/kg) and the reversal experiment (rats treated with SNP (2.0 mg/kg) 5 minutes prior to MK-801 administration (0.1 mg/kg), n=12). Due to the extensive training required to learn the task, squad 1 animals (n=22) were reused in drug testing: SNP dose-response curve only (n=7), reversal only (n=5), SNP and MK-801 dose-response curves (n=3), MK-801 dose-response curve and reversal (n=7). In squad 2, the preventative effect of SNP was explored. SNP (5.0 mg/kg, i.p.) was administered 3 h and 35 minutes prior to MK-801 (0.05 mg/kg, i.p.) and 4 h prior to the beginning of TUNL task.

2.4.7 Statistical Analysis

The fully automated nature of the touchscreen procedure and data collection eliminates the potential for researcher bias. All graphs present the data as group means plus the standard

error of the mean (SEM). The dependent measures analyzed include overall accuracy (% correct on selection trials), accuracy on 2 s delay trials, accuracy on 6 s delay trials, accuracy on large separation trials (Fig 1C; minimum of 4 squares between sample and choice stimuli), accuracy on small separation trials (Fig 1C; 1-2 squares between sample and choice stimuli), number of selection trials completed (number of different stimuli presentations), number of correction trials completed (trials replicating selection trials), total trials completed (selection trials plus correction trials), mean reward collection latency, mean correct response latency, and mean incorrect latency. Statistics were calculated using *Statistical Package for the Social Sciences (SPSS) Version 21*. The MK-801 and SNP dose-response data was analyzed using a one-way repeated measures ANOVA. Post-hoc analysis was performed with simple contrast tests, making comparisons only to saline. Two-way repeated measures ANOVA were used to analyze all other data sets (MK-801+SNP). One rat failed to complete any trials when treated with 4.0 mg/kg of SNP and was removed from the SNP dose-response curve analysis and not used for other testing. The final number of rats included in the analysis was 9 for the SNP dose-response curve. Corrections for sphericity violations, as indicated by Mauchly's test, were made using the Greenhouse-Geisser correction. Partial η^2 was calculated as a measure of effect size and represents the total variability in each dependent variable that can attributed to the intervention. Partial η^2 values of 0.01, 0.06, and 0.14 are considered small, medium, and large effect sizes respectively.

2.5 Results

2.5.1 MK-801 impaired TUNL performance in a dose-dependent manner

MK-801 (0.05, 0.075, 0.1 mg/kg) or saline was administered 25 minutes prior to TUNL testing. A repeated measures ANOVA revealed a dose-dependent effect of the drug on several dependent variables. Overall accuracy was altered following MK-801 treatment (Fig 2A; $F(3,27)=4.28$, $p=0.014$, partial $\eta^2=0.322$) with a post-hoc analysis identifying reduced performance following 0.1 mg/kg of MK-801 compared to saline treatment. Trials were grouped according to their pattern separation. MK-801 had an effect on large distance trials (Fig 2B; $F(3,27)=3.83$, $p=0.021$, partial $\eta^2=0.299$), with 0.1 mg/kg of MK-801 impairing performance compared to saline. Although MK-801 trended toward impairing accuracy on small distance trials, the effect was not significant (Fig 2B; $p=0.058$, partial $\eta^2=0.239$). The number of selection trials completed was unaffected by MK-801 (Fig 2C; $p>0.05$) but a main effect was observed for correction trials (Fig 2D; $F(3,27)=4.13$, $p=0.016$, partial $\eta^2=0.315$) and total trials (Fig 2E; $F(3,27)=3.39$, $p=0.032$, partial $\eta^2=0.274$). Subsequent post-hoc analysis revealed significant increase in correction and total trials completed following 0.05 and 0.1 mg/kg of MK-801 compared to saline treatments. Reward latency was altered following MK-801 treatment (Fig 2F; $F(3,27)=6.59$, $p=0.002$, partial $\eta^2=0.423$) with post-hoc analysis revealing all MK-801 treatments reduced latency compared to saline. The time to make a correct selection did not vary among the treatments (Fig 2F; $p>0.05$). MK-801 reduced incorrect latency (Fig 2F; $F(3,27)=3.98$, $p=0.018$, partial $\eta^2=0.307$), with rats treated with 0.05 and 0.1 mg/kg of MK-801 being significantly faster than saline.

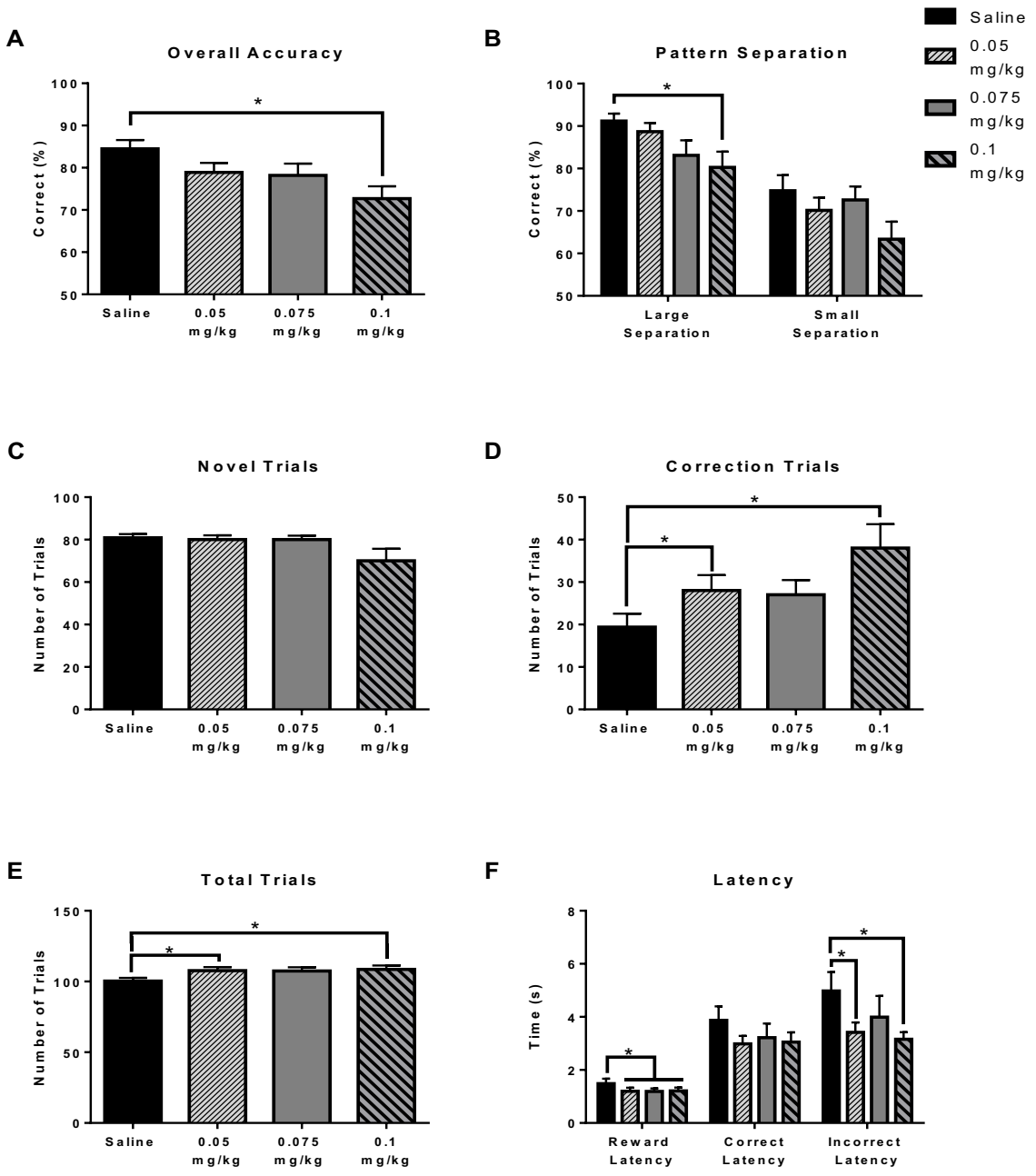

Fig 2. Effects of MK-801 (0.05, 0.075, 0.1 mg/kg) on TUNL. **A**. Accuracy as measured by the percentage of selection trials correctly completed. **B.** Accuracy broken down according to the difficulty of pattern separation. Separate repeated measures ANOVAs were completed for each pattern. **C.** Number of unique trials completed by the rats. **D**. Number of correction trials completed by rats. **E**. Sum of selection and correction trials completed by the rat. **F**. Response latencies for reward collection, correct trials and incorrect trials. Separate ANOVAs were used for each latency. * $p<0.05$ between groups as indicated.

2.5.2 SNP impaired TUNL performance in a dose-dependent manner

SNP (0.5, 1.0, 2.0, 4.0 mg/kg) or saline were administered 25 minutes prior to TUNL task initiation. SNP impaired performance on overall accuracy (Fig 3A; $F(2.10, 16.81)=6.64$, $p=0.007$, partial $\eta^2=0.454$) with subsequent post-hoc analysis revealing a significant difference between saline and 4 mg/kg SNP treatments. SNP did not alter performance on maximum distance trials (Fig 3B; $p>0.05$). There was a main effect of SNP on minimum separation trial accuracy (3B; $F(4,31)=4.67$, $p=0.004$, partial $\eta^2=0.368$) such that 4.0 mg/kg of SNP treatment impaired performance compared to saline treatment. SNP reduced the number of selection trials completed (Fig 3C; $F(1.10, 8.81)=15.98$, $p=0.003$, partial $\eta^2=0.666$) and post-hoc analysis identified fewer selection trials were completed following 4.0 mg/kg of SNP compared to saline treatment. No significant effect of SNP was observed on correction trials (Fig 3D; $p>0.05$). SNP had a significant effect on the number of total trials completed (Fig 3E; $F(1.28, 10.22)=12.33$, $p=0.004$, partial $\eta^2=0.606$). Subsequent post-hoc analysis revealed a significantly fewer total trials were completed after 4.0 mg/kg of SNP compared to saline treatment. Subsequent analysis demonstrated a main effect of SNP on reward latency (Fig 3F; $F(1.90, 15.27)=10.38$, $p=0.002$, partial $\eta^2=0.565$) and correct latency (Fig 3F; $F(2.06, 23.06)=5.56$, $p=0.014$, partial $\eta^2=0.410$) with a difference between saline and treatment with 2 mg/kg or 4 mg/kg of SNP on both. SNP increased incorrect latency (Fig 3F; $F(2.03, 16.22)=4.10$, $p=0.036$, partial $\eta^2=0.339$) with a significant difference between saline and 4 mg/kg SNP treatments.

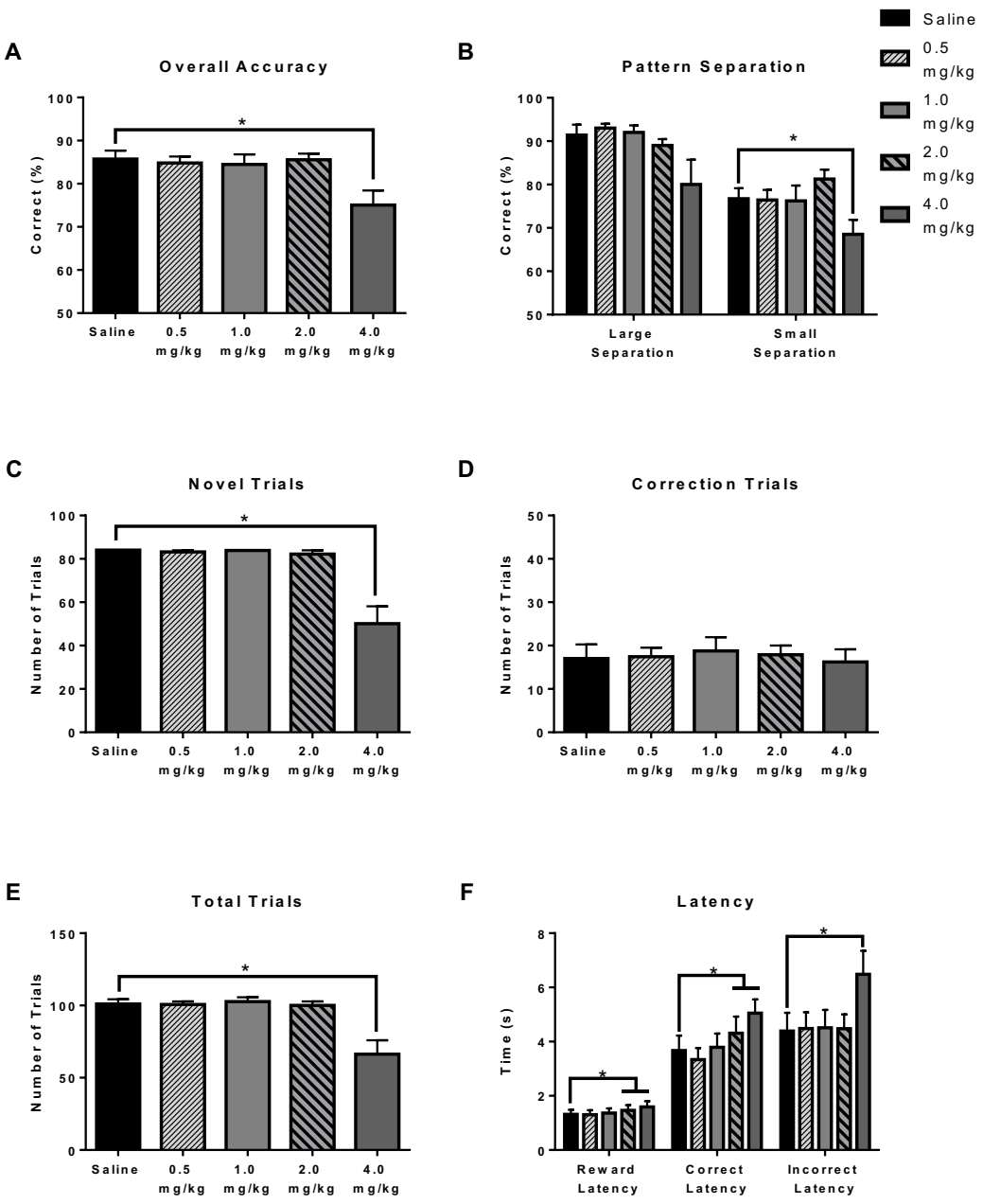

Fig 3. Effects of SNP (0.5, 1.0, 2.0, 4.0 mg/kg) on TUNL. **A**. Accuracy as measured by the percentage of selection trials correctly completed. **B.** Accuracy broken down according to the difficulty of pattern separation. Separate repeated measures ANOVAs were completed for each pattern. **C.** Number of unique trials completed by the rats. **D**. Number of correction trials completed by rats. **E**. Sum of novel and correction trials completed by the rat. **F**. Response latencies for reward collection, correct trials and incorrect trials. Separate ANOVAs were used for each latency. * $p<0.05$ between groups as indicated.

2.5.3 SNP improved pattern separation but failed to alleviate MK-801 impairments on the TUNL task

MK-801 and SNP doses was chosen based on the collected dose-response curve performance data. The 0.1 mg/kg dose of MK-801 was used because it produced the most robust impairment in TUNL task without increasing latency. The 2.0 mg/kg dose of SNP was the highest tested dose that did not impair TUNL performance or dramatically increase latency. The SNP (2.0 mg/kg) or saline was administered 5 minutes prior to MK-801 (0.05 mg/kg) or saline to observe whether SNP would rescue TUNL performance. A 2 (MK-801, saline) by 2 (SNP, saline) repeated measures ANOVA revealed no significant interactions between MK-801 and SNP treatment on any of the variables (statistics not shown). There was a main effect of MK-801 ($F(1,11)=15.63$, $p=0.002$, partial $\eta^2=0.587$) but not SNP ($p>.05$) on overall accuracy (Fig 4A). Trials were further divided according to working memory (2 s, 6 s) or pattern separation (large, small). MK-801 impaired trials that contained a 2 s delay (Fig 4C; $F(1,11)=7.21$, $p=0.021$, partial $\eta^2=0.396$), 6 s delay (Fig 4C; $F(1,11)=14.36$, $p=0.003$, partial $\eta^2=0.566$), or large distances (Fig 4B; $F(1,11)=26.83$, $p<0.001$, partial $\eta^2=0.709$) while SNP did not affect these variables ($p>0.05$). In contrast, SNP trended toward improving performance during small distance trials (Fig 4B; $p=0.058$, partial $\eta^2=0.288$) but MK-801 had no effect on these trials ($p>.05$). MK-801 significantly reduced the number of selection trials completed by the rats (Fig 4E; $F(1,11)=11.47$, $p=0.006$, partial $\eta^2=0.510$). Furthermore, MK-801 increased the number of correction trials (Fig 4F; $F(1,11)=23.00$, $p=0.001$, partial $\eta^2=0.676$) and total trials (Fig 4G; $F(1,11)=12.84$, $p=0.004$, partial $\eta^2=0.539$) completed. SNP did not alter the number of selection trials or correction trials completed (Fig 4E,F; $p > 0.05$). However, it is worth noting that SNP total trials trended toward being significantly increased (Fig 4G; $p=0.053$, partial $\eta^2=0.300$).

Analysis of latency found that MK-801 decreased reward (Fig 4H; $F(1,11)=9.73$, $p=0.010$, partial $\eta^2=0.469$), correct (Fig 4H; $F(1,11)=14.41$, $p=0.003$, partial $\eta^2=0.567$), and incorrect latency (Fig 4H; $F(1,11)=9.67$, $p=0.010$, partial $\eta^2=0.468$). In contrast, SNP increased reward (Fig 4H; $F(1,11)=35.35$, $p<0.001$, partial $\eta^2=0.763$) and incorrect latency (Fig 4H; $F(1,11)=6.94$, $p=0.023$, partial $\eta^2=0.387$) but did not influence correct latency (Fig 4H; $p>0.05$).

To further investigate the effects of SNP on the TUNL task, trials were categorized into four groups based on their delay and pattern. MK-801 significantly impaired performance at the 6 s delay regardless of whether the separation was large (Fig 4D; $F(1,11)=18.64$, $p=0.001$, partial $\eta^2=0.629$) or small (Fig 4D; $F(1,11)=5.93$, $p=0.033$, partial $\eta^2=0.350$). MK-801 also reduced accuracy on 2 s delay and large separation (Fig 4D; $F(1,11)=10.60$, $p=0.008$, partial $\eta^2=0.491$) but not 2 s delay and small separation trials ($p>0.05$). In contrast, SNP had no effect on trials with a 2 s delay and large separation, 6 s delay and small separation, or 6 s delay large separation but facilitated performance on trials with a 6 s delay and small separation (Fig 4D; $F(1,11)=7.79$, $p=0.018$, partial $\eta^2=0.414$).

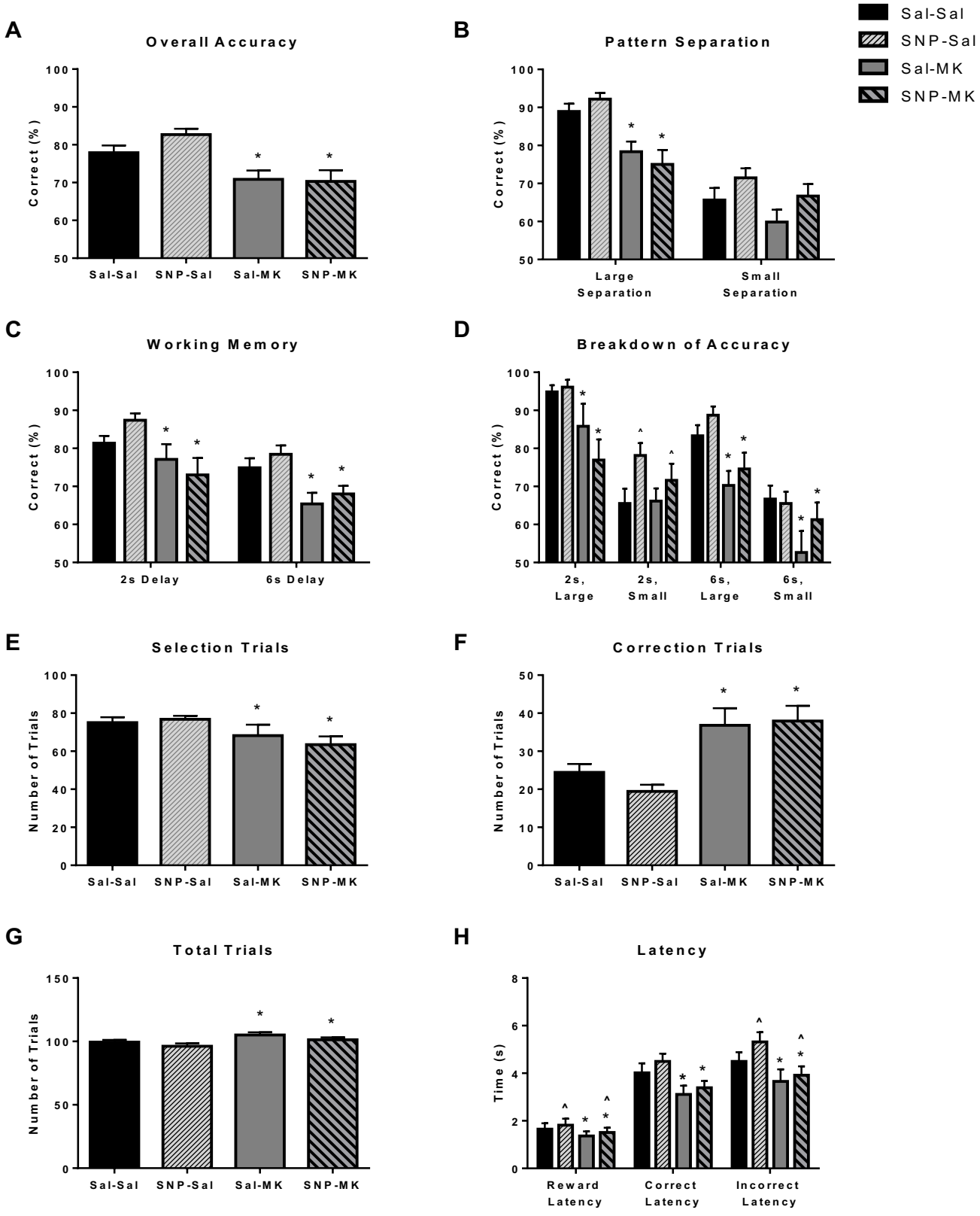
A
Overall Accuracy
Correct (%)
Sal-Sal
SNP-Sal
Sal-MK
SNP-MK
B
Pattern Separation
Large Separation
Small Separation
C
Working Memory
2s Delay
6s Delay
D
Breakdown of Accuracy
2s, Large
2s, Small
6s, Large
6s, Small
E
Selection Trials
Number of Trials
F
Correction Trials
G
Total Trials
H
Latency
Time (s)
Reward Latency
Correct Latency
Incorrect Latency

Fig 4. Effects of SNP (2.0 mg/kg) and MK-801 (0.1 mg/kg) on TUNL. **A.** MK-801 significantly reduced accuracy, while SNP had no effect. **B.** MK-801 reduced accuracy when the stimuli had a large separation and had no effect when they were closer together. SNP did not alter performance for large separations but facilitated performance for small separations (p=0.058). **C.** MK-801 reduced the number of correct responses at the 2 s and 6 s delay, while SNP had no effect. **D**. Trials were broken down according to separation and delay. MK-801 impaired performance on 2 s delay and large separation, 6 s delay and large separation, and 6 s delay small separation trials. SNP significantly improved performance on trials with a 2 s delay and small separation. **E**. MK-801 reduced selection trials completed while SNP had no effect. MK-801 increased the number of correction **(F)** and total **(G)** trials completed while SNP did not change the number of completed trials. **H.** MK-801 reduced reward, correct and incorrect latency while SNP increased reward and incorrect latency but did not affect correct latency in this sample. * indicate a significant effect of MK-801 (p<0.05). ^ indicate a significant effect of SNP (p<0.05).

2.5.4 SNP does not prevent MK-801-induced impairments in the TUNL task

Previous studies have examined the potential of SNP to prevent the cognitive impairments observed in NMDA dysfunction models of schizophrenia (Maia-de-Oliveira et al. 2015b). A previously published experiment using MK-801 in TUNL found that the 0.05 mg/kg dose of MK-801 was sufficient to reduce performance compared to saline, as higher doses led to high omission rates (Kumar et al. 2015a). Our dose-response curve showed 0.05 mg/kg of MK-801 was sufficient to change performance on correction trials, thus the lower dose was used in the following experiment. SNP (5 mg/kg) or was administered 3 hours and 35 minutes prior to MK-801 (0.05 mg/kg) or saline and 4 h prior to TUNL task initiation. No interactions were significant following a 2 (MK-801, saline) by 2 (SNP, saline) repeated measures ANOVA (statistics not shown). MK-801 reduced overall accuracy on the TUNL task (Fig 5A; $F(1,15)=10.14$, $p=0.006$, partial $\eta^2=0.404$) on the TUNL task. The impairment was observed whether the delay was 2 s (Fig 5B; $F(1,15)=7.75$, $p=0.014$, partial $\eta^2=0.341$) or 6 s (Fig 5B; $F(1,15)=5.517$, $p=0.038$, partial $\eta^2=0.256$). After grouping trials according to pattern separation, it was observed that MK-801 impaired performance on small distance trials (Fig 5B; $F(1,15)=8.38$, $p=0.011$, partial $\eta^2=0.359$) but not large distance performance (Fig 5C; $p=0.061$, partial $\eta^2=0.215$). SNP did not influence overall, pattern separation, or delay accuracy (Fig 5A,B,C; $p>0.05$). As a result of the null effect of SNP on accuracy, an investigation of SNP following the categorization of trials according to working memory and pattern separation was not conducted. Neither SNP nor MK-801 influenced the number of selection trials the rat completed (Fig 5D; $p>0.05$). However, MK-801 increased the number of correction trials (Fig 5E; $F(1,15)=18.38$, $p<0.001$, partial $\eta^2=0.551$) and total trials (Fig 5F; $F(1,15)=40.65$, $p<0.001$, partial $\eta^2=0.730$) the rat completed. There was no significant main effect of SNP on total trials

(Fig 5F; Fig p>.05) but the completion of fewer correction trials trended toward significance (Fig 5E; p=0.063, partial η^2=0.212). MK-801 significantly reduced reward (Fig 5G; $F(1,15)$=18.35, p<0.001, partial η^2=0.550), correct (Fig 5G; $F(1,15)$=26.78, p<0.001, partial η^2=0.641), and incorrect latency times (Fig 5G; $F(1,15)$=38.31, p<0.001, partial η^2=0.719). SNP did not influence reward and correct latencies (Fig 5G; p>0.05) but significantly increased incorrect latency (Fig 5G; $F(1,15)$=8.70, p=0.010, partial η^2=0.367).

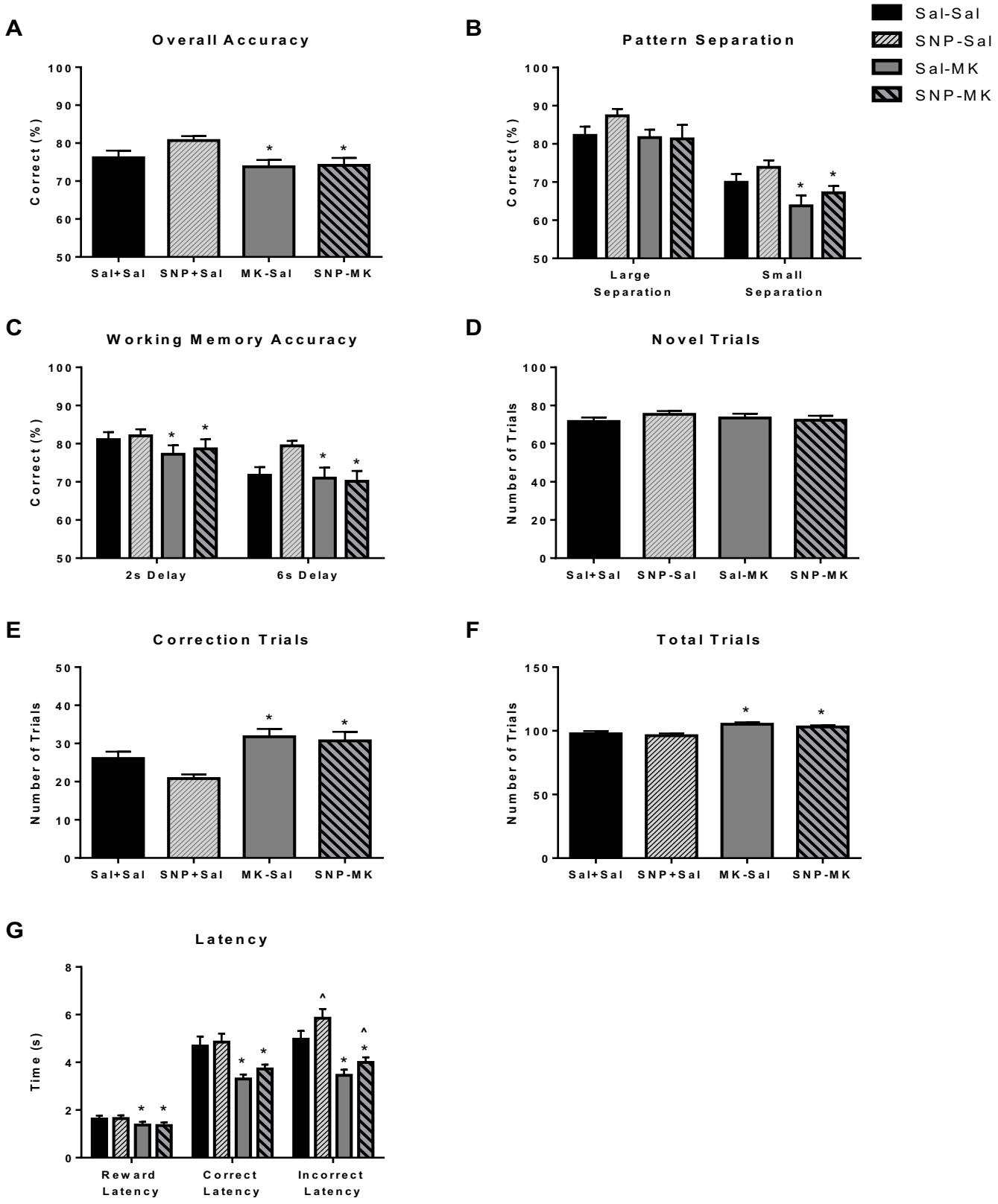
A
Overall Accuracy
Correct (%)
100
90
80
70
60
50
Sal+Sal
SNP+Sal
MK-Sal
SNP-MK
B
Pattern Separation
Correct (%)
Large Separation
Small Separation
Sal-Sal
SNP-Sal
Sal-MK
SNP-MK
C
Working Memory Accuracy
Correct (%)
2s Delay
6s Delay
D
Novel Trials
Number of Trials
E
Correction Trials
Number of Trials
F
Total Trials
Number of Trials
G
Latency
Time (s)
Reward Latency
Correct Latency
Incorrect Latency

Fig 5. Effects of SNP (5 mg/kg) when administered prior to MK-801 (0.05 mg/kg) on TUNL. **A.** MK-801 significantly reduced performance on overall accuracy across all selection trials but SNP had no effect. **B.** MK-801 impaired performance on small separation trials but did not influence performance on large separation trials. SNP did not affect performance regardless of pattern separation. **C**. MK-801 impaired performance on both delays but SNP had no effect on either. **D.** Drug treatment did not affect the number of selection trials completed. MK-801 but not SNP increased the number of correction **(E)** and total **(F)** trials completed. **G**. MK-801 reduced latency on reward, correct, and incorrect latencies while SNP only affected incorrect latency by increasing it. * indicate a significant effect of MK-801 ($p<0.05$). ^ indicate a significant effect of SNP ($p<0.05$).

2.6 Discussion

The cognitive symptoms of schizophrenia are not alleviated by conventional antipsychotics (Marder and Fenton 2004). Recently, SNP has demonstrated promise as an adjunct treatment to reduce these symptoms, specifically reducing working memory impairments (Maia-de-Oliveira et al. 2015a). In the current experiments, we examined the effects of SNP in the acute MK-801 model of schizophrenia within the TUNL task. MK-801 (0.05 and 0.1 mg/kg) impaired TUNL performance by reducing accuracy and increasing the number of correction trials completed. SNP did not attenuate (2.0 mg/kg) or prevent (5.0 mg/kg) MK-801-induced performance changes. Interestingly, SNP independently improved accuracy on trials with small pattern separations.

2.6.1 MK-801-induced disruption of TUNL

NMDA receptor dysfunction contributes to symptom manifestation in patients with schizophrenia (Adell et al. 2012; Coyle 2012). In rodents, acute MK-801 treatment demonstrates face validity as a model of schizophrenia as its administration produces behavioral changes similar to those observed in the disease (Nestler and Hyman 2010). The acute administration of MK-801 results in locomotor hyperactivity (Howland et al. 2012; Mahmood et al. 2016), reduced sociability (Morales and Spear 2014), and performance deficits on touchscreen-based (Kumar et al. 2015b; Kumar et al. 2015a; Lins et al. 2015; Lins and Howland 2016) and working memory (Homayoun et al. 2004; Galizio et al. 2012) tasks. One published study has explored the changes following acute MK-801 (0.05 mg/kg) administration in the TUNL task (Kumar et al. 2015a). In that study, working memory performance was assessed following 1 s and 20 s delays. In the present study, delays with less variability (2 s and 6 s) were used in order to detect subtle changes in performance due to delay. We observed that, regardless of delay, MK-801 (0.05 and

0.1 mg/kg) reduced overall accuracy compared to saline. This suggests that the TUNL task is vulnerable to NMDA receptor antagonism regardless of working memory demand. In Kumar et al. (2015), TUNL task accuracy following a 20 s was only examined using large pattern separations. In contrast, the present study included large and small separations during both delays. Overall, we observed that MK-801 (0.05 mg/kg) reduces performance on large and small separation trials. Accuracy on most trials, with the exception of 2 s delay and small pattern separation, was impaired following MK-801 (0.1 mg/kg). The present study is the first to analyze the effects of MK-801 on correction trials. Previous studies utilizing touchscreen tasks in rodents have identified correction trials as a measure of perseveration (Lins et al. 2015; Lins and Howland 2016), a behavior frequently observed in patients with schizophrenia (Szoke et al. 2008; Ortuño et al. 2009). We observed MK-801 treatment increased the number of correction trials completed by the rats, suggesting MK-801 hindered the ability to inhibit incorrect response selection. These findings replicate MK-801-induced perseveration in a variety of other rodent paradigms (Cohn et al. 1992; Tuplin et al. 2015; Lins et al. 2015).

Previous studies have reported that MK-801 (0.05 mg/kg) has no effect on latency although MK-801 (0.075 mg/kg) increases reward and correct latencies on the TUNL task (Kumar et al. 2015a). We expanded the latency variables to include incorrect latency and our data show MK-801 (0.05 and 0.1 mg/kg) reduces reward, correct, and incorrect latencies. This finding contrasts previously reported MK-801-induced increase in latency on the TUNL task (Kumar et al. 2015a). Reduced latency may be indicative of increased impulsive behavior following acute MK-801 treatment. Taken together, reduced latency and an increased number of total trials completed suggests performance impairments on the TUNL task were not due to impediment of the physical ability to perform the task. Other studies have determined MK-801

does not impair visual perception at doses similar to those used here (Talpos et al. 2012), thus it is unlikely performance deterioration was a consequence of perceptual changes. The present experiment suggests acute MK-801 administration in rodents is capable of reducing the ability to resolve spatial patterns and maintain information across a delay. Acute MK-801 administration may also lead to increased perseverative behavior and impulsivity. Our findings are similar to the performance impairments that would be expected in patients with schizophrenia, providing additional support for the face validity of the MK-801 model of schizophrenia (Mesholam-Gately et al. 2009; Das et al. 2014).

2.6.2 SNP did not block MK-801-induced impairments but improved pattern separation

There are no available antipsychotics to alleviate the cognitive symptoms of schizophrenia. The lack of a positive control has limited the assessment of predictive validity in rodent models of schizophrenia, impeding the translatable development and evaluation of treatments for these symptoms (Markou et al. 2009). However, when SNP was administered as an adjunct treatment to patients with schizophrenia, it reduced working memory impairments on the n-back task (Maia-de-Oliveira et al. 2015a). Previous rodent studies have mirrored these findings as SNP reduces ketamine-induced cognitive deficits (Trevlopoulou et al. 2016). In the present study, SNP neither prevented (Fig 4; 5.0 mg/kg; 3 h and 35 min prior to MK-801) nor rescued (Fig 3; 2.0 mg/kg; 5 min prior to MK-801) MK-801-induced impairments in the TUNL task. Our findings fail to replicate previous reports of reduced cognitive impairments in patients (Maia-de-Oliveira et al. 2015a) and ketamine-treated rats (Trevlopoulou et al. 2016) following SNP, suggesting that acute MK-801 administration in the TUNL task may not be an appropriate paradigm to study the effects SNP in schizophrenia.

To the best of our knowledge, this is the first study to explore the effect of SNP on pattern separation and perseverative behaviors. When SNP (2 mg/kg) was administered 30 min prior to TUNL task initiation, accuracy on small separation trials increased. Although the p-value failed to meet the threshold for significance ($p=0.058$), our measure of effect size was large ($\eta=0.288$) suggesting the lack of significance may simply be a result of a small sample size. When accuracy during small separation trials accounted for delay, the facilitated pattern separation appeared to be driven by significantly improved performance during 2 s delays and small separation trials. Similarly, the administration of SNP 4 h prior to the TUNL task trended toward reducing the number of correction trials ($p=0.063$, $\eta=0.212$), suggesting that SNP may reduce perseverative behavior (Lins et al. 2015). Taken together, this study is the first to demonstrate SNP may have intrinsic properties that facilitate pattern separation and reduce perseveration. Future research should further explore the effects of SNP in these realms of cognition.

2.7 Conclusion

The results of this study indicate that acute MK-801 administration impairs performance on the TUNL task. Treatment with SNP did not reduce MK-801-induced accuracy impairments, suggesting the strengths of the acute MK-801 model of schizophrenia may not be high predictive validity. SNP trended toward improving pattern separation and reducing perseveration but future research is required to support this assertion.

3.0 GENERAL DISCUSSION

The effects of SNP on MK-801-induced impairments in the TUNL task were tested in the present study. MK-801 dose-dependently impaired performance on working memory and pattern separation. SNP did not prevent or rescue these impairments. Interestingly, SNP did improve performance on trials with difficult to discriminate patterns. This is the first study to explore the effects of SNP on pattern separation suggesting it may have intrinsic cognitive enhancing capabilities. Taken together, these results suggest that SNP does not alleviate the working memory impairments produced by acute MK-801 administration in the TUNL task.

3.1 MK-801-Induced Impairments

MK-801 is a non-competitive NMDA receptor antagonist that induces a physiological and behavioral state in rodents analogous to some aspects of schizophrenia (Moghaddam and Krystal 2012). In our study, MK-801 reduced overall accuracy and detailed analysis showed that working memory and pattern separating abilities were impaired. Further, MK-801 reduced latency measures and increased the number of correction trials completed, which may be indicative of impulsivity and perseveration respectively (Lins et al. 2015; Lins and Howland 2016). By antagonising NMDA receptors, MK-801 reduced NMDA receptor function. Beyond the primary effects of MK-801 on NMDA receptors, this drug has secondary effects that mimic some of the GABAergic changes that occur in schizophrenia. Specifically MK-801 preferentially binds to NMDA receptors on fast-spiking PV+ interneurons, preventing the influx of calcium and reducing the inhibitory effects of these cells on glutamatergic pyramidal neurons (Lewis and Moghaddam 2006).

PV+ interneurons make up about 25% of the interneurons within the PFC (Hashimoto et al. 2003), yet altered GABAergic function in schizophrenia appears to be relatively specific to this subset of neurons (Lewis et al. 2005). The 67-kDa isoform of glutamate acid decarboxylase (GAD67), an enzyme involved in GABA synthesis, messenger ribonucleic acid (mRNA) levels are markedly reduced in the dlPFC of patients with schizophrenia (Guidotti et al. 2000; Kimoto et al. 2014). This reduction is primarily localized to PV+ interneurons (Curley et al. 2011). Therefore, the reduced GABA neurotransmission in PV+ neurons is secondary to altered NMDA receptor function. The alterations in patient GAD67 levels are not improved by long-term haloperidol treatment (Volk et al. 2000). Further, antagonism of NMDA receptors may mirror working memory impairments observed in schizophrenia through downstream GABAergic changes. Information maintenance across a delay is believed to depend upon the coordinated and sustained firing of prefrontal pyramidal neurons (Goldman-Rakic 1995). This synchronization relies on normal GABA functioning and the activation of fast-spiking interneurons within the dlPFC during the delay phase of working memory tasks (Wilson et al. 1994). PFC interneurons have been implicated in mnemonic and sensory-motor working memory (Rao et al. 2000). Further support for the role of GABA in working memory comes from pharmacological studies where infusion of a $GABA_A$ antagonist into the dlPFC in monkeys or the mPFC of rats impairs working memory performance (Sawaguchi et al. 1989; Auger and Floresco 2015). In summary, reduced GABA neurotransmission in PV+ neurons is secondary to altered NMDA receptor function. Therefore, NMDA receptor antagonists pose the potential to model schizophrenia by incorporating GABA and glutamate dysfunction of schizophrenia (Lewis and Moghaddam 2006).

3.2 Nitric Oxide, Sodium Nitroprusside, and Schizophrenia

The present study found that SNP, a nitric oxide donor, did not prevent or rescue MK-801-induced performance impairments in the TUNL task. This experiment did not replicate previous rodent or human studies that demonstrated SNP ability to reduce psychomimetic or symptom presentation respectively (Hallak et al. 2013; Maia-de-Oliveira et al. 2015a; Maia-de-Oliveira et al. 2015b; Trevlopoulou et al. 2016). There are several explanations for the differences between previous studies and the present one. First, this was the first rodent experiment to measure the effects of SNP in the acute MK-801 model. Previous research has chosen to use ketamine as non-competitive NMDA receptor antagonists (Maia-de-Oliveira et al. 2015b; Trevlopoulou et al. 2016). Although all bind to NMDA receptors, different NMDA receptor antagonists may have divergent effects on downstream messengers and transcripts, producing variable behavioral changes (de Bartolomeis et al. 2013; Hillhouse and Porter 2014). Further, the varying structures, potencies, and binding profiles of these drugs may contribute to the differing psychomimetic behaviors induced (Hevers et al. 2008). Secondly, this was the first study using the TUNL task as a measure of working memory. Previous studies have observed SNP restores novelty preference and recognition memory in rodents (Trevlopoulou et al. 2016). However, this is the first rodent task examining cognition following SNP treatment using an operant conditioning paradigm. Lastly, this is the only study using Long Evans rats, while others have primarily used the Sprague Dawley strain. Strain has been shown to influence learning and memory as well as NMDA receptor binding and distribution (Lei et al. 2009; Kumar et al. 2015b). All three of these factors should be considered in future studies.

Interestingly, we observed SNP improved performance on small separation trials. The effect did not reach the threshold of statistical significance but this may be a power issue.

Following subsequent trial breakdown, it was observed that these improvements were specific to small separation trials with a short delay. To the best of our knowledge, this is the first study to explore the effects of SNP on pattern separation in any context. The mechanism underlying SNPs antipsychotic and cognitive properties are unknown. This may have implications in multiple disease pathologies where reduced pattern separation is believed to lead to disrupted episodic memory, such as schizophrenia (Tamminga et al. 2010) and Alzheimer's disease (Palmer and Good 2011).

Nitric oxide (NO) is produced by enzymes within the nitric oxide synthase (NOS) family. There are three types of NOS enzymes: endothelial, cytokine-inducible, and neuronal. Neuronal NOS (nNOS), is widely expressed in the brain including the hippocampus and frontal cortex (Blum-Degen et al. 1999) and is of particular importance in the study of schizophrenia (Freudenberg et al. 2015). This enzyme is calcium-calmodulin dependent and is localized within central and peripheral neurons. nNOS is physically coupled to the NMDA receptor complex. This NMDA receptor-nNOS coupling is believed to increase the efficacy of nNOS in response to glutamate-induced calcium influx following NMDA activation (Nedvetsky et al. 2002). This enzyme catalyzes the conversion of L-arginine to NO (Szabó 1996). NO diffuses across lipid membranes and into the cellular cytosol where it is primarily received by guanylate cyclase (GC). The majority of mechanisms, including vasodilation and the inhibition of platelet aggregation, are mediated by GC (Szabó 1996). Linkage analysis, candidate gene and genome-wide association studies have supported nNOS as a risk gene for schizophrenia (reviewed in (Freudenberg et al. 2015)). Interestingly, schizophrenia patients homozygous for a single nucleotide polymorphism in the nNOS gene, rs6490121, have significantly worse working memory and verbal IQ than other genotype groups (Donohoe et al. 2009). This polymorphism

has also been correlated with altered patterns of activation in schizophrenia patient PFC during spatial working memory tasks in fMRI (Rose et al. 2012) and EEG (O'Donoghue et al. 2012) studies. Therefore, disturbed nNOS function may relate to the cognitive impairments of the disease and pharmacologically altering the NO-GC pathway may reduce cognitive symptoms of schizophrenia.

In patients, SNP treatment often occurs via titration at low doses to allow for the careful monitoring (Friederich and Butterworth 1995; Hottinger et al. 2014). The metabolism of SNP results in cyanide anion production of cyanide anions (Cn^-) (Bisset et al. 1981). Administration of SNP at too large of a dose or too quick of an infusion rate can lead to cyanide toxicity, producing metabolic acidosis, CNS dysfunction and cardiovascular instability (Bisset et al. 1981). Previous rodent studies using non-competitive NMDA receptor antagonists, such as PCP and ketamine, have demonstrated a reversal of the behavioral changes that are induced by these drugs following SNP (Bujas-Bobanovic et al. 2000a; Maia-de-Oliveira et al. 2015b; Trevlopoulou et al. 2016). The precise mechanism that produces these behavioral changes is not clear. N(G)-nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor, potentiates the PCP-induced behavior and c-fos expression, reducing expression within the frontal cortex. This suggests the involvement in the NO system in PCPs mechanism of action (Bujas-Bobanovic et al. 2000b). The expression of c-fos, an immediate early gene, is indicative of a metabolically active neuronal population. In contrast to saline, immunostaining identifies more predominant neuronal activation globally, with specifically high levels of increased expression in the cortical regions of the brain following PCP treatment. 2 mg/kg of SNP was sufficient to reduce the c-fos activation in the frontal cortex neurons and 6 mg/kg reduced overall expression of c-fos to levels not significantly different from saline+saline or SNP (2, 4, or 6)+saline treatments (Bujas-

Bobanovic et al. 2000a). PCP interacts with many other neurotransmitter systems, therefore other receptors and pathways may be involved in increased expression of c-fos. This is supported by the c-fos expression not being symmetrical to the distribution of NMDA receptors, with low expression observed in the hippocampus and striatum. However, the activation could be a consequence of reduced inhibitory tone in the brain due to the inactivation of GABA receptors (Bujas-Bobanovic et al. 2000a).

Several mechanisms relating to SNPs cognitive alleviating properties have been proposed. One mechanism suggests that the alleviation in cognitive impairments is due to increased blood flow. Vasodilation is induced by donated nitric oxide diffusing into vascular smooth muscle and activating guanylate cyclase directly. This results in the production of cyclic guanosine monophosphate (cGMP) which inhibits calcium from entering the cell and may increase the reuptake of calcium into the smooth endoplasmic reticulum (Friederich and Butterworth 1995). The second mechanism suggests that SNP improves cognition through its activation of cyclic guanosine monophosphate. The nNOS converts L-arginine to NO, thus increased levels of L-arginine may increase nNOS dysfunctional in schizophrenia. However, the administration of L-arginine does not interfere with PCP-induced behavioral effects nor does it alter PCP-induced cfos expression (Bujas-Bobanovic et al. 2000a). But this could be due to NO production not being dependent upon the extracellular substrate (Szabó 1996). Thus, it is proposed that SNP blocks PCP effects by bypassing nNOS and activating the NO-guanylyl cyclase signalling pathway. Another prediction is that SNP works through its mediation of tissue plasminogen factor release by endothelial cells (Hoirisch-Clapauch and Nardi 2015). Ultimately, the mechanism that allows the amelioration of schizophrenic symptoms remains largely

unknown and future research should aim at understanding this pathway and verifying previous pilot study findings (Maia-de-Oliveira et al. 2015a).

There are several ways to direct future research related to this thesis. One initial objective may be to expand this methodology by incorporating changes that increase the alignment between our study and human pilot studies of SNP. This may be done by injecting an antipsychotic in conjunction to SNP or using a pump instead of a single injection to administer the SNP dose over a longer period of time (Hallak et al. 2013; Maia-de-Oliveira et al. 2015a). In addition, future studies may investigate the predictive validity of the MK-801 model. Our study was the first to explore the effects of SNP in the MK-801 model of schizophrenia in a working memory task in rodents. This novelty makes it difficult to determine whether our null findings were a consequence of the model of schizophrenia chosen or the task utilized. Therefore, it would be beneficial to take a rodent behavioral paradigm that has been shown the positive effects of SNP (e.g., ketamine in the novel object recognition task) and replace ketamine with MK-801 (Trevlopoulou et al. 2016). If SNP reduced the recognition memory impairments induced by MK-801 treatment, it would suggest the TUNL task may not be appropriate in the study of SNP. Similarly, it would be beneficial to use ketamine as the model of schizophrenia in the TUNL task because SNP has been effective in reducing other cognitive impairments in this model (Kandratavicius et al. 2015; Trevlopoulou et al. 2016). If SNP was able to alleviate ketamine-induced TUNL task impairments, ketamine treatment may hold greater predictive validity than MK-801 as a model of schizophrenia.

3.3 General Conclusion

This thesis presents novel findings regarding the treatment of cognitive deficits of schizophrenia within a rodent model. The data demonstrates the efficacy of acute MK-801 treatment in inducing robust working memory impairments, similar to those observed in patients with schizophrenia. Previous rodent and patient studies have demonstrated SNP's capacity to alleviate cognitive impairments but this was not replicated in our experiment. This thesis is the first to suggest SNP may improve pattern separation. These findings provide valuable insight into the face and predictive validity of the MK-801 model within the TUNL task.

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