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DISCRIMINATIVE STIMULUS PROPERTIES OF PD149163

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DISCRIMINATIVE STIMULUS PROPERTIES OF PD149163

By

Candice J. Schuck

THESIS

Submitted to
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ABSTRACT

DISCRIMINATIVE STIMULUS PROPERTIES OF PD149163

By

Candice J. Schuck

Schizophrenia is a life-long debilitating disease that currently affects approximately 1% of the world population, including 3.2 million Americans. Currently available pharmacotherapeutics are mainly effective for the positive symptoms, but are minimally effective for negative symptoms and cognitive impairment. A promising new class of drugs currently in the experimental phase of development is called neurotensin analogs and are thought to produce effects similar to antipsychotic drugs, but the precise mechanisms that lead to the behavioral effect of neurotensin in the brain is unknown. The current study used a behavioral procedure called drug discrimination in rats in an attempt to elucidate the mechanisms that mediate the potential therapeutic effects of neurotensin analogs. The discriminative stimulus properties of PD149163 were not robust, and after several changes in training dose, and change to administration route, 6 out of 10 rats met training criteria to discrimination of 0.0625mg/kg PD149163 dose from vehicle. Full stimulus generalization occurred to a 0.0312mg/kg and 0.125mg/kg dose of PD149163. Full stimulus generalization occurred to the D₂ receptor-preferring antagonist haloperidol, at the 0.1mg/kg dose. These findings provide evidence that the stimulus effects produced through NT₁ receptor agonism may be similar to D₂ receptor antagonism.

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Candice J. Schuck

2010

This work is dedicated to my children, Cassandra, Wyatt, John, and Madeline,
whom I love so very much.

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I would like to thank my family for being there with me through thick and thin through the last two grueling years. I don't know what I would have done without all your love to give me something to strive for and pull me through.

Thanks to my God, for hearing all my prayers!

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LIST OF ABBREVIATIONS

NT: Neurotensin

NTR: Neurotensin receptor (NT₁, NT₂, NT₃)

APD: Antipsychotic Drug

DA: Dopamine

D₂: Dopamine D2 receptor

EPS: Extrapiramidal Side Effects

5HT: Serotonin

5HT_{2A}: Serotonin 2A receptor

VTA: Ventral Tegmental Area

SN: Substantia Nigra

PPI: Pre-pulse Inhibition

DD: Drug Discrimination

DS: Discriminative Stimulus

INTRODUCTION

Schizophrenia

Schizophrenia is a life-long, debilitating disease of the brain that currently affects over 1% of the world population including more than 3.2 million Americans. The disease is characterized by positive and negative symptoms and cognitive impairments. Positive symptoms consist of unusual thoughts or perceptions, including hallucinations, delusions and severe thought disorganization, and disrupted motor movement. Negative symptoms represent a reduction in normal emotional and behavioral states and include flat affect, anhedonia, social withdrawal, and impoverished speech. Cognitive impairment may consist of disrupted working memory, attention deficits, and poor executive function.

There are several different forms of schizophrenia, each differing in the type and level of severity of symptoms. The DSM-IV defines schizophrenia as a disorder characterized by a deteriorating ability to function in everyday life and by some combination of hallucinations, delusions, thought disorder, movement disorder and inappropriate emotional expressions (American Psychiatric Association, 1994). Cognitive impairment is not included in the DSM-IV, but according to Keefe and Fenton (2007), patients with schizophrenia score 1.5-2.0 standard deviations below healthy controls on neurocognitive tasks involving memory, working memory, attention, problem solving, cognitive processing time and social cognition.

There are many speculations as to the etiology of the schizophrenia. These include, but are not limited to, multiple convergent factors such as gene expression, viruses, toxins, nutrition, birth insult and psychological experience involved in the

development of the brain from the time of conception to early adulthood (Andreasen, 1999). Studies suggest both genetic and environmental influences, and a large body of evidence (Meltzer, and Stahl, 1976; Pycock, Kerwin, & Carter, 1980; Seeman, 1988; Carlsson, Waters, Holm-Waters, Tedroff, Nilsson & Carlsson, 2001; Hirvonen, Van Erp, Huttunen, Aalto, Nagren, Huttunen, Lonnqvist, Hietala, & Cannon, 2005; Guillin & Laruelle, 2005) suggests that there is an alteration in a number of different neurotransmitter systems (e.g. dopamine, GABA, glutamate, serotonin) but the exact cause of schizophrenia remains elusive.

History of schizophrenia

In 1887 German physician Emil Kraepelin first named this disease dementia praecox related to the rapid cognitive degeneration that was seen beginning in late teen and early adulthood years. Cognitive degeneration impairs memory, attention, and the ability to organize behavior appropriately. In 1911, Swiss psychiatrist Eugene Bleuler renamed this same mental illness schizophrenia, meaning in Greek SCHIZO (split) and PHRENE (mind). He categorized the symptoms associated with the disease and coined the terms positive and negative when describing them. Throughout the late 19th and early 20th century, treatments for people suffering from schizophrenia focused mainly on calming their abnormal behaviors and episodes of psychosis. Everything from strait jackets and solitary confinement in padded rooms to ice baths, induced fever, and insulin or electric shock were used. Additionally, a neurosurgical procedure that severed the neuronal connections between the frontal lobes and the rest of the brain began to be used in the late 19th century.

In 1888, Gottlieb Burckhardt performed the first psychosurgery of the brain and presented a paper on his work in 1889 at the Berlin Medical Conference. His work and writings met criticism from his medical colleagues and eventually led Burckhardt to discontinue his investigation of cortical lesioning.. Throughout the years to follow, Burckhardt's psychosurgery continued to be investigated and his ideas were revitalized by 1910, developing into a neurosurgical specialty (Manjila, Rengachary, Xavier, Parker, & Guthikonda, 2008). By 1935 ideas that originated with Burckhardt had become a neurosurgical procedure called the lobotomy. A lobotomy consisted of severing the neural connections between the frontal lobes and the rest of the brain. Physician Walter Freeman refined the lobotomy procedure and in 1946 began performing transorbital lobotomies in the United States. The transorbital lobotomy involved utilizing a piece of equipment known as a leucotome, which resembled an ice pick, to access the brain via the eye socket of the patient. The procedure allowed for lobotomies to be performed outside of a surgical suite, therefore making it affordable to those patients he saw as needing it the most but who could least afford it. The purpose of a lobotomy was to calm psychosis, but all too frequently patients were left completely incapacitated. Eventually the risks involved proved to outweigh the benefits and by the early 1970's the overall use of the lobotomy procedure had all but ceased. The struggle to improve the lives of patients with schizophrenia and other mental disorders was ongoing and the search for more humane and effective measures to treat this disease continued.

Antipsychotic drugs

The year 1950 brought about the accidental discovery of the first antipsychotic drug (APD) chlorpromazine. Chlorpromazine is a phenothiazine and was initially used as an adjunct treatment with anesthetics, to reduce the amount of anesthesia needed prior to surgical procedures. The drug produced a state of calm and conscious sedation and eventually was utilized by Henri Lashborit and psychiatric researchers in France on patients with schizophrenia, effectively alleviating symptoms of psychosis in those patients it was administered to. Chlorpromazine began to be used as an APD and led to a significant decrease in the amount of patients needing to be permanently hospitalized. Between the late 1950's and early 1980's the more than 500,000 patients in psychiatric facilities was reduced to less than 220,000.

The discovery of the beneficial use of chlorpromazine as an APD ignited the search for additional APDs for the treatment of schizophrenia. The butyrophenones were the next class of APDs developed. These included drugs such as haloperidol, perphenazine and fluphenazine, which are considered first generation or typical APDs. Typical APD's effectively alleviate positive symptoms in schizophrenia via blockade of dopamine D₂ receptors in the brain. Through blockade of the D₂ receptors in the nigrostriatal dopamine pathway in the brain, typical APD's also produce undesirable side effects known as extrapyramidal side effects (EPS). EPS resemble symptoms of Parkinson's disease, including muscle rigidity, involuntary motor movements and tremors. The clinical doses of APDs are directly related to D₂ receptor occupancy and it is noted that a minimum of 50% receptor occupancy is required for clinical response to APDs. EPS is produced when receptor occupancy reaches 80% or above

in the nigrostriatal pathway (Kapur, Zipursky, Jones, Remington & Houle, 2000; Meltzer, 2007). Additionally, long term use of typical APDs may result in a more serious disorder known as tardive dyskinesia which can persist even after discontinuation of the medication. Despite the side effects, these medications provided relief and were used consistently until the early 1990's at which time a new class of APDs began to be developed. These new drugs were labeled second generation or atypical APDs.

Atypical APDs rarely produce EPS and are not only effective in treating the positive symptoms associated with the schizophrenia, but also reduce the negative symptoms as well. The behavioral effects of atypical APDs may be produced via blockade of dopamine D₂ receptors and serotonin (5HT_{2A}) receptors in the brain. The negative symptoms of schizophrenia are thought to result from an imbalance of these neurochemicals in the mesocortical and mesolimbic dopaminergic pathways in the brain. It is believed that there is a hyperfunction of dopamine in the mesolimbic pathway and a hypofunction of dopamine in the mesocortical pathway. These pathways originate in the ventral tegmental area and project through the limbic system and prefrontal cortex (Guillin, & Laruelle, 2005). According to a hypothesis put forth by Meltzer, Matsubara, and Lee (1989), a 3 fold higher affinity for serotonin (5HT_{2A}) over dopamine (D₂) receptors may be indicative of the absence of EPS as well as improved clinical response, such as effectiveness in treating both positive and negative symptoms of schizophrenia. Another hypothesis is that atypical APDs are thought to dissociate much quicker from the D₂ receptor than typical APDs. This is known as the "fast off" effect, which allows for binding of the drug to the D₂ receptor

for just long enough to be effective for positive and negative symptoms but not long enough to cause EPS. (See Table 1).

Table 1. Receptor binding affinities (expressed a Ki, nM) for PD149163, neurotensin, and selected antipsychotic drugs

DRUG	NT1	NT2	D2	5-HT2A
PD149163	159	>10,000	>10,000	>10,000
Haloperidol	n/a	n/a	1.4	25
Clozapine	n/a	n/a	150	3.3
Neurotensin	0.25	7	n/a	n/a

Ki values (nM)

(PD149163 data are from Petrie et al. 2004; Haloperidol and Clozapine data are from Schotte et al. 1996; Neurotensin data are from Pettibone et al. 2001.)

The first atypical APD was clozapine. For a number of years clozapine was not considered an APD due to its lack of production of EPS-like effects in preclinical animal models. The production of EPS-like symptoms during trials with animal models was thought to be a key indicator of a drug's ability to produce APD effects. Clozapine did not display EPS but it was effective in treating not only the positive symptoms of schizophrenia, but negative symptoms as well. An estimated 20-60% of patients with schizophrenia are treatment resistant (Meltzer, & Kostacoglu, 2001; Miller, McEvoy, & Jeste 2006) and although 40-60% of the treatment resistant patients are also resistant to clozapine, it is considered the gold standard drug for use in patients with treatment resistant schizophrenia. Although clozapine was highly effective in treating schizophrenia it had a very dangerous side effect called agranulocytosis. Agranulocytosis involves the loss of the white blood cells that fight

off infection and can be deadly if the patient's blood count is not monitored very closely. In 1976, 17 of approximately 3,000 patients in Finland suffered from agranulocytosis after taking clozapine, 8 of whom had fatal reaction. This led to a full investigation and brief halt to the use of clozapine. With the reinstatement of clozapine as an APD came the requirement of weekly blood count monitoring in effort to prevent this devastating side effect (Idanpaan-Heikkila, Alhava, Olkinuora, & Palva, 1977).

Over the last two decades other atypical APDs have been developed, such as risperidone, olanzapine, quetiapine, sertindole and ziprasidone. These drugs are thought to be equally as effective as clozapine and rarely produce EPS or agranulocytosis, but may cause metabolic disturbances associated with diabetes mellitus and hypercholesterolemia. They may also produce a cardiac abnormality called QT interval prolongation, where the length of time in the heartbeat is longer. Generally this is not a severe complication unless it progresses into a condition called *torsades de pointes* which causes sudden cardiac arrest ultimately causing death.

A large clinical trial study entitled CATIE (Clinical Antipsychotic Trials of Intervention Effectiveness) funded by the National Institute of Mental Health compared and contrasted both efficacy and side effects of 1 typical (perphenazine) and 4 atypical (olanzapine, quetiapine, risperidone and ziprasidone) APDs (Lieberman, Stroup, McEvoy, Swartz, Rosenheck, Perkins, Keefe, Davis, Davis, Lebowitz, Severe, & Hsiao, 2005). A total of 1493 patients suffering from schizophrenia, from 57 different clinical locations in 24 states, participated in the CATIE study. The sampling of patients included participants of academic and mental

health treatment facilities and were representative of the population being investigated. The objective of the study was to investigate ways to maximize the benefits and minimize the side effects in use of APDs.

A major issue with APD use is non-compliance by the patient. Patients generally will not comply with medication regime related to the severity of side effects and/or lack of significant improvement of symptoms. Poor medication compliance leads to poor control of symptoms and relapse. Throughout the course of the CATIE study, over 74% of the participants were switched to a different medication than the one they were started on, related to intolerable side effects. Those patients who were started on olanzapine most consistently followed the medication regime and had less frequent instances involving hospitalization but were subject to metabolic side effects that were not as severe with the other APDs tested. Overall, the older typical APD perphenazine was just as effective as the new atypicals and all of the atypicals were comparable to one another (Lieberman, et al., 2005; Keefe, Palmer, Capuano, Rosenheck, & Lieberman, 2007). Considering cost, effectiveness and tolerability, this study provided comprehensive information in regards to the comparison of different APDs that are currently on the market for schizophrenia, giving patients and doctor's information needed to make appropriate decisions regarding the use of APDs.

The main goal in developing APDs is to provide therapeutics that improve the patients overall ability to function (i.e. physically, emotionally and cognitively) on a daily basis. For the most part, APDs currently on the market are primarily effective for positive symptoms and have less of an effect for the negative symptoms and

cognitive impairment. Thus, there continues to be a need for APDs that not only improve positive and negative symptoms, but cognitive impairments as well.

Dopamine

Dopamine (DA) is a catecholamine neurotransmitter which may mediate the efficacy of all APDs. There are currently 5 known DA receptors. They are divided into two families, the D₁ receptor family (D₁ and D₅) and the D₂ receptor family (D₂, D₃ and D₄). All DA receptors are G-protein coupled receptors that are located both on DA neurons (autoreceptors, D₂ and D₃) and on postsynaptic cells, including those which are cholinergic, GABA-ergic, glutamatergic, peptidergic and serotonergic (all subtypes, D₁-D₅). 80-90% of DA neurons are located in the mid brain and project throughout the brain along the nigrostriatal, mesolimbic and mesocortical pathways (see Binder, Kinkead, Owens, & Nemeroff, 2001, for review). The DA midbrain nuclei consist of the substantia nigra (SN), ventral tegmental area (VTA) and the retrorubral field. The nigrostriatal dopaminergic pathway begins in the SN and projects to the neostriatum (caudate nucleus and putamen). This area is involved in motor function. The mesolimbic dopaminergic pathway begins in the VTA and projects to several areas of the limbic system which is involved in reward and integration of time and space. The mesocortical pathway also begins in the VTA but projects out to the motor, premotor and prefrontal cortices, areas which are involved in working memory and executive function.

Neurotensin

Recent research has focused more closely on other neurochemicals that interact with the dopaminergic systems within the brain, such as neurotensin (Binder,

et al. 2001; McMahon, Boules, Warrington, & Richelson, 2002; Dobner, 2005).

Neurotensin is a neuropeptide that was first isolated by Carraway and Leeman in 1973. Neuropeptides act as chemical mediators in neuron to neuron communication, integrating complex behaviors. Peptides are located in vesicles in axon terminals and the cell bodies for neurotensin are co localized in several areas of the brain with dopamine. At least three neurotensin receptors have been discovered thus far. Neurotensin has the highest affinity for NT₁, a 10 fold less affinity for NT₂ and 1,000 fold less affinity for NT₃, which is also the least studied. When neurotensin binds to the NT₁ receptor, it has been shown to act through several different mechanisms which inhibit dopamine release and decrease D₂ receptor agonist binding affinity (see Binder et al. 2001 for review).

Dopamine and neurotensin interactions

Neurotensin co localizes with DA throughout the brain. 80-90% of neurotensin receptors (NTR) are expressed on DA neurons and most DA neurons in the ventral tegmental area and substantia nigra have NTR, especially NT₁. The mechanism of action by which NT affects DA, results from NT binding to NT₁. When NT binds it may regulate gene expression, exhibit receptor/receptor allosteric interactions by activating 2nd messenger systems or alter cell firing and ion channels. These actions in turn inhibit D₂ receptor binding. Furthermore, activating NT₁ in the midbrain DA neurons may cause cells to depolarize which creates opposition of DA agonists. It can also induce auto inhibition of the cells firing rate and alter the activity of DA neurons (Binder et al., 2001).

In patients with schizophrenia who displayed a greater degree of psychotic symptoms, neurotensin levels in cerebrospinal fluid were significantly decreased. With the use of APDs, neurotensin levels were brought back to a level comparable to those of normal controls (Wilderlov, Lindstrom, Besev, Mansberg, Nemeroff, Breese, Kizer, & Prange, 1982). Additionally, patients with low cerebral spinal fluid concentrations of neurotensin had a greater degree of thought disorder, delusions and hallucinations, behavioral disorganization and impaired functioning. Sharma, Janicak, Bisette, and Nemeroff (1997) performed a study which compared level of psychopathological behavior to levels of neurotensin in cerebral spinal fluid of 42 participants with schizophrenia (N=29) or schizoaffective disorder (N=13) prior to treatment with an APD. Following four weeks of treatment with APDs, 18 of the subjects (schizophrenia N=14, schizoaffective disorder N=4) agreed to a second cerebral spinal fluid analysis. Pretreatment psychopathology was significantly higher for those subjects with the lowest levels of neurotensin in CSF. In addition, they found that a decrease in negative symptoms and psychopathological behaviors was correlated to higher levels of neurotensin in CSF. Findings such as these suggest that neurotensin is somehow involved in the etiology of the chemical imbalance in schizophrenia and has behavioral, physiological and pathological significance.

The possible influence that dopamine and neurotensin systems may have on one another has initiated many studies that have implicated neurotensin in the pathophysiology of certain central nervous system disorders, including schizophrenia (McMahon et al., 2002; Seutin, 2005; Dobner, 2005). Several laboratory tests using animal models have shown that neurotensin and neurotensin analogs produce effects

similar to that of antipsychotic drugs (Tanganelli, O'Conner, Ferraro, Bianchi, Beani, Ungerstedt, & Fuxe, 1994; Meltzer, & McGurk, 1999; Austin, 2000; Hamid, Hyde, Ehan, Wolf, Herman, Nemeroff, & Kleinman, 2002; Feifel, Melendez, & Shilling, 2003; Petrie, Bubser, Casey, Davis, Roth, & Deutch, 2004; Stowe, Landry, Tang, Owens, Kinkead, & Nemeroff, 2005; Azmi, Norman, Spicer, & Bennett, 2006; Prus, Huang, Li, Dai, & Meltzer, 2007; Norman, Beckett, Spicer, Ashton, Langlois, & Bennett, 2008). Tanganelli et al. (1994) showed that microinfusion of neurotensin facilitated GABA release and was associated with a decrease in dopamine in the rat nucleus accumbens while results from a study by Stowe et al. (2005) investigating the electrophysiological effects of neurotensin on spontaneously active neurons in the rat nucleus accumbens indicate that neurotensin does in fact mediate therapeutic effects of APDs. Azmi et al. (2006) was able to reverse the effects of memory disruptive scopolomine with the use of PD149163 in a novel object recognition task and Feifel et al. (2003) was able to block disruption of prepulse inhibition that was produced by a serotonin (5HT_{2A}) agonist with the systemic administration of the neurotensin agonist PD149163. In addition, Petrie et al. (2004) investigated FOS expression in the prefrontal cortex of rats and found that PD149163 increased the FOS expression, suggesting that neurotensin plays a role in the activation of interneurons in the prefrontal cortex. These studies support the hypothesis that neurotensin analogs improve neurochemical levels in ways that may offer superior efficacy for treating patients with schizophrenia.

PD149163, a novel analog of neurotensin

When given systemically neurotensin is unable to penetrate the blood-brain barrier. In order to investigate neurotensin thoroughly researchers needed to develop a neurotensin mimetic (neurotensin analog) which would cross the blood-brain barrier (Binder et al., 2001; McMahon et al., 2002; Dobner, 2005). Several neurotensin analogs are now available and are currently in the experimental phase of development.

The experimental compound PD149163 is a neurotensin analog. Studies have been done that support the hypothesis that neurotensin interacts with dopamine and has effects similar to those of APDs. Feifel, Melendez and Shilling (2004) used an animal model called pre-pulse inhibition, which is used for screening drugs for APD effects, to test the effects of the typical APD haloperidol, the atypical APD clozapine and PD149163 on sensorimotor gating. The study used Brattleboro rats which have a genetic mutation that creates a deficit in pre-pulse inhibition (PPI) that is comparable to people with schizophrenia. They found that with acute administration of all three drugs, haloperidol 0.05mg/kg had no effect on PPI with acute administration but had effect with chronic administration as a previous study by the same researchers. Clozapine 10mg/kg and 15mg/kg, and PD149163 1.0, 3.0 and 5.0mg/kg doses all were effective in reversing the PPI deficit in these genetically mutant rats. This evidence supports the hypothesis that the neurotensin analog PD149163 has atypical APD-like properties. In contrast, Norman et al. (2008) completed another behavioral model used for screening APDs that involves amphetamine induced hyperlocomotion. PD149163 was administered centrally and subchronically for 7 days and chronically

for 21 days. Previous studies had shown attenuation of amphetamine induced hyperlocomotion with acute administration, but Normans 2008 study showed no effect on amphetamine induced hyperlocomotion after 7 days administration of PD149163 and potentiation after 21 days administration. These findings are indicative of the need to further elucidate the behavioral effects of PD149163. A number of studies have been done to investigate the putative APD effects of PD149163, but none have yet been done to investigate the receptor properties that mediate the behavioral effects of neurotensin or neurotensin analogs, including PD149163. One way researchers have of determining how the effects of certain drugs in the brain produce their behavioral effects is by using an animal model called the drug discrimination (DD) task.

Drug Discrimination

The drug discrimination (DD) task and training procedure is analogous to procedures that are used to establish sensory discriminations (e.g., turn right when the cue light is on and left when it is off) except that the differential sensory conditions are replaced by drug conditions which are said to have stimulus effects. In rodent models the subject is said to have learned to discriminate and the drug is said to be discriminable when the response appropriate to the current drug state is reliably performed. Utilizing this task allows researchers to compare new experimental drugs with other known APDs to determine what properties they hold and what their functional mechanisms are.

Drug discrimination is used in preclinical development of APDs to evaluate pharmacological mechanisms that mediate discriminative stimulus properties and the

distinguishable differences in typical and atypical APDs. Numerous studies have been done using the DD task (Stewart, 1962, Colpaert, Niemegeers, & Janssen, 1976; McElroy, Stimmel, & O'Donnell, 1989; Prus, Baker, & Meltzer, 2004; Prus, Philibin, Pehrson, Stephans, Cooper, Wise, & Porter, 2005; Porter, & Prus, 2009) and several APDs (chlorpromazine, haloperidol, clozapine, olanzapine, quetiapine, ziprasidone) over the last 40 years.

Chlorpromazine was the first APD to be successfully discriminated by animals by Stewart in 1962 in a shock avoidance task, and haloperidol was then found to be discriminable in a two lever operant discrimination task with food reward by Colpaert et al. (1976) and McElroy et al.(1989). Colpaert et al. (1976) reported much difficulty training with haloperidol 0.04mg/kg, requiring in excess of 80 training sessions to establish the discrimination. Typically, 30-40 training sessions enables accurate discriminative responding in the DD task. The study done by McElroy et al. (1989) established discrimination over a mean of 45 training sessions for haloperidol 0.05mg/kg. Although Colpaert did not test for generalization, McElroy did and found that chlorpromazine fully generalized to haloperidol with 83% drug lever responding. Both haloperidol and chlorpromazine are D₂ receptor-preferring antagonists, so stimulus generalization between these two drugs shows evidence of D₂ antagonism. Additional support that the discriminative stimulus properties of haloperidol include antagonism of dopamine receptors was obtained by testing with cocaine and amphetamine which are dopamine agonists. Haloperidol's discriminative stimulus was completely blocked by these drugs.

The atypical APD clozapine was first established as a training drug in DD by Goas and Boston (1978). Clozapine displays robust discrimination stimulus properties time and again and has since become the prototypical or gold standard atypical APD by which other APDs and potential APDs are compared, to evaluate for putative APD properties and receptors mechanism of action (see Porter and Prus, 2009 for review). According to the literature, few APDs have been used as training drugs in the drug discrimination paradigm, but existing studies have provided valuable information about the differences and similarities between typical and atypical APDs. By using APDs as training drugs in the drug discrimination paradigm and testing stimulus generalization between the training drug and other compounds, we can draw an inference about what mechanistic properties a new compound or existing APD may have.

RATIONALE

Drug discrimination is one of the most informative methods used by researchers for investigating behavioral and neuropharmacological effects of drugs. By training an animal to identify the characteristic effect of a drug (DS) we can measure the effects in a precise, reliable and quantifiable manner. We can compare drugs already investigated and FDA approved with experimental compounds to investigate the putative APD effects and elucidate the receptor mechanisms involved in the drugs action.

Recent studies using animal models have lent support to the hypothesis that neurotensin analogs produce behavioral effects similar to APDs, yet the precise receptor mechanisms responsible for these effects are unknown. Drug discrimination was therefore used in the current study in an attempt to evaluate and clarify the receptor mechanisms involved in the discriminative stimulus effects of the neurotensin analog PD149163.

METHODS

Subjects

Ten male Sprague Dawley rats (Charles River, Portage, Michigan, USA) obtained at approximately 90 days old were used for this study. The subjects were housed in the rodent colony located at Northern Michigan University, Marquette, Michigan. The rodent colony was kept on a 12 hour light/dark schedule with lights on at 0700. Temperature and humidity were held at a constant and subjects were housed individually in transparent polysulfone flat-bottom cages with free access to water. Due to the fact that the subjects would be working for food reward during training sessions food access was restricted to maintain 85% of each subject's free-feeding weight. All procedures were approved in advance by the Northern Michigan University Institutional Animal Care and Use Committee (protocol 113) and were consistent with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Apparatus

Six rat operant chambers equipped with two retractable levers, a food receptacle, 45mg pellet dispenser and a 28V light were used (Med-Associates Inc., St. Albans, Vermont, USA). The levers were located equidistantly on either side of the food pellet receptacle. The chambers were housed in sound-attenuating cubicles with fans for ventilation and masking noise. The chambers were controlled by and data were recorded using, Med-PC version 4 (Med-Associates Inc.)

Drugs

PD149163 (NIMH Chemical Synthesis and Drug Supply Program, National Institute of Mental Health, Bethesda, Maryland, USA), and haloperidol HCl (Tocris, Ellisville, Missouri, USA) were in salt form and administered in 1ml/kg volume.

PD149163 was dissolved in sterile water and haloperidol HCl was dissolved in sterile water with a few drops of lactic acid. The number and range of doses were determined based upon behaviorally effective doses that had previously been used in other research studies involving these drugs. The number and range of doses were additionally tested in order to form a dose-effect curve that began at a dose with no effect and ended with a dose with full effect or highly suppressed response rate.

Drugs were injected in a 1ml/kg volume either subcutaneously (SC) or intraperitoneally (IP) 30 minutes prior to a session.

Drug Discrimination Training

There were several stages to the training process in the drug discrimination procedure: lever press training, fixed ratio training, errorless training and drug discrimination training. Test sessions began after drug discrimination training was complete. Only one training or test session was completed each day (see figure 1).

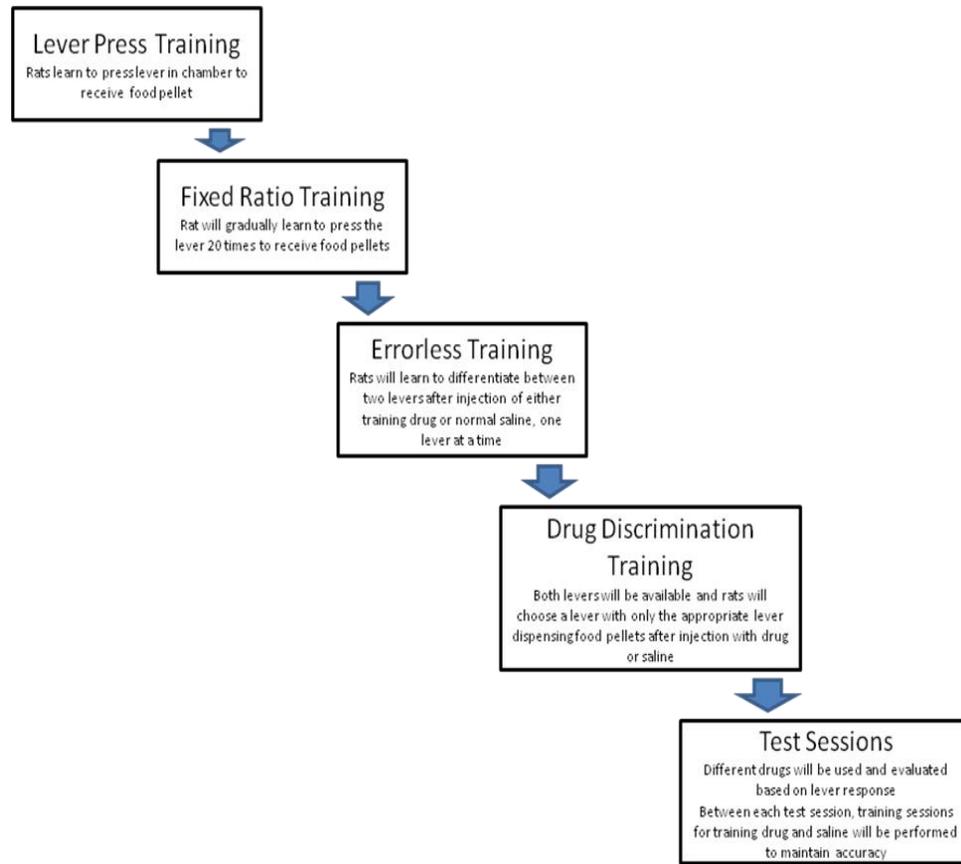


Figure 1. Steps to Drug Discrimination Training.

Subjects were initially trained to lever press using a fixed-ratio (FR) 1 schedule. Training began with the saline appropriate lever. Lever assignment was counterbalanced for left or right positioning within the group of subjects. After lever press training the FR was gradually increased, based on performance, until subjects responded on a FR20 schedule. Once trained to lever press on the FR20 schedule, 5 consecutive errorless daily 20 minute training sessions of saline and then the training dose of PD14916 (see RESULTS below for the training dose used) were conducted with only the condition-appropriate lever present. Following errorless training sessions, two-lever drug discrimination training began, with saline (S) and PD149163

(D) training days assigned based on a single-double alternation pattern (i.e., SDSSDSDDS etc.). During two-lever training, every lever press reset the FR20 counter for the opposite lever. Subjects were tested once the following criteria were met for 5 out of 6 consecutive training sessions: 1) first FR20 occurring on the condition-appropriate lever, 2) 80% or greater condition-appropriate responding during the first FR20, 3) 80% or greater condition-appropriate responding for the total session, and 4) at least 0.1 responses per second for the total session.

Before every test session, subjects had to meet the above four criteria for both a saline and a PD149163 training session. Test sessions ended after an FR20 occurred on either lever and no food pellet reward was delivered. Otherwise, the session ended after 20 minutes. As with training sessions, every lever press reset the FR20 counter on the opposite lever.

Data Analysis

Percent PD149163-lever responding was expressed as a percentage based on the number of PD149163-appropriate responses emitted divided by the total number of PD149163 and saline-appropriate responses emitted. In order to be included in the percent PD149163-lever responding calculation, rats were required to emit at least 10 responses during the test session. Percent PD149163-lever responding and responses per second were reported as means +/- the standard error of the mean (SEM). Full stimulus generalization from the PD149163 discriminative stimulus was defined as 80% or greater PD149163-appropriate responding, based on the criteria used in training sessions. No stimulus generalization was defined as 20% or less PD149163-appropriate responding, and partial stimulus generalization was defined as 20-80%

PD149163-appropriate responding. Statistically significant differences in response rates across doses and control points were determined using a repeated measures one way analysis of variance (ANOVA). Statistically significant differences were followed by a Dunnett's post hoc test to compare response rates for each dose to saline control. Regression methods were used to calculate the ED₅₀ dose (+/- 95% confidence intervals [CI]) for percent PD149163-appropriate responding for drugs that produced full stimulus generalization. All analyses were conducted using GraphPad Prism for Windows, version 5.02 (GraphPad Software, San Diego, California, USA).

RESULTS

After completion of magazine training (2 sessions) each subject completed 5 sessions of lever press training. Once subjects learned to lever press on a fixed-ratio (FR) of one the FR was gradually increased to reach an FR20. Subjects 1-8 completed FR20 training in 8 sessions, subject 9 took 29 sessions and subject 10 took 9 sessions. Following FR training subjects then completed 5 sessions of errorless training in which only the saline-appropriate lever was present. After 5 saline-appropriate lever errorless training sessions were complete, 5 errorless training sessions with PD149163-appropriate lever present were completed. The beginning dose of PD149163, 0.5mg/kg, was administered via intraperitoneal injection (IP) 30 minutes prior to training session. A reduction in responding was seen with this dose and after 4 sessions the training drug dose was decreased to 0.25mg/kg via IP injection. Each subject (with the exception of 9) completed 7-9 sessions alternating with saline as not to exceed 3 drug days in a row at the 0.25mg/kg IP dose. This dose also was found to create significant response rate suppression. The drug dose was again decreased. Subjects 1, 3, and 10 were trained for 4-6 sessions at the 0.125mg/kg IP dose before beginning 2-lever drug discrimination training at a dose of 0.1mg/kg IP and all other subjects began 2-lever drug discrimination training at this time on 0.1mg/kg IP. The PD149163 0.1mg/kg training dose was utilized for an additional 5 sessions for subjects 1-8 and 2 sessions for 9 and 10 before re-evaluation of data. The training dose was at that time (approximately trial 32 of DD training across subjects except #9) decreased to 0.05mg/kg IP. Response rates improved to a range acceptable to set criteria. This dose was used and subject responses evaluated over approximately 30

sessions (60, including double single alternating design with saline) but accuracy was not consistently above 80% for first fixed ratio (FFR). Criteria set calls for 80% or greater FFR accuracy for 5 of 6 consecutive training sessions. Training dose was then changed to PD149163 0.25mg/kg, and route changed to subcutaneous (SC) injection. A total of 8 drug training sessions were conducted at this dose. Response rate suppression was seen once again. Dose was then decreased to 0.125 mg/kg SC. After 15-19 training sessions 6 of the 10 subjects were able to finally meet criteria for stimulus generalization testing. The remaining 4 animals (1, 2, 3 and 5) continued to train on the 0.125mg/kg SC dose for a total of 32 additional drug training sessions before the dose was decreased once again to 0.0625mg/kg SC for all subjects, including the 6 subjects who met criteria at an average of 121 total saline/drug drug discrimination training sessions. Subjects 1, 2, 3 and 5 needed to have the training drug dose reduced again after 3 drug training sessions at PD 0.0625mg/kg to PD 0.0312mg/kg. Subjects 1,2 and 3 were then able to meet set criteria with an average of 212 total DD training sessions. Although these subjects were finally able to progress to stimulus generalization testing they were unable to complete the dose response curve for the training drug and their data are therefore excluded from the studies final analysis. Subject 5 never met training criteria and was excluded from the study completely after a total of 312 DD training sessions. (For review, see Table 2)

Table 2. Drug discrimination training trials to meet criteria (values in each cell equal the number of sessions conducted for that condition)

SUBJECT	1	2	3	4	5	6	7	8	9	10
FR TRAIN	8	8	8	8	8	8	8	8	29	9
ERRORLESS SALINE	5	5	5	5	5	5	5	5	5	5
ERRORLESS DRUG TRAINING/DOSES										
PD0.5mg/kg IP	4	4	4	4	4	4	4	4		
PD0.25mg/kg IP	8	7	9	7	7	7	7	7		8
PD0.125mg/kg IP	6		5							4
DRUG DISCRIMINATION TRAINING/DOSES										
PD0.1mg/kg IP	5	5	5	5	5	5	5	5	2	2
PD0.05mg/kg IP	30	31	30	31	30	31	27	31	30	27
PD0.25mg/kg SC	8	8	8	8	8	8	8	8	8	8
PD0.125mg/kg SC	32	31	32	16	32	19	15	16	17	16
PD0.0312mg/kg SC	17	17	17		17					
PD0.0625mg/kg SC	3	3	3		3					
TOTAL SALINE SESSIONS	80	95	98	59		58	55	55	33	43
TRIALS TO CRITERIA	195	210	233	130		132	121	126	110	108

Stimulus generalization grouped testing results for PD149163 are shown in figure 2. PD149163 produced full stimulus generalization to itself (80% PD-drug

lever responding or greater) at the 0.0625mg/kg SC training dose as well as the 0.125mg/kg SC dose. (ED50= 0.041mg/kg, 95% confidence-interval (CI)= 0.030-0.054mg/kg). Although a significant suppression in response rates (relative to Saline) occurred at both of these doses ($F[5,25]= 7.63, p<0.001$) (see figure 3). No substitution was seen at PD149163 0.0156mg/kg or 0.0312mg/kg doses and partial substitution was seen in 3 subjects for the 0.25mg/kg SC dose although response rates were greatly suppressed at this dose with 3 subjects having no response at all. (See figures 4 and 5 for individual results).

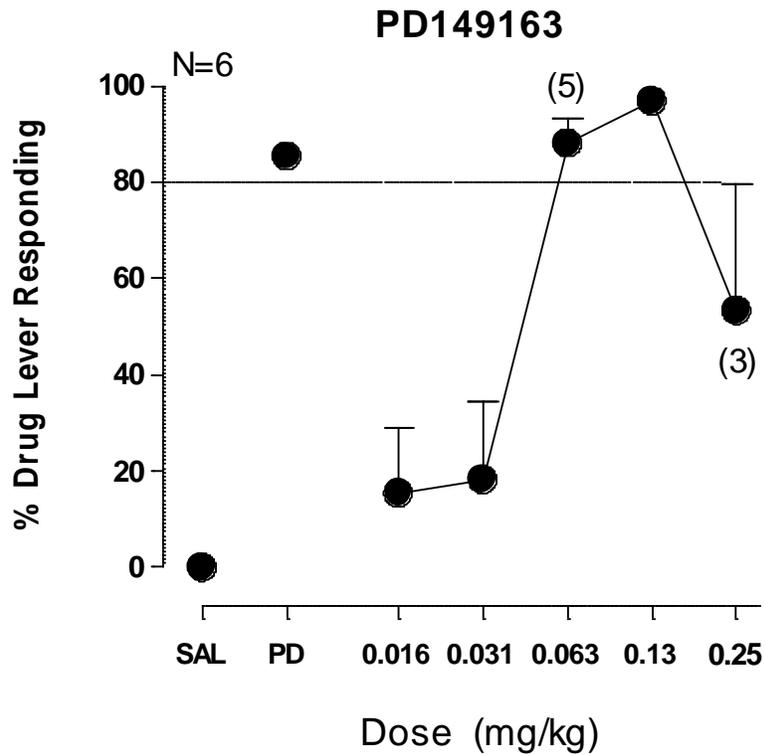


Figure 2. Percent drug lever responding for PD149163

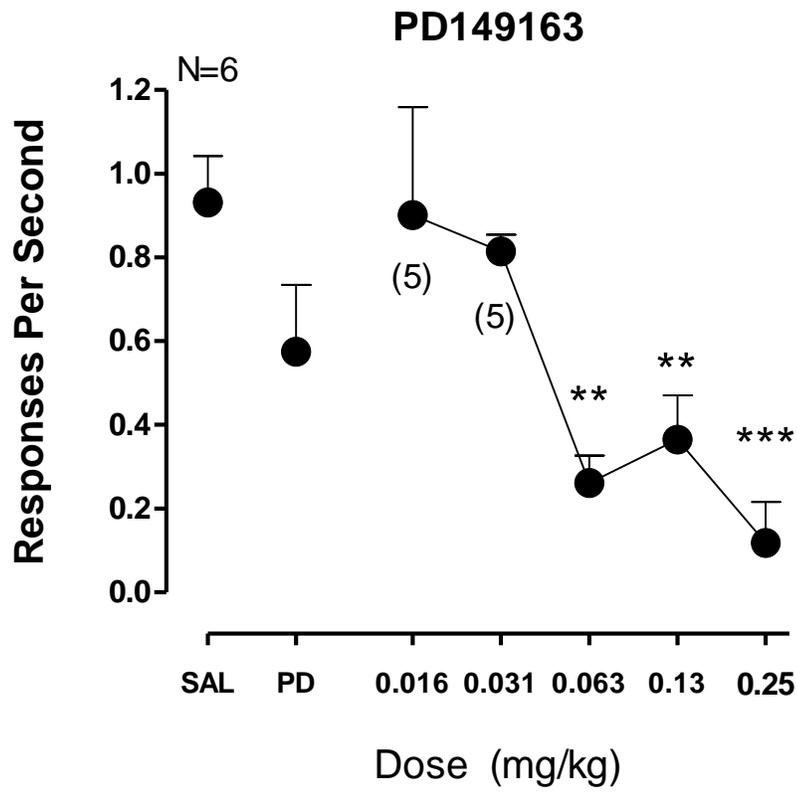


Figure 3. Responses per second for PD149163

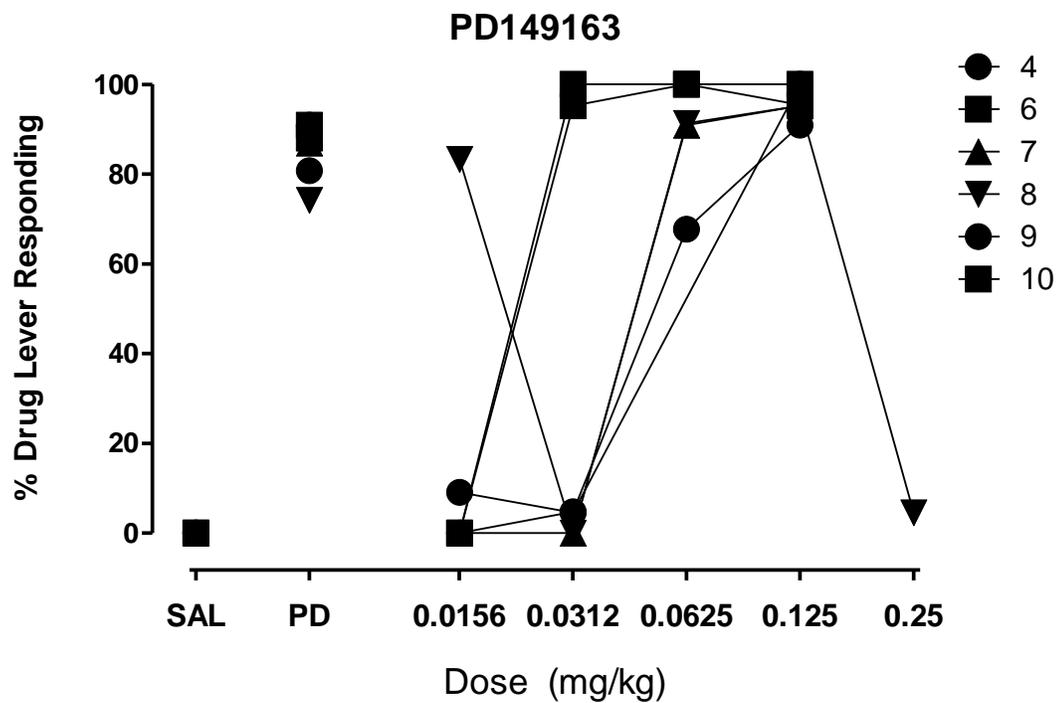


Figure 4. Percent drug lever responding for PD149163 for individual subjects

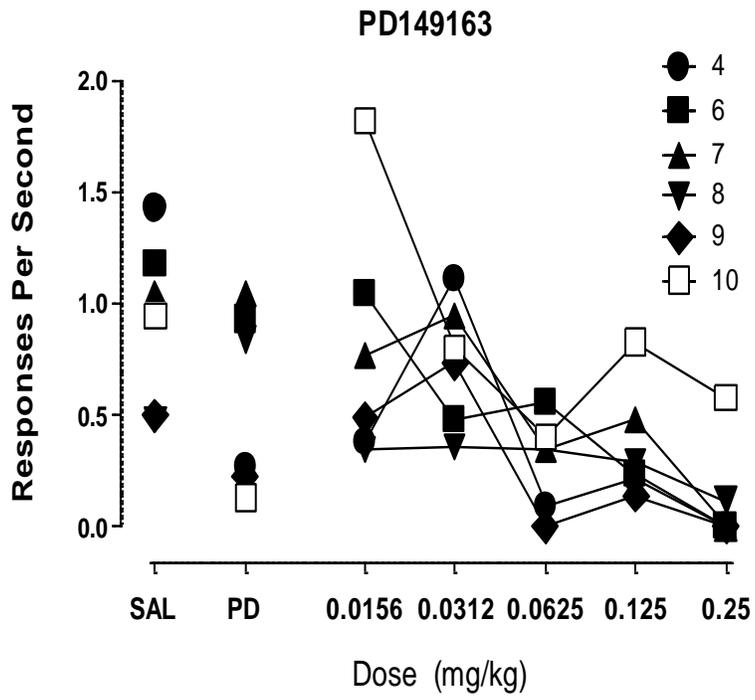


Figure 5. Responses per second for PD149163 for individual subjects

Stimulus generalization testing to D₂ receptor preferring antagonist and typical APD haloperidol was completed following the dose response curve for PD149163. Group results are shown in figure 6. Haloperidol was tested at the following four doses, 0.025, 0.05, 0.1 and 0.2mg/kg SC. Full stimulus generalization occurred at the 0.1mg/kg SC dose of haloperidol. (ED₅₀= 0.051mg/kg, 95% CI= 0.034-0.077mg/kg). Partial generalization occurred to a dose of 0.05mg/kg and no stimulus generalization was seen at the 0.025mg/kg SC dose. Figure 7 shows response rates for haloperidol as a function of dose. Response rates were significantly suppressed compared to those from saline, across all doses except 0.025mg/kg SC (F[4,16]= 3.153, p<0.01). Haloperidol 0.2mg/kg proved to be too behaviorally disruptive to obtain sufficient test results. (See figures 8 and 9 for individual results)

It should be noted that through casual observation, at approximately three minutes post injection of training drug PD149163, subjects consistently displayed a repetitive, purposeless digging behavior that would last approximately 4-5 minutes. Following this behavior the subjects would transition into a sedated state. With doses above 0.0625mg/kg of the training drug PD149163 the sedation persisted throughout training/test sessions.

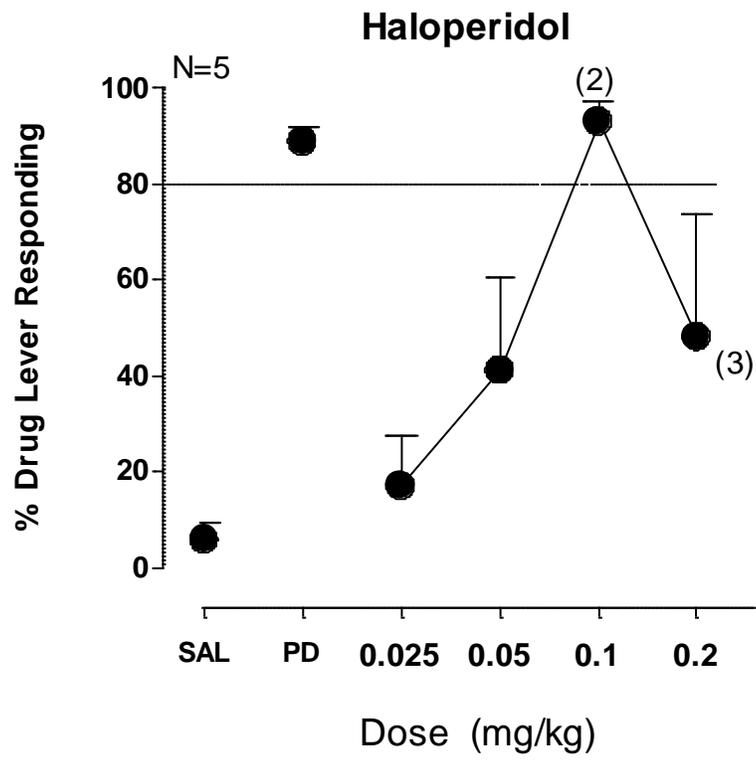


Figure 6. Percent drug lever responding for haloperidol

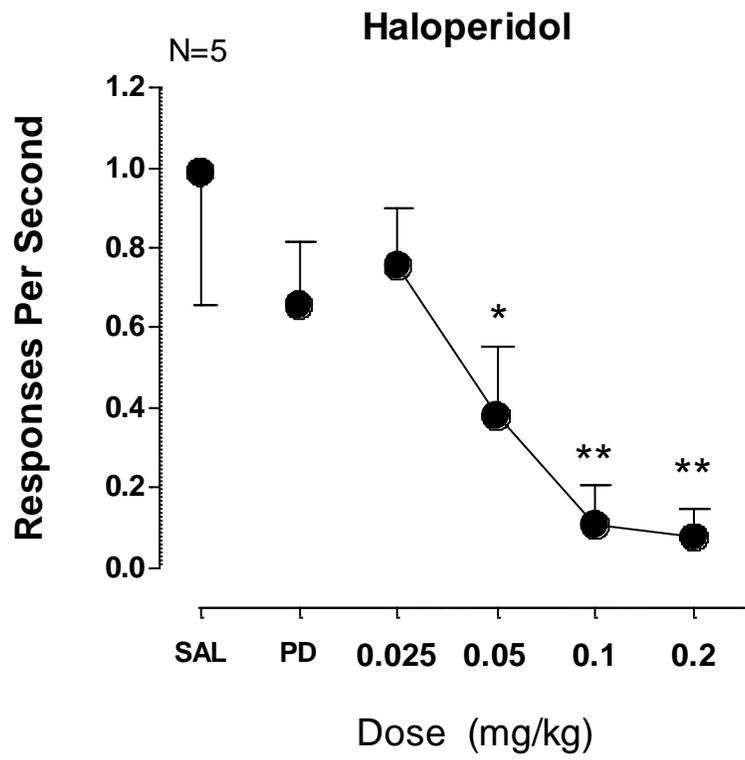


Figure 7. Responses per second for haloperidol

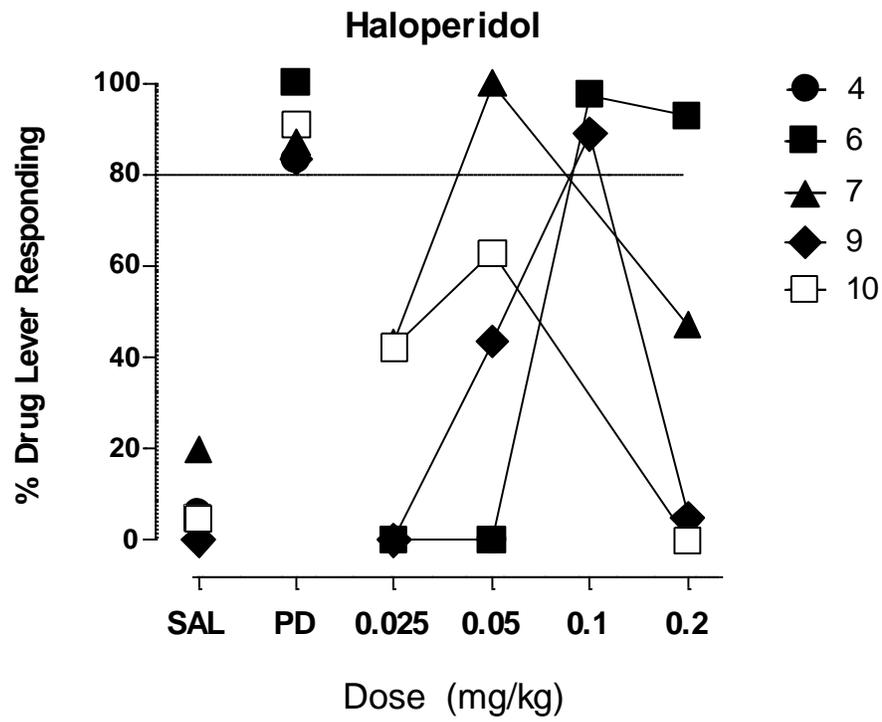


Figure 8. Percent drug lever responding for haloperidol for individual subjects

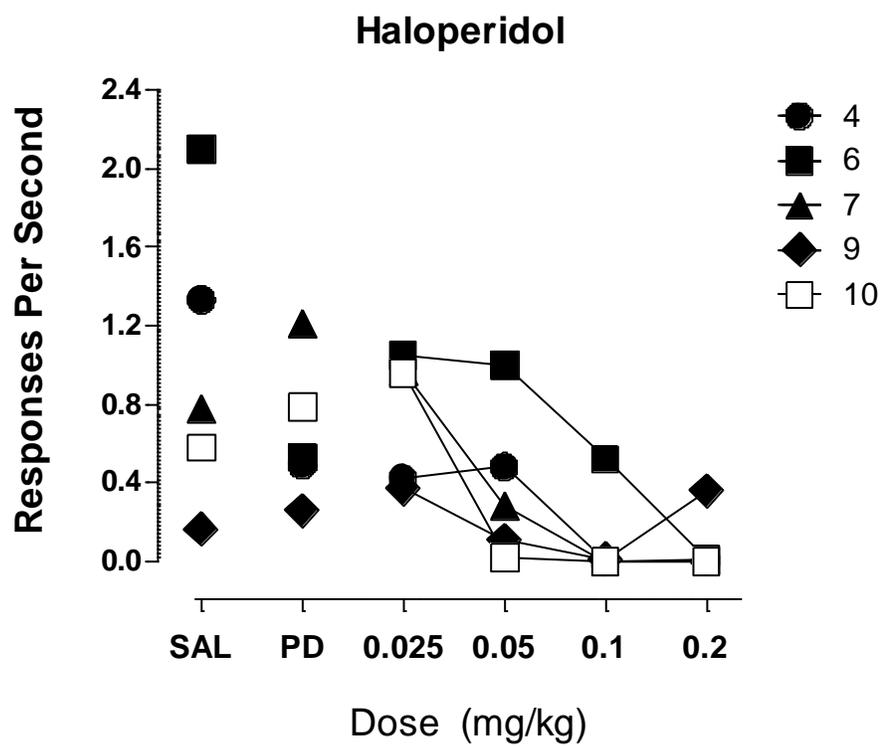


Figure 9. Responses per second for haloperidol for individual subjects

DISCUSSION

The present study utilized the drug discrimination paradigm in an attempt to assess the discriminative stimulus properties of PD149163. The discriminative stimulus properties of PD149163 were not robust and after several changes in training drug dose and administration route, 6 out of 10 subjects finally met training criteria to discriminate a PD149163 0.0625mg/kg dose from saline. Overall training took a mean of 121 twenty minute training sessions with one training session per day. Full stimulus generalization occurred to a 0.0625mg/kg and 0.125mg/kg dose of PD149163. Full stimulus generalization to the D₂ receptor-preferring antagonist and typical APD haloperidol was shown at the 0.1mg/kg haloperidol dose but with significant rate response reduction.

Since DD has never been established with neurotensin or a neurotensin analog, doses of PD149163 that were used in other studies were used as a guide when deciding upon a training dose for the current study. Feifel et al.(2004) was able to use 1.0, 3.0 and 5.0 mg/kg doses of PD149163 without behavioral disruption and Shilling and Feifel (2008) used 1.5 and 0.5mg/kg doses effectively. The current study began training at the PD149163 0.5mg/kg training dose. This dose proved to have severe rate suppressant effects, leading to a progressive systematic decrease in doses used for training these rats. These disruptions in response rates in DD are not uncommon for APDs. For example, Goudie and Smith (2002) established the atypical APD quetiapine as a discriminative stimulus, but only at a dose that significantly suppressed response rates compared to vehicle. Furthermore, Porter, McCallum, Varvel, and Vann (2000) established a 10.0 mg/kg dose of the atypical APD clozapine as a discriminative stimulus, but only have

repeated administration of this dose to invoke tolerance. Similar effects have been reported for a 5.0 mg/kg clozapine training dose by Prus et al (2005). These effects are not unlike those seen clinically, although tolerance generally develops to the sedating effects of APDs after several weeks. For 6 of the subjects in the present study, it took an average of 121 training sessions, several changes in dose and one change in route, in order to meet set criteria for drug discrimination. The dose for these 6 subjects was PD149163 0.0625mg/kg. Although this is the lowest dose used thus far in published literature involving PD149163, this dose proved to be too potent still for the remaining 4 subjects in the current study. Although the remaining 4 subjects continued on at a lower dose, they were still unable to maintain criteria after excessive trials, with one subject being eliminated from the study completely after over 300 training sessions.

The drug discrimination paradigm has been used for decades as a preclinical investigative tool in studying psychoactive drugs. Literature dating back to the early 70's has reported difficulty establishing discriminative stimulus properties of typical APDs (Colpaert et al. 1976; McElroy, et al. 1989). Colpaert et al. (1976) reported discrimination of the typical APD haloperidol at a dose of 0.04mg/kg by rats but with much difficulty, requiring over 80 sessions to drug discrimination training. McElroy et al. (1989) was able to establish discrimination with haloperidol at a 0.04mg/kg dose with lengthy training and then show stimulus generalization to chlorpromazine, but that was the only drug tested for stimulus generalization in that study. Both drugs are D₂ receptor- preferring antagonists and typical APDs. In the current study not only was it very difficult to establish the discriminative stimulus cue for PD149163, full stimulus generalization to the typical APD haloperidol was shown, suggesting

that the neurotensin analog PD149163 holds similar stimulus properties to D₂ receptor antagonists. This is curious considering the amount of research that gives support to the notion that PD149163 is more like an atypical APD, which are known to not only exhibit their behavioral effects through blockade of D₂ receptors, but through 5-HT_{2A} receptors as well.

The current findings of stimulus generalization from PD149163 to haloperidol suggest that the discriminative stimulus effects of PD149163 may be produced by indirect inhibition of D₂ receptor function. Neurotensin NT1 receptor activation has been shown to decrease the affinity for dopamine to the D₂ receptors, which functionally may act like a D₂ receptor antagonist. This may be the basis for stimulus generalization to haloperidol, which is a highly selective D₂ receptor antagonist. Yet, unlike D₂ receptor antagonists, PD149163 appears to be completely devoid of EPS liability. In animals, doses of PD149163 as high as 1.0 mg/kg (Fiefel et al. 2004) and 8.0 mg/kg (Holly, Ebrecht, and Prus, submitted) have entirely failed to produce catalepsy in rats. Similarly, the neurotensin analog NT69L not only fails to elicit catalepsy in rats, but also prevented haloperidol-induced catalepsy in rats (Cusack, Boules, Tyler, Fauq, McCormick, & Richelson, 2000). Thus, while PD149163 so far appears to share discriminative stimulus effects with haloperidol, PD149163 does not appear to exhibit a typical APD-like behavioral profile.

CONCLUSIONS

The present study provides the first characterization of the discriminative stimulus properties of a neurotensin analog. Based on the present findings, the discriminative stimulus properties of PD149163 appear similar to those elicited by D₂ receptor blockade. This, however, is inconsistent with the atypical APD-like properties shown in other animal models. Thus, further evaluation of the discriminative stimulus properties of PD149163 and other neurotensin analogs is warranted. An important next step should be to further evaluate effective training doses in order to identify a dose that elicits a more robust discriminative stimulus. Future directions of this research should include 1) stimulus generalization testing with a negative control, such as the D₂ agonist apomorphine, 2) stimulus generalization testing to other neurotensin analogs, 3) blockade of the discriminative stimulus effects of PD149163 by a NT receptor antagonist, and 4) stimulus generalization testing to atypical APDs, and 5) stimulus generalization testing to ligands that are selective for receptors that atypical APDs bind to (e.g., 5-HT_{2A} receptors). Methodological variations might also be pursued, such as using a variable interval reinforcement schedule.

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APPENDIX A

Northern Michigan University IACUC approval letter.



Northern
Michigan
University

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

MEMORANDUM

February 23, 2009

TO: Dr. Adam Prus
Department of Psychology

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

RE: **Application to use Vertebrate Animals**
Application # IACUC 113
Approval Period: 02/20/2009-04/23/2010

The Institutional Animal Care and Use Committee have approved your application to use vertebrate animals in research, "Discriminative stimulus properties of PD149163".

If you have any questions, please contact me.

kjm