2012

EFFECTS OF PD149163 ON WORKING MEMORY IN A DELAYED NON-MATCH TO POSITION TASK

Jennifer L. Thornton
Northern Michigan University

EFFECTS OF PD149163 ON WORKING MEMORY IN A DELAYED NON-MATCH TO POSITION TASK

By

Jennifer L. Thornton

THESIS

Submitted to
Northern Michigan University
In partial fulfillment of the requirements
For the degree of

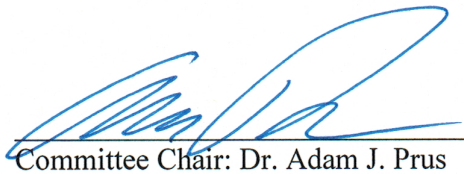
MASTER OF SCIENCE IN PSYCHOLOGY

Graduate Studies Office

2012

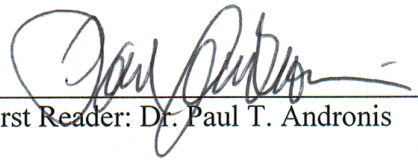
SIGNATURE APPROVAL FORM

This thesis by Jennifer L. Thornton is recommended for approval by the student's Thesis Committee and Department Head in the Department of Psychology and by the Dean of Graduate Studies.



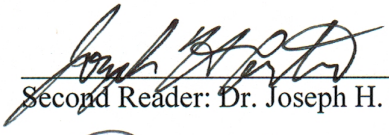
4/5/12

Committee Chair: Dr. Adam J. Prus — Date



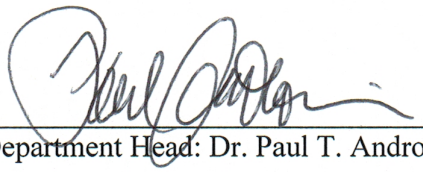
5.IV.12

First Reader: Dr. Paul T. Andronis — Date



4/2/12

Second Reader: Dr. Joseph H. Porter — Date



5.IV.12

Department Head: Dr. Paul T. Andronis — Date

Assistant Provost for Graduate Education and Research: Dr. Brian Cherry — Date

OLSON LIBRARY
NORTHERN MICHIGAN UNIVERSITY

THESIS DATA FORM

In order to catalog your thesis properly and enter a record in the OCLC international bibliographic data base, Olson Library must have the following requested information to distinguish you from others with the same name or similar names and to provide appropriate subject access for other researchers.

NAME:
Thornton, Jennifer Lynn

DATE OF BIRTH:
May 12, 1986

ABSTRACT

EFFECTS OF PD149163 ON WORKING MEMORY IN A DELAYED NON-MATCH TO POSITION TASK

By

Jennifer L. Thornton

Schizophrenia is a chronic and debilitating disorder that affects approximately 1 percent of the population. Cognitive deficits have been recognized one of the core features of schizophrenia, and have also been linked to functional outcome. Working memory is among the cognitive deficits observed in patients with schizophrenia and is thought to be one of the underlying mechanisms of other cognitive functions. Current antipsychotics mainly address positive symptoms, and do little for negative symptoms or cognitive deficits. Neurotensin is a hypotensive peptide that has been implicated as a possible antipsychotic mechanism. In preclinical trials, neurotensin agonists have been shown to have a pharmacological profile similar to atypical antipsychotic drugs. The present study assessed the effects of neurotensin-1 agonist PD149163 (0.0625-0.25 mg/kg) in a delayed non-match to position working memory task as well as typical antipsychotic haloperidol (0.025-0.20 mg/kg), atypical antipsychotic risperidone (0.125-1.0), atypical antipsychotic clozapine (0.625-5.0 mg/kg) and NMDA antagonist MK-801 (0.025-0.10). The present study revealed that both typical and atypical antipsychotics, haloperidol and risperidone respectively, impair working memory. PD149163 appears to be similar to clozapine in that it does not alter percent accuracy. The results of this study suggest that PD149163 may be similar clozapine regarding efficacy for working memory impairments.

AKNOWLEDGEMENTS

The author would like to thank Dr. Adam Prus for his copious advice and support; Dr. Paul Andronis, and Dr. Joseph Porter for their support and encouragement throughout this project.

Additional thanks are required to all of the members of the psychopharmacology lab, especially Katelin Matazel and Ashley Schmeling for their technical assistance.

Finally, the author would like to thank her family, especially her parents, Chris and Roger Thornton, as well as her fiancé, Richard Smith for their love and support.

This thesis follows the format prescribed by the *Publication manual of the American Psychological Association*. Washington, D.C.: American Psychological Association, 6th Edition.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Schizophrenia is a chronic and debilitating disorder that affects approximately 1 percent of the population. Schizophrenia consists of a wide array of symptoms that fall into three categories: positive symptoms, negative symptoms and cognitive deficits (American Psychiatric Association, 2000; Mueser & Jeste, 2008; Weinberger & Harrison, 2011).

Symptoms of Schizophrenia

Positive Symptoms. Positive symptoms include delusions, auditory hallucinations, disorganized speech, and catatonia (American Psychiatric Association, 2000). Among the positive symptoms, delusions and auditory hallucinations are most common (Weinberger & Harrison, 2011). Delusions occur as beliefs that are implausible and not derived from everyday experiences. Hallucinations describe experiences involving sensory information that is not present. Auditory hallucinations often consist of distinct voices from that of one's own thoughts (American Psychiatric Association, 2000).

Disorganized thinking has been emphasized as a diagnostic characteristic in the DSM-IV. Disorganized thinking consists of thoughts that are hard to follow, and individuals with disorganized thinking tend to jump around from idea to idea. Disorganized speech also includes speech that is incomprehensible. Disorganized behavior can be apparent in a variety of ways, but oftentimes leads to difficulties performing everyday activities (American Psychiatric Association, 2000). Catatonic

behavior is a lack of reactivity to the environment. This can also be expressed as rigidity, an inability to move on command, or random movement.

Negative Symptoms. Negative symptoms are symptoms that are absent from normal functioning (American Psychiatric Association, 2000). These symptoms include affective flattening, alogia, and avolition. One of the most common negative symptoms is affective flattening, where a person experiences and displays a diminished range of emotions and emotional responses. Alogia, which is a lack of speech, is characterized by short, concise, vacant replies, and the person seems to have a reduction of thoughts, which results in less productivity of speech as well as diminished fluency. Avolition is characterized by the inability to make and carry out goal-oriented actions.

Cognitive Deficits in Schizophrenia. Though not a diagnostic criteria in the DSM-IV, neurocognitive deficits are another array of symptoms that are central to schizophrenia (Gold, 2004; Green 1996; Green, Kern, Braff, & Mintz, 2000; Green, Kern & Heaton, 2004). Neurocognitive deficits have been reliably demonstrated in the literature. There is a broad range of neurocognitive deficits that can vary in degrees of severity. Some of the neurocognitive deficits include verbal and nonverbal memory, general intelligence, motor performance, visual and auditory attention, spatial ability, executive function, language and interhemispheric tactile-transfer (Heinrichs & Zakzanies, 1998).

Cognitive deficits seem to be stable and are not progressive (Heinrichs & Zakzanies, 1998). Reichenberg et al. (2009) found that when compared with normal controls, individuals with schizophrenia patients exhibited significant impaired neurocognitive performance on verbal ability, verbal declarative memory, visual

declarative memory, abstractive-executive function, attention and processing speed, simple motor skills and language ability. They were also significantly impaired in many of these areas compared to patients with psychotic bipolar disorder, schizoaffective disorder and psychotic depressive disorder. Furthermore, they found that 15 percent of the schizophrenic patients they studied were considered neuropsychologically normal, while 81.9 percent were neuropsychologically impaired. Other literature suggests that although there is variability in the magnitude of neurocognitive deficits, the majority of schizophrenic patients display neurocognitive impairment in memory, executive function, motor function, tactile transfer, attention, general intellectual ability and language (Reichenberg & Harvey, 2007).

Working Memory in Schizophrenia

Many studies suggest that working memory is a core feature of neurocognitive deficits in schizophrenia (Silver, Feldman, Bilker, & Gur, 2003; Goldman-Rakic, Castner, Svensson, Siever, & Williams, 2004; Barch & Ceaser, 2012). Working memory is a type of memory that holds small amounts of information, including goal-related information, during problem solving. Working memory consists of a phonological loop and a visiospacial pad that briefly store specialized auditory and visual information, respectively, in the prefrontal cortex. Working memory tasks involve a delay component where neurons in the prefrontal cortex are stimulated and the information is sustained during the delay (Durztewitz, Seamans, & Senjowski, 2000; Goldman & Rosvold, 1970; Silver et al., 2003). Because of this delay component, much of the research on working memory available uses some variation of a delayed task paradigm. In animal models, delayed-non-match-to-position tasks have been used to assess working memory in several

studies (Ballard & McAllistar, 1999; Ballard & McAllistar 2000; Gemperle, McAllister, & Olpe, 2002; Goldman & Rosvold, 1970). Because working memory is thought to be the underlying mechanism in executive function, it is vital for normal functioning.

Deficits in working memory in schizophrenic patients are linked with functional outcomes (Silver et al., 2003; Goldman-Rakic et al., 2004). One of the important aspects of functional outcome is employment (Green, 1996). Schizophrenia diagnosis is associated with the biggest decrease in employment rates among people with long-term mental health issues. Perkins and Rinaldi (1999) report that from 1990 to 1999 unemployment among people with schizophrenia increased from 88 percent to 96 percent. These factors suggest that neurocognitive deficits should be a target for treatment of schizophrenia (Gold, 2004; Green et al., 2000).

Dopamine. It is thought that dopamine in the prefrontal cortex plays a critical role in working memory function (Aalto, Brück, Laine, Någren, & Rinne, 2005; Abi-Dargham et al., 2002; Durstewitz et al., 2000; Goldman-Rakic et al., 2004; Li, Kellendonk, Simpson, Kandel, Gao, 2011; Sawaguchi & Goldman-Rakic, 1991). In monkeys, depletion of dopamine in the prefrontal cortex causes impairments in working memory (Sawaguchi & Goldman-Rakic, 1991). Wantanabe, Kodama, and Hikosaka (1997) found that during working memory tasks in nonhuman primates, there was an increase in extracellular dopamine in the dorsolateral prefrontal cortex. Studies done with nonhuman primates suggest that working memory is reliant on stimulation of D_1 receptors in the dorsolateral prefrontal cortex, which are much more abundant than D_2 receptors (Abi-Dargham et al., 2002; Durstewitz, et al. 2000; Goldman-Rakic et al., 2004). Other studies have shown that D_1 antagonists injected into the prefrontal cortex impair working

memory, and it has also been suggested that there are alterations in D_1 dopamine receptors in the prefrontal cortex of schizophrenic patients (Goldman-Rakic et al., 2004).

Dopamine pathways start in the ventral tegmental area and then go to the mesocortical and mesolimbic dopamine systems. In schizophrenia, psychotic symptoms are associated with over-activity of dopamine in the mesolimbic pathway, and cognitive and negative symptoms are associated with hypoactivity of dopamine in the mesocortical pathway (Diaz-Mataiz et al., 2005). In working memory tasks, dopaminergic neurons are activated in the midbrain and the dopamine levels in the prefrontal cortex increase during delay-task performance (Phillips, Ahn, & Floresco, 2004). Blocking dopaminergic function in the prefrontal cortex disrupts this performance. Research has indicated that in schizophrenic patients, dopamine activity is increased in the mesolimbic pathways, but that there is dopamine hypoactivity in the prefrontal cortex (Rubeša, Gudelj & Kubinska, 2011).

Glutamate. It has been suggested that there is a hypofunction of NMDA receptors in the glutamate pathway impair cognitive functioning in schizophrenia. In normal brains, NMDA receptor activation causes the release of GABA, which inhibits dopamine release from mesolimbic dopamine neurons. If the NMDA receptors become under activated there is no inhibition of dopamine release in the mesolimbic dopamine pathway, thus producing overexpression of this dopamine pathway. In the mesocortical pathway, deficient NMDA receptor activation reduces dopamine neuron activity, resulting in diminished dopamine levels in cerebral cortex. Subsequently, diminished cortical dopamine levels likely impairs working memory function (Rubeša et al., 2011).

Studies using the NMDA receptor antagonists ketamine, phencyclidine (PCP), and dizocilpine (MK-801) support this hypothesis for NMDA receptors in working memory function. This class of drugs is often used in animal models of schizophrenia because of their ability to induce effects similar to both positive and negative symptoms as well as the cognitive deficits associated with schizophrenia. Their ability to impair working memory makes them a useful model for finding treatments with the ability to reverse these deficits, but also gives some insight on the possible mechanisms involved with working memory impairment in schizophrenia (Green, 2003; Levin, Bettegowda, Weaver & Christopher, 1998; Meltzer, Horiguchi, & Massey, 2011).

Serotonin. Serotonin (5-HT_2) receptors may also play a role in cognitive function in schizophrenia. Various studies have implicated the role of 5-HT_{2A} receptors in mechanisms of actions of hallucinogens (Rubeša et al., 2011). Atypical antipsychotics also act as 5-HT_{2A} receptor antagonists, further implicating serotonin's involvement in schizophrenia (Mueser & Jeste, 2008). The 5-HT_{2A} pathway interacts with both the glutamate and dopamine pathways in the brain. Meltzer et al. (2011) postulate that that the 5-HT_{2A} blockade from atypical antipsychotics is the mechanism behind the ability of atypical antipsychotics to reverse the effects of NMDA antagonists better than typical antipsychotics. There has also been evidence that 5-HT_{2A} receptor-mediated activation may be insufficient in patients with schizophrenia. Other studies indicate that there are higher levels of platelet serotonin in individuals with schizophrenia compared to healthy individuals (Rubeša et al., 2011).

History of Schizophrenia

Emil Kraepelin conceptualized the modern concept of schizophrenia in the early 20th century. Kraepelin coined the term "dementia praecox," which was meant to emphasize that this type of dementia had an early onset, unlike other types of dementia such as Alzheimer's disease. Kraepelin defined symptoms of dementia praecox as chronic and progressive, and identified negative symptoms as the most important feature of the disorder (Shean, 2004; Weinberger & Harrison, 2011).

Eugene Blueler coined the term schizophrenia. Bleuler admired Kraepelin's work, however he did not think that schizophrenia was inevitably chronic and progressive. Like Kraepelin, Bleuler thought that negative symptoms, including fragmented thought, were the most important feature of the disorder. Thus Bleuler renamed dementia praecox as "schizophrenia;" "schiz" meaning fragmenting or splitting, and "phren" meaning mind. Schizophrenia eventually became the predominant term used to describe the disorder (Mueser & Jeste, 2008; Shean, 2004; Weinberger & Harrison, 2011).

John Hughlings-Jackson was one of the first psychiatrists to apply a characterization of "positive" and "negative" symptoms toward diagnosing patients with schizophrenia. He believed that negative symptoms represented a loss of function, and that positive symptoms represented an augmentation of normal function (Weinberger & Harrison, 2011). For example, affective flattening is defined as a stunted range of emotions, which is a loss of the normal range of emotions. Hallucinations, on the other hand, are present in schizophrenic patients, whereas in normal individuals hallucinations are not present.

During the years of modern medicine there have been changes in what symptoms characterized schizophrenia. For many years the focus was on positive symptoms, because these symptoms were easier to assess, and that is what clinicians were able to treat (Weinberger & Harrison, 2011). In recent years treatment has shifted from simply positive symptoms toward also addressing negative symptoms and cognitive deficits. Research is being geared towards new drugs that affect both positive and negative symptoms, as well as cognitive deficits. This shift has also led to incorporating cognitive function assessments in clinical drug trials (Gold, 2004).

Antipsychotic Drugs

Typical Antipsychotics. In 1952, a clinical study was conducted that resulted in a paradigm shift in the treatment of schizophrenia – the discovery of the first antipsychotic drug chlorpromazine (Delay & Deniker, 1952).. Psychiatrists found that chlorpromazine was able to reduce the psychotic symptoms associated with schizophrenia. After chlorpromazine was discovered, many other antipsychotic drugs were synthesized. This class of drugs became known as typical antipsychotics (also called classic antipsychotics or first-generation antipsychotics). Typical antipsychotics have a high affinity to block D_2 receptors (Green, 2003; Meyer & Simpson, 1997; Mailman & Murthy, 2010; Mueser & Jeste, 2008; Shean, 2004).

Typical antipsychotic drugs cause both mild and severe adverse effects. The severe side effects include a menagerie of movement problems, which appear in the first few days or first few weeks of use. They consist of extrapyramidal side effects; which are Parkinson-like symptoms that include tremor, slowed movements, rigidity, as well as

acute dystonic reactions, including akathisia, and restlessness (Meyer & Simpson, 1997; Shean, 2004).

Tardive dyskinesia is the most severe adverse effect caused by typical antipsychotic drugs. Tardive dyskinesia is characterized by involuntary movements that usually involve the mouth, face, and tongue, and occurs in about 20-30 percent of patients taking typical antipsychotics. These effects are usually seen after long-term use of typical antipsychotics and can last for years, and in some cases are irreversible (Bishnoi, Chopra, & Kulkarni, 2007). All of these movement side effects are thought to be caused by blocking dopamine D_2 receptors in the basal ganglia (Meyer & Simpson, 1997; Mailman & Murthy; 2010).

While effective for their ability to reduce positive symptoms of schizophrenia, the extrapyramidal side effects are difficult to live with and are associated with patient noncompliance (Meyer & Simpson, 1997; Mueser & Jeste, 2008; Shean, 2004). Another problem with typical antipsychotics is that they are ineffective, or only partially effective in 25-60 percent of patients (Mailman & Murthy, 2010). Typical antipsychotic drugs also do not seem to be effective in treating the negative symptoms or cognitive deficits associated with schizophrenia (Meyer & Simpson, 1997). In fact, typical antipsychotics can impair or limit improvements in cognitive functioning (Terry et al., 2008; Carli, Calcagno, Mainolfi, Mainini, & Invernizzi, 2011; Gemperle, et al. 2003). Research has found that chlorpromazine can cause impairments in learning, encoding and retrieval (Terry et al., 2008). Studies have shown that at high doses, haloperidol cannot reverse the cognitive impairments induced by NMDA antagonist 3-(R)-2-carboxypiperazin-4-propyl-

1-phosphonic (Carli et al., 2011). In fact, haloperidol, particularly at high doses, impairs most types of memory function (Gemperle et al., 2003).

Atypical Antipsychotics. The introduction of clozapine changed the pharmacology of antipsychotics. Clozapine was originally synthesized in 1958 and was structurally derived from tricyclic antidepressants (Meyer & Simpson, 1997). While laboratory studies eventually indicated it did not have a high affinity for D_2 blockade, which was a marker for typical antipsychotic drugs, it was shown to be effective in treating schizophrenia in clinical trials. Unlike typical antipsychotics, clozapine did not cause extrapyramidal side effects and also showed efficacy in the treatment of negative symptoms. Clozapine also showed a response rate of about 30-60 percent; and had a 30 percent response rate in typical antipsychotic-resistant patients, compared with 4 percent for chlorpromazine (Kane, Honigfeld, Singer, & Meltzer, 1988; Meyer & Simpson, 1997; Mueser & Jeste, 2008; Shean 2004). McEvoy et al. (2006) found that in patients who had previously not responded to atypical antipsychotic medication, clozapine was more effective than switching to another newer atypical antipsychotic such as olanzapine, quetiapine or risperidone. Clozapine was approved by the FDA in the United States in 1990 for treating treatment-resistant schizophrenic patients, nearly 40 years after its discovery due to the potential for clozapine to cause serious blood disorders such as agranulocytosis, leukopenia, neutropenia, leukocytosis, anaemia, eosinophilia, thrombocytopenia and thrombocythaemia (Herceg, Mužinić, & Jukić, 2010). After its release, patients were required to have weekly white blood cell counts to monitor for these potential side effects (Meyer & Simpson, 1997; Mailman & Murthy, 2010; Mueser & Jeste, 2008).

Clozapine was the first of a new class of drugs known as atypical antipsychotics (second-generation antipsychotics) that was pharmacologically defined as antagonizing serotonin 5-HT_2 receptors with a greater affinity than antagonizing D_2 receptors (Meyer & Simpson, 1997; Meltzer, Horiguchi, & Massey, 2011; Mueser & Jeste, 2008). It has been suggested that antagonism of 5-HT_2 receptors increases dopamine in the prefrontal cortex, potentially alleviating negative symptoms and cognitive deficits associated with schizophrenia (Mueser & Jeste, 2008). However, the effect of clozapine in treating cognitive deficits remains unclear. Some studies have shown that clozapine improves cognitive functioning in schizophrenia, while others show that it fails to effect on cognitive functioning (Rezvani et al. 2008).

Risperidone was the second atypical antipsychotic drug approved for use in the United States (Shean, 2004). Risperidone did not have the same adverse effects on white blood cells as clozapine (Meyer & Simpson, 1997). Risperidone also appeared to cause extrapyramidal side effects if the dose was higher than 8.0 mg/day, however the normal dose range is between 0.5-6.0 mg/day, with an average of 4 mg/day (Meltzer & McGurk, 1999; Meyer & Simpson, 1997; Mueser & Jeste, 2008).

In a meta-analysis conducted by Houthoofd, Morrens, & Sabbe (2008), risperidone was associated with improvements in several cognitive domains such as processing speed, attention, learning and memory, and reasoning and problem solving. However, no improvement in verbal and nonverbal working memory was found. Houthoofd et al. (2008) also found that haloperidol and risperidone had similar effects on cognition except for verbal learning and memory, reasoning and problem solving, and

verbal fluency for which results were ambivalent, and social cognition for which there were no data for comparison.

One of the major adverse effects associated with most atypical antipsychotics is significant weight gain (Allison et al, 1999). Significant weight gain is defined as an increase of seven percent from original weight (Farwell et al., 2004). Typical antipsychotics can also cause weight gain, but they do not appear to cause as much weight gain as some of the atypical antipsychotics. Drugs that have been shown to cause significant weight gain include, but are not limited to haloperidol, risperidone and clozapine. Clozapine is associated with the highest weight gain with mean increases of 4.42kg (Allison, et al., 1999). Twenty five percent of people taking atypical antipsychotics experienced significant weight gain, 40 percent of people taking olanzapine had significant weight gain and 37 percent of people taking risperidone experienced significant weight gain within the first year of treatment (Farwell et al. 2004). Obesity is associated with many health risks including metabolic syndrome, type 2 diabetes, hypertension, coronary artery disease, stroke, sleep apnea, cancers, reproductive function, osteoarthritis and liver and gall bladder disease (Kopelman, 2006).

Neurotensin

Neurotensin is a hypotensive peptide found in the central nervous system as well in the peripheral nervous system. It was isolated from bovine hypothalami by Carraway and Leeman (1973). Since then, it has been indicated as a possible new target for antipsychotic drugs (Boules, Shaw, Fredrickson, & Richelson, 2007).

In the peripheral nervous system, neurotensin acts as a paracrine and endocrine peptide in digestion and cardiovascular function, and is also believed to have effects on

growth hormones affecting normal and cancerous cells (McMahon, Boules, Warrington & Richelson, 2002). In the central nervous system, neurotensin has hypothermic effects, antinociception effects, stimulates anterior pituitary hormone secretion and modulates dopamine neurotransmission (McMahon et al., 2002; Cacéda et al., 2006).

The neurotensin peptide cannot cross the blood brain barrier, however, there have been several analogs, which have been developed that do cross the blood brain barrier and deliver neurotensin to the brain (McMahon et al., 2002; Cacéda et al., 2006). Concentration levels of neurotensin are highest in the amygdala, lateral septum, bed nucleus of the stria terminalis, substantia nigra and the ventral tegmental area (Cacéda et al., 2006).

There are three types of neurotensin receptors: NT_1, NT_2, and NT_3. Most research has focused on the first two types of receptors, and the functional response of NT_3 remains. The affinity of neurotensin for the NT_3 receptor is over 1000 fold weaker than for NT_1 and NT_2 receptors (McMahon et al., 2002). High densities of NT_1 and NT_2 receptors have been found in the substantia nigra, the entorhinal cortex, cingulate cortex and prefrontal cortex in normal postmortem human brain tissues (Lahti, Cochrane, Roberts, Conley, & Tamminga, 1998).

Neurotensin and dopamine. Research has shown that neurotensin interacts with several different neuronal systems in the brain associated with schizophrenia and antipsychotic drug effects. These include the dopaminergic, serotonergic, cholinergic and noradrenergic systems. It has been postulated that neurotensin's interaction with the dopaminergic system explains many of the antipsychotic effects of neurotensin analogs (McMahon et al., 2002). Neurotensin appears to inhibit dopamine D_2 receptor function,

through either second messenger pathways or direct receptor-receptor interactions between NT_1 and D_2 receptors (Cacéda et al., 2006). The reduction of D_2 activation resembles effects seen by typical and atypical antipsychotic drugs, which function as antagonists for D_2 receptors. Yet, neurotensin does not induce catalepsy, which is an animal model of extrapyramidal side effects related to D_2 receptor antagonism. Further, neurotensin prevents haloperidol-induced catalepsy (Cacéda et al., 2006; McMahon et al., 2002).

Research on postmortem brains of schizophrenic patients found that there are decreased neurotensin receptors in prefrontal cortex, the caudate nucleus and the cingulate cortex (Lahti et al., 1998). The neurotensin agonist NT69L has also been found to increase extracellular dopamine levels in the medial prefrontal cortex, and neurotensin administration has also been shown to increase dopamine levels in the prefrontal cortex (Prus, Huang, Li, Dai, & Meltzer, 2007). Petkova-Kirova et al. (2008) found that injections of neurotensin into prefrontal cortex produced significant, long lasting, and concentration-dependent increase in extracellular release of both dopamine and serotonin. Injections of neurotensin into the ventral tegmental area have also been shown to increase dopamine in the prefrontal cortex (Sotty et al., 2000). *In vivo* studies of neurotensin showed that neurotensin affects extracellular dopamine regulation by reducing dopamine D_2 and enhancing D_1 receptor sensitivity (Fuxe et al., 1992). Since increased dopamine in the prefrontal cortex is associated with working memory function, this suggests that neurotensin has the potential to improve working memory deficits (Sotty et al., 2000; Sawaguchi & Goldman-Rakic, 1991).

Herve et al., (1986) found that neurotensin binding is increased in the prefrontal and entorhinal cortices after treatment with antipsychotic drugs. There have also been reports that neurotensin concentration levels are lower in the cerebral spinal fluid of schizophrenics; however, after administration of antipsychotic drugs, neurotensin concentration levels are normalized (Binder, Kinkead, Owens & Nemeroff, 2001). There has been some indication that increased neurotensin concentration levels in cerebral spinal fluid are associated with improvements in negative symptoms (Sharma, Janikac, Bissette & Nemeroff, 1997).

Behavioral effects of neurotensin. In antipsychotic drug screening tests, neurotensin agonists have been shown to have a pharmacological profile similar to atypical antipsychotic drugs (Cusack, Boules, Tyler, Fauq, McCormick, & Richelson, 2000; Boules et al., 2007). Feifel, Melendez, Murray, Tina Tran, Rullan, & Shilling (2008) showed the neurotensin-1 receptor agonist PD149163 reduced amphetamine induced locomotor activity. The neurotensin agonist NT69L has been shown to reduce amphetamine and cocaine induced hyperactivity (Boules et al., 2001). Feifel, Melendez and Shilling (2004) showed that acute administration of PD149163 and clozapine were able to reverse prepulse inhibition deficits in Brattleboro rats; however, acute administration of haloperidol did not exhibit these effects, suggesting that neurotensin agonists exhibit effects similar to atypical antipsychotic drugs. The NT_1 agonist PD149163 was also able to block the prepulse inhibition deficits induced by the 5-HT_{2A} agonist and hallucinogen 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (Feifel, Melendez, & Shilling, 2003). Feifel et al. (2011) showed that clozapine and PD149163 both facilitated prepulse inhibition in Brown Norway rats,

whereas haloperidol did not. PD149163 does not produce cataleptic effects in rats, which is an animal model of extrapyramidal side effects (Holly, Ebrecht, & Prus, 2011). Holly et al. (2011) also found that PD149163 significantly decreased conditioned avoidance response.

In screening models of antipsychotic drug effects, PD149163 has been shown to produce behaviors similar to other antipsychotic drugs. Azmi, Norman, Spicer and Bennett, (2006) found that when given PD149163, rats were able to discriminate objects and were also able to reverse scopolamine-induced deficits in novel object discrimination. SR142948A (a neurotensin antagonist) blocked the reversal of scopolamine-induced deficits by PD149163. Another neurotensin antagonist, SR48692, was found to increase working memory errors in a food search task looking at spatial learning in Long-Evans rats (Tirado-Santiago, Muñoz, Rodríguez-González, & Maldonado-Vlaar, 2006). In a trace conditioning with an aversive procedure, PD149163 showed a dose related dissociation between trace and contextual conditioning, whereas SR142948A did not (Grimond-Billa, Norman, Bennett, & Cassaday, 2008). In a study looking at the effects of PD149163 on associative learning, Norman, Grimond-Billa, Bennet, & Cassaday, (2010) found that PD149163, at the 0.125 mg/kg dose, reduced associative learning. There was some overall reduction in associative learning at the 0.25mg/kg dose. PD149163 has also been shown to improve social discrimination in Brattelboro rats as well as in Long Evans rats. (Feifel, Mexal, Melendez, Liu, Goldenberg, & Shilling, 2009). Petrie et al. (2004) found that PD149163 increased Fos expression in the prefrontal cortex, suggesting potential alleviation of working memory deficits in schizophrenia.

RATIONALE

Cognitive deficits have been shown to play a critical role in functional outcome for schizophrenic patients (Green, 1996). Of these cognitive deficits it has been suggested that working memory impairments might be one of the most critical aspects of these cognitive deficits, as it is central in executive functioning and planning (Silver, et al. 2003). Current antipsychotic drugs do little to alleviate these deficits that play such an important role in the ability of schizophrenic patients to live a normal life after the onset of symptoms (Meyer & Simpson, 1997). Studies have indicated that neurotensin has possible antipsychotic efficacy for treating schizophrenia and that it also might have the ability to improve cognitive deficits, or at least not further impair them, which would be an improvement as compared to many of the current antipsychotic medications. Since the neurotensin peptide cannot cross the blood brain barrier, neurotensin receptor agonists and antagonists have been developed (Cacéda et al., 2006). One of these analogs, PD149163, is a NT_1 receptor agonist. Current research suggests PD149163 has a drug profile similar to atypical antipsychotic drugs and that it may be able to improve or not further disrupt cognitive deficits (Feifel et al., 2003; Norman et al., 2010; Norman, Becket, Spicer, Ashton, Langlois, Bennet, 2008). Working memory is often tested using delayed response tasks. An animal model of working memory that has been used in many studies is a delayed non-match to position (DNMTP) task (Ballard & McAllistar, 1999; Ballard & McAllistar 2000; and Gemperle et al., 2002). Therefore, the present study will assess the effects of the neurotensin agonist PD149163 on a DNMTP working memory

task and compare it to the typical antipsychotic haloperidol and to the atypical antipsychotics risperidone and clozapine.

METHODS

Animals

In this study, twelve male Sprague-Dawley rats (390-480g) were used. The rats were housed in a vivarium maintained on a 12-hour light/dark (light on at 06:00) schedule and constant temperature of 20-22°C. Rats were individually housed and had free access to water. Food was restricted in order to maintain the rats at 85 percent of their free-feeding body weights. All procedures were approved by the Institutional Animal Care and Use Committee at Northern Michigan University.

Apparatus

This experiment used six commercially built rat operant chambers (Med-Associates, St. Albans. VT). Chambers were made of acrylic and stainless steel and had a grid floor with a removable waste pan. The chambers were located in sound attenuating cabinets that were equipped with fans for ventilation and noise control. Each chamber contained three levers. Two of the levers were located on either side of the food tray. The third lever was located on the center of the opposite wall (the back wall). A light was located approximately 10 centimeters above the center lever and approximately 2.5 centimeters below the ceiling. Acrylic partitions were located on each side of the center lever. Each partition touched both the ceiling and the grid floor on either side of the center lever and extended outward approximately 15 centimeters. A mechanical food dispenser dispensed a 45mg dustless food pellet (Bio-Serve, Frenchtown, NJ) into the food tray.

Drugs

The drugs tested in this study were the NT_1 receptor agonist PD149163 (0.0625, 0.125, and 0.25mg/kg, sc; Gift from the NIMH Drug Repository, Bethesda, MD), the atypical antipsychotic drugs risperidone (0.125, 0.25, 0.05mg/kg, ip; Sigma-Aldrich, St. Louis, MO) and clozapine (0.625, 1.25, and 2.5mg/kg, ip; NIMH Drug Repository), the typical antipsychotic drug haloperidol (0.025, 0.05, 0.1 mg/kg, ip; Sigma-Aldrich),and the non-competitive NMDA receptor antagonist (+)-MK-801 maleate ((5S,10R)-(+)-5-Methyl-10,11-dihydro -5H-dibenzo[a,d]cyclohepten-5,10-imine maleate, 0.025, 0.05, 0.01 mg/kg, sc; Sigma-Aldrich).

Risperidone clozapine and haloperidol were dissolved in water with a few drops of 85 percent lactic acid. PD149163 and MK-801 were dissolved in 0.9 percent physiological saline. Drugs were prepared for a 1ml/kg injection volume and were injected 30 minutes prior to testing either subcutaneously (PD149163 and MK-801) or intraperitoneally (risperidone, clozapine, and haloperidol). The order of the drugs for each animal was assigned in a quasi-random order to provide a different drug testing order for each subject.

Delayed Non-Match To Position Training

The first training session was conducted to habituate animals to the operant chambers. Animals were placed in the chamber and received a food pellet on a fixed interval schedule (60s) of reinforcement. In the second training session, animals were presented with one lever and received a food pellet every time the lever was pressed. The third training session consisted of alternating the extension of either the left or right levers, where presses on either lever resulted in a food pellet when pressed.

The fourth training session was the introduction of the center lever on the back wall. First, the animals were presented with either the left or the right levers on the front wall. After the first lever was pressed, the center lever was presented. Once the center lever was pressed, animals were rewarded with a food pellet.

Errorless Training. The fifth training session was errorless training. For 84 trials animals were presented with either a right or left lever, which served as the sample lever. After the sample lever was pressed, the center lever was presented and sample lever retracted. Once the center lever was pressed, it retracted and then the opposite lever of the sample lever extended and pressing it resulted in a food pellet.

Non-Match To Position Training. After six sessions of errorless training, no delay non-match to position training was started. First, either the left or right lever was presented. Once the lever was pressed the center lever was then presented and the sample lever was retracted. Once animals pressed the center lever it retracted and both the left and right levers were immediately extended. In order to receive a food pellet the animal had to press the opposite lever of the sample lever. If a correct choice was made, levers retracted, a food pellet was given, and the next trial began after five seconds. If an incorrect choice was made the levers retracted and a "timeout" occurred. A timeout consisted of retracting both levers and deactivating the house light for 20 seconds. After the 20 seconds, the light was activated and the next trial began.

0-20 second Delayed Non-Match To Position Training. After consistent high accuracy performances (≥90% accuracy) in no delay-non-match-to-position training, a 0-20 second delay was introduced. This training was the same as the no delay non-match to position training, except that after the first lever was pressed there was a 0-20 (0, 2.5, 5,

7.5,10, 15 and 20s) second delay presented in random order. Animals were trained on this task until they achieved three consecutive sessions at the following criteria: a) at least 80 percent accuracy on the zero second delay, b) at least 50 percent accuracy on the twenty second delay, c) 40-60 percent lever preference and d) fewer than 10 percent trial omissions.

Testing Sessions

Test sessions were identical to training session, except that longer delays were used. After meeting training criteria, rats were tested with different time delay ranges. Based upon these results, randomized delays ranging between 0 – 40 seconds (0, 5, 10, 15, 20, 30, and 40s) were used.

For drug testing, each rat was tested using a repeated measures design. The animals were tested two days a week with at least 48 hours in between testing doses of the same drug, with no training sessions in between. After completing all test sessions for a particular drug, rats were given three training sessions with 0-20 second delays, as described earlier. After meeting training criteria, test sessions were conducted with a different drug.

Data Analysis

This study was a repeated measures design with two independent variables. The first independent variable was the drug and the second independent variable was the delay. There were five dependent variables, which included percent accuracy, omissions, sample latency, center lever press rate and correct choice latency. Percent accuracy was calculated by dividing the number of correct trials by the number of trials completed and then multiplying by 100. Center lever response rate was calculated by dividing the

number of center lever presses by the delay length. Sample latency was the amount of time it took the animal to press the sample lever after it was extended. Correct latency was the amount of time it took the animal to make a correct choice after both the left and right levers were extended. With the exception of analyses for trial omission, analyses were not conducted when animals omitted more than 8 trials for a given delay. If a particular animal's data had to be excluded for more than two doses per drug, that animal was excluded from the entire analysis of that drug. A two factor repeated measures analysis of variance was used to analyze each dependent variable; percent accuracy, center rate, sample latency, and correct latency. A one factor repeated measures analysis of variance was used to analyze omissions data and time delay data. Tukey HSD post hoc multiple comparisons tests were conducted when appropriate. Analyses were conducted using GB-stat (GB-Stat v10; Dynamic Microsystems, Inc., Silver Spring, MD).

RESULTS

Training

Eleven of the animals met criteria in an average of 29.27 (+/- SEM=0.0935) training sessions. One animal was removed from the study during training after failure to advance beyond errorless training after 24 sessions. Three animals were removed during the course of this study due to poor health.

Time delay

Percent Accuracy. The percent accuracy data for time delay are shown in figure 1. Time delay significantly decreased percent accuracy ($F[6, 76]=9.77$, $p<0.0001$). Tukey post hoc multiple comparisons tests revealed significant decreases in percent accuracy between 0 second delay and 20, 30 and 40 second delays.

Omissions. The omissions data for time delay can be found in table 1. There was no significant effect of time delay on number of omissions ($F[6,76]=1.68$, $p>0.05$).

Center Lever Response Rate. The center lever response rate data for time delay can be found in table 1. There was a significant effect of time delay on center lever response rate ($F[6,76]=15.90$, $p<0.0001$). Tukey post hoc multiple comparisons tests revealed significant increases in center lever response rate between 0 second delay and 5, 10, 15, 20, 30 and 40 second delays.

Sample latency. The sample latency data for time delay can be found in table 1. No significant effect of time delay on sample latency was found ($F[6,76]=1.01$, $p>0.05$).

Correct latency. The correct latency data for time delay can be found in table 1. No significant effect of time delay on correct latency ($F[6,76]=1.53$, $p>0.05$) was found.

Table 1. Mean and standard error of the mean (SEM) of time delay for a) numbers of omissions, b) center lever response rate, c) sample latency, and d) correct latency.

Delay (s)/Variable		**0**	**5**	**10**	**15**	**20**	**30**	**40**
Omissions	Mean	0.200	0.100	0.200	0.400	0.200	0.400	0.300
	SEM	0.133	0.100	0.200	0.267	0.133	0.221	0.213
Center Rate	Mean	0.581	1.006	1.063	1.013	1.020	1.014	1.036
	SEM	0.042	0.128	0.126	0.108	0.107	1.010	0.109
Sample Latency	Mean	2.222	1.876	2.027	1.851	1.863	2.044	1.887
	SEM	0.531	0.371	0.508	0.392	0.417	0.432	0.315
Correct Latency	Mean	2.299	2.068	2.14	2.269	2.193	2.087	2.065
	SEM	0.127	0.107	0.122	0.214	0.107	0.126	0.117

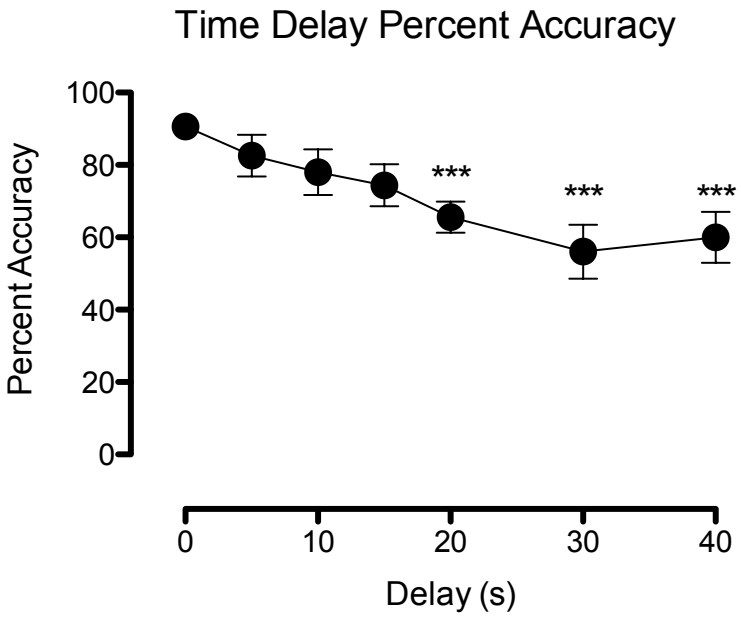


Figure 1. Effects of 0-40 second delays on percent accuracy. Percent accuracy data expressed as mean (+/- [SEM], n=11). ***p<0.001 versus 0 second delay.

PD149163

Percent accuracy. The percent accuracy data for PD149163 are shown in figure 2, top left panel. A statistically significant main effect was found across delays although differences between delays verses 0 second delay were not revealed by Tukey post hoc multiple comparisons ($F[6,24]=11.33$, $p<0.001$). PD149163 failed to alter percent accuracy ($F[3,12]=0.87$, $p>0.05$). There was a significant interaction between delay and PD149163 ($F[18,72]=1.99$, $p<0.05$), however multiple comparisons tests failed to reveal statistical differences between doses of PD149163 versus vehicle across each time delay ($F[18,72]=1.99$, $p<0.05$).

Omissions. Omissions data for PD149163 are shown in figure 2, top right panel. PD149163 had a significant effect on number of omissions ($F[3, 24]=8.71$, $p<0.001$). Post hoc Tukey multiple comparisons revealed a significant increase in number of omissions from vehicle to 0.125 mg/kg, and vehicle to 0.25 mg/kg.

Center Lever Response Rate. Center lever response rate data for PD149163 are shown in figure 2, center left panel. A significant main effect of time delay was found ($F[6,24]=24.02$, $p<0.0001$). Tukey post hoc multiple comparisons tests revealed that there was a significant increase in center lever response rate between 0 second delay and 5, 10, 15, 20, 30, and 40 second delays. There was no significant main effect of dose ($F[3,12]=0.41$, $p>0.05$) . There was no significant interaction effect found between PD149163 and time delay ($F[18,72]=0.62$, $p>0.05$)

Sample Latency. Sample Latency data for PD149163 are shown in figure 2, center right panel. There was no significant main effect of time delay ($F[6,24]=1.95$,

p>0.05.) There was no significant main effect of PD149163 ($F[3,12]=3.43$, $p=0.05$). No interaction effect between PD149163 and delay was found ($F[18, 72]=0.95$, $p>0.05$).

Correct Latency. Correct latency data for PD149163 are shown in figure 2, bottom panel. No significant main effects were found for neither drug dose ($F[3,12]=1.21$, $p>0.05$) nor time delay ($F[6,24]=0.26$, $p>0.05$). There was no significant interaction between PD149163 and time delay ($F[18,72]=1.18$, $p>0.05$).

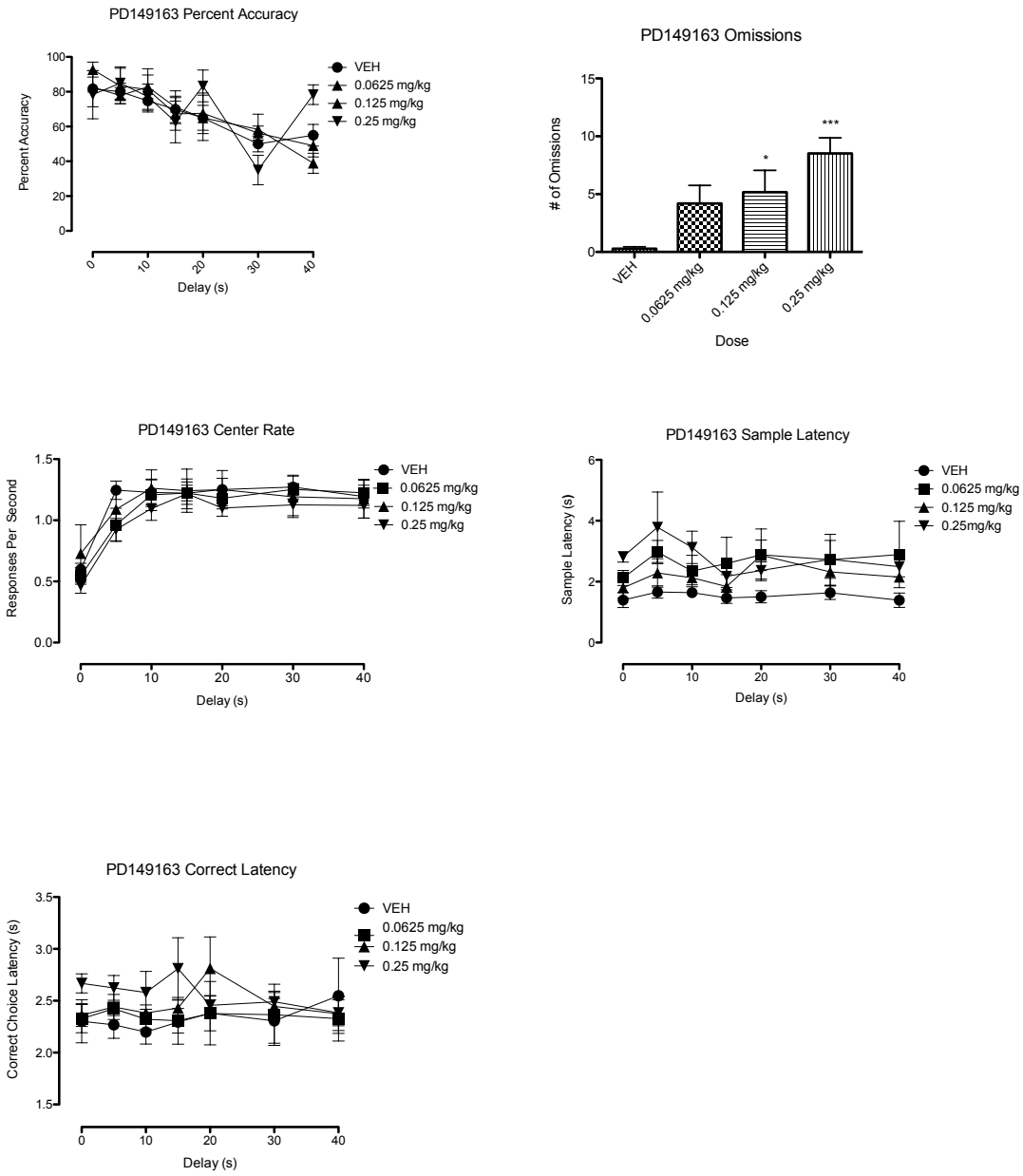


Figure 2. Effects of the neurotensin-1 receptor agonist PD149163 and time delay expressed as mean (+/- SEM) a) percent accuracy, n=5 (top left), b) number of omissions, n=9 (top right), c) center rate, n=5 (center left), d) sample latency, n=5 (center right) and e) correct choice latency, n=5 (bottom). *p<0.05, **p<0.0001 versus vehicle.

Risperidone

Percent Accuracy. Percent Accuracy data for risperidone are shown in figure 3, top left panel. Due to high numbers of trial omissions, risperidone 1.0 mg/kg was excluded from analysis, with the exception of omissions data. A significant main effect of time delay on percent accuracy was also found ($F[6,42]=10.5$, $p<0.0001$). Tukey post hoc multiple comparisons tests revealed a significant decrease in percent accuracy from 0 second delay to 40 second delay. A significant main effect of risperidone on percent accuracy was found, although Tukey post hoc multiple comparisons tests failed to reveal differences between doses and vehicle($F[3,21]=7.41$, $p<0.01$). No significant interaction effect between risperidone and time delay on percent accuracy was revealed ($F[18,126]=1.25$, $p>0.05$).

Omissions. The numbers of omissions for risperidone are shown in figure 3, top right panel. Risperidone had a significant effect on number of omissions ($F[4, 28]=22.02$, $p<0.0001$). Post hoc Tukey multiple comparisons tests revealed a significant increase of trial omissions from vehicle to 1.0 mg/kg risperidone.

Center Lever Response Rate. Center lever response rate data for risperidone are shown in figure 3, center left panel. A significant main effect of time delay on center rate ($F[6,42]=3.80$, $p<0.01$) and a main effect of risperidone on center lever response rate ($F[3, 21]=6.78$, $p<0.01$) were found, however Tukey post hoc multiple comparisons test failed to show differences between doses or time delays. No significant interaction between risperidone and time delay on center lever response rate was found ($F[18,126]=1.34$, $p>0.05$).

Sample Latency. Sample latency data for risperidone are shown in figure 3, center right panel. No main effect of time delay on sample latency was found ($F[6,42]=2.06$, $p>0.05$). A significant main effect of risperidone on sample latency was found, although Tukey post hoc multiple comparisons tests failed to show differences between doses ($F[3,21]=17.51$, $p<0.0001$). No significant interaction between risperidone and time delay on sample latency was revealed ($F[18,126)=1.33$, $p>0,05$).

Correct Latency. Correct latency data for risperidone are shown in figure 3, bottom panel. There was a significant main effect of time delay on correct latency, however Tukey post hoc multiple comparisons failed to show differences between time delays ($F[6,42]=3.20$, $p<0.05$). A significant main effect of dose on correct latency was found, however Tukey post hoc multiple comparisons tests failed to reveal differences between doses ($F[3,21]=19.52$, $p<0.0001$). A significant interaction between risperidone and time delay on correct latency was found ($F[18,126]=2.88$, $p<0.01$). Tukey multiple comparisons tests revealed increased correct latencies between vehicle and 0.50 mg/kg risperidone across all delays.

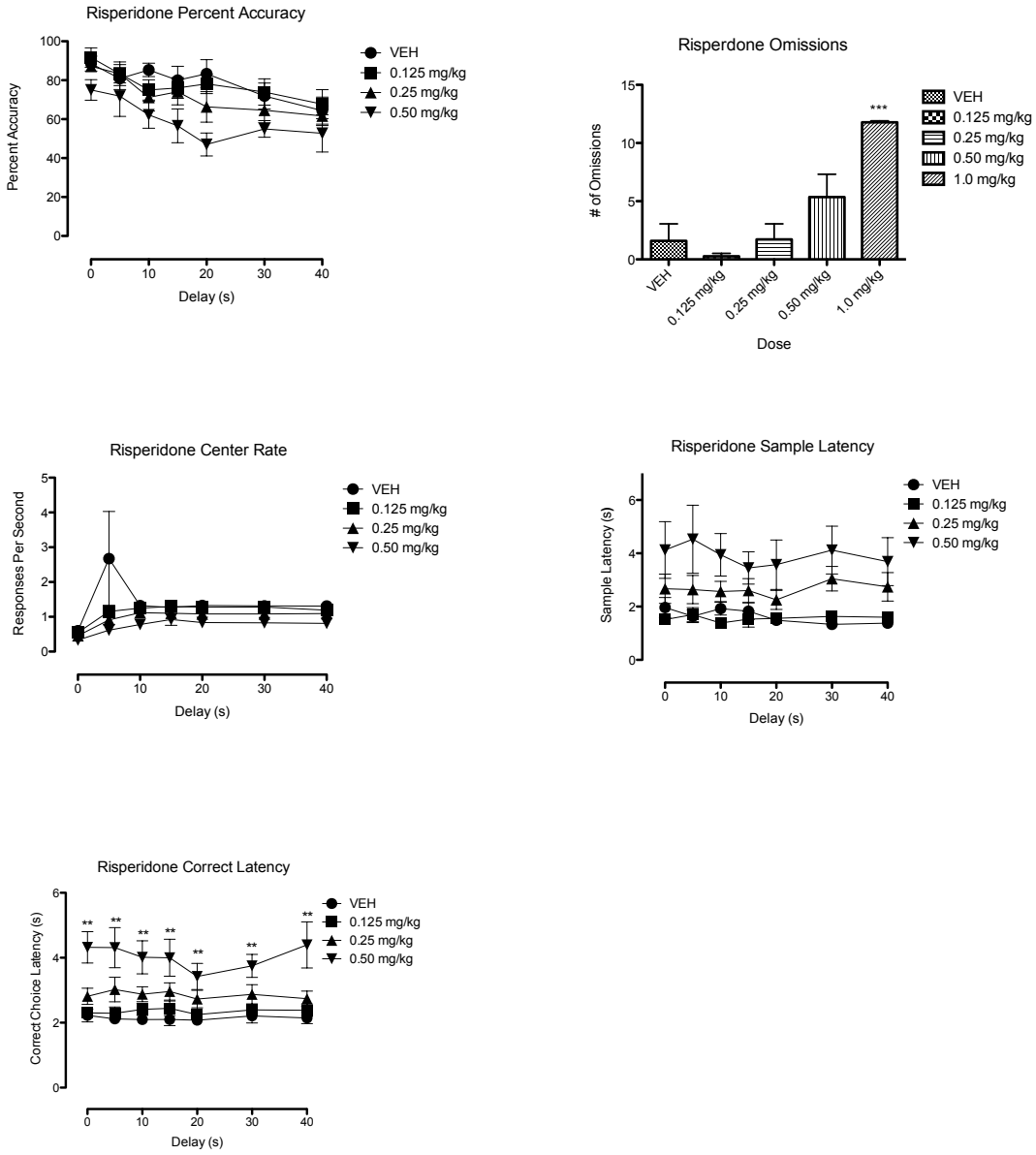


Figure 3. Effects of the atypical antipsychotic risperidone and time delay on the mean (+/- SEM) a) percent accuracy, n=8 (top left), b) number of omissions, n=8 (top right), c) center rate, n=8 (center left), d) sample latency, n=8 (center right) and e) correct choice latency, n=7 (bottom). **p<0.01 versus vehicle.

Clozapine

Percent Accuracy. Percent accuracy data for clozapine are shown in figure 4, top left panel. Due to high numbers of trial omissions, 5.0 mg/kg clozapine was not analyzed with the exception of omissions data. A significant main effect of time delay on percent accuracy was found ($F[6,60]=18.64$, $p<0.0001$). Tukey post hoc multiple comparisons showed a significant decrease in accuracy from 0 second delay to 30 and 40 second delays. No main effect of clozapine on percent accuracy was revealed ($F[3,30]=2.50$, $p>0.05$). There was no interaction between clozapine and time delay on percent accuracy ($F[18, 180)=0.98$, $p>0.05$).

Omissions. The numbers of omissions data for clozapine are shown in figure 4, top right panel. Clozapine had a significant effect on numbers of omissions ($F[4,24]=13.07$, $p<0.0001$). Tukey post hoc multiple comparisons tests revealed significant increases in number of omissions from vehicle to 5.0 mg/kg clozapine.

Center Lever Response Rate. Center lever response rate data for clozapine are shown in figure 4, center left panel. A main effect of time delay on center rate was found ($F[6,60]=32.38$, $p<0.0001$). Tukey post hoc multiple comparisons tests revealed increased center lever response rate between 0 second delay and 5, 10, 15, 20, 30 and 40 second delays. No main effect of clozapine on center lever response rate was found ($F[3,30]=2.21$, $p>0.05$). A significant interaction between clozapine and time delay on center lever response rate was found ($F[18,180]=1.84$, $p<0.05$). Tukey post hoc multiple comparisons tests revealed increased center lever response rates between vehicle and 0.625 mg/kg at 15 second delay, vehicle and 1.25 mg/kg at 30 second delay, and vehicle and 2.5 mg/kg at 30 second delay.

Sample Latency. Sample latency data for clozapine are shown in figure 4, center right panel. No main effect of time delay on sample latency was revealed ($F[6,60]=0.57$, $p>0.05$). There was no main effect of clozapine on sample latency ($F[3,30)=0.53$, $p>0.05$). No significant interaction between clozapine and time delay on sample latency was found ($F[18,180]=1.25$, $p>0.05$).

Correct Latency. Correct latency data for clozapine are shown in figure 4, bottom panel. There was no significant main effect of time delay on correct latency ($F[6,60]=1.55$, $p>0.05$). No significant main effect of clozapine on correct latency was found ($F[3,30]=0.525$,$p>0.05$). A significant interaction between clozapine and time delay on correct latency was found, although Tukey post hoc multiple comparisons failed to reveal any differences ($F[18,180]=1.83$, $p<0.05$).

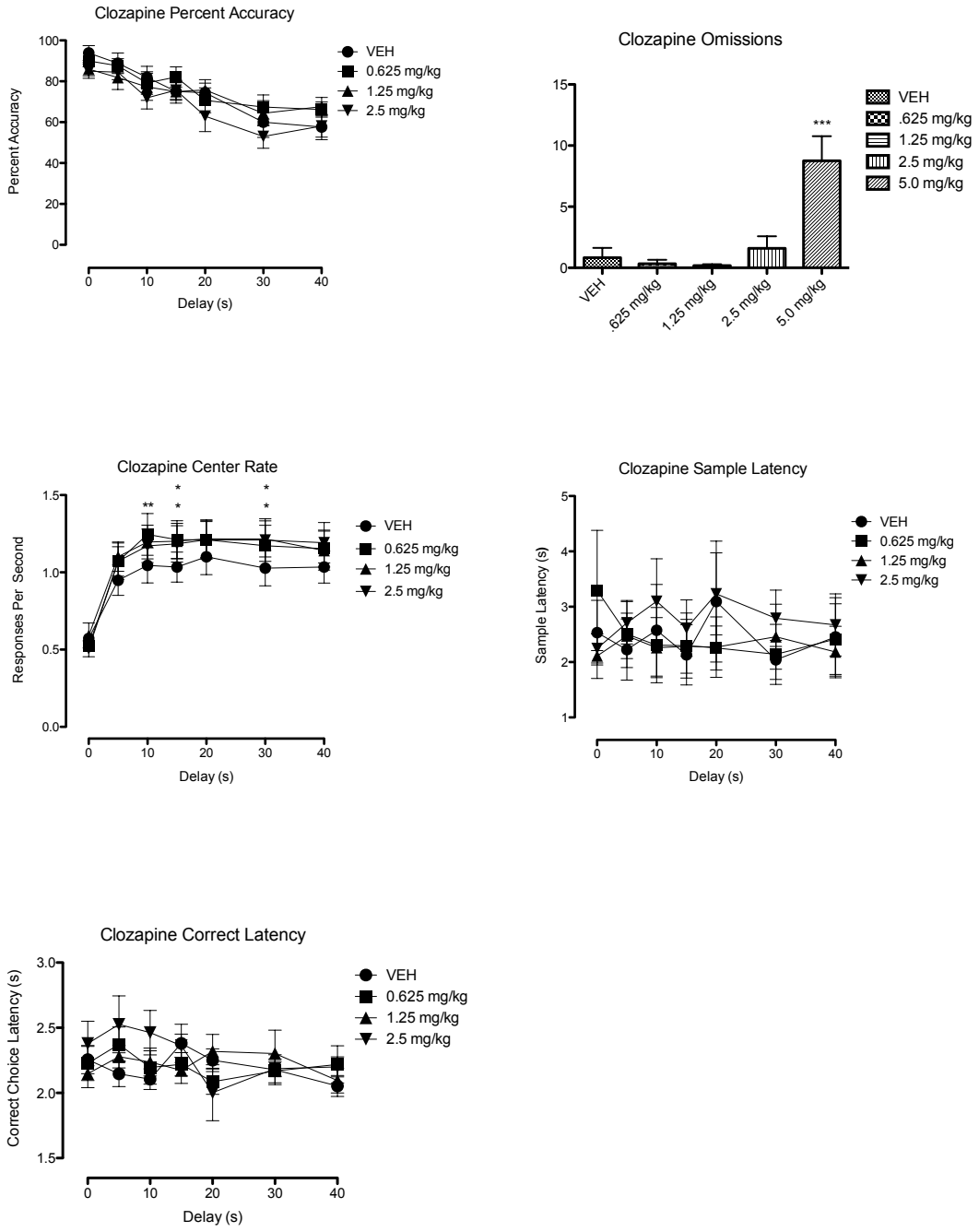


Figure 4. Effects of the atypical antipsychotic clozapine and time delay on the mean (+/- SEM) a) percent accuracy, n=10 (top left), b) number of omissions, n=7 (top right), c) center rate, n=10 (center left), d) sample latency, n=10 (center right) and e) correct choice latency, n=10 (bottom). *$p<0.05$, **$p<0.01$ versus vehicle.

Haloperidol

Percent Accuracy. The percent accuracy data for haloperidol are shown in figure 5, top left panel. Due to high numbers of omissions 0.20 mg/kg was removed from analysis with the exception of omissions data. A significant main effect of time delay on percent accuracy was found ($F[6,36]=18.76$, $p<0.0001$). Tukey post hoc multiple comparisons tests revealed a significant decrease in accuracy between 0 second delay and 30 second delay. There was a significant main effect of haloperidol on percent accuracy, although Tukey post hoc multiple comparisons tests failed to reveal differences between doses ($F[3,18]=4.83$, $p<0.05$). There was no significant interaction between haloperidol and time delay on percent accuracy ($F[18, 108]=0.70$, $p>0.05$).

Omissions. The numbers of omissions data for haloperidol are shown in figure 5, top right panel. Haloperidol had a significant effect on number of omissions ($F[4, 24]=9.16$, $p<0.0001$). Post hoc Tukey multiple comparisons revealed a significant increase in number of omissions from vehicle to 0.10 mg/kg and vehicle to 0.20 mg/kg haloperidol.

Center Lever Response Rate. Center lever response rate data for haloperidol are shown in figure 5, center left panel. There was no main effect of time delay on center rate ($F[6,36]=1.90$, $p>0.05$). No main effect of haloperidol on center lever response rate was revealed ($F[3,18]=2.55$, $p>0.05$). There was no significant interaction between haloperidol and time delay on center lever response rate ($F[18,108]=1.10$, $p>0.05$).

Sample Latency. Sample latency data for haloperidol are shown in figure 5, center right panel. No main effect of time delay on sample latency ($F[6,36]=0.65$, $p>0.05$). A main effect of haloperidol on sample latency was found, however Tukey post

hoc multiple comparisons tests failed to reveal differences between doses ($F[3,18]=4.14$, $p<0.0214$). There was no significant interaction between haloperidol and time delay on sample latency ($F[18,108]=0.87$, $p>0.05$).

Correct Latency. Correct latency data for haloperidol are shown in figure 5, bottom panel. There was no main effect of time delay on correct latency ($F[6,36]=0.92$, $p>0.05$).There was no significant main effect of haloperidol on correct latency ($F[2,18]=2.72$, $p>0.05$). No interaction of haloperidol and time delay on correct latency was found ($F[18,108]=1.17$, $p>0.05$).

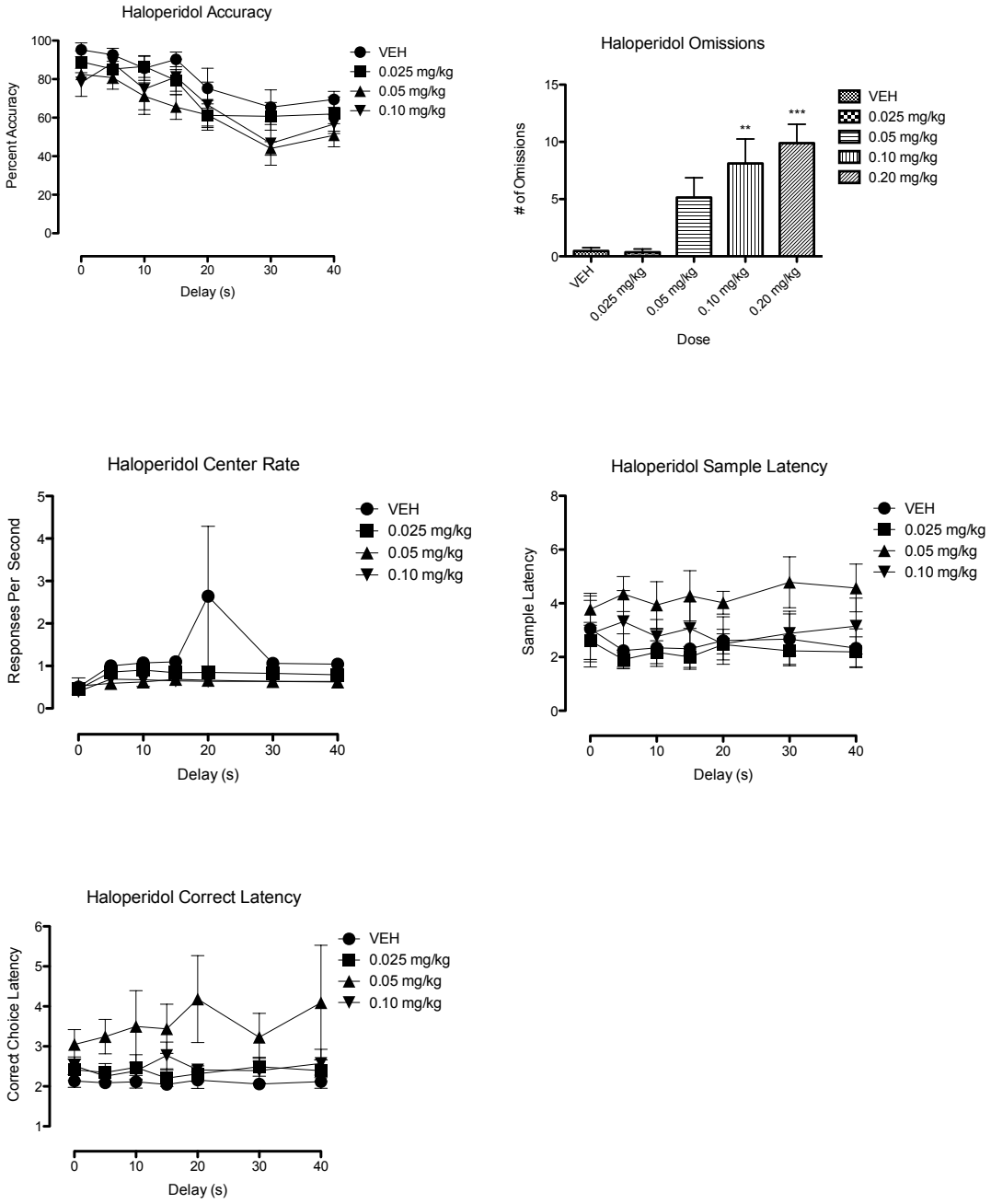


Figure 5. Effects of the typical antipsychotic haloperidol and time delay on the mean (+/- SEM) a) percent accuracy, n=7 (top left), b) number of omissions, n=7 (top right), c) center rate, n=7 (center left), d) sample latency, n=7 (center right) and e) correct choice latency, n=7 (bottom). **p<0.01 and ***p<0.001 versus vehicle.

MK-801

Percent Accuracy. Percent accuracy data for MK-801 are shown in figure 6, top left panel. Due to high numbers of omissions, data for 0.10 mg/kg MK-801 was not analyzed with the exception of omissions data. There was a significant main effect of time delay on percent accuracy, although Tukey post hoc multiple comparisons test did not find differences between delays ($F[6,24]=0.44$, $p<0.0001$). MK-801 had a significant main effect on percent accuracy, although Tukey post hoc multiple comparisons did not reveal differences between doses ($F[2,8]=18.30$, $p<0.001$). No interaction effect between MK-801 and time delay on percent accuracy was found ($F[12,48]=1.03$, $p>0.05$).

Omissions. The numbers of omissions data for MK-801 are shown in figure 6, top right panel. MK-801 had a significant effect on number of omissions ($F[3, 12]=114.6$, $p<0.0001$). Post hoc Tukey multiple comparisons tests revealed a significant increase from vehicle to 0.10 mg/kg MK-801.

Center Lever Response Rate. Center lever response rate data for MK-801 are shown in figure 6, center left panel. A main effect of time delay on center lever response rate was found ($F[6,24]=24.37$, $p<0.0001$). Tukey post hoc multiple comparisons tests revealed an increase in center lever response rates between 0 second delay and 5, 10, 20, 30 and 40 second delays. No main effect of MK-801 on center lever response rate was revealed ($F[2,8]=0.82$, $p>0.05$). There was no significant interaction between MK-801 and time delay on center lever response rate ($F[12,48]=0.47$, $p>0.05$).

Sample Latency. Sample latency data for MK-801 are shown in figure 6, center right panel. No main effect of time delay on sample latency was observed ($F[6,24]=1.50$, $p>0.05$). There was no significant main effect of MK-801 on sample latency

($F[2,8]=4.08$, $p>0.05$). An interaction effect between MK-801 and time delay on sample latency was found ($F[12,48]=2.03$, $p<0.05$). Tukey post hoc multiple comparisons tests revealed an increase in sample latency from vehicle to 0.05 mg/kg following 30 and 40 second delays.

Correct Latency. Correct latency data for MK-801 are shown in figure 6, bottom panel. There was no significant main effect of time delay on correct latency ($F[6,24]=0.61$, $p>0.05$). No significant main effect of MK-801 on correct latency was observed ($F[2,8]=1.31$, $p>0.05$). There was no significant interaction effect between MK-801 and time delay on correct latency ($F[12,48]=1.64$, $p>0.05$).

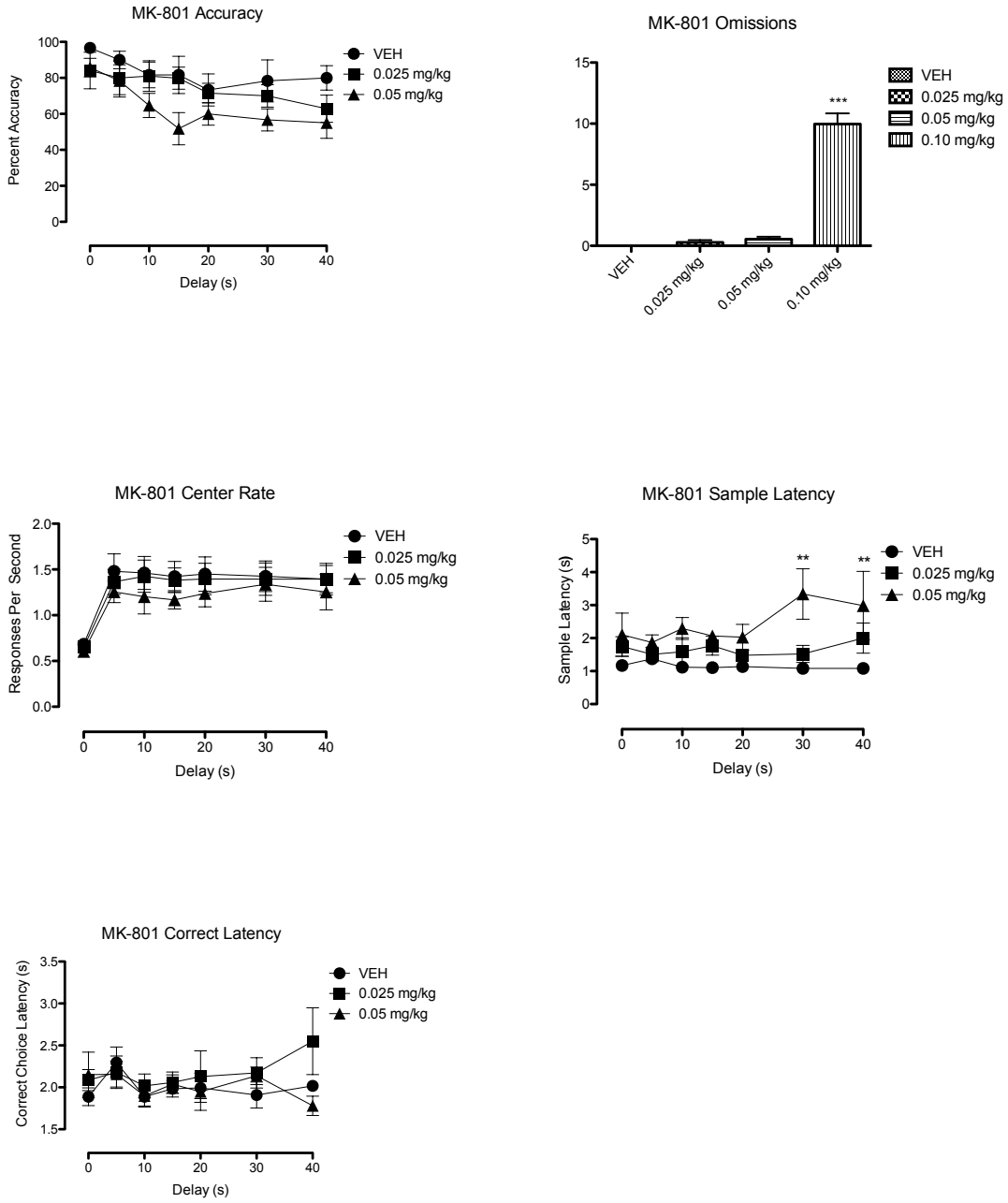


Figure 6. Effects of the NMDA receptor antagonist (+)-MK 801 maleate and time delay on the mean (+/- SEM) a) percent accuracy, n=5 (top left), b) number of omissions, n=5 (top right), c) center rate, n=5 (center left), d) sample latency, n=5 (center right) e) correct choice latency, n=5 (bottom) **p<0.01 and ***p<0.001 versus vehicle.

DISCUSSION

The present study was conducted to assess the effects of the neurotensin NT_1 receptor agonist PD149163, antipsychotic drugs risperidone, clozapine, haloperidol, and non-competitive NMDA receptor antagonist MK-801 on working memory using a delayed-non-match to position task. The present study revealed that time delay significantly decreased percent accuracy, especially at longer delays. PD149163 and time delay had a significant interaction on percent accuracy; however, there was no main effect of PD149163 on working memory. Risperidone and haloperidol both significantly decreased percent accuracy, while clozapine did not have an effect on percent accuracy. All drugs significantly increased numbers of omissions, mainly at high doses, with the exception of PD149163, which increased numbers of omissions at both medium and high doses.

Percent Accuracy

PD149163 and time delay had a significant interaction, although multiple comparisons failed to reveal where the interaction occurred. However, PD149163 did not have a significant main effect of dose on percent accuracy. Even with the exclusion of high doses due to high numbers of omissions, risperidone, haloperidol and MK-801 all significantly decreased percent accuracy, although post hoc multiple comparisons tests failed to reveal at what doses for all cases. Clozapine failed to significantly alter percent accuracy. Clozapine's lack of effect on percent accuracy is consistent with other studies that have found clozapine either produces an improvement or has no effect on working memory (Gemperle et al., 2003). These results indicate that PD149163 is most similar to

clozapine, as both do not alter percent accuracy, and suggests that PD149163 is a potential atypical antipsychotic that does not further impair working memory.

The present study revealed that both typical and atypical antipsychotics impair working memory. While clozapine did not impair working memory and does not cause extrapyramidal side effects, it does have the potential to cause serious blood disorders such as agranulocytosis, leukopenia, neutropenia, leukocytosis, anaemia, eosinophilia, thrombocytopenia and thrombocythaemia, which are potentially fatal (Herceg et al., 2010). The National Institute of Mental Health started an initiative called the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) that focuses on stimulating the development of cognition-enhancing drugs for the treatment of schizophrenia (Green et al., 2004; Marder & Fenton, 2004; Meyer & Simpson, 2007). While the focus is on cognitive enhancing drugs, the development of drugs that have no effect on cognition are a step in the right direction. While the ultimate goal may be to enhance cognition, finding drugs that do not worsen cognition would be an improvement. The results from the present study suggest that PD149163 is a possible atypical antipsychotic that does not impair working memory, and also does not appear to cause extrapyramidal side effects in preclinical models. Since the cognitive deficits associated with schizophrenia are related to functional outcome it is imperative that these deficits are addressed as treatment targets (Green, 1996; Green et al., 2000; Green et al. 2004; Marder & Fenton, 2004).

Omissions

One of the limitations of this study was the inability to test higher doses due to the high numbers of omissions. In the present study, PD149163 produced a significant main

effect on omissions at the 0.125 mg/kg and 0.25 mg/kg doses. Due to high numbers of omissions, four animals were excluded from data analysis, which was more than the number of animals excluded for risperidone, clozapine or haloperidol. A possible explanation for this could be that the doses for PD149163 were too high for this food-motivated task. It has been suggested that PD149163 decreases food intake in rats, especially at 0.25 mg/kg and higher doses and a lack of appetite could be an explanation for the high numbers of omissions since the present study relied on a food motivated task (Feifel, Goldenberg, Melendez, & Shilling, 2010; Norman, Grimond-Billa, Bennett, & Cassaday, 2010). Norman, Grimond-Billa, Bennett, & Cassaday (2010) found that in an appetitive trace conditioning procedure, PD149163 decreased unconditioned stimulus responses at 0.25 mg/kg, and inter-trial-interval responses at 0.125 mg/kg and 0.25 mg/kg. However, the neurotensin antagonist SR142948A increased unconditioned responding for food as well as inter-trial-interval responses.

It is also unlikely that these effects were due to motor side effects. In a study conducted by Holly et al. (2010), PD149163, even at doses as high as 8.0 mg/kg, did not produce catalepsy in rats. Since there was no significant increase of sample latency, center lever press rate, or correct latency, it is reasonable to conclude that omissions were due to lack of motivation rather than inability to perform the task. Future studies with PD149163 might use working memory tasks that utilize water or other non-food motivators, since there appears to be a lack of motivation to work for food. Using a non-food motivated working memory task may yield more accurate results.

Risperidone also had a significant effect on number of omissions at the 1.0 mg/kg dose. However, unlike PD149163, risperidone also significantly increased sample latency

and center lever response rates, suggesting that the reasons behind omissions may be due to different mechanisms. Risperidone (0.5 mg/kg) also produced a significant increase in correct latency suggesting that it took the animals longer to make a correct choice compared to vehicle. Marston et al. (2009) reported similar effects with risperidone in a delayed non-match to position task.

While lower doses of clozapine did not have a significant effect on number of omissions, the highest dose of 5.0 mg/kg did. Although sample latency was not affected, clozapine also significantly increased center lever response rate. Clozapine and time delay had a significant interaction on correct latency. This suggests that while clozapine does not alter working memory, it might take longer for a correct decision to be made.

Haloperidol also significantly increased the number of omissions at the 0.10 mg/kg and 0.20 mg/kg doses. Gemperle et al. (2003) found that haloperidol increased the number of omissions at higher doses. Haloperidol also significantly increased sample latency. However, haloperidol did not significantly increase center lever response rate or correct latency. These findings are similar to those reported by Gemperle et al. (2003), and suggest that the mechanisms behind omission rates are different than PD149163, clozapine or risperidone.

The NMDA non-competitive antagonist MK-801 significantly increased number of omissions at the 0.1 mg/kg dose. Due to the high number of omissions, the high dose of MK-801 (0.1 mg/kg) was excluded from the data analysis of percent accuracy, center lever response rate, sample latency, and correct latency. Despite the exclusion of the high dose of MK-801 (0.1 mg/kg), a main effect of MK-801 revealed a significant decrease in percent accuracy. There was also a significant interaction between MK-801 and time

delay on sample latency and post hoc multiple comparisons revealed that there was an increase in sample latency between vehicle and the 0.05 mg/kg dose following 30 and 40 second delays. One of the major limitations experienced during testing with MK-801 was the small sample size. Other studies using MK-801 have demonstrated working memory deficits (MacQueen, Bullard, & Galizio, 2011).

Time Delay

Time delay had a significant effect on percent accuracy, and post hoc multiple comparisons revealed significant differences between 0 second delay and the 20, 30 and 40 second delays. This is consistent with the concept that working memory temporarily holds goal-related information and involves a delay component where neurons in the prefrontal cortex are stimulated and the information is sustained during the delay. However, because the prefrontal cortex only temporarily holds the information, longer time delays cause the information to fade and percent accuracy decreases because the information necessary to complete the task is no longer available (Durztewitz et al., 2000).

Time delay had no significant effect on omissions, sample latency or correct latency. There was, however, a significant effect of time delay on center lever response rate. In this task, during the time delay the center lever remained presented for the duration of the time delay. For 0 second delay, as soon as animals pressed the center lever the choice levers were presented. During the other delays animals continually pressed the center lever until the delay was over. The longer the delay was the animals were less likely to continually pressed the lever as they were not immediately being rewarded.

Time delays in the present study were similar to those found in other studies (Ballard & McAllister, 1999; Ballard & McAllister, 2000; Germperle et al., 2003; Marston et al., 2009). Ballard and McAllister (1999) showed that there was a decrease in choice accuracy across delays. Gemperle et al. (2003) also showed drug-delay interactions with decreased choice accuracy for iloperidone and haloperidol at the highest doses; however, there was no dose-delay interaction with clozapine. Marston et al. (2009) reported dose-delay interactions that decreased choice accuracy with olanzapine and risperidone at longer delays. Talpos, McTighe, Dias, Saksida, and Bussey (2010) compared rats with a hippocampal lesion to controls in a delayed non-match to location paradigm. Longer delays decreased accuracy in both controls and lesioned rats. They also found that while rats with lesioned hippocampi were able to perform tasks, they were more susceptible to delay effects. Béracochéa and Jaffard (1995) also showed time delay had a significant effect on accuracy using a delayed non-match to position task using a t maze.

Time delay also had a significant main effect on percent accuracy across testing with PD149163, risperidone, clozapine, haloperidol and MK-801. There was no significant main effect of time delay on number of omissions across testing with any drugs. There was a significant main effect of time delay in testing sessions with PD149163, risperidone, clozapine and MK-801, but not haloperidol for center lever response rate. The results for the main effect of time delay on center rate during the clozapine testing session were identical to the results for center lever response rate for time delay with no drugs. There was no main effect of time delay on sample latency across testing sessions with PD149163, risperidone, clozapine, haloperidol or MK-801.

There was no significant main effect of time delay on correct latency during testing sessions with PD149163, clozapine, haloperidol or MK-801. There was, however, a significant main effect of time delay on correct latency during testing with risperidone, although post hoc Tukey multiple comparisons failed to reveal where the differences occurred. With a couple exceptions, overall the main effects of time delay during testing sessions was similar to the effects of time delay when no drug was given across all dependent variables.

CONCLUSION

In conclusion, results for risperidone, clozapine, haloperidol and MK-801 are consistent with findings from previous studies; while clozapine had no significant effect on working memory, risperidone, haloperidol and MK-801 all reduced accuracy (Gemperle, McAllister, & Olpe, 2003; Houthoofd, Morrens, & Sabbe, 2008; MacQueen, Bullard, & Galizio, 2011; Rezvani, 2008). PD149163 appeared to be similar to clozapine in that there was no significant main effect of dose on percent accuracy, however there was a significant interaction between dose and time delay with PD149163.

Due to the high number of omissions at the 0.125 mg/kg and 0.25 mg/kg doses, several animals had to be excluded from this study. This exclusion, along with the removal of 2 animals from the study resulted in a small sample size, which is a limitation of the present study. The doses of PD149163 may have been too high for this food motivated task, and one limitation of the present study was the inability to test higher doses due to the high number of omissions. Because of the suggested appetite suppressant effects of PD149163, using a working memory paradigm that is not a food-motivated task, such as a water-motivated task or a light/dark motivated t maze, might yield more accurate results (Feifel, Goldenberg, Melendez, & Shilling, 2010; Norman, Grimond-Billa, Bennett, & Cassaday, 2010). Future studies should consider using non-food motivated tasks while testing with neurotensin-1 agonist PD149163. This would help with the suggested appetite suppressant effects of PD149163 and allow for higher doses to be tested.

This is the first study showing the effects of a neurotensin agonist using a delayed non-match to position paradigm. The NT_1 agonist PD149163 appears to be similar to clozapine as neither of these compounds altered percent accuracy, suggesting that NT_1 agonist PD149163 may be no worse than atypical antipsychotic clozapine. There is potential for NT_1 receptor agonists to be effective antipsychotic drugs that fail to impair or limit gains in cognitive function.

REFERENCES

Aalto, S., Brück, A., Laine, M., Någren, K., & Rinne, J.O. (2005). Frontal and temporal dopamine release during working memory and attention tasks in healthy humans: a positron emission tomorgraphy study using the high-affinity dopamine D2 receptor ligand [11C]FLB 457. *Journal of Neuroscience, 25*(10)*,* 2471-2477.

Abi-Dargham, A., Mawlawi, O., Lombardo, I., Gil, R., Martinez, D., Huang, Y., Hwang, D.R., Keilp, J., Kochan, L., Van Heertum, R., Gorman, J.M., & Laruelle, M. (2002). Prefrontal dopamine D1 receptors and working memory in schizophrenia. *Journal of Neuroscience, 22*(9)*,* 3708-3719.

Allison, D.B., Mentore, J.L, Heo, M., Chandler, LP., Cappelleri, J.C., Infante,M.C., & Weiden, P.J. (1999). Antipsychotic-induced weight gain: a comprehensive research synthesis. *The American Journal of Psychiatry, 156,* 1686-1696.

American Psychiatric Association (2000). Diagnostic and statistical manual of mental disorders (4th ed., Text Revision). Washington, DC: American Psychiatric Association.

Azmia, N., Norman, C., Spicer, C.H. & Bennett, G.W. (2006). Effects of neurotensin analogue (PD149163) and antagonist (SR142948A) on the scopolamine-induced deficits in a novel object discrimination task. *Behavioural Pharmacology*, *17*, 357-362.

Barch, D.M. & Ceasar, A. (2012). Cognition in schizophrenia: core psychological and neural mechanisms. *Trends in Cognitive Sciences, 15*(6)*,* 246-253.

Ballard, T.M. & McAllister, K.H. (1999). The acetylcholinesterase inhibitor, ENA 713 (Exelon), attenuates the working memory impairment induced by scopolamine in an operant DNMTP task in rats. *Psychopharmacology*, *146*, 10-18.

Ballard, T.M. & McAllister, K.H. (2000). The NMDA antagonist EAA 494 does not impair working memory in an operant DNMTP task in rats. *Pharmacology Biochemistry and Behavior, 65*(4)*,* 725-730.

Bearden, C.E., Rosso, I.M., Hollister, J.M., Sanchez, L.E., Hadley, T., Cannon, T.D. (2000). A prospective cohort study of childhood behavioral deviance and language abnormalities as predictors of adult schizophrenia. *Schizophrenia Bulletin, 26*(2), 395-410.

Béracochéa, D.J. & Jaffard, R. (1995). The effects of mammillary body lesions on delayed matching and delayed non-matching to place tasks in the mice. *Behavioral Brain Research, 68,* 45-52.

Binder, E.B., Kinkead, B., Owens, M.J., & Nemeroff, C.B. (2001). Neurotensin and dopamine interactions. *Pharmacological Reviews, 53*(4), 453-486.

Bishnoi, M., Chopra, K., & Kulkarni, S.K. (2007). Theophylline, adenosine receptor antagonist prevents behavioral, biochemical and neurochemical changes associated with an animal model of tardive dyskinesia. *Pharmacological reports, 59,* 181-191.

Boules, M., McMahon, B., Warrington, L., Stewart, J., Jackson, J., Fauq, A., McCormick, D., & Richelson, E. (2001). Neurotensin analog selective for hypothermia over antinociception and exhibiting atypical neuroleptic-like properties. *Brain Research, 919*(1), 1-11.

Boules, M., Shaw, A., Fredrickson, P., & Richelson, E. (2007). Neurotensin agonists: potential in the treatment of schizophrenia. *CNS Drugs, 21*(1)*,* 13-23.

Cacéda, R., Kinkead, B., & Nemeroff, C.B. (2006). Neurotensin: Role in Psychiatric and neurological diseases. *Peptides, 27,* 2385–2404

Carli, M., Calcagno, E., Mainolfi, P., Mainini, E., & Invernizzi, R.W. (2011). Haloperidol and clozapine have dissociable effects in a model of attentional performance deficits induced by blockade of NMDA receptors in the mPFC. *Psychopharmacology, 196*(2)*,* 269-280.

Carraway, R., & Leeman, S.E. (1973). The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. *The Journal of Biological Chemistry, 248(*19)*,* 6854-6851.

Cusack, B., Boules, M., Tyler, B.M., Fauq, A., McCormick, D.J. & Richelson, E. (2000). Effects of novel neurotensin peptide analog given extracranially on CNS behaviors mediated by apomorphine and haloperidol. *Brain Research, 856,* 48-54

Delay, J. & Deniker, P. (1952) Trente-huit cas de psychoses traitees par la cure prolongee et continue de 4560 R. P. *Compte Rendu du Congres Alien Neurol France, 50*, 503-513.

Diaz-Mataix, L., Scorza, M.C., Bortolozzi, A., Toth, M., Celada, P., & Artigas, F. (2005). Involvement of 5-HT1A receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical antipsychotic action. *Journal of Neurosciences, 25*(47),10831-10843.

Durstewitz, D., Seamans, J.K., & Sejnowski, T.J. (2000). Dopamine-mediated stabilization of delay-period activity in a network model of prefrontal cortex. *Journal of Neurophysiology, 83*(3)*,* 1733-1750.

Farwell, W.R., Stump,T.E., Wang, J., Tafesse E., L'Italien, G., Tierney, W.M.(2004). Weight Gain and New Onset Diabetes Associated with Olanzapine and Risperidone. *Journal of General Internal Medicine, 19,* 1200–1205.

Feifel, D., Melendez, G., & Shilling, P.D. (2003). A systematically administered neurotensin agonist blocks disruption of prepulse inhibition produced by a serotonin-2A Agonist. *Neuropsychopharmacology, 28*, 651-653

Feifel, D., Melendez, G. & Shilling, P.D. (2004). Reversal of sensorimotor gating deficits in brattleboro rats by acute administration of clozapine and a neurotensin agonist, but not haloperidol: a potential predictive model for novel antipsychotic affects. *Neuropsychopharmacology, 29*(4)*,* 731-738. .

Feifel, D., Mexal, S., Melendez, G., Liu, P.Y.T., Goldenberg, J., and Shilling, P.D. (2009). The Brattleboro rat displays a natural deficit in social discrimination that is restored by clozapine and a neurotensin analogue. *Neuropsychopharmacology, 34*(8)*,* 2011-2018.

Feifel, D., Schilling, P.D., Melendez, G. (2011). Clozapine and PD149163 elevate prepulse inhibition in brown norway rats. *Behavioral Neuroscience*, *125*(2)*,* 268-272.

Fuxe, K., O'Conner, W. T., Antonelli, T., Osborne, P.G., Tanganelli, S., Agnati, L.F., & Ungersted, U. (1992). Evidence for a substrate of neuronal plasticity based on

pre-and postsynaptic neurotensin—dopamine receptor interactions in the neostriatum. *National Academy of Sciences. 89*(12), 5591-5595.

Gemperle, A.Y., McAllister, K.H., & Olpe, H.R. (2003). Differential effects of iloperidone, clozapine, and haloperidol on working memory of rats in the delayed non-matching-to-position paradigm. *Psychopharmacology, 169,* 354-364.

Green, M.F. (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *The American Journal of Psychiatry, 153*(3)*,* 321-330.

Green, M.F., Kern, R.S., Braff, D.L., & Mintz, J. (2000). Neurocognitive deficits and functional outcome in schizophrenia: are we measuring the "right stuff"?. *Schizophrenia Bulletin, 26*(1)*,* 119-136.

Green, M.F., Kern, R.S. & Heaton, R.K. (2004). Longitudinal studies of cognition and functional outcome in schizophrenia: implications for MATRICS. *Schizophrenia Research, 72*(1)*,* 41-51.

Grimond-Billa, S.K., Norman, C., Bennet, G.W., & Cassaday, H.J. (2008). Selectively increase trace conditioning under the neurotensin agonist PD149163 and an aversive procedure in which SR142948A was without intrinsic effect. *Journal of Psychopharmacology, 22*(3)*,* 290-299.

Gold, J.M. (2004). Cognitive deficits as treatment targets in schizophrenia. *Schizophrenia Research, 72*, 21-28.

Goldman, P.S. & Rosvold, H.E. (1970). Localization of function within the dorsolateral prefrontal cortex of the rhesus monkey. *Experimental Neurology, 27*(2), 291-304.

Goldman-Rakic, P.S., Castner, S.A., Svensson, T.H., Siever, L.J., & Williams, G.V. (2004). Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. *Psychopharmacology, 174*(1), 3-16.

Heinrichs, R.W. & Zakzanies, K.K. (1998). Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. *Neuropsychology, 12*(3), 426-445.

Herceg, M., Mužinić, L., & Jukić, V. (2010). Can we prevent blood dyscrasia (leucopenia, thrombocytopenia) and epileptic seizures induced by clozapine. *Psychiatria Danubina, 22*(1), 85-89.

Herve, D., Tassin, J.P., Studler, J.M., Dana, C., Kitabgi, P., Vincent, J.P., Glowinski, J., & Rostene, W. (1986). Dopaminergic control of ^{125}I-labeled neurotensin binding site density in corticolimbic structures of the rat brain. *National Academy of Sciences, 83*(16), 6203-207.

Holly, E.N., Ebrecht, B., & Prus, A.J. (2011). The neurotensin-1 receptor agonist PD149163 inhibits conditioned avoidance responding without producing catalepsy in rats. European *Neuropsychopharmacology, 21,* 526-531.

Houthoofd, S.A.M.K., Morrens, M., Sabbe, B.G.C. (2008). Cognitive and psychomotor effects of risperidone in schizophrenia and schizoaffective disorder. *Clinical Therapeutics, 30,* 1565-1589.

Kane, J., Honigfeld, G., Singer, J., Meltzer, H. (1988). Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Archives in General Psychiatry, 45*(9), 789-196.

Kopelman, P. (2007). Health risks associated with overweight and obesity. *Obesity Reviews 8,* 13–17

Kroeze, W.K., Hufeisen, S.J., Popadak, B.A., Renock, S.M., Steinberg, S., Ernsberger P., Jayathilake K., Meltzer, H.Y., & Roth, B.L. (2003). H1-Histamine Receptor Affinity Predicts Short-Term Weight Gain for Typical and Atypical Antipsychotic Drugs. *Neuropsychopharmacology, 28,* 519-526.

Kuroki, T., Meltzer, H.Y., & Ichikawa, J. (1999). Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. *Journal of Pharmacology and Experimental Therapeutics, 288*(2), 774-781.

Lahti, R. A., Cochrane, E. V., Roberts, R. C., Conley, R. R., & Tamminga, C. A. (1998). [3H]Neurotensin receptor densities in human postmortem brain tissue obtained from normal and schizophrenic persons: an autoradiographic study. *Journal of Neural Transmission, 105,* 507-516.

Levin, E.D., Bettegowda, C., Weaver, T., & Christopher, N.C. (1998). Nicotine-dizocilpine interactions and workingand reference memory performance of rats in the radial-arm maze. *Pharmacology Biochemistry and Behavior, 61*(3), 335–340.

Li, Y.C., Kellendonk, C., Simpson, E.H., Kandel, E.R., & Gao, W.J. (2011). D2 receptor overexpression in the striatum leads to a deficit in inhibitory transmission and dopamine sensitivity in mouse prefrontal cortex. *Proceedings of the National Academy of Sciences, 108*(29)*,* 12107-12112.

MacQueen, D.A., Bulard, L., & Galizio, M. (2011). Effects of dizocilpine (MK801) on olfactory span in rats. *Neurobiology of Learning and Memory, 95,* 57-63.

Mailman, R.B. & Murthy, V. (2010). Third generation antipsychotic drugs: partial agonism or receptor functional selectivity? *Current Pharmaceutical Design*, *16*(5), 488-501.

Marder, S.R., & Fenton, W. (2004). Measurement and treatment research to improve cognition in schizophrenia: NIMH MATRICS initiative to support the development of agents for improving cognition in schizophrenia. *Schizophrenia Research, 72*, 5-9. .

Marston, H.M., Young, J.W., Martin, F.D.C., Serpa, K.A., Moore, C.L., Wong, E.H.F., Gold, L., Meltzer, L.T., Azar, M.R., Geyer, M.A., & Shahid, M. (2009). Asenapine effects in animal models of psychosis and cognitive function. *Psychopharmacology, 206,* 699-714.

McEvoy, J.P., Lieberman, J.A., Stroup, T.S., Davis, S.M., Meltzer, H.Y., Rosenheck, R.A., Swartz, M.S., Perkins, D.O., Keefe, R.S.E., Davis, C.E., Severe, J., & Hsiao, J.K. (2006). Effectiveness of clozapine versus olanzapine, quetiapine, and risperidone in patients with chronic schizophrenia who did not respond to prior atypical antipsychotic treatment. *American Journal of Psychiatry, 163*, 600-610.

McMahon, B.M., Boules, M., Warrington, L., & Richelson, E. (2002). Neurotensin analogs: indications for use as potential antipsychotic compounds. *Life Sciences, 70,* 1101-1119.

Meltzer, H.Y. & McGurk, S.R. (1999). The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. *Schizophrenia Bulletin, 25,* 233-255.

Meyer, J.M. & Simpson, G.M. (1997). From chlorpromazine to olanzapine: a brief history of antipsychotics. *Psychiatric Services, 48*(9), 1137-1139

Mueser, K.M. & Jeste, D. (2008). Clinical handbook of schizophrenia. New York, NY: The Guilford Press.

Norman, C., Becket, S., Spicer, CH., Ashton, D., Langlois, X., Bennet, GW. (2008). Effects of chronic infusion of neurotensin and a neurotensin NT1 selective analogue PD149163 on amphetamine-induced hyperlocomotion. *Journal of Psychopharmacology, 22,* 300-307.

Norman, C., Grimond-Billa, S.K., Bennet, G.W., & Cassaday, H.J. (2010). A neurotensin agonist and antagonist decrease and increase activity, respectively, but do not preclude discrete cue conditioning. *Journal of Psychopharmacology, 24*(3), 373-381.

Paz, R.D., Tardito, S., Atzori, M., & Tseng, K.Y. (2008). Glutamergic dysfunction in schizophrenia: from basic neuroscience to clinical psychopharmacology. *European Neuropsychopharmacology, 28,* 773-786.

Perkins, R., & Rinaldi, M. (2002). Unemployment rates among patients with long-term mental health problems: a decade of rising unemployment. *Psychiatric Bulletin, 26,* 295-298.

Petrie, K.A., Bubser, M., Casey, C.D., Duff Davis, M., Roth, B.L., & Deutch, A.Y. (2004). The neurotensin agonist PD149163 increases fos expression in the prefrontal cortex of the rat. *Neuropsychopharmacology, 29*, 1878-1888.

Petkova-Kirova, P., Rakovska, A., Zaekova, G., Ballini, C., Corte, D.L., Radomirov, R., & Vágvölgyi, A. (2008). Stimulation by neurotensin of dopamine and 5-

hydroxytryptamine (5-HT) release from rat prefrontal cortex: possible role of NTR1 receptors in neuropsychiatric disorders. *Neurochemistry International, 53,* 355-361.

Phillips, A.G., Ahn, S., & Floresco, S.B. (2004). Magnitude of dopamine release in medial prefrontal cortex predicts accuracy of memory on a delayed response task. Journal of Neuroscience, 24, 547-553.

Prus, A.J., Huang, M., Li, Z., Dai, J., Meltzer, H.Y. (2007). The neurotensin analog NT69L enhances medial prefrontal cortical dopamine and acetylcholine efflux: potentiation of risperidone-, but not haloperidol-, induced dopamine efflux. *Brain Research, 1184,* 354-364.

Reichenberg, A. & Harvey, P.D. (2007). Neuropsychological impairments in schizophrenia: integration of performance based and brain imaging findings. *Psychological Bulletin, 133*(5), 833-858.

Reichenberg, A., Harvey, P.D., Bowie, C., Mojtabai, R., Rabinowitz, J., Heaton, R.K., & Bromet, E. (2009). Neuropsychological function and dysfunction in schizophrenia and psychotic affective disorders. *Schizophrenia Bulletin, 35*(5), 1022-1029.

Rezvani, A.H. & Levin, E.D. (2004). Nicotine-antipsychotic drug interactions and attentional performance in female rats. *European Journal of Pharmacology, 486,* 175-182.

Rezvani, A.H., Tizabi, Y., Getachew, B., Hauser, S.R., Caldwell, D.P., Hunter, C., & Levin, E.D. (2008). Chronic nicotine and dizocilpine effects on nicotinic and NMDA glutamatergic receptor regulation: interactions with clozapine actions and

attentional performance in rats. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *32*, 1030-1040

Rubeša, G., Gudelj, L, & Kubinska, N. (2011). Etiology of schizophrenia and therapeutic options. *Psychiatria Danubina, 23*(3), 308-315.

Sawaguchi, T. & Goldman-Rakic, P.S. (1991). D1 Dopamine Receptors in Prefrontal Cortex: Involvement in Working Memory. *Science, 251,* 947-950.

Schilling, P.D. & Feifel, D.(2008). The neurotensin-1 agonist PD149163 blocks fear-potentiated startle. *Pharmacology Biochemistry and Behavior, 90,* 748-752.

Sharma, R.P., Janikac, P.G., Bissette, G. & Nemeroff, C.B. (1997). CSF neurotensin concentrations and antipsychotic treatment in schizophrenia and schizoaffective disorder. *The American Journal of Psychiatry, 154*(7), 1019-1021.

Shean, G. (2004). *Understanding and treating schizophrenia: contemporary research, practice, and theory.* Binghampton, NY: The Haworth Clinical Practice Press.

Silver, H., Feldman, P., Bilker, W., & Gur, R.C. (2003). Working memory deficit as a core neuropsychological dysfunction in schizophrenia. *American Journal of Psychiatry, 160*(10), 1809-1816.

Sotty, F., Brun, P., Leonetti, M., Steinberg, R., Soubrie, P, Renaud, B., & Saud-Chagny, M.F. (2000). Ccomparative effects of neurotensin, neurotensin (8-13) and [D-Tyr11]neurotensin applied into the ventral tegmental area on extracellular dopamine in the rat prefrontal cortex and nucleus accumbens. *Neuroscience, 98*(3), 485–492.

Talpos, J.C., McTighe, S.M., Dias, R., Saksida, L.M., & Bussey, T.J. (2010). Trial-unique, delayed nonmatching-to-location (TUNL): A novel, higholy

hippocampus-dependent automated touchscreen test of location memory and pattern separation. *Neurobiology of Learning and Memory, 94*(3-2), 341-342

Tirado-Santiago, G., Lazaro-Munoz, G., Rodriguez-Gonzalez, V., & Maldonado-Vlaar, C.S. (2006). Microinfusions of neurotensin antagonist SR 48692 within the nucleus accumbens core impair spatial learning in rats. *Behavioral Neuroscience, 120*(5), 1093-1102.

Wantanabe, M., Kodama, T., & Hikosaka, K. (1997). Increase of extracellular dopamine in primate prefrontal cortex during a working memory task. *Journal of Neurophysiology, 78*(5), 2795-2798.

Weinberger, D. R. & Harrison, P.J. (2011). *Schizophrenia* (3rd ed.). West Sussex, UK: Blackwell Publishing Ltd.

APPENDIX A

IACUC APPROVAL

Northern Michigan University

Continuing Education
1401 Presque Isle Avenue
Marquette, MI 49855-5301

MEMORANDUM

November 4, 2010

TO: Dr. Adam Prus
Department of Psychology

FROM: Terrance Seethoff, Ph.D.
Dean of Graduate Studies & Research

RE: **Application to use Vertebrate Animals**
Application # IACUC 158
Approval Period: 10/11/10-10/11/2012

The Institutional Animal Care and Use Committee, has approved the application to use vertebrate animals in research, "Assessment of PD149163 on delayed non-match to position performance".

If you have any questions, please contact me.

kjm

E-mail: conteduc@nmu.edu ■ Web site: www.nmu.edu/ce

APPENDIX B

MEDPC CODE FOR TEST PROCEDURE

```
\Delayed non-match to position
\
\This is the DNMTP procedure w\ to test longer delays of 0, 10, 20, 30, 40, 50 and 60 seconds
\August 18, 2009
\Written by Adam Prus
\
\
\Skematic
\ Input (Output)
\
\     ****************************
\     *                1(1)*
\     *LIGHT (7)             *
\     *                *****
\  *****                  *
\  * 2 (4)                ** PELLET (3)
\  *                      **
\  *****                  *
\     *                *****
\     *                3(2)*
\     *                   *
\     ****************************
\
\
\
\
\R1=LEFT
\R2=CENTER
\R3=RIGHT
^LEFT=1
^CENTER=4
^RIGHT=2
^PELLET=3
^HOUSELIGHT=7
^FAN=15
```

\
\
\
\A=DELAY
\B=CORRECT TRIALS 1=CORRECT
\C=LEFT LEVER PREFERENCE CALCULATION
\D=CENTER PRESSES
\E=CENTER RATE R/SEC
\F=
\G=
\H=TIME VARIABLES FOR LATENCY
\H(1)=START TRIAL TIME (FOR LATENCY TO SAMPLE LEVER PRESS)
\H(2)=CENTER LEVER START TIME
\H(3)=NONMATCH LEVER START TIME (FOR LATENCY TO PRESSING MATCH OR NONMATCH LEVER)
\H(4)=TOTAL NUMBER OF LEFT LEVER SAMPLE TRIALS (WHERE RIGHT LEVER PRESS IS THE NONMATCH LEVER)
\H(5)=TOTAL NUMBER OF MATCH ERRORS FOR LEFT LEVER SAMPLE TRIALS (WHERE LEFT LEVER PRESS WAS THE MATCH LEVER)
\H(6)=
\H(7)=
\H(8)=CENTER LEVER PRESSES
\I=USED TO ACCESS DELAYS
\J=USED IN SUMARRAY CALCULATION FOR INCORRECT CHOICE LATENCY CALCULATION
\L=SAMPLE LEVER 1=LEFT 3=RIGHT
\M=SAMPLE LATENCY (TIME BETWEEN TRIAL START AND SAMPLE PRESS)
\N=CENTER LATENCY (TIME BETWEEN SAMPLE PRESS AND CENTER PRESS)
\O=NONMATCH LATENCY-CORRECT (TIME BETWEEN CENTER PRESS AND NONMATCH LEVER PRESS)
\P=ACCESS TO LIST X
\Q=LATENCY CALCULATIONS; DIM INTO FOUR VARIABLES
\Q(1)=MEAN FIRST PRESS LATENCY
\Q(2)=MEAN CENTER PRESS LATENCY
\Q(3)=MEAN INCORRECT CHOICE LATENCY
\Q(4)=MEAN CORRECT TRIAL LATENCY
\Q(5)=MEAN TOTAL TRIAL LATENCY
\Q(6)=DUMMY LABEL DURING SESSION
\Q(7)=PERCENT CORRECT TRIALS
\R=MATCH LATENCY-INCORRECT
\S=OMISSIONS BY TRIAL (DIM BELOW)
\U=TRIAL COUNTER
\V=
\W=
\X=LIST FOR RANDOM LEVER CHOICE
\Y=SECONDS

```
\
List X = 0,1 \1=LEFT SAMPLE 0=RIGHT SAMPLE
LIST W = 0.1",5",10",15",20",30",40" \DELAYS
VAR_ALIAS MEAN FIRST PRESS LATENCY=Q(1)
VAR_ALIAS MEAN CENTER LATENCY=Q(2)
VAR_ALIAS MEAN CORRECT CHOICE LATENCY=Q(3)
VAR_ALIAS MEAN INCORRECT CHOICE LATENCY=Q(4)
VAR_ALIAS MEAN TRIAL LATENCY=Q(5)
DIM A=84
DIM B=84
DIM D=84
DIM E=84
DIM H=10 \EXPERIMENTAL VARIABLES
DIM L=84 \SAMPLE LEVER PER TRIAL
DIM O=84  \CORRECT CHOICE LATENCY
DIM N=84 \CENTER LATENCY
DIM M=84
DIM S=84 \OMISSIONS
DIM R=84 \INCORRECT CHOICE LATENCY
DIM Q=6
\
\
\
\**********************************************************************
**********\
\
\1. SECONDS (Y) 2. TRIALS (U)  3. DELAY (I)  4.  STATUS  5. LEFT PREF
\
\**********************************************************************
**********
\
\
S.S.1
S1,
  #START:ON ^HOUSELIGHT,^FAN;SET P=0, U=0--->S2
S2, \TRIAL START
  0.1":ADD U;RANDI P=X;RANDD I=W;SET H(1)=Y, A(U)=I, L(U)=P; SHOW
2,TRIALS,U, 3,DELAY,I/100; IF P=1 [@LEFT,@RIGHT] \EITHER THE LEFT OR
RIGHT LEVER PROTRACTS
    @LEFT: ON ^LEFT--->S3
    @RIGHT: ON ^RIGHT--->S3
S3,
  #R1!#R3:OFF ^LEFT,^RIGHT;ON ^CENTER;SET M(U)=Y-H(1),H(2)=Y;SHOW
4,CENTER,Q(6)--->S4
  20":SET S(U)=1;OFF ^LEFT,^RIGHT,^HOUSELIGHT;SHOW 4,TIMEOUT,Q(6)---
>S9
```

```
S4,
  I#T:--->S5
  #R2:ADD H(8)--->SX
S5,
  #R2:ADD H(8);SET D(U)=H(8),E(U)=H(8)/(Y-H(2)),H(8)=0;SET H(3)=Y;ON
^LEFT,^RIGHT;OFF ^CENTER;SHOW 4,CHOICE,Q(6)--->S6
  20":SET S(U)=1;OFF ^CENTER,^HOUSELIGHT;SHOW 4,TIMEOUT,Q(6)--->S9
S6,
  #R1:IF P=1 [@MATCH,@NONMATCH]
     @MATCH:OFF ^LEFT,^RIGHT,^HOUSELIGHT;SET R(U)=Y-H(3);ADD
H(4),H(5);SHOW 4, TIMEOUT, Q(6)--->S9 \GO TO TIMEOUT AND ADD
VARIABLE FOR LEFT LEVER PREFERENCE CALCULATION
     @NONMATCH:ON ^PELLET; SET B(U)=1,O(U)=Y-H(3); SHOW 4, CORRECT,
Q(6)--->S7  \CORRECT CHOICE
  #R3:IF P=0 [@MATCH,@NONMATCH]
     @MATCH:OFF ^LEFT,^RIGHT,^HOUSELIGHT;SET R(U)=Y-H(3);SHOW 4,
TIMEOUT,Q(6)--->S9 \GO TO TIMEOUT
     @NONMATCH:ON ^PELLET; SET B(U)=1,O(U)=Y-H(3);ADD H(4);SHOW 4,
CORRECT,Q(6)--->S7     \CORRECT CHOICE
  20":SET S(U)=1;OFF ^LEFT,^RIGHT,^HOUSELIGHT;SHOW 4, TIMEOUT,
Q(6);IF U>=84 [@STOP, @KEEPGOING]
     @STOP:SET  C=H(5)/H(4)*100;SHOW 5,LEFT_PREF,C, 6, RIGHT_ERROR
TRIALS, H(5), 7, RIGHT_TRIALS, H(4)--->STOPABORTFLUSH
     @KEEPGOING:--->S9
S7,
  0.5":OFF ^PELLET,^LEFT,^RIGHT; IF U>=84 [@STOP, @KEEPGOING]
     @STOP:SET  C=H(5)/H(4)*100;SHOW 5,LEFT_PREF,C, 6, RIGHT_ERROR
TRIALS, H(5), 7, RIGHT_TRIALS, H(4)--->STOPABORTFLUSH
     @KEEPGOING:--->S8
S8,
   5":--->S2
S9,
   20":ON ^HOUSELIGHT;SET P=0;SHOW 3,SAMPLE,Q(6);IF U>=84 [@STOP,
@KEEPGOING]
     @STOP:SET  C=H(5)/H(4)*100;SHOW 5,LEFT_PREF,C, 6, RIGHT_ERROR
TRIALS, H(5), 7, RIGHT_TRIALS, H(4)--->STOPABORTFLUSH
     @KEEPGOING:--->S2

S.S.2
S1,
   #START:--->S2
S2,
   0.1":SET Y=Y+0.1; SHOW 1, SECONDS, Y--->SX
```