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EFFECTS OF PD149163 ON SPATIAL WORKING AND REFERENCE MEMORY IN RATS PERFORMING A RADIAL ARM MAZE TASK

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EFFECTS OF PD149163 ON SPATIAL WORKING AND REFERENCE MEMORY IN RATS PERFORMING A RADIAL ARM MAZE TASK

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ABSTRACT

EFFCTS OF PD149163 ON SPATIAL WORKING AND REFERENCE MEMORY IN RATS PERFORMING A RADIAL ARM MAZE TASK

By

Maureen Suzanne Donegan

The current study evaluated the cognitive-symptom efficacy of the neurotensin analog PD149163 for the treatment of schizophrenia. Schizophrenia is a life-long debilitating mental illness that affects 1% of the population world wide. The symptoms of schizophrenia include positive and negative symptoms, as well as cognitive deficits. Current drug treatments fail to improve these cognitive deficits. The current study used 20 male Sprague-Daley rats and a radial arm maze task to compare the effects of PD149163, clozapine, and haloperidol on working memory and spatial reference memory. To create deficits in working memory, all rats were given the memory impairing drug dizocilpine (MK-801) alone and in combination. The number of working, reference, and total memory errors was calculated. The results show that MK801 did significantly increase the number of working, reference, and total memory errors while PD149163 had no effect on memory. Haloperidol had a trend of increasing reference memory errors and clozapine had a trend of increasing working memory errors. None of the drugs were able to reverse the strong memory impairing effect of MK801. The fact that PD149163 did not increase errors in the maze suggest that the effects of PD149163 should be explored in other areas of cognition, such as attention.

DEDICATION

This thesis is dedicated to my parents, Scott and Susan Donegan for their continuous support and love.

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INTRODUCTION

Schizophrenia is a debilitating psychological disorder that often requires intensive care. The disorder is not only detrimental to the people who have it but also to the society at large, because most individuals with schizophrenia are unable to keep a job and end up relying on government welfare and healthcare (Perkins & Rinaldi, 2002). The number of people affected with schizophrenia is large, two to three million individuals in the U.S., and roughly 1% of the global population (Cáceda, Kinkead, & Nemeroff, 2006; Regier et al., 1993). The symptoms of schizophrenia first show up during the late teens or early 20's and they must be managed for the rest of one's life, since there is no cure for this devastating illness.

The symptoms of schizophrenia are labeled as both positive and negative. Positive symptoms refer to the presence of abnormal behavior, while negative symptoms refer to the absence of normal behavior. Psychologists in the United States use the Diagnostic and Statistics Manual IV-Text Revision (2000) to diagnosis patients with schizophrenia. Positive symptoms listed in the DSM-IV-TR include hallucinations and delusions. Negative symptoms include social withdrawal, reduced affect or emotional responsiveness, and reduced movement. Positive symptoms, such as hallucinations, are thought to occur in schizophrenia patients due to distortions in mental imagery and errors in sensory gating. These errors lead to difficulty in distinguishing between internally generated and externally presented information (Aleman, Böcker., Hijman, de Haan, & Kahn, 2003). Aleman et al. (2003) also believe perception in schizophrenia is heavily influenced by top-down sensory processes which could alter incoming sensory

information and cause hallucinations. On the other hand, negative symptoms can be a result of co-occurring depression and have been attributed to a variety of causes such as structural brain abnormalities, enlarged ventricles (Andreasen, Flaum, Swayze, Tyrrell, & Arndt, 1990), attenuation of the excitatory glutamate neurotransmission (Heresco-Levy et al., 1999), and even chronic use of antipsychotic drugs.

Types of Schizophrenia

Schizophrenia is listed as a spectrum disorder with five sub classifications in the DSM-IV-TR. These sub classifications depend on both positive and negative symptoms and to what degree they are exhibited in the patient. The five sub classifications of schizophrenia are: Paranoid type, Catatonic type, Disorganized type, Undifferentiated type, and a Residual type. Individuals with paranoid schizophrenia exhibit mainly positive symptoms, which could include auditory hallucinations and delusions about people trying to hurt them or plot against them. A patient with catatonic schizophrenia exhibits negative symptoms and suffers from extreme disturbances in movement. These disturbances consist of periods of little movement, called catatonic stupor, followed by hyperactivity. A person with catatonic schizophrenia may also display stereotypic behavior by repeatedly making the same movements. People diagnosed with disorganized schizophrenia display chaotic behaviors and have trouble completing simple tasks. Their behaviors consist of positive and negative symptoms. Disorganized schizophrenia patients also suffer from impairments in communication and emotional expression and often act inappropriately. Undifferentiated schizophrenia is a diagnosis for people who do not fit into any of the other groups and their symptoms may fluctuate over time. Residual type schizophrenia is a diagnosis that is used when a patient does not exhibit

positive symptoms of schizophrenia but still shows some of the negative symptoms, such as decreased emotional affect (Bengston., 2006).

Cognitive Impairment

Although the diagnosis of schizophrenia is based on the presence of positive or negative symptoms, people diagnosed with schizophrenia also have severe cognitive impairments (Goldman-Rakic, Castner, Svensson, Siever, & Williams, 2004; Green, 1996; Kaneda, Jayathilak, & Meltzer, 2010; Meltzer & McGurk, 1999; Silver, Feldman, Bilker, & Gur, 2003; Woodward, Purdon, Meltzer, & Zald, 2005). Key cognitive impairments occur for attention, perception, and executive functioning (Green & Nuechterlein, 1999). These cognitive impairments can be observed in tests of verbal learning and memory, verbal fluency, visual learning and memory, and working memory (Silver et al., 2003). In a meta-analysis of over forty studies, Woodward et al. (2005) stated that schizophrenia patients typically score more than one standard deviation below the mean on many neuropsychological tests. In addition, several studies have concluded that improvement in cognition is the best predictor of functional outcomes, which include the ability to gain employment, daily living skills, social problem solving, and psychosocial skill acquisition (Green, Kern, & Heaton, 2004; Kaneda et al., 2010; Meltzer & McGurk, 1999; Woodward et al., 2005)

Functional outcomes like employment are a big concern for schizophrenia patients because studies report that over 75% of schizophrenia patients are unemployed (Kaneda et al., 2010; Mueser, Salyers, & Mueser, 2001). In one clinical study of 82 schizophrenia patients, only 15 patients or 18.3% were employed full-time (Meltzer,

Thompson, Lee, & Ranjan, 1996). In addition, a 10 year longitudinal study of people with schizophrenia reported that the unemployment rate increased from 88% in 1990 to 96% in 1999, despite the number of work programs for mental health patients in the area increasing (Perkins & Rinaldi, 2002). These studies show that unemployment is a serious problem for people diagnosed with schizophrenia. In addition to a lack of income, unemployment can cause more stress and leave the patient without status, identity, daily structure, or social support (Perkins & Rinaldi, 2002). Treatment for schizophrenia needs to be improved so that patients seeking part or full-time employment may obtain it. If more patients with schizophrenia are able to maintain employment, then less frequent hospital stays and lower rates of suicide are expected (Kaneda et al., 2010). Therefore new drug development for schizophrenia needs to focus on restoring cognitive function to schizophrenia patients so that they may improve their quality of life and succeed in society (Goldman-Rakic et al., 2004).

Drug Treatment

Typical antipsychotics. Chlorpromazine became the first antipsychotic drug after its antipsychotic effects were fortuitously observed. It was developed in 1950 as a pre-anesthetic drug, but when given to psychiatric patients, it was found to have a calming effect on those with schizophrenia (Meyer & Simpson, 1997). Several other drugs similar to chlorpromazine were developed in the following years and these drugs became known as "typical" antipsychotics. All typical antipsychotics are dopamine D_2 receptor antagonists. Although these drugs reduce positive symptoms of schizophrenia by decreasing dopamine activity they also can cause adverse side effects through the same mechanism.

Adverse side effects of typical antipsychotics. In schizophrenia, dopamine neurons in the mesolimbic pathway, which runs from the ventral tegmental area to various limbic structures, are suspected to be over active. The resulting over stimulation of numerous limbic structures, including the amygdala and nucleus accumbens, is thought to be the cause of positive symptoms. However, the nigrostriatal dopamine pathway is normal in schizophrenia patients. This pathway releases dopamine into the basal ganglia which is an area important for controlling movement. Thus, the blockade of D_2 receptors in this pathway causes extrapyramidal side effects (EPS).

EPS is characterized by motor symptoms such as, muscle tremors, rigidity, slurred speech and slowed movements (Kamin, Manwani, & Hughes, 2000; Meyer & Simpson, 1997). Akathisia is another characteristic of this disorder, resulting in restlessness and pacing or rocking. Yet, these side effects were considered to be minimal when compared to the reduction of positive symptoms in schizophrenia patients, and typical antipsychotics continued to be widely prescribed in the 1950s and 1960s. These symptoms are a direct result of antipsychotic drug therapy and can be eliminated if drug treatment is discontinued (Kamin et al., 2000). However, when long-term use of typical antipsychotics is stopped, schizophrenia patients may experience an EPS related condition called tardive dyskinesia. Tardive dyskinesia is a hyperkinetic motor disorder, characterized by repetitive, involuntary, and purposeless movements of the face and mouth (Casey., 1990; Meyer & Simpson, 1997). Facial spasms are commonly observed in patients with tardive dyskinesia, including movements of the jaw, tongue, or lips, such as chewing, lip smacking, and puckering. This motor disorder can last for years after a

patient stops taking typical antipsychotics and may be irreversible in a minority of patients (Casey., 1990; Kane, 1988).

In addition, typical antipsychotic drugs may also produce neuroleptic malignant syndrome. Symptoms of neuroleptic malignant syndrome include high fever, rapid heart rate, changes in blood pressure, sweating, muscle rigidity and altered consciousness (Levenson, 1985). These symptoms usually subside after a few days but can last as long as a couple of weeks. Less severe adverse effects of typical antipsychotics include infertility, disruptions in menstrual cycles, reduced lactation, loss of libido, and erectile dysfunction (Halbreich, Kinon, Gilmore, & Kahn, 2003). These hormonal and sexual side effects are caused by hyperprolactinemia, which is caused by elevated levels of prolactin in the blood stream for long periods of time. This occurs during drug therapy with typical antipsychotics because the glands from which prolactin is released are normally inhibited by the activation of dopamine D_2 receptors. However when these receptors are blocked the glands are never inhibited and elevated prolactin levels result (Gudelsky & Porter, 1980; Meltzer & Stahl, 1976). Studies using rats have shown that the typical antipsychotic, haloperidol, elevated prolactin levels for up to 24 hours while the atypical antipsychotic, clozapine, was shown to elevate prolactin levels for only 1-2 hours (Gudelsky, 1981) indicating that typical antipsychotics may induce hyperprolactinemia more easily in humans than atypical antipsychotics. Typical antipsychotics are effective at reducing psychosis in schizophrenia patients but they create a multitude of other adverse effects, some life-threatening, and therefore are less frequently prescribed than the more recently developed class of "atypical" antipsychotic drugs.

Atypical Antipsychotics. The first atypical antipsychotic, or second generation antipsychotic, to be developed was clozapine in 1959. Clozapine was considered atypical simply because it did not produce EPS. Clozapine's behavioral profile is thought to be a result of its high affinity for the serotonin 5-HT_{2A} receptor and relatively weaker affinity for the dopamine D_2 receptors (Meltzer, Matsubara, & Lee, 1989). In addition, atypical antipsychotics also bind to other receptors including adrenergic, cholinergic, and histaminergic receptors (Schotte et al., 1996). Therefore it is evident that atypical antipsychotic drugs have much more complex mechanisms of action than typical antipsychotic drugs. The diversity in receptor affinities causes atypical antipsychotics to have varying effects on positive and negative symptoms and cognitive functions. As a result, some atypical antipsychotic drugs are best at alleviating positive symptoms while others are best at treating negative symptoms and some aspects of cognitive impairment. Once it was realized that this class of drugs not only reduced the positive symptoms of schizophrenia but also minimized the negative symptoms with decreased side effects, it became the pharmacological model for other antipsychotics (Meyer & Simpson, 1997). Other common atypical antipsychotics developed with similar binding profiles to clozapine include risperidone, olanzapine, ziprasidone, and quetiapine (Roth, Sheffler, & Kroeze, 2004).

A new class of antipsychotic drugs is also being developed. The prototype drug for this class is aripiprazole (Abilify) and is considered a third generation antipsychotic drug because instead of acting as a dopamine D_2 receptor antagonist it acts as a partial agonist at dopamine D_2 receptors (Burris et al., 2002). In the past, antagonistic drugs were binding to the dopamine receptors and preventing other neurotransmitters from

binding there but not activating the receptor. Aripiprazole will bind to some of the dopamine D2 receptors as a partial agonist and some of these receptors will be activated causing changes in the brain. A similar drug, bifeprunox is also being studied in clinical trials as a third generation antipsychotic drug for schizophrenia (Newman-Tancredi, Cussac, & Depoortere, 2007). It remains to be seen how these drugs do in clinical trials and on the pharmacology market.

Adverse side effects of atypical antipsychotics. Although atypical antipsychotics are associated with a reduced risk of extrapyramidal side effects and tardive dyskinesia, they still have other adverse effects. The first atypical antipsychotic, clozapine, unexpectedly disrupted the immune system of several patients when it was introduced to the European market in the 1970's (Alvir, Lieberman, Safferman, Schwimmer, & Schaaf, 1993). This potentially fatal adverse side effect is called agranulocytosis, which is a reduction in white blood cell counts and reduced ability to fight off infections. The occurrence of this side effect is rare, seen in 1-2% of patients, and can be reversed if administration of clozapine is stopped but the risk was enough to keep the FDA from allowing clozapine to be prescribed in the United States until 1990 (Alvir et al., 1993; Meyer & Simpson, 1997). Clozapine is now prescribed in the U.S. but patients must have their white blood cell counts tested every week for the first six months of treatment and biweekly thereafter (Kamin et al., 2000; Sedky, Shaughnessy, Hughes, & Lippmann, 2005). One of the reasons clozapine is still on the market is because it has shown to be superior to other antipsychotics in treating schizophrenia patients who are treatment-resistant and/or suicidal (Meltzer et al., 2003).

Atypical antipsychotic drugs, especially clozapine and olanzapine, also can cause weight gain (Kroeze et al., 2003; Lambert, Chou, Chang, Tafesse, & Carson, 2005; Wirshing et al., 1999). Kroeze et al. (2003) discovered that the strong affinity for the H_1 histamine receptor as seen in clozapine and other antipsychotics is positively correlated with weight gain in schizophrenia patients. Increased weight increases the risk for more severe side effects such as hypertension, hyperglycemia, and in some cases type II diabetes which is a serious health concerns for patients (Kroeze et al., 2003; Lambert et al., 2005). In addition to weight gain, several atypical antipsychotics have an adverse effect on the cardiovascular system called "QT interval prolongation" which is an elongation of the heartbeat intervals. The QT prolongation is not life-threatening but it can lead to a more serious condition called torsades de pointes which can cause sudden cardiac arrest (Stollberger, Huber, & Finsterer, 2005; Zareba & Lin, 2003). These side effects are serious and often have a negative effect on patients' quality of life and therefore alternative drug therapy with fewer side effects should be explored.

Cognitive impairments and antipsychotic drugs. An additional reason to look for alternative treatment for schizophrenics, besides adverse side effects, is that the current antipsychotic drugs fail to significantly improve cognitive impairments. Yet, as a class, atypical antipsychotics have been reported to improve cognitive function more than typical antipsychotics. Meltzer and McGurk (1999) assessed how the atypical antipsychotic drugs, clozapine, risperidone, and olanzapine affected cognitive domains. They found that while the atypical antipsychotic drugs where able to improve some aspects of cognition in humans, none showed improvements for all cognitive domains. Risperidone was shown to improve working memory, executive function and attention.

Clozapine improved attention and verbal fluency. Olanzapine improved verbal learning and memory. However, neither clozapine nor olanzapine improved working memory. This construct is considered to be a key factor in cognitive impairment in schizophrenia and a good indicator for functional outcomes (Goldman-Rakic., 1991; Silver et al., 2003). Research conducted with atypical antipsychotics have shown that they can improve cognitive function but these improvements are modest and do not lead to improvements in daily life skills or long-term employment (Green, Kern, Braff, & Mintz, 2000; Woodward et al., 2005). The search for a medication that will treat all symptoms of schizophrenia and rehabilitate patients must continue, but a difficulty for creating new drug treatments is the unknown cause of schizophrenia.

Disease Models

While the cause of schizophrenia is not known, it has been determined to have a genetic factor (Kendler, 1983, 1997; Petronis, Paterson, & Kennedy, 1999). As stated in the introduction there is a 1% rate of prevalence or risk of having schizophrenia. However this risk increases to about 2-4% when a close relative (aunt, uncle, cousin, nephew or niece) has schizophrenia. Having a parent with schizophrenia increases the risk to 13% and if both parents have schizophrenia their children have a 50% chance of also suffering from the disorder (Gottesman, 1991). However, the probability of one identical twin having schizophrenia if the other twin is diagnosed with schizophrenia is only 50%, so the cause is not solely genetic but environmental as well. Environmental factors, such as stress or illness, are considered to be triggers for individuals who are already genetically predisposed for schizophrenia (Corcoran et al., 2003).

Neurodevelopmental hypothesis. One hypothesis for the cause of schizophrenia is the neurodevelopmental hypothesis (Petronis et al., 1999; Weinberger, 1987). According to this hypothesis, the brain develops abnormally in schizophrenia patients. These abnormalities include enlarged ventricles and reduced cortical volumes. In addition many cortical regions are hypoactive in schizophrenia patients (Petronis et al., 1999). Genetic researchers have shown that the DISC1 gene (disrupted in schizophrenia 1) may be responsible for abnormalities in the brains of schizophrenia patients. This gene modulates second messenger proteins which are responsible for neural progenitor proliferation during embryonic brain development and in the adult hippocampus (Mao et al., 2009). A mutation in this gene may cause abnormal neurodevelopment, leading to schizophrenia.

The dopamine hypothesis. The dopamine hypothesis states the symptoms of schizophrenia are caused by hyperdopaminergic activity. This hypothesis is based on studies showing that antipsychotics drugs exert their antipsychotic effects by reducing dopamine transmission in the brain. The origins of the dopamine hypothesis came from reports of acute psychosis after amphetamine overdose that was indistinguishable from paranoid schizophrenia. (Beamish & Kiloh, 1960; Greenwood, 1957; McConnell, 1961; O'Flanagan & Taylor, 1950). Symptoms reported by McConnell (1961) included disorders of thought, paranoid delusions, and auditory and visual hallucinations. Amphetamine induces these symptoms by blocking the reuptake of monoamines in the synapse causing an overall increase in the amount of monoamines in the brain.

In a discrimination study with rats, researchers demonstrated that the interoceptive cues of amphetamine administration are regulated by the dopamine system

(Schechter & Cook, 1975). These researchers therefore suggest that dopamine mediates amphetamine psychosis and paranoid schizophrenia. Other researchers confirmed that haloperidol has a strong affinity for dopamine receptors specifically in the caudate nucleus, putamen, globus pallidus, nucleus accumbens and amygdala (Creese, Burt, & Snyder, 1975) . Furthermore, researchers established that antipsychotics, act as antagonists, inhibiting the release of dopamine in rat striatal slices (Seeman & Lee, 1975). In a review of the dopamine hypothesis, Meltzer and Stahl (1976) suggest that schizophrenia is caused by too much dopamine in brain, particularly in the mesolimbic dopamine pathway, which originates in ventral tegmental area and projects to various regions in the limbic system (Meltzer & Stahl, 1976).

Revisions to the dopamine hypothesis added that schizophrenia is caused by increased dopamine in the limbic system but decreased dopamine in the prefrontal cortex (Davis, Kahn, Ko, & Davidson, 1991; Howes & Kapur, 2009). Decreased activity in the prefrontal cortex is called hypofrontality, and may account for many of the cognitive deficits found in schizophrenia, such as impairments in working memory and attention (Brozoski, Brown, Rosvold, & Goldman, 1979; Goldman-Rakic et al., 2004). The revised dopamine hypothesis includes the idea that the mesocortical dopamine pathway, which originates in the ventral tegmental area and projects to the prefrontal cortex, has decreased dopamine release in people with schizophrenia (Davis et al., 1991). This hypothesis predicts an inverse relationship between hyperdopaminergic activity in the mesolimbic pathway and hypodopaminergic activity in the mesocortical pathway, resulting in a surplus of dopamine in the limbic pathway and a lack of dopamine in the prefrontal cortex. Using microdialysis, researchers found that administration of the

typical antipsychotic haloperidol had little effect on the dopamine concentration in the prefrontal cortex, but administration of the atypical antipsychotic drug clozapine elevated dopamine levels in the prefrontal cortex (Kuroki, Meltzer, & Ichikawa, 1999). These findings possibly explain why atypical antipsychotic drugs are more effective than typical antipsychotic drugs at reducing negative symptoms and cognitive impairment in schizophrenia.

The glutamate hypothesis. The glutamate hypothesis evolved from case studies of individuals who exhibited psychotic symptoms similar to schizophrenia after overdosing on phencyclidine or ketamine (Allen & Young, 1978; Cohen, Rosenbaum, Luby, & Gottlieb, 1962; Javitt & Zukin, 1991; Krystal et al., 1994). These drugs noncompetitively block NMDA glutamate receptors and induce both positive and negative symptoms of schizophrenia, as well as cognitive impairments, resulting in a drug state closely resembling schizophrenia. The glutamate hypothesis of schizophrenia proposes the pathology of schizophrenia is due to the hypofunction of NMDA glutamate receptors (Marek et al., 2010; Paz, Tardito, Atzori, & Tseng, 2008; Tsai & Coyle, 2002). As a result of NMDA receptor hypofunction there is decreased glutamate transmission which could be responsible for dysregulation of the local circuits and long-loop pathways between the prefrontal cortex and limbic structures (Marek et al., 2010).

Animal Models

Since schizophrenia is a uniquely human disorder, models have been made to predict the antipsychotic effects of a drug in humans using rats that do not have schizophrenic behavior. Other models induce psychotic-predictive behavior in rats that

are similar to the behaviors exhibited by schizophrenics such as sensory gating deficits, social withdrawal, and purposeless or stereotyped behavior. These models use drugs that increase dopamine release (e.g. amphetamine) or block NMDA glutamate receptors (e.g. PCP, Ketamine). A commonly used drug for induced psychosis in rats is dizocilpine (MK-801) which is a NMDA glutamate receptor antagonist. It has been shown to significantly impair sensorimotor gating in rats as well as working and reference memory in radial arm maze tasks (Levin, Bettegowda, Weaver, & Christopher, 1998; Zhang et al., 2005).

The radial arm maze is an apparatus used with rats or mice to asses working memory, spatial reference memory, and long-term memory all of which are key factors in cognitive functioning (Ortega-Alvaro, Gibert-Rahola, & Micó, 2006). The type of memory being tested depends on the task. Most radial arm mazes consist of a center platform and eight arms with food cups at the end. The walls around the maze typically have visual cues to aid the rats in spatial navigation. The rats used in this task are normally food deprived and the arms can be baited with food pellets in various patterns. Two commonly used tasks are the 4x8 (win-stay) and the non-match to sample (win-shift) task.

In the 4x8 task, the same four arms are baited each trial and a rat must remember which arms are baited and enter only those arms. Re-entries to arms already visited are considered errors of working memory and entries into arms not baited during training are considered reference memory errors (Levin et al., 1998; Zhang et al., 2005).

In the non-match to sample task the rats only have access to four arms during the first trial and in the second trial they must remember what arms they already went to and

only enter the other four arms. Working and reference memory errors are counted the same as in the 4x8 task. Drugs that have both positive and negative effects on memory can be tested in the radial arm maze by using a test-retest paradigm and comparing the number of errors made in each trial.

The typical antipsychotic, haloperidol, has been tested in the radial arm maze alone and in combination with memory impairing drugs. When tested alone haloperidol had no effect on acquisition of a radial arm task (Terry et al., 2007) or on retention in a delayed non match to sample radial arm maze task (Wolff & Leander, 2003). When tested in combination with another drug, haloperidol enhanced the disruptive effect of alcohol and failed to reverse the memory deficits created by amphetamine (de Oliveira & Nakamura-Palacios, 2003; Nagai et al., 2007).

Some atypical antipsychotics, which have shown to improve cognitive function, have also been tested in the radial arm maze, but their effects on working memory and reference memory have been inconclusive. In a different study, olanzapine and clozapine were shown to have a negative effect on working memory following acute administration, but the effect diminished with chronic administration (Ortega-Alvaro et al., 2006). However in another study using a delayed non-match to sample task, olanzapine (3 and 5 mg/kg) and risperidone (0.1mg/kg) reduced the number of errors in a retention trial when administrated immediately after the information phase, while clozapine and ziprasidone had no effect on memory retrieval (Wolff & Leander, 2003). Researchers have continued to search for new antipsychotics which will consistently improve multiple domains of cognitive functioning. Many new drugs have been tested using these animal models of cognitive functioning. Although some promising results

have been found, a treatment to improve all symptoms of schizophrenic, including cognitive impairments, has not been found.

Neurotensin

A novel approach for treating schizophrenia. Neurotensin is a central nervous system neuropeptide that has received interest for its potential antipsychotic and cognitive enhancing abilities. Three different neurotensin receptors have been found in the brain and have been named the NT1, NT2, and NT3 receptors (Binder, 2001; Vincent JP, 1999). Researchers have found reduced numbers of neurotensin receptors in the caudate nucleus, cingulate cortex, and prefrontal cortex of the postmortem brains of schizophrenia patients (Lahti, Cochrane, Roberts, Conley, & Tamminga, 1998). In addition, it has been reported that the concentration of neurotensin is deceased in the cerebral spinal fluid of schizophrenia patients but increases after administration of antipsychotic drugs (Binder, 2001; Cáceda et al., 2006; Sharma, Janicak, Bissette, & Nemeroff, 1997). Sharma et al. (1997) also reported that an increase of neurotensin in cerebral spinal fluid is positively correlated with improvements in negative symptoms of schizophrenia. Based on these findings, the neurotensin system should be a target of novel antipsychotics that seek to treat negative symptoms and cognitive deficits.

Enhancing the activity of neurotensin in schizophrenia patients by giving them an analog of the peptide that can cross the blood-brain barrier may be clinically useful in treating the negative symptoms and cognitive impairment in schizophrenia (Prus, Huang, Li, Dai, & Meltzer, 2007). Neurotensin has been shown to have a regulatory role in the release of dopamine and serotonin in the prefrontal cortex similar to atypical antipsychotics. In one study, researchers demonstrated that local administration of

neurotensin through microdialysis increases dopamine levels in the prefrontal cortex (Petkova-Kirova et al., 2008). Other studies using local administration of neurotensin have shown that microinjections of neurotensin directly into the ventral tegmental area also increased dopamine levels in the prefrontal cortex (Sotty et al., 2000) as well as increased dopamine metabolism in the NAC (Kalivas & Taylor, 1985). These effects are similar to the response seen after the administration of atypical antipsychotic drugs (McMahon, 2002). The ability of antipsychotic drugs to increase dopamine levels in the prefrontal cortex is significant because it is believed to be a contributing factor for how they improve cognitive functioning in schizophrenia patients (Meltzer & McGurk, 1999; Woodward et al., 2005). Besides increasing the level of dopamine in the prefrontal cortex, neurotensin also acts on the serotonin system in a similar way to atypical antipsychotics.

Behavioral effects of neurotensin. The neurotensin analog PD149163, has been designed as a neurotensin receptor agonist that can cross the blood-brain barrier and is being tested in rats for its antipsychotic properties. The acoustic startle reflex is one of many behavioral assays that test the effects of neurotensin or its analog on behavior. This assay examines sensorimotor gating, which is the ability to filter incoming stimuli, by measuring prepulse inhibition. It also has clinical relevancy because schizophrenia patients have deficits in sensorimotor gating, which can be alleviated after treatment with atypical antipsychotics (Cáceda et al., 2006). Researchers have shown that neurotensin improves sensory gating when infused into the nucleus accumbens of rats and reverses disruptions of prepulse inhibition caused by administration of amphetamine (Feifel, Minor, Dulawa, & Swerdlow, 1997). Researchers have also used genetically modified

rats, such as the Brattleboro strain, to observe deficits in PPI and test novel antipsychotics for their ability to restore sensorimotor gating. In one study, researchers found that PD149163 (1.0 and 3.0 mg/kg, s.c.) reversed the PPI deficits found in Brattleboro rats in the same manner as clozapine (10 and 15 mg/kgl, s.c.) but not haloperidol (0.1, 0.5, 1.0 mg/kg, s.c.), which failed to improve PPI behavior and also produced catalepsy in the rats at the 1 mg/kg dose (Feifel, Melendez, & Shilling, 2004).

RATIONALE

The overall goal of the study was to determine if PD149163 should continue to be explored as a novel antipsychotic that may have better functional outcomes than the current drug treatments for schizophrenia .In order to evaluate the effects PD149163 on working and reference memory, rats were trained to perform a 4x4 radial arm maze task. The first goal of this investigation was to determine if PD149163 had any effect on cognition in the radial arm maze. The second goal of this investigation was to determine if PD149163 could reverse the cognitive deficits created by the administration of MK801. The third goal of this investigation was to compare the effects of PD149163 to a typical and atypical antipsychotic, haloperidol and clozapine, respectively.

METHODS

Subjects

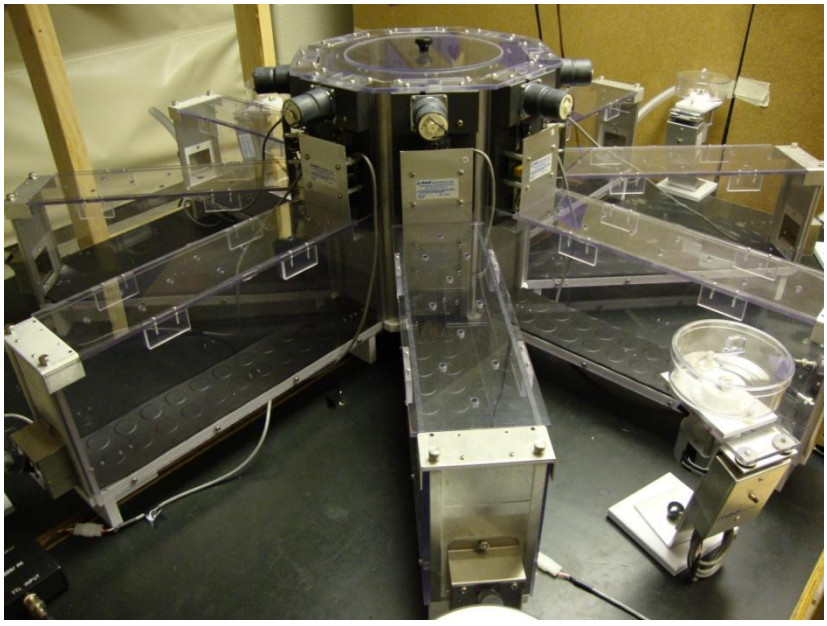
The experiment used twenty male Sprague-Dawley rats (Charles River Inc., Portage, MI). All subjects were individually housed in plastic cages in a room kept under constant temperature conditions and a 12 hour light/dark cycle. The animals had free access to water in their cages and food was restricted to 85% of their free feeding weights in order to motivate the rats to perform the behavioral task for food rewards. All procedures were approved by the Institutional Animal Care and Use Committee at Northern Michigan University and were consistent with the recommendations provided in the Guide for the Care and Use of Laboratory Animals.

Apparatus

A radial arm maze (Med Associates, St. Albans, VT) was used for the experiments. The radial arm maze (RAM) had 8 arms (45.7cm x 10.16cm) and eight automatic guillotine doors that projected from an octagonal central platform (152.4cm x152.4 cm, height 45.7cm). The maze was elevated 79 cm above the ground. The maze was made of clear polycarbonate, white polypropylene runway bases and an aluminum frame. Black rubber was placed on top of the runways and in the center platform so that the contrast between the maze and the rats was greater, thus allowing for better detection and tracking of the rat by video camera. At the end of each arm was a food cup that could be baited with food pellets (45mg). Visual cues, such as paper cut outs of shape, were placed within and outside the maze to act as cues for spatial navigation. The trials were

controlled by EthoVision XT Trial & Hardware Control Module (Noldus, Leesburg, VA). Behavioral activity was tracked by a camera mounted above the maze and recorded by Noldus video software. The researchers controlled the maze from the computer module behind a blind.

Figure 1. A side view of the radial arm maze.



Training

Rats were habituated to the maze for two consecutive days. During habituation, each rat was placed in the center platform and allowed to freely explore all of the arms. Food pellets were randomly scattered throughout the maze and in the food cups located at the end of each arm. Each habituation session lasted 10 minutes. After this, training sessions began.

Each daily training session consisted of two identical trials. There was a 10 second delay between when the rat was placed in the center platform and when the trial started to

prevent any placement bias. During each trial every door was open but only four of the eight arms were baited with a single food pellet in the food cup. The configuration of baited arms was different (pseudorandom) for every rat to control for spatial and olfactory cues but kept consistent for each individual animal through out training and testing. The trial ended once the rat had either obtained every food pellet or five minutes elapsed, whichever occurred first. In between trials the maze was cleaned with 20% isopropyl alcohol to eliminate olfactory cues. The training criterion consisted of 3 consecutive trials with no more than one reference or working memory error (see table for definitions).

Table 1: The dependent variables recorded are listed below

Variables	**Definition**
Working Memory Errors	Number of re-entries to an arm previously visited during the same trial
Reference Memory Errors	Number of entries into unbaited arms
Total Memory Errors	Number of working + reference memory errors
Path Length	Distance travelled in centimeters
Velocity	Distance (cm) divided by total time (sec)
Test session time	Total time taken to complete test (sec)
Percent of time spent immobile	Time spent immobile (sec) divided by test time (sec)

Testing and Treatment Design

After meeting training criteria, test sessions began. A test session was only one trial, but otherwise identical to a training session. Drug tests occurred on Tuesdays and Fridays, with no experimental procedures occurring between test sessions. For testing, the rats were randomly divided into two groups with ten rats each. Group one consisted for rats 1-10 and group two consisted of rats 11-20. Group one was administered MK801 and PD149163 and their respective vehicles in a counterbalanced design to test the effects of the drugs alone and in combination. In group two, a typical and atypical antipsychotic, haloperidol and clozapine, were tested alone and in combination with MK-801 as a comparison.

Table 2: Drugs and doses that were used in this experiment

Drug Name	Dose Pre-Injection time	Mechanism	Rationale
MK-801	.07 mg/kg s.c. + 30 min	NMDA receptor antagonist	Produces memory deficits. The NT analog will be tested to see if it can reverse these memory-disruptive effects.
PD149163	0.0625, 0.125, 0.25 mg/kg s.c. +30 min	Neurotensin analog	Treatment being studied
Haloperidol	0.03125, 0.0625 mg/kg s.c. + 30 min	Typical antipsychotic	Comparison drug
Clozapine	0.3125, 1.25 mg/kg s.c. + 30 min	Atypical antipsychotic	Comparison drug

Data Analysis

The dependent variables in this study are similar to those assessed in other studies. In most studies a reference memory error is recorded as an entry into an arm that was not baited in the training period (Levin et al., 1998; Terry et al., 2007). In other words, memory of the baited arms from the first trial is indicated by the rat entering only those arms in the consecutive trials. Working memory errors are counted by the number of times a rat re-enters an arm already visited in the same trial (Wolff & Leander, 2003). The other dependent variables of this study that were also record in other studies were the path length and test session time (Ortega-Alvaro et al., 2006; Wolff & Leander, 2003). In addition to those two locomotor variables, the percent of time spent immobile and velocity was also recorded using EthoVision tracking software. All variables were expressed as means (+/- standard error of the mean [SEM]). There were two factors assessed in every analysis, and the levels for each factor corresponded to the number of doses, and vehicle, for each drug. One factor was the treatment drug, which consisted of either PD149163, haloperidol, or clozapine. The other factor was MK801, which always consisted of two levels, vehicle and a 0.07 mg/kg dose. Both factors were repeated measures variables, and therefore a two-factor repeated measures analysis of variance test was conducted. Statistically significant differences for either factor main effect or for an interaction effect were further analyzed using a Tukey HSD multiple comparisons post hoc test. All analyses were conducted using PASW Statistics version 18 (Chicago, Illinois) for Microsoft Windows.

RESULTS

Training

Of the initial ten animals obtained for each group only nine in group 1 and seven in group 2 met the training criteria and were used for testing. Again, the training criteria required each rat to make no more than one error over three consecutive trials. In group 1 the mean number of training days required to meet criteria was 30.89 days +/- 2.08 standard error of the mean [SEM]). In group 2 the mean number of training days required to meet criteria was 36.29 days +/- 3.35 SEM). Rats that failed to meet the training criteria after 50 days of training were removed from the study. This number of days exceeded over two standard deviations from the mean number of trials needed to reach criteria.

PD149163 + MK801

Working memory errors. The effects of MK807 (vehicle [VEH] and 0.07 mg/kg) and PD149163 (VEH, 0.0625, 0.125, and 0.25 mg/kg) are shown in figure 2. Treatment with a 0.07 mg/kg dose of MK801 produced a statistically significant increase in working memory errors (mean [M] = *2.7 +\- 0.60*) compared to vehicle (M= 0.64 +/- *0.19*), (*F[1, 8]=14.29, p<0.01, eta^2=0.64).* Moreover, MK801 treatment had a large effect on working memory errors, accounting for 64% of the total variance. There was not a statistically significant effect shown for the main effect of PD149163 treatment (*F[3, 24]=0.71, p>0.05, eta^2=0.08*) nor for the interaction effect (*F[3, 24]=1.24, p>0.05, eta^2=0.13*).

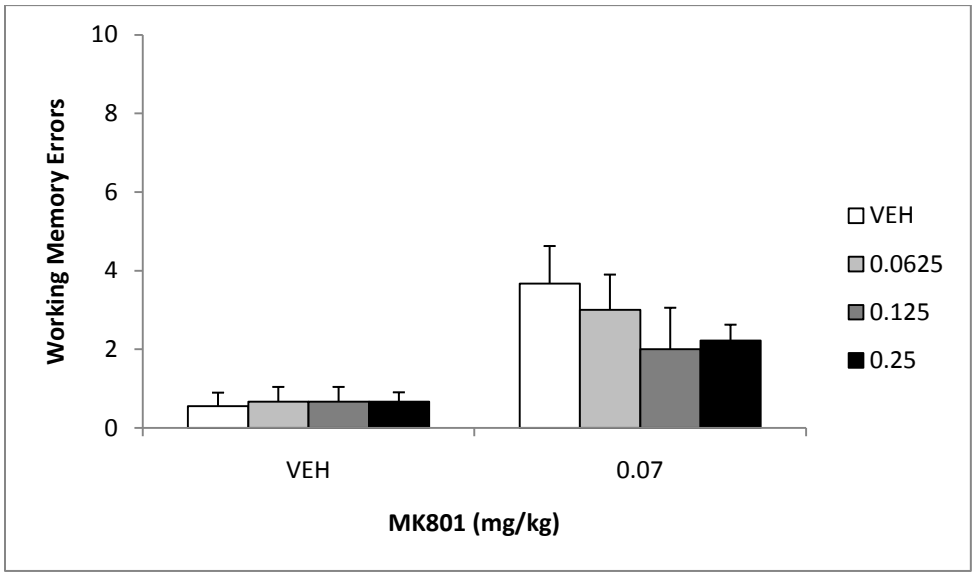


Figure. 2 The effects of PD149163 + MK801 on mean (+/- SEM) number of working memory errors.

Reference memory errors. The effects of MK807 (VEH and 0.07 mg/kg) and PD149163 (VEH, 0.0625, 0.125, and 0.25 mg/kg) are shown in figure 3. Treatment with a 0.07 mg/kg dose of MK801 produced a statistically significant increase in reference memory errors (M = *2.47 +\- 0.27*) compared to vehicle (M= 1.50 +/- *0.2*), (*F[1, 8]=15.8, p<0.01, eta^2=0.66).* Moreover, MK801 treatment had a large effect on reference memory errors, accounting for 66% of the total variance. There was not a statistically significant effect shown for the main effect of PD149163 treatment (*F[3, 24]=0.66, p>0.05, eta^2=0.08*) nor for the interaction effect (*F[3, 24]=1.32, p>0.05, eta^2=0.14*).

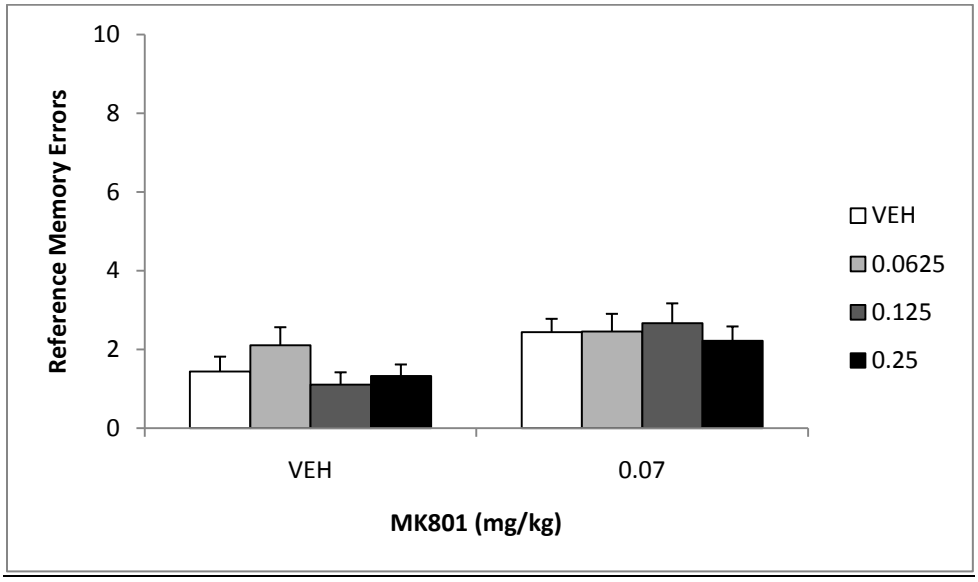


Figure 3: The effects of PD149163 + MK801 on mean (+/- SEM) number of reference memory errors.

Total errors. The effects of MK807 (VEH and 0.07 mg/kg) and PD149163 (VEH, 0.0625, 0.125, and 0.25 mg/kg) are shown in figure 4. Treatment with a 0.07 mg/kg dose of MK801 produced a statistically significant increase in total memory errors (M = *4.92 +\- 0.73*) compared to vehicle (M= 2.14 +/- *0.26*), (*F[1, 8]=17.13, p<0.01, eta^2=0.68).* Moreover, MK801 treatment had a large effect on total memory errors, accounting for 68% of the total variance. There was not a statistically significant effect shown for the main effect of PD149163 treatment (*F[3, 24]=0.55, p>0.05, eta^2=0.06*) nor for the interaction effect (*F[3, 24]=0.07, p>0.05, eta^2=0.008*).

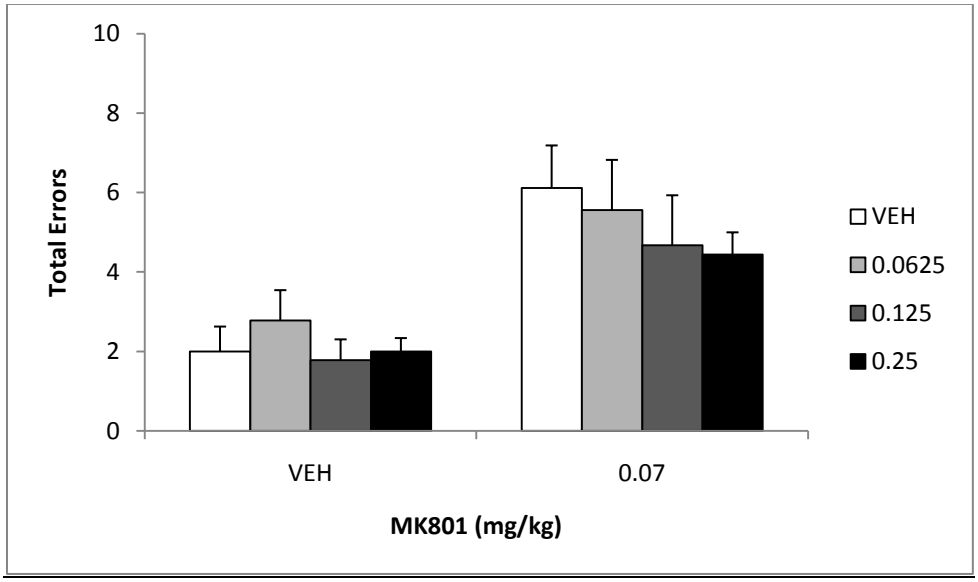


Figure 4: The effects of PD149163 + MK801 on mean (+/- SEM) number of total memory errors.

Path length. The effects of MK807 (VEH and 0.07 mg/kg) and PD149163 (VEH, 0.0625, 0.125, and 0.25 mg/kg) are shown in table 3. Treatment with a 0.07 mg/kg dose of MK801 produced a statistically significant increase in path length (*M = 741.96 cm +\- 79.70 cm*) compared to vehicle (*M= 426.83 cm +/- 17.39 cm*), (*F[1, 8]=15.75, p<0.01, eta^2=0.66).* Moreover, MK801 treatment had a large effect on path length, accounting for 66% of the total variance. There was not a statistically significant effect shown for the main effect of PD149163 treatment (*F[3, 24]=0.53, p>0.05, eta^2=0.06*) nor for the interaction effect (*F[3, 24]=0.74, p>0.05, eta^2=0.08*).

Test session time. The effects of MK807 (VEH and 0.07 mg/kg) and PD149163 (VEH, 0.0625, 0.125, and 0.25 mg/kg) are shown in table 3. There was not a statistically significant effect shown for the main effect of MK801 (*F[1,8] =0.77, p>0.05, eta^2=0.09* nor PD149163 treatment (*F[3, 24]=0.88, p>0.05, eta^2=0.10*). There was a statistically significant effect shown for the interaction effect (*F[3, 24]=3.65, p<0.05, eta^2=0.31*). Based upon a comparison between simple effect means using a Tukey post hoc multiple comparisons test, statistically significant differences in test session times were not found between these groups.

Velocity. The effects of MK807 (VEH and 0.07 mg/kg) and PD149163 (VEH, 0.0625, 0.125, and 0.25 mg/kg) are shown in table 3. Treatment with a 0.07 mg/kg dose of MK801 produced a statistically significant increase in velocity (*M = 14.98 cm/s +\- 0.83 cm/s*) when compared to vehicle (*M= 11.47 cm/s +/- 0.75 cm/s*), (*F[1, 8]=20.83, p<0.01, eta^2=0.72).* Moreover, MK801 treatment had a large effect on velocity, accounting for 72% of the total variance. There was not a statistically significant effect shown for the main effect of PD149163 treatment (*F[3, 24]=2.97, p>0.05, eta^2=0.27*).

There was a statistically significant interaction effect (*F[3, 24]=4.15, p<0.05, eta^2=0.34*). Based upon a comparison between simple effect means using a Tukey post hoc multiple comparisons test, significantly greater velocity was observed when rats were treated with MK801 + PD149163 (0.0625mg/kg) (*M= 14.36 cm/s*) compared to when rats were treated with VEH + PD149163 (0.0625 mg/kg) (*M= 10.78 cm*). Significantly greater velocity also was shown, when rats were treated with MK801 + PD149163 (0.125 mg/kg) (*M=15.18 cm/s*) compared to VEH + PD149163 (0.125 mg/kg) (*M=11.33cm/s*), and when rats were treated with MK801 + PD149163 (0.125 mg/kg) (*M=15.26 cm/s*) compared to VEH + PD149163 (0.125 mg/kg) (*M=9.24 cm/s*). In addition, significantly greater velocity was observed when rats were treated with VEH + VEH (*M=14.52 cm/s*) compared to VEH + PD149163 (0.125 mg/kg) (*M=9.24 cm/s*). The interaction had a large effect on velocity, accounting for 34% of the total variance.

Percent of time spent immobile. The effects of MK807 (VEH and 0.07 mg/kg) and PD149163 (VEH, 0.0625, 0.125, and 0.25 mg/kg) are shown in table 3. There was not a statistically significant effect shown for the main effect of MK801, (*F[1, 8]=4.35, p>0.05, eta^2=0.35).* There was a main effect of PD149163 treatment (*F[3, 24]=3.33, p<0.05, eta^2=0.29).* Based on the results from the ANOVA, statistically greater percent of time spent immobile was observed when rats were treated with PD149163 (0.25 mg/kg) (*M= 76.8% +\- 4.20%*) compared to when rats were treated with PD149163 VEH (*M= 66.52% +/- 1.45%*). Moreover, PD 149163 treatment had a large effect on time spent immobile, accounting for 29% of the total variance. There was not a statistically significant interaction effect (*F[3, 24]=0.14, p>0.05, eta^2=0.02*). No other statistically significant effects were found.

Table 3.The effects of PD149163 and MK801 on locomotor activity.

		Path Length (cm)		Test session time (s)		Velocity (cm/s)		Percent of time spent immobile (%)	
MK Dose (mg/kg)		VEH	0.07	VEH	0.07	VEH	0.07	VEH	0.07
PD Dose (mg/kg)	VEH	412.21	789.86	29.75	56.70	14.52	15.11	68.74	64.30
	0.0625	520.97	780.50	49.27	56.95	10.78	14.36	74.96	70.44
	0.125	455.31	736.40	42.25	51.06	11.33	15.18	73.21	67.26
	0.25	462.82	661.08	61.55	44.43	9.24	15.26	80.41	73.21

Haloperidol + MK801

No omissions occurred during testing with dose combinations of haloperidol and MK801.

Working memory errors. The effects of MK807 (VEH and 0.07 mg/kg) and haloperidol (VEH, 0.03125, and 0.0625 mg/kg) are shown in figure 5. Treatment with dose combination of haloperidol and MK801 did not produced statistically significant effects on working memory errors in this task, (main effect of haloperidol, *F[2,12] = 0.36, p>0.05, eta²=0.06*; main effect of MK801, *F[1, 6]=5.22, p>0.05, eta^2=0.47*; interaction effect, *F[2,12]=1.49, p>0.05, eta^2=0.20*). Although no statistically significant effects were found there were still large effect sizes for MK801 and the interaction, which accounted for 47% and 20% of the variance, respectively.

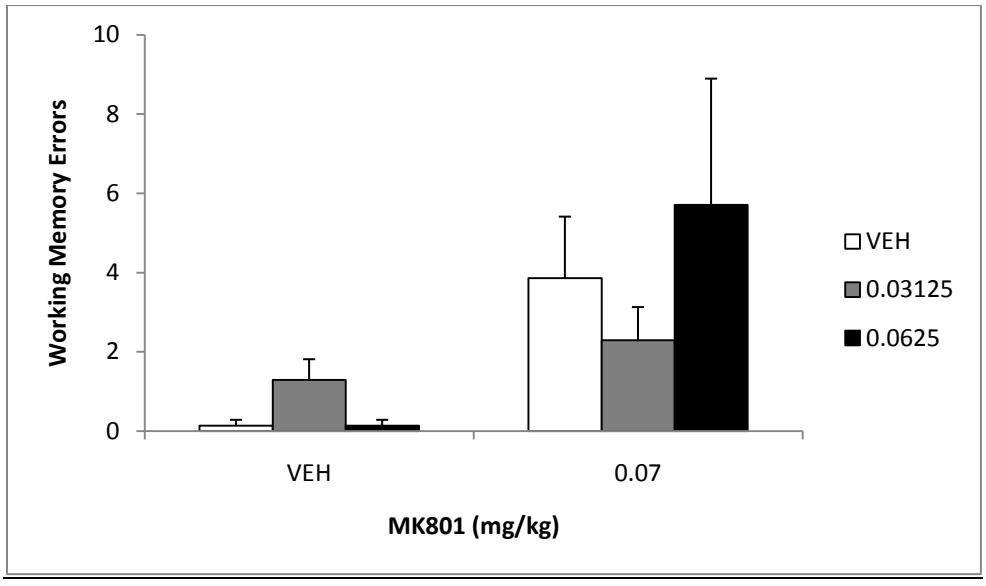


Fig. 5: The effects of haloperidol + MK801 on mean (+/- SEM) number of working memory errors.

Reference memory errors. The effects of MK807 (VEH and 0.07 mg/kg) and haloperidol (VEH, 0.03125, and 0.0625 mg/kg) are shown in figure 6. Treatment with a 0.07 mg/kg dose of MK801 produced a statistically significant increase in reference memory errors (*M=2.71 +\- 0.14*) compared to vehicle (*M=1.52 +/- 0.12*), (*F[1, 6]=55.15, p<0.001, eta^2=0.90).* Moreover, MK801 treatment had a large effect on reference memory errors, accounting for 90% of the total variance. There was not a statistically significant effect shown for the main effect of haloperidol treatment (*F[2,12] =0.33, p>0.05, eta²=0.05*) nor for the interaction effect (*F[2,12]=0.27, p>0.05, eta^2=0.04*).

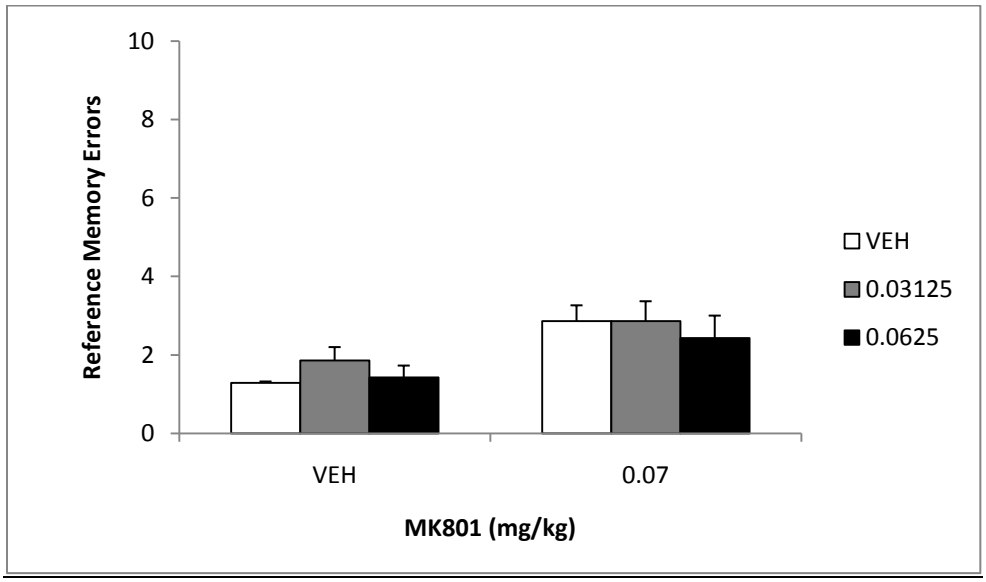


Fig. 6: The effects of haloperidol + MK801 on mean (+/- SEM) number of reference memory errors.

Total memory errors. The effects of MK807 (VEH and 0.07 mg/kg) and haloperidol (VEH, 0.03125, and 0.0625 mg/kg) are shown in figure 7. Treatment with a 0.07 mg/kg dose of MK801 produced a statistically significant increase in total memory errors (*M=6.29 +\- 1.50*) compared to vehicle (*M=1.95 +/- 0.22*), (*F[1, 6]=7.30, p<0.05, eta^2=0.55).* Moreover, MK801 treatment had a large effect on total memory errors, accounting for 55% of the total variance. There was not a statistically significant effect shown for the main effect of haloperidol treatment (*F[2,12] =0.33, p>0.05, eta²=0.05*) nor for the interaction effect (*F[2,12]=0.77, p>0.05, eta^2=0.11*).

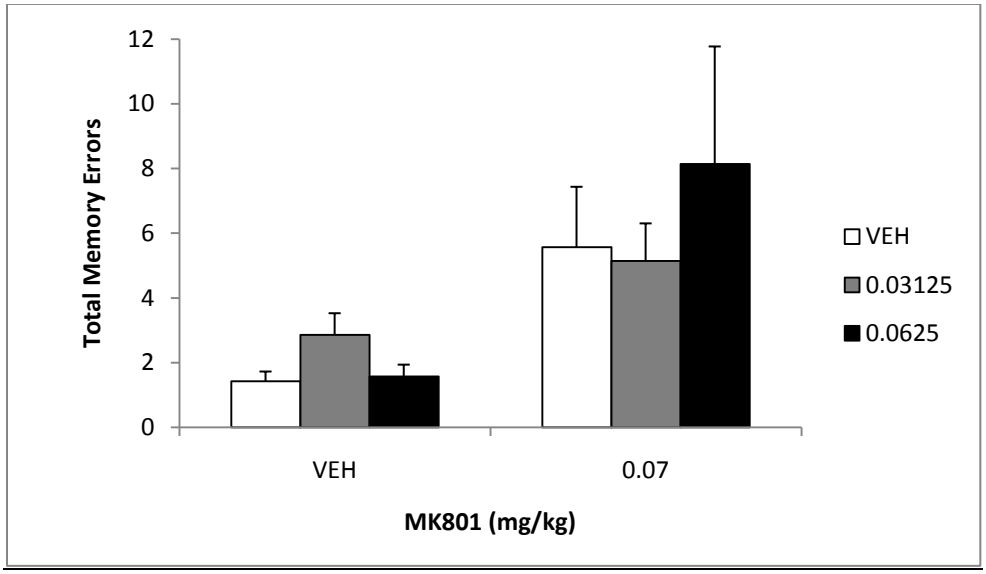


Fig.7: The effects of haloperidol + MK801 on mean (+/- SEM) number of total memory errors.

Path length. The effects of MK807 (VEH and 0.07 mg/kg) and haloperidol (VEH, 0.03125, and 0.0625 mg/kg) are shown in table 4. Treatment with a 0.07 mg/kg dose of MK801 produced a statistically significant increase in path length (*M= 858.08 cm +\- 123.54 cm*) compared to vehicle (*M=477.43 cm +/- 20.52 cm), F[1, 6]=10.24, p<0.05, eta^2=0.63).* Moreover, MK801 treatment had a large effect on path length, accounting for 63% of the total variance. There was not a statistically significant effect shown for the main effect of haloperidol treatment (*F[2, 12]=0.16, p>0.05, eta^2=0.03*) nor for the interaction effect (*F[2, 12]=0.74, p>0.05, eta^2=0.11*).

Test session time. The effects of MK807 (VEH and 0.07 mg/kg) and haloperidol (VEH, 0.03125, and 0.0625 mg/kg) are shown in table 4. Treatment with a 0.07 mg/kg dose of MK801 produced a statistically significant increase test session time (*M= 59.09 s +\- 6.70 s)* compared to vehicle (*M=33.43 s +/- 2.72 s*), *(F[1, 6]=10.53, p<0.05, eta^2=0.64).* Moreover, MK801 treatment had a large effect on session time, accounting for 64% of the total variance. There was not a statistically significant effect shown for the main effect of haloperidol treatment (*F[2, 12]=0.06, p>0.05, eta^2=0.01*) nor for the interaction effect, *F[2,12]= 1.10, p>0.05, eta^2=0.36*).

Velocity. The effects of MK807 (VEH and 0.07 mg/kg) and haloperidol (VEH, 0.03125, and 0.0625 mg/kg) are shown in table 4.Treatment with dose combinations of haloperidol and MK801 did not produce statistically significant effects on velocity in this task (main effect of MK801, *F[1, 6]= 0.045, p > 0.05, eta^2=0.01;* main effect of haloperidol, *F[2, 12]=0.32, p>0.05, eta^2=0.05*; interaction effect, *F[2, 12]= 0.17, p>0.05, eta^2=0.03*). Based on the results from the ANOVA no statistically significant differences in velocity were observed.

Percent of time spent immobile. The effects of MK807 (VEH and 0.07 mg/kg) and haloperidol (VEH, 0.03125, and 0.0625 mg/kg) are shown in table 4. Treatment with dose combinations of haloperidol and MK801 did not produce statistically significant effects on percent of time spent immobile in this task, (main effect of MK801, $F[1, 4] = 0.004$, $p > 0.05$, $eta^2 = 0.001$; main effect of haloperidol, $F[2, 8] = 0.511$, $p > 0.05$, $eta^2 = 0.113$; interaction effect, $F[2, 8] = 0.08$, $p > 0.05$, $eta^2 = 0.02$). Based on the results from the ANOVA no statistically significant differences in percent of time spent immobile were observed.

Table 4: The effects of haloperidol and MK801 on locomotor activity.

		Path Length (cm)		Test session time (s)		Velocity (cm/s)		Percent of time spent immobile (%)	
MK Dose (mg/kg)		VEH	0.07	VEH	0.07	VEH	0.07	VEH	0.07
Hal Dose (mg/kg)	VEH	471.99	841.49	30.16	58.82	15.25	14.60	66.25	68.28
	0.03125	529.80	730.92	41.93	52.50	14.04	14.57	73.32	69.96
	0.0625	430.49	1001.82	28.22	65.94	15.72	15.19	67.93	67.80

CLOZAPINE+ MK801

No omissions occurred during testing with dose combination of haloperidol and MK801

Working memory errors. The effects of MK807 (VEH and 0.07 mg/kg) and clozapine (VEH, 0.3125, and 1.25 mg/kg) are shown in figure 8. Treatment with a 0.07 mg/kg dose of MK801 produced a statistically significant increase in working memory errors (*M=3.00 +\- 0.72*), compared to vehicle (*M=1.24 +/- 0.58*), (*F[1, 6]=15.16, p<0.01, eta^2=0.72).* Moreover, MK801 treatment had a large effect on working memory errors, accounting for 72% of the total variance. There was not a statistically significant effect shown for the main effect of clozapine treatment (*F[2,12] = 0.32, p>0.05, eta²=0.05*) nor for the interaction effect (*F[2,12]=0.84, p>0.05, eta^2=0.12).*

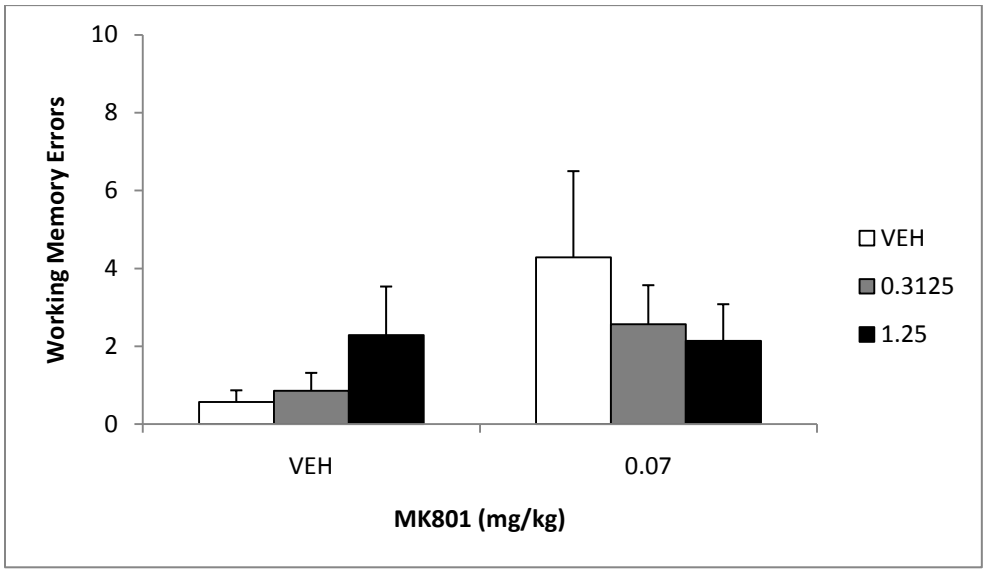


Fig. 8: The effects of clozapine + MK801 on mean (+/- SEM) number of working memory errors.

Reference memory errors. The effects of MK807 (VEH and 0.07 mg/kg) and clozapine (VEH, 0.3125, and 1.25 mg/kg) are shown in figure 9. Treatment with dose combinations of MK801 and clozapine did not produce statistically significant effects on reference memory errors in this task, (main effect of MK801, *F[1, 6]=3.69, p>0.05,* main effect of clozapine, *F[2,12] = 2.44, p>0.05, eta²=0.29*; *eta²=0.38*; interaction effect, *F[2,12]=2.87, p>0.05, eta²=0.32).* No statistically significant effects were found.

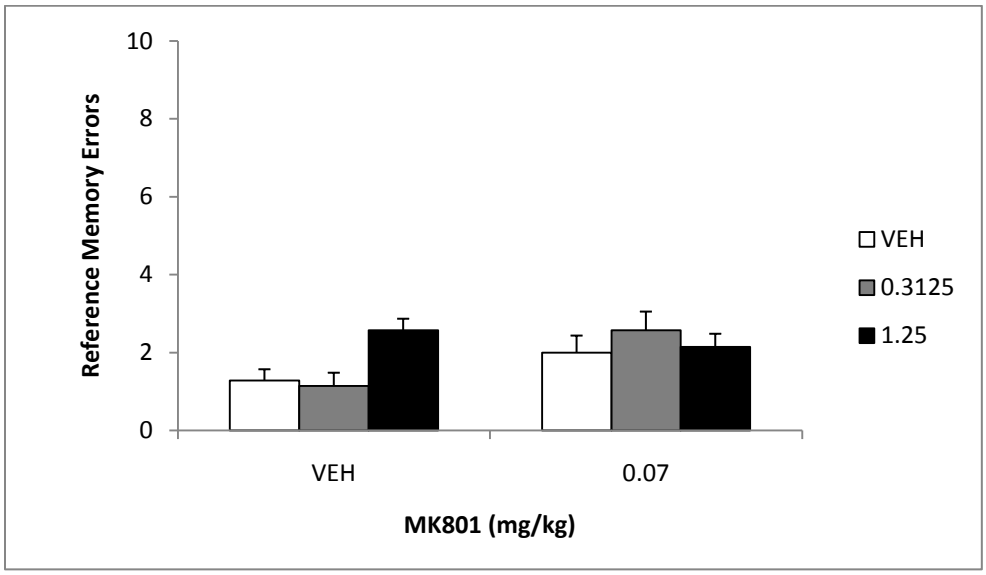


Fig. 9: The effects of clozapine + MK801 on mean (+/- SEM) number of reference memory errors.

Total memory errors. The effects of MK807 (VEH and 0.07 mg/kg) and clozapine (VEH, 0.3125, and 1.25 mg/kg) are shown in figure 10. Treatment with a 0.07 mg/kg dose of MK801 produced a statistically significant increase in total memory errors (*M=5.76+\- 0.81*), compared to vehicle (*M= 2.81+/- 0.78*), (*F[1, 6]=17.45, p<0.01, eta^2=0.74).* Moreover, MK801 treatment had a large effect on total memory errors, accounting for 74% of the total variance. There was not a statistically significant effect shown for the main effect of clozapine treatment (*F[2,12] = 0.80, p>0.05, eta²=0.12*) nor for the interaction effect (*F[2,12]=1.73, p>0.05, eta^2=0.22).*

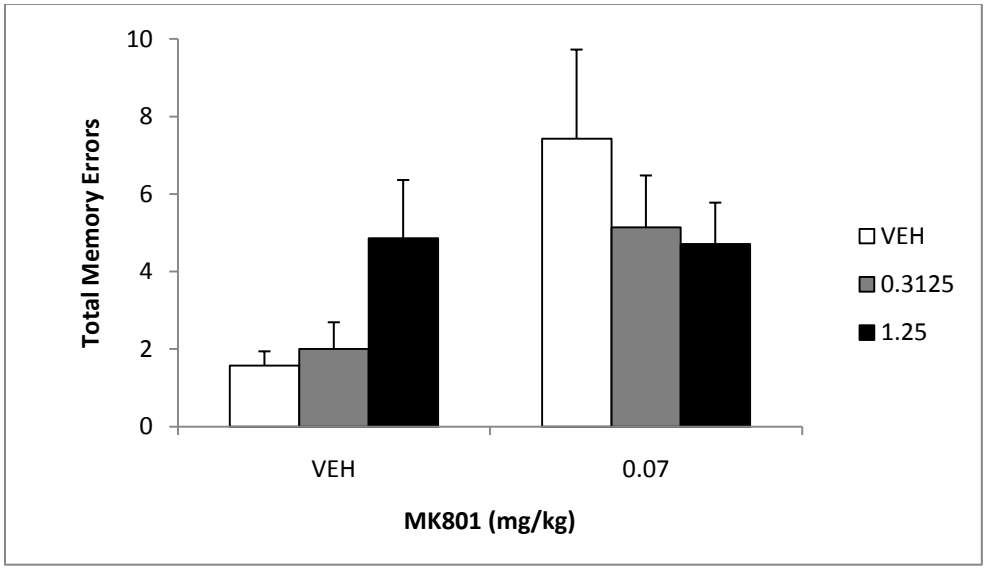


Fig. 10: The effects of clozapine + MK801 on mean (+/- SEM) number of total memory errors.

Path length. The effects of MK807 (VEH and 0.07 mg/kg) and clozapine (VEH, 0.3125, and 1.25 mg/kg) are shown in table 5. Treatment with a 0.07 mg/kg dose of MK801 produced a statistically significant increase in path length (*M= 700.62 cm +\- 43.20 cm*) compared to vehicle (*M=530.40 cm +/- 63.59 cm), F[1, 6]=6.01, p=0.050, eta^2=0.50).* Moreover, MK801 treatment had a large effect on path length, accounting for 50% of the total variance. There was not a statistically significant effect shown for the main effect of clozapine treatment (*F[2, 12]=2.01, p>0.05, eta^2=0.25*) nor for the interaction effect (*F[2,12]= 1.01, p>0.05, eta^2=0.14*).

Test session time. The effects of MK807 (VEH and 0.07 mg/kg) and clozapine (VEH, 0.3125, and 1.25 mg/kg) are shown in table 5. Treatment with dose combinations of clozapine and MK801 did not produce statistically significant effects on test session time in this task, (main effect of MK801, *F[1, 6]=0.16, p>0.05, eta^2=0.03*; main effect of clozapine, *F[2, 12]=3.28, p>0.05, eta^2=0.35*; interaction effect, *F[2,12]= 1.60, p>0.05, eta^2=0.21*).

Velocity. The effects of MK807 (VEH and 0.07 mg/kg) and clozapine (VEH, 0.3125, and 1.25 mg/kg) are shown in table 5. Treatment with dose combinations of clozapine and MK801 did not produce statistically significant effects on velocity in this task, (main effect of MK801, *F[1, 6]=0.09, p>0.05, eta^2=0.02*; main effect of clozapine, *F[2, 12]=2.99, p>0.05, eta^2=0.33*; interaction effect, *F[2,12]= 0.88, p>0.05, eta^2=0.13*).

Percent of time spent immobile. The effects of MK807 (VEH and 0.07 mg/kg) and clozapine (VEH, 0.3125, and 1.25 mg/kg) are shown in table 5. Treatment with dose combinations of clozapine and MK801 did not produce statistically significant effects on percent of time spent immobile in this task, (main effect of MK801, *F[1, 6]=0.015, p>0.05, eta^2=0.003*; main effect of clozapine, *F[2, 12]=0.66, p>0.05, eta^2=0.10*; interaction effect, *F[2,12]= 0.17, p>0.05, eta^2=0.03*).

Table 5: The effects of clozapine and MK801 on locomotor activity.

		Path Length (cm)		Test session time (s)		Velocity (cm/s)		Percent of time spent immobile (%)	
MK Dose (mg/kg)		VEH	0.07	VEH	0.07	VEH	0.07	VEH	0.07
Clozapine Dose (mg/kg)	VEH	425.09	653.61	12.77	25.27	15.76	14.04	70.57	71.57
	0.3125	445.77	739.42	5.11	12.69	13.97	15.10	73.30	71.87
	1.25	720.33	708.83	7.79	11.92	12.20	13.60	75.45	76.80

DISCUSSION

This study evaluated the effects of MK801, PD149163, haloperidol and clozapine on performance in the radial arm maze task. The study demonstrated that administration of MK-801 creates deficits in working, reference, and total errors made in a radial arm maze task. These deficits proved to be hard to reverse. No drug combination with MK-801 significantly reduced errors when compared to the number of errors made when MK-801 was administered by itself. PD149163 failed to significantly improve or impair memory in the radial arm maze. Haloperidol and clozapine also did not significantly effect working, reference, or total memory errors.

Training

The training criteria for this study were similar to those used other studies (de Oliveira & Nakamura-Palacios, 2003; Wolff & Leander, 2003; Zhang et al., 2005). The rats were trained twice a day and could not make more than one error in three consecutive trials before moving on to testing. The average number of training days required to meet criteria was about 36 days. This was a greater number of training days required to meet criteria than reported by Zhang (2005) who train rats to perform the same task in 15-20 sessions (2 trials per day). They used a similar radial arm maze but had a transparent plastic octagonal hub blocking the arms instead of metal doors during the first 30 seconds of each trial (Zhang & O'Donnell, 2000). This would have allowed the rats to see down the arms before entering them and might have helped them orientate in the maze and prevent making incorrect entries. In the first group only one rat, #7, failed to meet the training criteria, but in the second group three rats, #12, #15, and #16, failed to meet the

training criteria. The removal of these animals reduced the sample size to nine for group one and only seven for group two.

MK801

The positive and negative symptoms and cognitive impairment in schizophrenia have been suggested to be caused by glutamate hypofunction, particularly in prefrontal cortex (Marek et al., 2010; Paz et al., 2008; Tsai & Coyle, 2002). MK801 is an NMDA glutamate antagonist and therefore blocks glutamate transmission causing glutamate hypofuntion. After administration of MK801 rodents show behaviors that mimic schizophrenic symptoms like stereotypy, social withdrawal, and deficits in prepulse inhibition (Labonte, Bambico, & Gobbi, 2009). Furthermore, MK801 induces severe cognitive deficits and has been used to model the symptoms of schizophrenia in rodents (Rezvani et al., 2008). Other researchers (de Oliveira & Nakamura-Palacios, 2003; Legault, Smith, & Beninger, 2006; Nagai et al., 2007) have used different drugs (i.e. alcohol, scopolamine, and methamphetamine, respectively) to create memory impairments in radial arm maze. Yet, the effects of MK801 on the glutamate system give it the most face and construct validity as a pharmacological model of psychosis in animals (Labonte et al., 2009) and it was chosen to model the cognitive deficits of schizophrenia in this study. Ketamine, another NMDA glutamate receptor antagonist, also produced MK-801-like deficits in cognition in animals (McGinnis, 2010) .

The results of this study show that MK801 produced a reliable deficit in working and reference memory in the radial arm maze task, as seen in other studies (Levin et al., 1998; Marcus et al., 2005; Zhang et al., 2005). Yet, none of the drugs tested in the current

study reversed these MK-801-induced deficits. The dose chosen for MK801in the current study, 0.07 mg/kg, is similar to the dose used in other cognition studies using rats. For example, Levin et al. (1998) attenuated working and reference memory deficits induced by a 0.1 mg/kg dose of MK801 using nicotine. Zhang (2005) on the other hand, used inhibitors of the enzyme, type four phosphodiesterase, to enhance working and reference memory that had been impaired by MK801 (0.1mg/kg) administration.

The effects of MK801 were not identical for each group tested in present study. In group one MK801 (0.07 mg/kg) significantly increased working, reference, and total errors. MK801 significantly increase path length and velocity in group one but not test session time or percent of time spent immobile. In group two MK801 significantly increased reference and total memory errors, but not working memory errors. MK801 significantly increased the path length and session time but did not effect velocity or percent of time spent immobile. MK801 has been observed to cause significant hyperactivity in some studies (Levin et al., 1998) but did not alter exploration time in other studies (Zhang et al., 2005). The data from the present study indicates that 0.07 mg/kg of MK801 did not have an overall effect on locomotor behavior.

PD149163

PD149163 is an analog of neurotensin, a neuropeptide neurotransmitter. Neurotensin binds to receptors in the brain that are co-localized with dopamine receptors and modulates dopaminergic transmission (Binder, 2001). More specifically, neurotensin acts as an antagonist at D_2 receptors through several possible mechanisms and may be an endogenous antipsychotic (Cáceda et al., 2006). In fact, patients with schizophrenia have

low concentrations of neurotensin in their CSF and the neurotensin system has been hypothesized to be dysregulated in schizophrenia (Cáceda et al., 2006; Sharma et al., 1997). When administered to rodents, neurotensin analogs like PD149163 have shown similar effects to antipsychotic drugs in several behavioral tasks. For example, neurotensin analogs and neurotensin itself have been shown to prevent condition avoidance responding in a similar manner to other atypical antipsychotics when administered systemically and intracerebroventrically, respectively (Holly, Ebrecht, & Prus, 2010; Luttinger, Nemeroff, & Prange, 1982) In addition, PD149163 reversed a pharmacologically induced impairment in prepulse inhibition which is a measure of sensorimotor gating, a core deficit in schizophrenia (Feifel et al., 2009), as well as blocking fear-potentiated startle (Shilling & Feifel, 2008). It also has been shown to reverse scopolamine-induced deficits in a novel object discrimination task of working memory (Azmi, Norman, Spicer, & Bennett., 2006). Based on these previous studies it was hypothesized that PD149163 might improve working and reference memory in a radial arm maze task. However, in the current study PD149163 did not significantly increase or decrease working, reference, or total errors in the radial arm maze, alone or in combination with MK801. The lack of evidence of PD149163's ability to enhance cognition might be a result of using the memory disruptive drug MK801 (see discussion above).

PD149163 had no effect on path length, test session time, or velocity but did significantly increase the percent of time spent immobile during a session. Even though percent of time spent immobile was significantly increased, the overall test session time was not increased and PD149163 was concluded to not have a significant effect on

locomotor behavior in this study. In a different study, Azmi et al. (2006) reported no difference in the total amount of time spent exploring the objects between drug groups in a novel object discrimination task suggesting that there was no effect of PD149163 on baseline exploratory behavior.

When comparing behavioral effects of neurotensin analogs to other antipsychotics they appear to have a profile more similar to atypical antipsychotics than classical antipsychotics, like haloperidol. For example, Hertel et al. (2002) tested the acute effects of the neurotensin analog, NT69L (0.08, 0.16, 0.31 mg/kg s.c.) on conditioned avoidance behavior, an assay with high predictive validity, and found it to be more similar to atypical antipsychotics. Atypical antipsychotics reduce the percentages of avoidances without increasing the number of escape failures, which is predictive of antipsychotic drug efficacy. In the same study a typical antipsychotic, haloperidol (0.04, 0.08, 0.16 mg/kg, s.c.), suppressed the conditioned avoidance behavior and increase the number of escape failures both acutely and chronically over a period of three weeks, producing negative motor side effects. (Hertel, Olsen, & Arnt, 2002). NT69L, on the other hand, failed to have an effect on conditioned avoidance behavior when administered chronically (twice a day for at least 7 days), indicating that tolerance to neurotensin analogs may develop after repeat administration (Hertel et al., 2002). Yet, the study still suggests that neurotensin receptor agonists may stimulate beneficial antipsychotic-like effects without creating adverse motor effects.

Haloperidol

The typical antipsychotic, haloperidol, was chosen as a comparison drug to PD149163 in the present study. Haloperidol modulates dopaminergic transmission but in a different way than PD149163. Instead of indirectly modulating dopamine transmission, haloperidol binds directly to the dopamine D_2 receptors acting as an antagonist (Schotte et al., 1996). By blocking D_2 receptors, haloperidol decreases dopaminergic activity in the prefrontal cortex and can negatively effect cognitive skills like processing speed and procedural learning in humans (Woodward et al., 2005).

In the present study there was no main effect of haloperidol on number of errors made alone or combination with MK801 Researchers using similar radial arm maze tasks also found no effect of haloperidol alone on working memory errors (de Oliveira & Nakamura-Palacios, 2003; Levin, 1997; Nagai et al., 2007; Terry et al., 2007; Wolff & Leander, 2003). However, when given in combination with different memory disruptive drugs haloperidol did increase memory errors. For example, haloperidol (3.2 mg/kg) increased the disruptive effect of alcohol on a 1-hour delayed radial arm maze task (de Oliveira & Nakamura-Palacios, 2003). Also, haloperidol (1 and 2 mg/kg) failed to reverse a methamphetamine induced impairment in working memory in a delayed radial arm maze task (Nagai et al., 2007).

Cognitive impairment by haloperidol may be related to motor depressive effects. Haloperidol has been shown to reduce locomotor activity in rodents and produce a significant increase in catalepsy when compared to vehicle at a relatively low dose (1.0 mg/kg) (Holly et al., 2010). Wolff and Leander (2003) found that haloperidol (1.0 and 3.0

mg/kg) increased the test session time during the retention phase of a radial arm maze task. Yet, in the current study haloperidol (0.03125 and 0.0625 mg/kg) did not significantly effect path length, test session time, velocity, or percent of time spent immobile. These results are important because they demonstrate that the doses of haloperidol administered did not significantly affect locomotor behavior in any way. Therefore, secondary effects of haloperidol on locomotor behavior can be eliminated as an explanation for the memory effects observed.

Clozapine

Clozapine is also a weak antagonist of the dopamine D_2 receptor and a strong antagonist for the serotonin, 5-TH_{2A} receptor, among many other receptors. This combination of lower D_2 / higher 5-HT receptor binding affinity and ability to increase dopamine levels in the PFC is thought to be responsible for the cognitive enhancement sometimes seen after the administration of atypical antipsychotics (Kuroki et al., 1999; Woodward et al., 2005). A receptor type that clozapine activates, which other atypical antipsychotics do not, are the M_1 and M_4 receptors (Roth et al., 2004). These receptors stimulate the release of acetylcholine when activated and have been shown to improve memory in animals (Woodward et al., 2005). A review of clinical studies with schizophrenia patients revealed that clozapine improved attention and verbal fluency in humans, but failed to improve working or spatial reference memory (Meltzer & McGurk, 1999).

In the present study there was no main effect of clozapine on the number of errors made when it was administered with vehicle and with MK801. Researchers using a

similar radial arm maze tasks also found no effect of clozapine on working memory errors (Marcus et al., 2005; Nagai et al., 2007; Wolff & Leander, 2003). One study did find that acute administration of clozapine (5, 10, 20 and 40 mg/kg, i.p.) significantly increased working memory errors but this effect was diminished with chronic treatment (Ortega-Alvaro et al., 2006). In addition, at least one study reported a reversal of MK801 induced deficits in working memory (counted as number of entries to repeat) by clozapine (5mg/kg i.p.) which the current study failed to observe (Marcus et al., 2005).

Atypical antipsychotics like clozapine have been shown, at best, to mildly improve some aspects of cognition in other human studies (Kaneda et al., 2010). In a longitudinal study of schizophrenia patients, Kaneda et al. (2010) reported that clozapine improved functioning on six out of nine cognitive measures. Meltzer and McGurk (1999) have stated that atypical antipsychotics are better at improving cognitive function in schizophrenia patients than typical antipsychotics. Yet, a review by Woodward et al. (2005) concluded that no one atypical antipsychotic medication was better at improving overall cognitive function than another and that even when improvements were made they were mild. Research and development of atypical antipsychotics needs to continue in search of a more holistic therapy option that treats all aspects of the disorder and not just one type of symptom.

In the current study, clozapine had no main effect on path length, test session time, velocity or percent of time spent immobile. These results demonstrate that the doses of clozapine which were administered (0.3125 mg/kg and 1.25 mg/kg, s.c.) did not significantly affect locomotor behavior in any way. This is important to note because some studies report increasing test session time (5, 10, 20 and 40 mg/kg, i.p) and errors of

omission with high doses (50 mg/kg, p.o) of clozapine (Ortega-Alvaro et al., 2006; Wolff & Leander, 2003). Ortega-Alvaro (2006) also reported a decrease in distance travelled after acute administration of clozapine (10, 20 and 40 mg/kg i.p.) These results suggest that clozapine has a sedating effect on the motor system, which could be a result of its affinity for H_1 histamine receptors (Roth et al., 2004), but these symptoms were not observed at the low doses of clozapine used in this study.

SUMMARY AND CONCLUSIONS

Although this study did not find a significant improvement in memory following administration of PD149163 it is worth noting that PD149163 did not impair any aspects of memory either. It has been suggested that since PD149163 has a similar profile of atypical antipsychotics it might be a novel antipsychotic that has therapeutic effects without negatively effecting aspects of cognition like working and reference memory. When evaluating novel antipsychotics researchers and doctors need to consider their ability to improve cognition since cognitive impairment is a core symptom of schizophrenia (Goldman-Rakic et al., 2004; Meltzer & McGurk, 1999; Silver et al., 2003; Woodward et al., 2005). Cognitive impairments can be observed as deficits in executive functions, working memory, verbal skills, processing speed and attention (Meltzer & McGurk, 1999; Silver et al., 2003; Woodward et al., 2005). Improvements in these cognitive domains are directly related to functional outcomes like employment and social interaction skills (Kaneda et al., 2010; Meltzer & McGurk, 1999). Deficits in cognition may predict long-term outcomes of people with schizophrenia and must be addressed (Wolff & Leander, 2003). In addition, the search for a medication that will treat all symptoms of schizophrenia and rehabilitate patients must continue.

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APPENDIX A

Northern Michigan University

Continuing Education
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MEMORANDUM

March 15, 2010

TO: Dr. Adam Prus
Maureen Donegan
Department of Psychology

FROM: Cynthia A. Prosen, Ph.D.
Dean of Graduate Studies & Research

RE: **Application to use Vertebrate Animals**
Revised Application # IACUC 140
Approval Period: 03/15/2010-05/01/2011

The Institutional Animal Care and Use Committee have approved your application to use vertebrate animals in research, "Effects of PD149163 on memory".

If you have any questions, please contact me.

kjm