2009

ROLE OF 5-HT1A RECEPTORS IN THE ABILITY OF IDAZOXAN AND RACLOPRIDE TO BLOCK CONDITIONED AVOIDANCE RESPONDING

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Thesis

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

MASTER OF SCIENCE

Graduate Studies Office

2009

SIGNATURE APPROVAL FORM

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Jacobson, Sarah Marie

DATE OF BIRTH:
March 5, 1982

ABSTRACT

ROLE OF 5-HT_{1A} RECEPTORS IN THE ABILITY OF IDAZOXAN AND RACLOPRIDE TO BLOCK CONDITIONED AVOIDANCE RESPONDING

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Sarah M. Jacobson

Atypical antipsychotic drugs (APD)s are regarded as more effective and safer than typical APDs for the treatment of schizophrenia. The hypothesis that combined blockade of α_2 and D_2 receptors produces atypical APD effects has been supported by the ability of the α_2 receptor antagonist idazoxan (IDX) combined with a low dose of the D_2 receptor antagonist raclopride (RAC) to block conditioned avoidance responding in rats. However, IDX is also a partial agonist at 5-HT_{1A} receptors. The present study sought to clarify the role of 5-HT_{1A} receptors in the effects of IDX combined with RAC, on conditioned avoidance responding in 16 male Sprague Dawley rats using a two-chamber shuttlebox equipped with a tilting grid floor. The α_2 adrenoceptor antagonist, yohimbine (YOH), was also tested in combination with RAC. RAC dose-dependently inhibited avoidance responding. IDX and YOH decreased avoidance responding when paired with an ineffective dose of RAC. Pretreatment with the 5-HT_{1A} receptor antagonist WAY100635 failed to significantly alter the avoidance rate of the IDX and RAC combination. The α2 adrenoceptor agonist, guanfacine, restored deficits in responding induced by the RAC+IDX treatment. The 5-HT_{1A} agonist 8-OH-DPAT reduced avoidance responding when paired with the ineffective dose of RAC. Based on these findings, α2 receptor blockade, not 5-HT_{1A} receptor stimulation, appears to mediate the ability of IDX and RAC to block conditioned avoidance responding.

AKNOWLEDGEMENTS

The author would like to thank Dr. Adam Prus for providing intellectual and financial support throughout the course of this study, Dr. Cynthia Prosen for her suggestions and encouragement, and Dr. Joe Porter for providing valuable input during the completion of this research and writing. This document was formatted in accordance with the American Psychological Association style guidelines.

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LIST OF ABBREVIATIONS

5-HT: Serotonin

APD: Antipsychotic Drug

CAR: Conditioned Avoidance Response

CAT: Catalepsy

DPAT: (+)8-OH-DPAT

DSM: Diagnostic and Statistical Manual of Mental Disorders

EPS: Extrapyramidal Symptoms

GF: Guanfacine

HAL: Haloperidol

IDX: Idazoxan

PPI: Prepulse Inhibition

RAC: Raclopride

WAY: WAY100635

YOH: Yohimbine

INTRODUCTION

Schizophrenia is a debilitating, lifelong mental illness diagnosed in approximately 1 percent of the worldwide population (Freedman, 2003), and is characterized by positive (e.g. hallucinations, delusions) and negative (e.g. alogia, anhedonia) symptoms and cognitive impairments (working memory and attention). Schizophrenia has been described as the world's most expensive mental illness, due to the progressive deterioration of patients over the course of a lifetime, which subsequently places a lasting financial burden on society. Much of the cost of the treatment of schizophrenia is due to poor adherence to pharmacological treatment programs, which in turn leads to hospitalization. It is estimated that repeated hospitalizations cost approximately $806 million annually (Marcus & Olfson, 2008). Many patients do not experience a significant recovery from symptoms, even with modern pharmacotherapeutic therapies.

The Diagnostic and Statistical Manual of Mental Disorders (DSM) lists comprehensive diagnostic criteria for the diagnosis of schizophrenia and defines specific subtypes of schizophrenia. The DSM specifies that patients must exhibit two or more of the following symptoms for a significant portion of a one-month period: delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior, or negative symptoms. Delusions or hallucinations alone, however, are sufficient to diagnose schizophrenia if the clinician notes that they are especially bizarre or disruptive of normal behavior. Patients who are diagnosed with schizophrenia per DSM criteria must also experience a significant decrease in social

functioning, including maintaining employment and personal relationships. Symptoms must be persistent for the duration of 6 months prior to treatment intervention without the occurrence of a major depressive or manic behavioral episode. Symptoms must not be directly related to the effects of substance abuse (American Psychiatric Association [*DSM-IV-TR*], 2000).

History of Treatment

Prior to the discovery of pharmacological treatments for schizophrenia, patients were commonly sedated, restrained or subject to electroconvulsive therapy, ice-baths or frontal lobotomies. These treatments offered very limited gains in improving the symptoms of schizophrenia. Psychiatric treatment was transformed by the introduction of chlorpromazine in 1952. This treatment represented the first typical antipsychotic and significantly reduced psychotic symptoms in 70% of patients (Meyer & Simpson, 1997).

Chlorpromazine and similar drugs developed later, now referred to as typical, or first generation antipsychotic drugs, only proved effective in reducing positive symptoms, and did not improve negative symptoms and cognitive impairments. Additionally, patients treated with typical antipsychotic drugs frequently developed movement disorders, referred to as extrapyramidal symptoms (EPS), which could be relieved by discontinuation of drug treatment. In some cases a class of movement disorders, called tardive dyskinesia, developed following chronic typical antipsychotic drug administration, which persisted after discontinuation of treatment (Meltzer & Stahl, 1976; Meyer & Simpson, 1997). Despite such setbacks, the discovery of a pharmacologically effective treatment for schizophrenia provided an

important foundation from which to base hypotheses regarding the causes of schizophrenia, the first being the dopamine hypothesis of schizophrenia.

Dopamine Hypothesis

A comprehensive review by Meltzer and Stahl (1976) describes the dopamine hypothesis of schizophrenia based on evidence from three categories: the ability of antipsychotic drugs to reduce positive symptoms, the phenomenon of amphetamine psychosis (which resembles the psychotic symptoms of schizophrenia) and pharmacological studies carried out in animal models of psychosis. These lines of evidence show that increases in dopamine activity are correlated with psychotic symptoms and that drugs that block the activity of dopamine alleviate psychotic symptoms in patients with schizophrenia.

Amphetamine Psychosis

Randrup and Munkvad (1967) demonstrated that a drug that stimulates dopamine receptors, amphetamine, induces behaviors termed stereotypy in rats, mice, guinea pigs and monkeys. Amphetamine-induced stereotypy is defined as a behavior that is performed continuously or purposelessly, such as continuous grooming of a single limb or purposeless searching head movements. Stereotypy interrupts normal grooming and exploring behaviors. In the absence of stereotypy, amphetamine increases the frequency of normal behaviors from that of baseline. Decreases in purposeful behaviors through a replacement by non-productive behaviors are associated with the agitated and disorganized behavior of patients with schizophrenia. Because stereotypy was produced through agonism of dopamine receptors, it was then hypothesized that psychotic symptoms are resultant from increases in dopamine

activity in the brain (e.g. see Carlsson & Lindqvist, 1963; Meltzer & Stahl, 1976 for review). Further animal studies in cats supported the dopamine hypothesis by showing that long- and short-term amphetamine treatment produced significant changes in brain chemistry, including a 95% increase in dopamine levels in the striatum (Trulson & Jacobs, 1979).

Studies in humans using amphetamine and other dopamine agonists also support the dopamine hypothesis of schizophrenia. Clinical measures of psychotic behavior are significantly increased by the administration of the synthetic dopamine precursor L-Dopa in patients with schizophrenia (Yaryura-Tobias, Diamond, & Merlis, 1970). Furthermore, L-Dopa treatment in patients with Parkinson's Disease increases dopamine levels in the nigrostriatal region and has the potential to induce psychotic symptoms over time (Meltzer & Stahl, 1976). Positron emission tomography research shows that patients with schizophrenia have greater dopamine release in response to amphetamine administration, termed amphetamine challenge, compared to healthy controls (Breier et al., 1997). Such increases in dopamine release in response to amphetamine administration are correlated with a significant worsening of positive symptamology, which is greater in patients with schizophrenia than in healthy control subjects (Laurelle et al., 1996).

Antipsychotic drugs

Typical antipsychotic drugs are used to support the hypothesis of dopamine overactivity in schizophrenia. Seeman (1975) showed a strong positive correlation between the strength of dopamine D_2 receptor blockade, a characteristic shared by nearly all typical antipsychotic drugs, and clinically effective dose of an

antipsychotic. These clinically prescribed doses are also effective in reversing amphetamine-induced psychosis in healthy controls. Specifically, the typical antipsychotic haloperidol significantly attenuates amphetamine-induced increases in psychotic behavior. In addition to alleviating psychosis in healthy controls, haloperidol significantly improves psychotic symptoms in amphetamine addicts as well as in schizophrenic patients and is effective in treating psychosis induced by long-term L-DOPA treatment in patients with Parkinson's disease (Angrist, Lee & Gershon, 1974).

Angrist and colleagues (1980) studied amphetamine treatment in unmedicated patients with schizophrenia to further explore the relationship between antipsychotic treatment response and sensitivity to amphetamine. Their research found that patients with a higher sensitivity to amphetamine also had a greater improvement in psychotic symptoms with antipsychotic treatment. Additionally, treatment-resistant patients showed less sensitivity to amphetamine. These findings indicated a need to explore dysfunction in neurotransmission beyond that of dopamine hyperfunction, especially in patients that are insensitive to typical antipsychotic drug treatment.

Negative symptoms and cognitive impairments

While chlorpromazine initiated a dramatic change in the treatment of patients with schizophrenia, the drugs synthesized after its discovery increased in potency but not in efficacy (Freedman, 2003). A population of patients still remained who were not effectively treated by dopamine D_2 receptor antagonists. Discoveries made by Angrist and colleagues (1980) regarding the relationship between amphetamine sensitivity and antipsychotic response were supported by further research showing

that patients insensitive to apomorphine treatment were also treatment-resistant when administered typical antipsychotic drugs (Garver, Zelman, Hirschowitz, Hitzemann, & Mavroidis, 1984). Evidence has been found indicating that dopamine hyperfunction in the mesolimbic system is also present in patients who have dopamine hypofunction in mesocortical regions (see Svensson, 2003 for review). In such cases, a pure dopamine receptor antagonist would be ineffective in treating all symptoms affecting patients with schizophrenia.

Persistent blockade of dopamine receptors can cause Parkinson-like movement disorders, often referred to as extrapyramidal side effects (EPS). Dopamine deficiencies caused by dopamine antagonism interrupt the functioning of the nigrostriatal (mesostriatal) dopamine tract, which regulates the extrapyramidal nervous system (Hornykiewicz, 1966; Klawans, 1973; Randrup & Munkvad, 1970). It follows that dopamine antagonists, typical antipsychotic drugs, are implicated in deterioration of normal motor control.

Adverse effects associated with typical antipsychotic drug treatment are not limited to movement disorders, and include decreases in cognitive functioning, which is often already abnormal in schizophrenia (Mehta, Montgomery, Kitamura, & Grasby, 2008). Depleting dopamine in the prefrontal cortex has cognitive effects similar to that of ablation of prefrontal cortical areas in rhesus monkeys. Reversal of cognitive deficits induced by dopamine depletion can be achieved by administration of the synthetic dopamine precursor L-DOPA, or the dopamine receptor agonist apomorphine, suggesting that dopamine activity is important for normal cognitive functioning (Brozoski, Brown, Rosvold, & Goldman, 1979). Therefore, antipsychotic

drugs that antagonize the effects of dopamine in areas important for cognition may worsen cognitive deficits in patients with schizophrenia.

Cognitive impairments are most strongly related to functional outcome in patients with schizophrenia. Measures of functional outcome include the ability to maintain meaningful personal relationships, carry out daily living routines and successfully retain a job. Impairments in cognitive functions, such as episodic memory, working memory and sustained attention are persistent across most published clinical studies of patients with schizophrenia (see Sharma & Antonova, 2003 for review). The presence of severe cognitive dysfunction negatively impacts patient functional outcomes. Milev and colleagues (2005) further supported these data by finding that impairments of verbal memory, processing speed and attention are negatively related to functional outcome, specifically, work performance. The typical antipsychotic, haloperidol, was found by Saeedi and colleagues (2006) to dose-dependently produce deficits in sustained attention and reaction time in healthy volunteers. Attention and working memory are impaired in healthy volunteers given the typical antipsychotic sulpiride at doses below what would be considered clinically effective (Mehta et al., 2008). Furthermore, high dopamine D_2 receptor occupancy is correlated with depression and decreases in self-control and emotional regulation (de Haan, Lavalaye, Linszen, Dingemans, & Booij, 2000).

For some time, researchers questioned whether or not dopamine receptor antagonism was an essential feature of antipsychotic action. Greenblatt and colleagues (1980) argued that dopamine antagonism is not necessary for antipsychotic effects. Their research found that the 5-$HT_{2A/2C}$ agonist, cis-5,6-Dimethoxy-2-

methyl-3-[2-(4-phenyl-1-piperazinyl)-ethyl]indoline (DHO), was more effective in inhibiting locomotor activity than chlorpromazine. DHO was not, however, as effective in protecting against lethal doses of amphetamine as chlorpromazine, and DHO caused increases rather than decreases in amphetamine-induced stereotypy. Thus, DHO is not a dopamine antagonist, but has characteristics that could be considered desirable in a typical antipsychotic drug.

Atypical antipsychotic drugs

The class of drugs developed to address the shortcomings of typical antipsychotic drugs are known as atypical antipsychotics, the first of which, clozapine, was approved for use in the United States in 1989 (Jibson & Tandon, 1998). Clozapine is the first known antipsychotic effective for both positive and negative symptoms and to also have a low EPS liability. Treatment with clozapine, however, occasionally results in seizures and dangerous declines in white blood cell counts, known as agranulocytosis (Meyer & Simpson, 1997). Despite these drawbacks, clozapine is still sometimes used in treatment-resistant patients in conjunction with frequent blood draws to monitor white blood cell counts (Kane, Honigfeld, Singer, & Meltzer, 1988). Patients treated with clozapine have improvements in memory, attention and executive function as well as improvements in positive symptamology (Hagger et al., 1993). Symptom improvements with clozapine are correlated with discharge from inpatient psychiatric facilities (Manschreck, Redmond, Candela, & Maher, 1999) and decreases in suicidal behavior (Meltzer et al., 2003).

The receptor binding profile of clozapine has been used in clarifying the characteristics found in effective antipsychotic drug treatment. Compared to typical antipsychotic drugs, clozapine has a much lower affinity for dopamine D_2 receptors. Furthermore, clozapine has actions at serotonin (5-HT) receptors as well as adrenoceptors in the brain. At the 5-HT_{1A} receptor subtype, clozapine acts as an agonist. Alternatively, clozapine acts as an antagonist at 5-HT_{2A} receptors and at α_2 adrenoceptors (for review, see Ashby & Wang, 1996).

Positive outcomes following clozapine treatment prompted the development of other antipsychotics with a receptor binding profile similar to that of clozapine but without the dangerous side effects. In 1994, risperidone was approved for use in the United States (Jibson & Tandon, 1998). Risperidone was the first atypical antipsychotic drug developed after clozapine, and had efficacy for both positive and negative symptoms. Patients treated with higher doses of risperidone, however, often develop extrapyramidal side effects. Similar to clozapine and risperidone, olanzapine has a diverse receptor binding profile, is effective for both positive and negative symptoms of schizophrenia and has low EPS liability. Olanzapine is effective at low doses, more effective than typical antipsychotics in reducing the positive and negative symptoms of schizophrenia and less likely to induce movement disorders compared to typical antipsychotics (Meyer & Simpson, 1997).

Theories of antipsychotic atypicality

D_2/5-HT_{2A} hypothesis

Animal models using amphetamine to induce psychotic stereotypy produce abnormalities not only in dopamine but also in serotonin. Short-term amphetamine

treatment greatly increases dopamine levels in the striatum, which may be responsible for producing psychotic stereotypy. Long-term amphetamine administration decreases serotonin and its metabolites in the hippocampus, striatum and diencephalon (Trulson & Jacobs, 1979). Treatment with LSD, a 5-HT agonist, produces hallucinations, a positive symptom of schizophrenia, which has been linked to 5-HT_{2A} stimulation (Fiorella, Rabin, & Winter, 1995).

Serotonin receptors are abnormal in patients with schizophrenia. Frontal cortical serotonin receptors are decreased in patients with schizophrenia as compared to age- and gender-matched controls and normal patients have age-related serotonin receptor decreases while patients with schizophrenia of all ages have decreases in serotonin receptors, specifically the 2A subtype (Dean & Hayes, 1996). These data are indicative of either a failure to synthesize 5-HT_{2A} receptors, or uninhibited pruning of the receptors in early life. In either case, this abnormality may be an important factor underlying the symptoms of schizophrenia. It is important to note, however, that the certain polymorphisms of the genes responsible for 5-HT_{2A} receptor expression in patients with schizophrenia have not been found to be significantly different from normal control subjects (Bertola, Cordeiro, Zung, Miracca, & Vallada, 2007).

Meltzer (1989) proposed the D_2/5-HT_{2A} hypothesis of antipsychotic atypicality based upon the observation that antipsychotic drugs with a higher affinity for 5-HT_{2A} receptors over D_2 receptors have higher clinical efficacy for treating positive and negative symptoms and have lower EPS liability. Studies using positron emission tomography in humans have found that patients treated with atypical versus

typical antipsychotic drugs have higher 5-HT_{2A} binding relative to D_2 binding. These observations include the prototypical atypical antipsychotic, clozapine (Goyer et al., 1996; Nordstrom, Farde, & Halldin, 1993). Currently, all known atypical antipsychotic drugs have a higher affinity for the 5-HT_{2A} receptor over the D_2 receptor (Jibson & Tandon, 1998; Meltzer, Li, Huang, & Prus, 2006), with the exception of amisulpride, which stimulates dopamine $D_{2/3}$ receptors and has no affinity for 5-HT receptors (Natesan, Reckless, Barlow, Nobrega, & Kapur, 2008) and aripiprazole, which has a greater affinity for $D_{2/3}$ receptors over 5-HT_{2A} receptors (DeLeon, Patel, & Crismon, 2004).

Seeman (2002) opposed this theory of antipsychotic atypicality in favor of the dopamine D_2 "fast-off" theory. Seeman noted that older antipsychotic drugs, such as chlorpromazine and haloperidol bind tightly to dopamine D_2 receptors and are not easily displaced by endogenous dopamine. Newer, atypical antipsychotics, however, are more easily dissociated from dopamine D_2 receptors, allowing endogenous dopamine to bind to and activate the receptor, thus reducing EPS liability.

5-HT_{2A} receptor involvement

While blockade of 5-HT_{2A} receptors alone does not have an antipsychotic effect, the addition of a 5-HT_{2A} antagonist to a typical antipsychotic drug treatment potentiates the effects of a typical antipsychotic in pre-clinical studies. This combination does not worsen measures of catalepsy which predict EPS in humans (Ellenbroek, Prinssen, & Cools, 1994; Wadenberg et al., 2000; Wadenberg, Hicks, Richter, & Young, 1998; Wadenberg, Salmi, Jimenez, Svensson, & Ahlenius, 1996). Therefore, administration of a 5-HT_{2A} antagonist in combination with a typical

antipsychotic produces a drug profile which resembles the effects of known atypical antipsychotic drugs. Efficacy may be improved without increasing the amount of dopamine antagonism, which in turn reduces the likelihood of inducing EPS. Blockade of 5-HT_{2A} receptors in mice reduces amphetamine-induced motor activity, further suggesting the protective effects of 5-HT_{2A} antagonism on motor systems in the brain (Moser, Moran, Frank, & Kehne, 1996). Reversing the 5-HT_{2A} antagonistic effects of atypical antipsychotics provides further evidence for the 5-HT_{2A}/D_2 hypothesis of atypicality. Stimulation of 5-HT_{2A} receptors in combination with clozapine treatment reduces measures in animal models that predict clinical efficacy, suggesting that 5-HT_{2A} antagonism accounts, at least in part, for the effects of clozapine (Ellenbroek et al., 1994).

Microdialysis studies by Marcus and colleagues (2000) show that 5-HT_{2A} antagonism potentiates dopamine release preferentially in non-motor associated regions, such as the prefrontal cortex, contributing to increased treatment efficacy and decreased EPS liability. Increases of dopamine in prefrontal cortical areas do not, however, occur with 5-HT_{2A} antagonism alone. When paired with a dopamine antagonist such as haloperidol, 5-HT_{2A} antagonism increases dopamine in the prefrontal cortex, while dopamine increases in motor regions induced by haloperidol are significantly attenuated (Liegeois, Ichikawa, & Meltzer, 2002).

5-HT 1A receptor involvement

Serotonin 1A receptor agonism has been suggested to contribute to an atypical antipsychotic drug profile. Specifically, 5-HT_{1A} agonism may reduce the occurrence of EPS induced by dopamine antagonism. Conversely, antagonism of 5-HT_{1A}

receptors increases the EPS liability of typical antipsychotic drugs. Depletion of serotonin, however, eliminates this enhancement of cataleptic effects (Prinssen, Colpaert, & Koek, 2002; Prinssen, Koek, & Kleven, 2000).

Research investigating neurochemicals in specific brain regions has supported the potential role of 5-HT_{1A} agonism in atypical antipsychotic drug effects. Serotonin 1A agonism has been shown by Ichikawa and colleagues (1995) to attenuate the effects of amphetamine on the release of dopamine in the striatum and the nucleus accumbens. Stimulation of 5-HT_{1A} receptors in combination with typical antipsychotic drug treatment decreases dopamine levels in these areas, presumably imparting a decreased EPS liability to the typical antipsychotic drugs tested (Ichikawa & Meltzer, 2002). Behavioral studies by Ellenbroek and colleagues (1994) support the neurochemical evidence by showing that administration of a 5-HT_{1A} agonist reduces typical antipsychotic-induced movement disorders while preserving drug efficacy. Alternatively, blocking 5-HT_{1A} receptors decreases the efficacy in treating positive and negative symptoms of atypical antipsychotic drugs such as clozapine. Clozapine-induced increases in prefrontal cortical dopamine release are inhibited by co-treatment with a 5-HT_{1A} antagonist (Rollema, Lu, Schmidt, & Zorn, 1997) while behavioral measures of the clinical efficacy of clozapine are enhanced by co-treatment with a 5-HT_{1A} agonist (Ellenbroek et al., 1994).

Adrenoceptors

α_1 adrenoceptor receptor involvement

The atypical antipsychotic drugs clozapine and risperidone both have an affinity for the α_1 adrenoceptor, and blockade of this receptor has been implicated in

mediating the activity of the mesolimbic dopamine system (Mathe, Nomikos, Hildebrand, Hertel, & Svensson, 1996; Svensson, 2003). Marcus and colleagues (2000) suggested that antagonism of α_1 receptors inhibits typical antipsychotic-induced dopamine increases in motor regions of the nucleus accumbens, thereby decreasing EPS liability.

Sensory-motor gating deficits, which are known to be impaired in schizophrenic patients, can be blocked in animal models by the α_1 antagonist prazosin (Bakshi & Geyer, 1997). Agonism of these receptors disrupts normal sensory-motor gating in animal models, providing further evidence that this receptor mechanism contributes to the efficacy of atypical antipsychotic drugs such as clozapine (Carasso, Bakshi, & Geyer, 1998). $Alpha_1$ adrenoceptor blockade does not, however, protect against EPS induced by typical antipsychotic drug treatment (Wadenberg & Hertel et al., 2000).

α_2 adrenoceptor receptor involvement

Increased affinity for α_2 adrenoceptor antagonism over 5-HT_{2A}/D_2 receptor antagonism has been suggested to contribute to an efficacious antipsychotic drug profile. The atypical antipsychotic olanzapine, which is 40 times more potent at α_2 adrenoceptors than clozapine, but has a similar 5-HT_{2A}/D_2 binding profile, is effective at much lower doses than clozapine (Meyer & Simpson, 1997). The protective effects of α_2 adrenoceptor antagonists on typical and atypical antipsychotic drug-induced EPS has been demonstrated by a number of researchers. Invernizzi and colleagues (2003) reversed the cataleptic effects of the typical antipsychotic drug, haloperidol, through α_2 adrenoceptor receptor blockade. Catalepsy induced by high

doses of atypical antipsychotic drugs, whose action is mediated through D_2/5-HT_{2A} antagonism, can also be reduced by α_2 antagonism (Kalkman, Neurmann, Hoyer, & Tricklebank, 1998). Increases in 5-HT in response to α_2 antagonism have been noted in *in vivo* neurochemical studies, which, given the evidence regarding the protective effects of 5-HT_{1A} agonism on dopamine-antagonist induced EPS, further supports the hypothesis that α_2 antagonism plays a role in antipsychotic atypicality (Hertel, Nomikos, Schilstrom, Arborelius, & Svensson, 1997).

α_2/D_2 hypothesis

Hertel, Nomikos, & Svensson (1999) first proposed the α_2/D_2 hypothesis of antipsychotic atypicality based upon behavioral tests as well as *in vivo* microdialysis in rats. They found that the effects of a typical antipsychotic drug, which blocks dopamine D_2 receptors, is enhanced by co-treatment with an α_2 adrenergic receptor antagonist. The research showed that dopamine levels in the prefrontal cortex are significantly higher in rats treated with an α_2/D_2 receptor antagonist combination compared to rats treated with either drug alone. Furthermore, behavioral tests showed this combination has greater antipsychotic efficacy than either drug given alone. The effects of the α_2/D_2 receptor blockade were compared to that of clozapine and even proposed to be more effective than clozapine in the treatment of schizophrenia.

Nearly all of the evidence used in support of the α_2/D_2 hypothesis of antipsychotic atypicality has been through the use of a drug called idazoxan. The distribution of idazoxan in the rat brain is consistent with that of known α_2 adrenoceptor distribution (Mallard, Hudson, & Nutt, 1992). Idazoxan has therefore been used in a variety of animal models exploring how antagonism of α_2 receptors

contributes to an atypical antipsychotic drug profile. It is important to note, however, that 5-HT_{1A} receptor stimulation has been implicated in the ability of idazoxan to produce an atypical antipsychotic profile when paired with a typical antipsychotic drug. Specifically, 5-HT_{1A} stimulation is thought be responsible for the ability of idazoxan to reduce the EPS liability of a typical antipsychotic drug (Kleven, Assie, Cosi, Barret-Grevoz, & Newman-Tancredi, 2005).

Combining idazoxan with a typical antipsychotic drug reverses drug-induced memory impairments in rats. These impairments can also be reversed by clozapine treatment alone, and the treatments share a similar level of receptor binding at the dopamine D_2 receptor and α_2 adrenoceptors (Marcus et al., 2005). Catalepsy induced by typical antipsychotic drugs can be reversed using idazoxan, and idazoxan alone, even at very high doses, does not induce catalepsy in rats (Wadenberg, Wiker, & Svensson, 2007). Anti-cataleptic effects are further shown in studies that indicate idazoxan treatment, both alone and in conjunction with typical antipsychotic drug treatment, prevents dopamine increases in areas that are implicated in the development of EPS (Invernizzi, Garavaglia, & Samanin, 2003). Furthermore, idazoxan treatment in conjunction with dopamine antagonism increases dopamine in the prefrontal cortex, implying that this treatment would be effective in reducing cognitive symptoms associated with schizophrenia (Hertel, Nomikos, & Svensson, 1999). By blocking α_2 adrenoceptors, clozapine-induced dopamine and norepinepherine increases in the prefrontal cortex are inhibited, suggesting that the α_2 antagonistic properties of clozapine contribute to its favorable clinical effects (Devoto et al., 2003). Smith, Wilson, Glue, & Nutt (1992) supported this hypothesis by

showing that idazoxan treatments in healthy human subjects did not affect memory, attention, or mood.

Table 1. Receptor binding affinities for typical and atypical antipsychotic drugs at dopamine, serotonin and adrenergic receptors in the brain. Binding results (K_i) for haloperidol, clozapine, risperidone and olanzapine were reported by Schotte et al. (1996). Alpha2 binding results (K_i) for olanzapine were reported by Bymaster et al. (1996). D_2 and 5-HT_{2A} results (K_d) for raclopride were reported by Seeman et al. (1997). Binding results for idazoxan, yohimbine, 8-OH-DPAT and chlorpromazine at D_2, 5-HT_{1A} and 5-HT_{2A} (K_i) were reported by Toll et al. (1998), α_1 and α_2 binding results (K_i) for idazoxan and yohimbine were reported by Doxey et al. (1984). Binding results (K_d) for WAY100635 were reported by Chemel et al. (2006). α_1 and α_2 results (K_i) for chlorpromazine and raclopride were reported by Hall et al. (1986) as well as 5-HT_{1A} results for raclopride. Binding results (K_i) for Guanfacine were reported by Boyajian et al. (1987).

Table 1. Receptor binding affinities for selected typical and atypical antipsychotic drugs

Antipsychotic	D_2	α_1	α_2	$5\text{-}HT_{1A}$	$5\text{-}HT_{2A}$
Chlorpromazine	3.0	14	3,050	3,115	3.6
Haloperidol	1.4	19	>5,000	3,080	25
Clozapine	150	23	160	180	3.3
Risperidone	3.3	2.3	7.5	250	0.16
Olanzapine	17	60	230	2,720	1.9
Raclopride	0.64	32,300	38,200	48,800	5,400
Idazoxan	>10,000	91	3.1	662	>10,000
Yohimbine	280	230	40	642	2,258
WAY100635	940	19.9	>10,000	2.2	6,2600
8-OH-DPAT	1,788	-	-	6.9	>10,000
Guanfacine	-	-	24.9	-	-

Models for studying potential antipsychotic drugs

Catalepsy test

The catalepsy test is the most frequently used test for the study of EPS. The test is performed by injecting an animal with the drug/s of interest, waiting a set period for onset of drug action and then placing the animal in an unusual position. The catalepsy score is based upon the amount of time the animal takes to correct its position (Sandberg, Bunsey, Giordano, & Norman, 1988). Catalepsy was considered at one time to be a desirable effect in potential antipsychotic drug treatments, but is now considered to be detrimental.

Measuring the effects of specific receptor agonists and antagonists on typical antipsychotic-induced catalepsy is a useful tool for developing atypical antipsychotic drugs with low EPS liability. Drugs that are known to induce catalepsy are paired with experimental therapeutics specific for a particular receptor in the brain to clarify the mechanisms involved in reducing EPS and developing atypical antipsychotic drugs. The catalepsy test is not, however, effective in discriminating between typical and atypical antipsychotic drugs. Researchers have also noted that typical antipsychotic-induced catalepsy appears to be similar to narcotic-induced catatonia. Therefore, the catalepsy test may not always be effective in determining whether or not a drug is an antipsychotic. To differentiate between typical antipsychotic and narcotic motor dysfunction, atropine may be given as a conjunctive treatment. In the case of typical antipsychotic-induced catalepsy, atropine acts as an antagonist to this effect, while narcotic-induced catatonia is not affected by atropine treatment (Costall

& Naylor, 1974). This evidence supports the idea that catalepsy, and therefore EPS, occurs due to a deficiency in dopamine function induced by chronic dopamine receptor antagonism in the form of typical antipsychotic treatment.

Although the catalepsy test cannot discriminate between atypical and typical antipsychotic drugs or narcotic-induced catatonia, it has high predictive validity for detecting EPS liability in humans. Positron emission tomography has been used by Wadenberg, Kapur, Soliman, Jones, and Vaccarino (2000) to show that antipsychotic drug doses that caused EPS in humans correlate with doses that caused catalepsy in rats.

Paw test

The paw test was developed as an alternative to the catalepsy test, which was ineffective in evaluating atypical antipsychotics such as clozapine that have a low EPS liability. The paw test measures muscle rigidity and compares the retraction times of both the fore- and hindlimbs of the rat. Ellenbroek, Peeters, Honig, and Cools (1987) found that typical antipsychotics inhibit forelimb and hindlimb retraction at equivalent doses, while atypical antipsychotics inhibit hindlimb retraction at lower doses more strongly than forelimb retraction. Furthermore, an increase in forelimb retraction time is correlated with increased catalepsy, thus predicting extrapyramidal symptom liability. The paw test is therefore an effective research tool not only for identifying potential typical antipsychotics, but also for differentiating between typical and atypical antipsychotic activity.

Prepulse inhibition (PPI)

Prepulse inhibition is purported to be a measure of sensorimotor gating, wherein a pre-pulse inhibits, or gates, the startle response to a stronger stimuli that immediately follows the pre-pulse. Therefore, deficits in PPI indicate a deficit in sensorimotor gating, which is linked to activity in the forebrain (Braff et al., 2001). PPI does not require training, as it occurs on the first exposure to pre-pulse and pulse stimuli (Blumenthal, Schicatano, Chapman, Norris, & Ergenzingerm, 1996), and can also be studied across species (Braff et al., 2001). In a review of clinical literature in schizophrenic patients, Braff, Geyer, and Swerdlow (2001) found deficits in pre-pulse inhibition (PPI) were consistently noted. Specifically, patients with early-onset schizophrenia have the most significant deficits in PPI (Kumari, Soni, Mathew, & Sharma, 2000).

Initial studies relating dopaminergic activity to PPI in rats found that stimulation of dopamine D_2 receptors inhibits PPI. This effect is attenuated by the administration of the dopamine D_2 receptor antagonist, haloperidol. Haloperidol alone, however, has no effect on PPI (Mansbach, Geyer, & Braff, 1988). Results from this study further supported the hypothesis that the symptoms of schizophrenia manifest as a result of overactivity of dopaminergic systems. The use of PPI in animals, however, does not appear to always be effective as a screening tool for antipsychotic agents in the absence of the pharmacological stimulation of dopamine activity. Phencycladine-induced PPI deficits, which manifest through glutamatergic activity, however, may reliably differentiate between typical and atypical antipsychotic drugs (see Geyer & Ellenbroek, 2003 for review). In human studies,

chronic treatment with typical antipsychotics, but not atypical antipsychotics, correlates with a reduction in PPI as compared to healthy controls (Kumari et al., 2000), suggesting that PPI may be useful as a tool for screening potential antipsychotic drugs in the case of chronic administration of experimental compounds, but not with acute drug administration.

Microdialysis

Detecting changes in neurotransmission in response to antipsychotic treatment is achieved through microdialysis. Researchers measure the activity in specific brain regions by inserting a microdialysis probe into a region of interest by stereotaxic surgery. Extracellular fluid is then sampled via the microdialysis probe and analyzed using high pressure liquid chromatography. Rollema and colleagues (1997) have demonstrated that clozapine increases dopamine levels in the prefrontal cortex. This area has been implicated in the negative symptoms and cognitive impairments of schizophrenia. Typical antipsychotic drugs increase dopamine levels in the striatum. Increases in dopamine in the striatum over the prefrontal cortex is thought to underlie the development of extrapyramidal symptoms as well as the aggravation of negative symptoms in schizophrenia (Kuroki, Meltzer, & Ichikawa, 1999). Therefore, experimental compounds that are evaluated pre-clinically for the treatment of schizophrenia would be expected to increase prefrontal cortical dopamine levels, while having little to no effect on dopamine levels in the striatum.

Conditioned Avoidance Response (CAR)

The conditioned avoidance response (CAR) task has been used to pre-clinically identify every known antipsychotic drug to date. In the CAR task, animals

are trained to avoid a noxious stimuli (e.g., a foot shock) by responding to a warning stimulus (e.g., a white noise) which precedes the noxious stimuli. Antipsychotic drugs reduce avoidance responding in the CAR task without inhibiting escape responses. Drugs that induce catalepsy or act as tranquilizers reduce avoidance and escape responses in the CAR task. That is, animals fail to respond to both the warning stimulus and the noxious stimuli. Tranquilizers, unlike antipsychotics, will persistently produce escape failures even when the shock stimulus is increased (Grilly, Johnson, Minardo, Jacoby, & LaRiccia, 1984). Both typical and atypical antipsychotics inhibit CAR, however, typical antipsychotics are far more potent in this regard, and the mechanism by which avoidance responses are decreased is hypothesized to be a result of dopamine D_2 receptor blockade. Further support for this hypothesis has been shown by the use of amphetamine, which restores antipsychotic-induced deficits in the CAR (Taboada, Souto, Hawkins, & Monti, 1979). Because both typical and atypical antipsychotic drugs have the potential to produce deficits in CAR, this task is inappropriate for differentiating typical from atypical antipsychotic drugs. Research using positron emission tomography in humans, as well as in vivo binding in rats, confirms that typical antipsychotic doses that produce CAR in rats have similar levels of dopamine receptor binding as those that have efficacy in humans. Also, doses that produce escape failures in CAR, a measure indicative of catalepsy, produce EPS in humans at similar levels of dopamine receptor occupancy (Wadenberg, Kapur et al., 2000).

Idazoxan and CAR

Hertel et al. (1999) found that idazoxan enhanced the effects of the typical antipsychotic, raclopride, in the CAR task and also potentiated raclopride-induced dopamine release in the prefrontal cortex. Wadenberg and colleagues (2007) have lent further support to the α_2/D_2 hypothesis of atypicality using idazoxan in the CAR task. Idazoxan enhanced the suppression of CAR when paired with a subthreshold dose of haloperidol as well as with a low dose of olanzapine. Idazoxan also reversed catalepsy induced by haloperidol, and potentiated haloperidol-induced dopamine increases in the prefrontal cortex, but not the nucleus accumbens. The use of α_2 adrenoceptor agonists in CAR was ineffective in restoring avoidance, rather, avoidance responses were decreased through this treatment, further suggesting that blockade of α_2 receptors contributes to an antipsychotic drug profile (Taboada et al., 1979).

Rationale

Because the α_2/D_2 hypothesis of atypicality has been supported nearly entirely by studies utilizing idazoxan as an α_2 adrenoceptor antagonist, there is a need to explore this hypothesis using other α_2 antagonists. Because idazoxan has been found to act as an agonist at 5-HT_{1A} receptors, it is unclear whether the action of α_2 adrenoceptor blockade is responsible for the antipsychotic action of idazoxan (Llado, Esteban, & Garcia-Sevilla, 1996).

These studies sought to determine if drugs that block of α_2 and D_2 receptors block a conditioned avoidance response, and if the effects of idazoxan at 5-HT_{1A} receptors may mediate antipsychotic effects. To test this hypothesis, a series of

compounds selective for α_2, D_2 and 5-HT_{1A} receptors were tested in the conditioned avoidance response task. First, the effects of dopamine D_2 receptor antagonism alone in the CAR task were evaluated. Dopamine D_2 receptor antagonists were then paired with an α_2 adrenoceptor antagonist to determine whether this combination would produce an antipsychotic effect in the CAR task. To block the 5-HT_{1A} receptor agonist properties of idazoxan, a 5-HT_{1A} receptor antagonist was given with the combined treatment of the D_2 receptor antagonist and idazoxan. The D_2 receptor antagonist and idazoxan treatment was also given in combination with an α_2 adrenoceptor agonist, in order to block the α_2 adrenoceptor antagonistic properties of idazoxan. A dopamine D_2 receptor antagonist was paired with a 5-HT_{1A} receptor agonist to determine whether combined dopamine D_2 receptor antagonism and 5-HT_{1A} receptor agonism is sufficient for producing an antipsychotic effect. It was hypothesized that 5-HT_{1A} agonism mediates the effects of idazoxan and a dopamine D_2 antagonist in the conditioned avoidance response task in rats.

Methods

Animals

Adult male Sprague-Dawley rats (Charles River, Inc, Portage, MI) were group housed in the Psychology Department rodent colony at Northern Michigan University for at least one week prior to experimental procedures. Animals' food rations were monitored so that excessive weight gain did not occur during the course of the study. Animals were not allowed to drop below 95% of their starting weight or exceed their healthy starting weight. The colony temperature and humidity were regulated with a 12 hour light/dark cycle (lights on at 7:00 a.m.). Rats had free access to water at all

times. All procedures were approved by the Northern Michigan University Institutional Animal Care and Use Committee (IACUC #094) and are consistent with the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996).

For the studies, eight subjects per group (16 subjects total) were sufficient to detect statistically significant effects (power = 0.80, $\alpha = 0.05$) given a medium magnitude of treatment effect (e.g., effect size = 0.30)(Jaccard and Becker, 1999, Statistics for the Behavioral Sciences). These studies used a blocked within-subjects research design, meaning that subjects served as their own experimental controls in 1 of 2 different treatment blocks. Dividing animals among the treatment blocks minimized discomfort by limiting the number of injections and test sessions to which the animals were exposed. Because 20% of subjects often fail to learn the procedures of the task, 20 subjects total (10 subjects per group) were used to insure that an adequate sample size would be available after the estimated failure rate.

Apparatus

A standard rat shuttle avoidance chamber with a tilting shock grid floor and guillotine dividing door was used for the Conditioned Avoidance Response task (Med Associates, model #ENV-010MC; 20.3 x 15.9 x 21.3 cm). The avoidance chamber was housed in a sound-attenuating chamber fixed with an exhaust fan which was on throughout all procedures to provide ventilation as well as to mask environmental noise. The apparatus was programmed and data was collected and recorded using MedPC software (version IV) provided by Med Associates.

Conditioned Avoidance Response

The procedures for conditioned avoidance response have been previously described (Wadenburg et al., 1998; Wadenburg et al., 2006). At the presentation of 80-dB white noise, animals had 10 seconds to move into the adjacent compartment of the shuttlebox. As this has a two-way avoidance procedure, animals could make an avoidance response from either side of the shuttlebox. If the rat remained for more than 10 seconds, a brief, low-intensity shock (0.3-0.5 mA of 0.5 second duration) was administered to the grid floor every 2.5 seconds until the rat escaped to the other compartment. However, if the rat failed to escape to the other compartment within 60 seconds, then the intermittent shock delivery was terminated (i.e., an escape failure) and the test session was terminated. Trials began at the onset of the white noise warning and were terminated when either 1) the rat successfully avoided the shock by crossing over into the adjacent compartment, 2) the rat failed to avoid the shock, but escaped the shock by crossing over to the adjacent compartment or 3) the rats failed to escape the shock after 60 seconds. The interval between trials varied randomly between 20 and 40 seconds. From this task, the behavioral variables of avoidance, escape, escape failure, before session crosses, and intertrial crosses were recorded by the MedPC software used to control the shuttlebox. Antipsychotic effects manifest as avoidance failures in the conditioned avoidance response task. That is, animals fail to move into the adjacent compartment at the presentation of the white noise. Instead, animals move to the adjacent compartment only when the shock is delivered. This failure is distinct from an escape failure wherein the shock fails to elicit an escape response and the animal remains in the chamber until the test session is terminated.

Escape failures are associated with tranquilizing effects or motor effects rather than antipsychotic effects.

Training

Animals were trained in the conditioned avoidance response task in daily sessions lasting 15 minutes, until a 90% successful avoidance rate was achieved over 3 consecutive sessions. The training session trials were identical to the procedures described above. Each training session consisted of 17 to 27 trials. Intertrial intervals varied randomly (VI 40).

Testing

Following completion of training, animals were given a 2 to 3 day rest period prior to the first test session. Afterwards, test sessions were conducted for one day followed by one day of rest and one day of training to ensure that drugs were no longer present from the previous test sessions. Thus, 2 test sessions and 2 training sessions were conducted per animal per week. At the beginning of each test day, all rats were given a 10 minute pretest session prior to drug or vehicle administration to insure that the animals were still performing the task accurately (at a 90% successful avoidance rate). Animals that failed to meet pretest criteria were not injected and given a day of rest, followed by additional training sessions as needed. After the pretest session, rats were given a subcutaneous injection of drug or vehicle, and then, after a 30 minute delay, a 10 minute test session consisting of 15 to 20 trials was conducted. The pretest and test session trials are identical to the procedures described above.

Treatments

Animals were assigned to one of two treatment blocks and received all treatments within their assigned block.

Table 2. Treatment blocks for 2 animal groups in the conditioned avoidance response task with the receptor mechanisms involved in drug action. Treatment orders were randomly assigned to each animal, with animals receiving all treatments within their assigned block. RAC = raclopride, HAL = haloperidol, IDX = idazoxan, YOH = yohimbine, WAY = WAY100635, GF = guanfacine, block = receptor blockade; antagonism, stim = receptor stimulation; agonism.

Table 2. Treatment groups for 2 animal groups in the conditioned avoidance response task. Treatments were randomized within groups.

TREATMENT GROUP	*RECEPTOR AFFINITY*
GROUP 1	
Dose response curves for all drugs	-
Vehicle	-
RAC + 8-OH-DPAT	D_2 block + 5-HT_{1A} stim
RAC + IDX + GF	D_2 block + α_2 block + α_2 stim
GROUP 2	
RAC + IDX	D_2 block + α_2 block
HAL + IDX	D_2 block + α_2 block
RAC + YOH	D_2 block + α_2 block
RAC + IDX + WAY	D_2 block + α_2 block + 5-HT_{1A} block

Treatment group one included dose-response curves for all compounds as well as one combination treatment aimed at exploring the possible role of 5-HT_{1A} agonism in antipsychotic action, along with the appropriate vehicle control groups. Treatment group two consisted of the α_2/D_2 combination groups, including the appropriate vehicle controls. Treatment group two also received a three-part treatment combination aimed at reversing the effects of the α_2/D_2 combination, along with the appropriate vehicle controls. Animals in both treatment groups were randomly assigned a treatment order within their block. Each treatment block was designed to have approximately the same number of treatments. All drugs, except

haloperidol and yohimbine, were dissolved in 0.9% saline. Haloperidol and yohimbine were dissolved in distilled water with a few drops of lactic acid and buffered back to a pH of 7 with sodium hydroxide.

The initial experiments performed in animal group 1 sought to identify an effective dose of a typical antipsychotic drug for reducing avoidance responding in the conditioned avoidance response task (CAR). Sub-effective (doses that fail to reduce CAR) and cataleptic (doses that result in escape failures) were also identified. Animals were randomly assigned the dose-response curves for the D_2 antagonists raclopride (0.025mg/kg, 0.05mg/kg, 0.075mg/kg) and haloperidol (0.0125mg/kg, 0.025mg/kg, 0.05mg/kg). To illustrate that α_2 adrenoceptor blockade alone does not produce an antipsychotic effect, both idazoxan (1.5mg/kg and 3.0mg/kg) and yohimbine (1.0mg/kg, 2.0mg/kg) were tested alone in the CAR task. Both a saline and a distilled water vehicle control were tested.

Once a dose-response curve was completed for raclopride and haloperidol, these drugs were paired with idazoxan in treatment group 2 to demonstrate that α_2/D_2 receptor blockade produces deficits in avoidance responding. Raclopride was also administered with the α_2 adrenoceptor antagonist, yohimbine, to further generalize the previous results. Therefore, previous studies using raclopride, haloperidol and idazoxan were replicated, and these results were generalized to the α_2 adrenoceptor antagonist, yohimbine. Appropriate vehicle controls for all treatment combinations were included in this treatment block. Animals were randomly assigned to a treatment schedule.

Treatment group two was also administered the 5-HT_{1A} antagonist, WAY100635 (WAY), with the raclopride/idazoxan combination. A very low dose of WAY (0.05mg/kg) as well as a higher dose (0.2mg/kg) was tested to explore the cataleptic effects of 5-HT_{1A} blockade when administered with the raclopride/idazoxan combination. Appropriate vehicle controls for these three-part combinations were included in this treatment block. Treatment group one received a three-part treatment combination to block the α_2 adrenoceptor component of idazoxan. This was achieved through the use of guanfacine (0.8mg/kg), an α_2 adrenoceptor agonist.

Finally, treatment group one included a combination treatment of a sub-effective dose of raclopride and the 5-HT_{1A} agonist, (+)8-OH-DPAT (0.04mg/kg and 0.08mg/kg) as well as the appropriate vehicle controls for this combination.

Data Analysis

Data were analyzed using GraphPad Prism version 5.01 (GraphPad Software, Inc., La Jolla, CA). Percent avoidance, percent escape, intertrial crosses and escape failures were obtained after every training, pretesting, and testing session. Percent values were calculated by dividing the number of avoidance or escape responses by the total number of trials. Percent avoidance, percent escape, and intertrial crosses for treatments within groups were compared using a repeated measures one-way analysis of variance (ANOVA) followed by a Tukey's multiple comparison test post-hoc analysis. Escape failures were analyzed using a Friedman test followed by Dunn's multiple comparison post-hoc analysis. Nonparametric analyses were used to evaluate escape failures, as only one escape failure could occur during each test session.

Results

Training

Of the 20 rats used in this study, 16 met the training criteria. Four animals were eliminated from the study after failing to meet training criteria following 30 days of training. Animals that had an escape failure on the first training day were given additional training wherein the experimenter moved the animal into the other compartment at the presentation of the white noise to facilitate responding. This training was discontinued when the animal had avoidance responses on three successive trials. Seven animals had escape failures during the first training session, while the remaining nine completed training without having escape failures. The animals that were retained for the study had 3 successive training periods of 90% avoidance response or better after an average of 7.3 days of training (+/- 2.1 standard error of the mean (SEM); Figure 1).

Figure 1. Acquisition of conditioned avoidance responding shown as mean percent avoidance responding (+/- SEM) over 12 consecutive training days for animals in the conditioned avoidance response task. Numbers in parentheses indicate N, otherwise, N = 16.

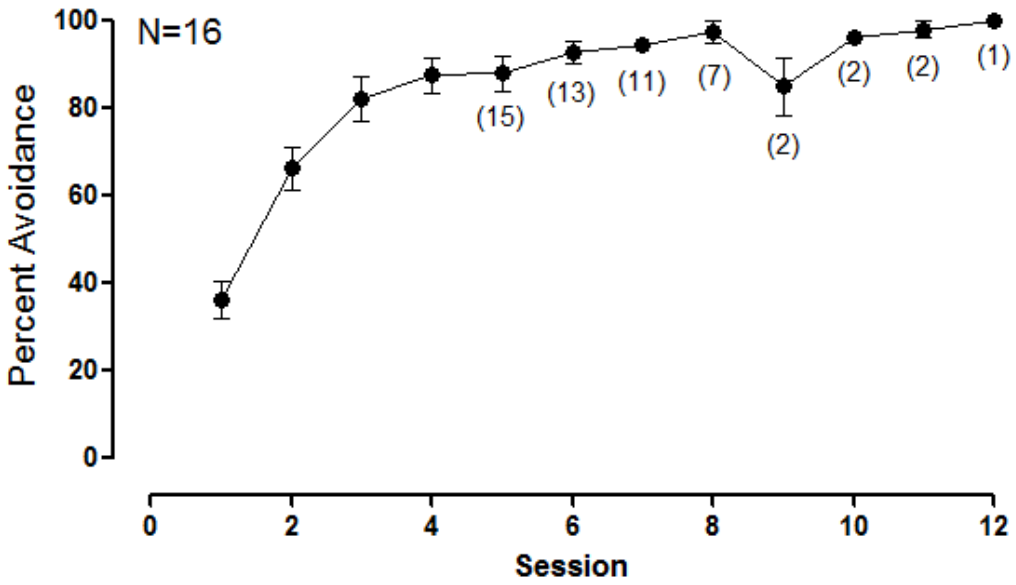

Figure 1. Conditioned avoidance response training over 12 consecutive training days.

Numbers in parentheses indicate N, otherwise, N = 16.

Testing

Dose Response Curves

Raclopride

The data for avoidance responding for raclopride (RAC; 0.025, 0.05, and 0.075 mg/kg) are shown in figure 2. Data for percent escape, escape failures and intertrial crosses for raclopride are shown in table 3. RAC reduced avoidance responding in a dose-dependent manner ($F(3, 21) = 20.29$, $p<0.0001$). RAC 0.05 mg/kg produced significantly lower percent avoidance than SAL ($p<0.01$). RAC 0.075 mg/kg also reduced avoidance significantly compared to SAL ($p<0.001$) as well as RAC 0.05 mg/kg ($p<0.05$). Percent escape increased in a dose-dependent manner ($F(3, 21) = 20.94$, $p<0.0001$). RAC 0.05 mg/kg produced significantly greater percent escape than SAL ($p<0.05$) and RAC 0.075 mg/kg produced significantly greater escape responses than SAL ($p<0.0001$). RAC 0.075 mg/kg produced significantly more escape responses than RAC 0.05 mg/kg ($p<0.05$). Escape failures were not significantly different overall (insert $\chi^2(3) = 4.800$, $p = 0.1870$). Intertrial crosses differed significantly overall ($F(3, 21) = 39.43$, $p<0.0001$), with all doses of RAC having significantly fewer intertrial crosses than SAL.

Haloperidol

The data for avoidance responding for haloperidol (HAL; 0.0125, 0.025, and 0.05 mg/kg) are shown in figure 3. Data for percent escape, escape failures and intertrial crosses for HAL are shown in table 4. HAL reduced avoidance responding in a dose-dependent manner ($F(3, 21) = 19.47$, $p<0.0001$). HAL 0.025 mg/kg and HAL 0.05 mg/kg produced significantly greater avoidance responding than H_20/LAC

($p<0.01$). HAL increased escape responses in a dose-dependent manner ($F(3, 21) = 34.59$, $p<0.0001$). HAL 0.025 mg/kg and HAL 0.05 mg/kg produced significantly more escape responses than H_20/LAC ($p<0.001$). HAL 0.025 mg/kg and HAL 0.05 mg/kg were no different in either percent avoidance or percent escape. There was an overall significance for number of escape failures across groups ($\chi^2(3) = 14.76$, $p<0.01$), however, Dunn's multiple comparisons post-hoc analysis found no significant difference between treatments. Overall, the number of intertrial crosses between groups was significant ($F(3, 21) = 35.82$, $p<0.0001$), with all HAL treatments having significantly fewer crosses than H_20/LAC ($p<0.001$).

Idazoxan

The data for avoidance responding for idazoxan (IDX; 1.5 and 3.0 mg/kg) are shown in figure 4. Data for percent escape, escape failures and intertrial crosses for IDX are shown in table 5. No statistical difference in percent avoidance was found between either IDX treatment and SAL ($F(2, 14) = 1.136$, $p = 0.3490$). Intertrial crosses differed significantly overall ($F(2, 14) = 5.081$, $p<0.05$), with both IDX treatments having fewer crosses than SAL ($p<0.05$). Each dose of IDX produced one escape failure.

Yohimbine

The data for avoidance responding for yohimbine (YOH; 1.0 and 2.0 mg/kg) are shown in figure 5. Data for percent escape, escape failures and intertrial crosses for YOH are shown in table 6. No significant difference in percent avoidance was found between either YOH treatment and SAL ($F(2, 14) = 0.4667$, $p=0.6365$), and no treatment had escape failures. No significant differences in intertrial crosses were

found between either YOH treatment and SAL ($F(2, 14) = 0.2946$, $p = 0.7493$). Neither dose of YOH produced escape failures.

WAY100635

The data for avoidance responding for WAY100635 (WAY; 0.05 and 0.2 mg/kg) are shown in figure 6. Data for percent escape, escape failures and intertrial crosses for WAY are shown in table 7. WAY and SAL did not differ significantly in percent avoidance ($F(2, 14) = 1.862$, $p = 0.1918$). Intertrial crosses differed significantly across treatments ($F(2, 14) = 7.527$, $p<0.01$) with WAY 0.05 mg/kg producing significantly fewer crosses than SAL ($p<0.05$) and WAY 0.2 mg/kg having significantly fewer crosses than SAL ($p<0.01$). Neither dose of WAY produced escape failures

Figure 2. Mean percent avoidance responding (+/- SEM) for raclopride (RAC) 0.025, 0.05, and 0.075 mg/kg and saline. RAC 0.05 mg/kg produced significantly lower percent avoidance than saline (*$p<0.05$). RAC 0.075 mg/kg produced significantly lower percent avoidance than saline (**$p<0.01$).

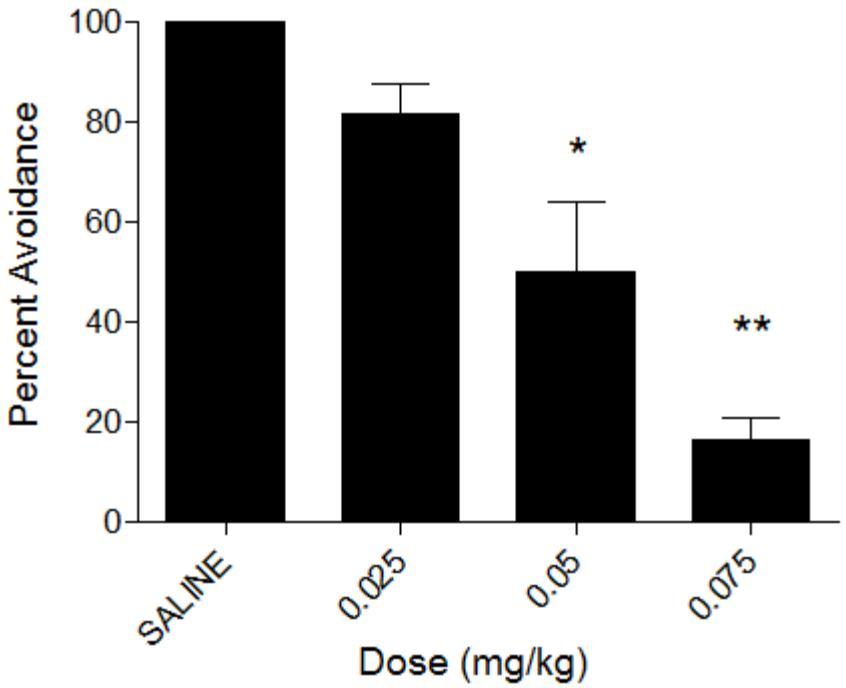

Figure 2. Mean percent avoidance responding (+/- SEM) for raclopride.

Table 3. Mean percent escape responding, number of escape failures and mean intertrial crosses for raclopride (RAC) 0.025, 0.05, and 0.075 mg/kg and saline

Treatment	*%Escape*	*Escape Failures*	*Intertrial Crosses*
Saline	0	0	17.8
RAC 0.025 mg/kg	17.6	0	3.1
RAC 0.05 mg/kg	45.5	2	0.1
RAC 0.075 mg/kg	80.4	2	0

Table 3. Mean percent escape, number of escape failures and intertrial crosses for raclopride.

Figure 3. Mean percent avoidance responding (+/- SEM) for haloperidol (HAL) 0.0125, 0.025, and 0.05 mg/kg and vehicle (H_20/LAC). HAL 0.025 and 0.05 mg/kg had significantly lower percent avoidance than H_20/LAC (***$p<0.001$).

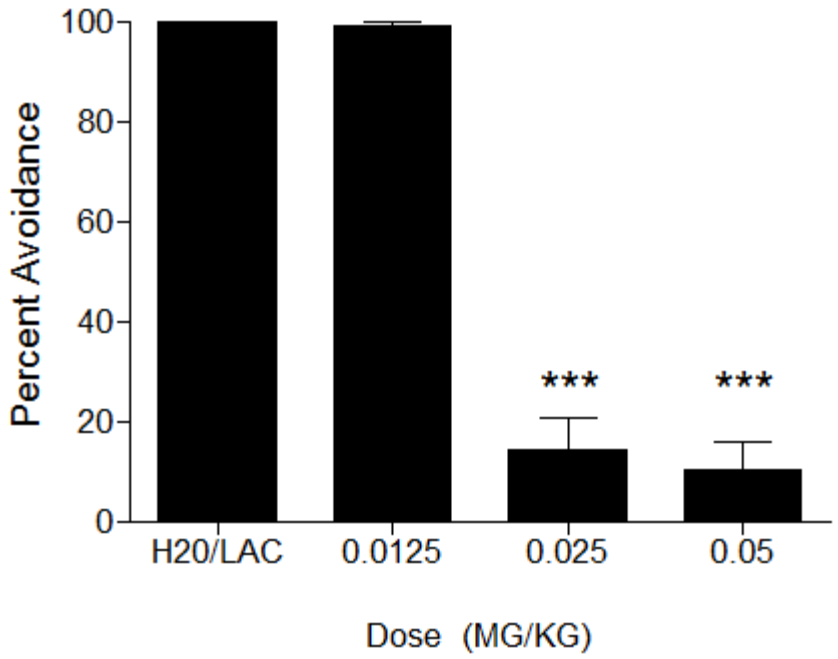

Figure 3. Mean percent avoidance responding (+/- SEM) for haloperidol.

Table 4. Mean percent escape responding, number of escape failures and mean intertrial crosses for haloperidol 0.0125, 0.025, and 0.05 mg/kg and vehicle (H_20/LAC).

Treatment	*%Escape*	*Escape Failures*	*Intertrial Crosses*
H_20/LAC	0	0	14.9
HAL 0.0125 mg/kg	0.8	0	4.1
HAL 0.025 mg/kg	69.9	5	0
HAL 0.05 mg/kg	64.8	6	0

Table 4. Mean percent escape, number of escape failures and intertrial crosses for haloperidol.

Figure 4. Mean percent avoidance responding (+/- SEM) for idazoxan (IDX) 1.5 and 3.0 mg/kg and saline.

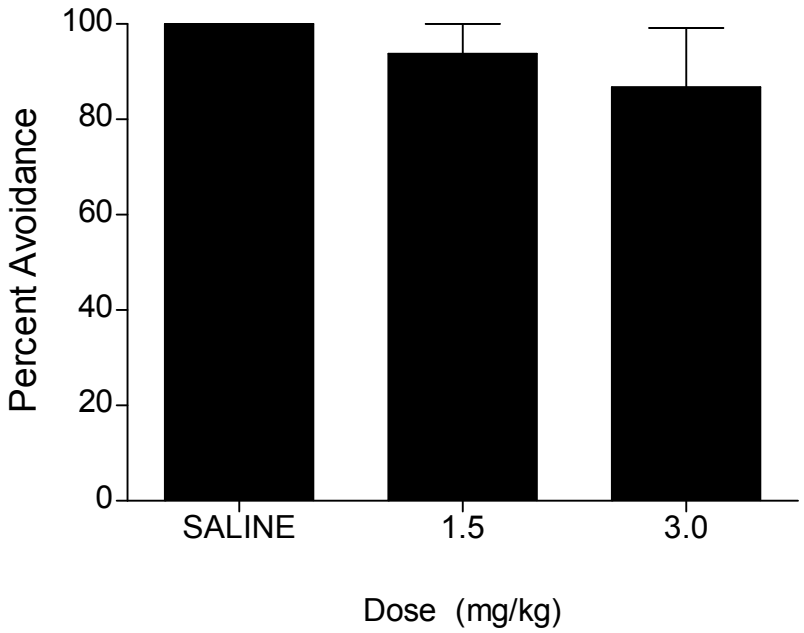

Figure 4. Mean percent avoidance responding (+/- SEM) for idazoxan.

Table 5. Shows mean percent escape responding, number of escape failures, and mean intertrial crosses for idazoxan (IDX) 1.5 and 3.0 mg/kg and saline.

Treatment	*%Escape*	*Escape Failures*	*Intertrial Crosses*
Saline	0	0	17.8
IDX 1.5 mg/kg	0	1	11.5
IDX 3.0 mg/kg	0.7	1	11.5

Table 5. Mean percent escape, number of escape failures and intertrial crosses for idazoxan.

Figure 5. Shows mean percent avoidance responding (+/- SEM) for yohimbine (YOH) 1.0 and 2.0 mg/kg and vehicle (H_20/LAC).

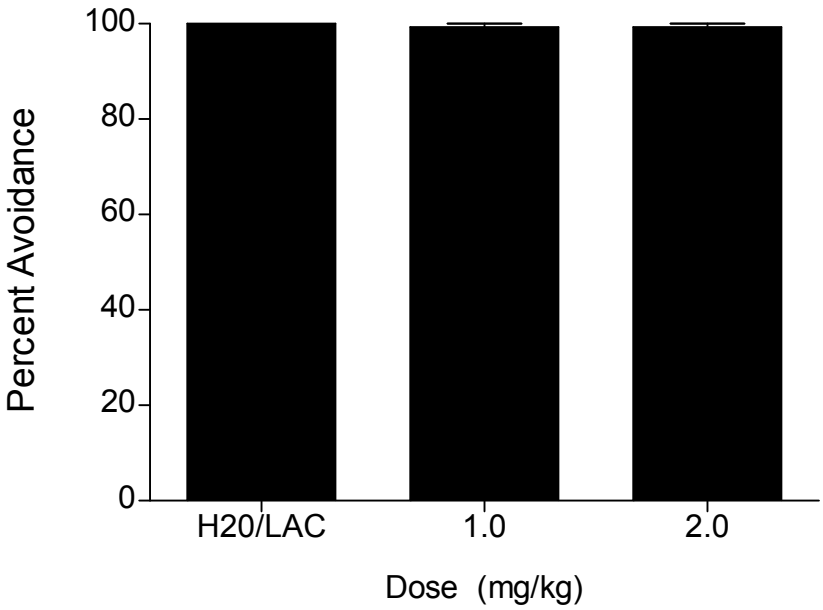

Figure 5. Mean percent avoidance responding (+/- SEM) for yohimbine.

Table 6. Mean percent escape responding, number of escape failures and mean number of intertrial crosses for yohimbine (YOH) 1.0 and 2.0 mg/kg.

Treatment	*%Escape*	*Escape Failures*	*Intertrial Crosses*
H_20/LAC	0	0	14.9
YOH 1.0 mg/kg	0.7	0	18
YOH 2.0 mg/kg	0.7	0	16.4

Table 6. Mean percent escape, number of escape failures and intertrial crosses for yohimbine.

Figure 6. Mean percent avoidance responding (+/- SEM) for WAY100635 (WAY) 0.05 and 0.2 mg/kg and saline.

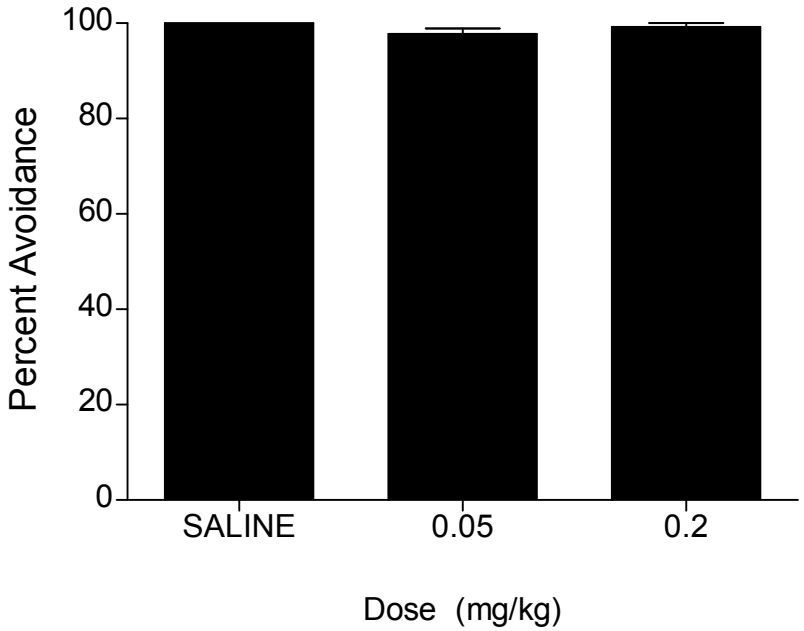

Figure 6. Mean percent avoidance responding (+/- SEM) for WAY100635.

Table 7. Mean percent escape responses, number of escape failures and mean number of intertrial crosses for WAY100635 (WAY) 0.05 and 0.2 mg/kg and saline.

Treatment	*%Escape*	*Escape Failures*	*Intertrial Crosses*
Saline	0	0	17.8
WAY 0.05 mg/kg	2.3	0	10.6
WAY 0.2 mg/kg	0.8	0	9.9

Table 7. Mean percent escape, number of escape failures and intertrial crosses for WAY100635.

Paired Treatments

Raclopride + Idazoxan

The data for avoidance responding for RAC+IDX (RAC = 0.025 mg/kg, IDX = 1.5 mg/kg) are shown in figure 7. Data for percent escape, escape failures and intertrial crosses for RAC+IDX are shown in table 8. Percent avoidance differed significantly across treatments ($F(3, 21) = 11.63$, $p<0.0001$). The vehicle controls did not differ significantly from one another. RAC+IDX produced lower percent avoidance than RAC+SAL ($p<0.01$), IDX+SAL ($p<0.001$) and SAL+ SAL ($p<0.001$). Percent escape differed significantly across treatments ($F(3, 21) = 10.84$, $p<0.001$). RAC+IDX produced significantly more escape responses than SAL+SAL ($p<0.001$), IDX+SAL ($p<0.001$), and RAC+SAL ($p<0.05$). Number of escape failures did not differ significantly across treatments ($\chi^2(3) = 6.000$, $p = 0.116$). Intertrial crosses differed significantly across groups ($F(3, 21) = 10.13$, $p<0.001$). RAC+IDX produced significantly fewer crosses than IDX+SAL ($p<0.001$).

Raclopride + Yohimbine

The data for avoidance responding for RAC+YOH (RAC = 0.025 mg/kg, YOH = 1.0 or 2.0 mg/kg) are shown in figure 8. Data for percent escape, escape failures and intertrial crosses for RAC+YOH are shown in table 9. Percent avoidance differed significantly overall ($F(5, 35) = 11.69$, $p<0.0001$). RAC+YOH 1.0 mg/kg produced significantly lower percent avoidance than SAL+H_20/LAC and SAL+YOH 1.0 mg/kg ($p<0.05$). RAC+YOH 2.0 mg/kg produced significantly lower percent avoidance than SAL+H_20/LAC ($p<0.001$) and SAL+YOH 2.0 mg/kg ($p<0.001$) Percent escape also differed significantly overall ($F(5, 35) = 11.38$, $p<0.0001$) with

RAC+YOH 1.0mg/kg producing significantly more escape responses than SAL+H_20/LAC and SAL+YOH 1.0 mg/kg ($p<0.05$). RAC+YOH 2.0 mg/kg produced significantly more escape responses than SAL+H_20/LAC ($p<0.001$) and SAL+YOH 2.0 mg/kg ($p<0.001$). Number of intertrial crosses differed significantly overall ($F(5, 35) = 8.709$, $p<0.0001$). RAC+YOH 1.0 mg/kg produced significantly fewer intertrial crosses than SAL+YOH 1.0 mg/kg ($p<0.05$) and RAC+YOH 2.0 mg/kg produced significantly fewer intertrial crosses than SAL+YOH 2.0 mg/kg ($p<0.001$).

Haloperidol + Idazoxan

The data for avoidance responding for HAL+IDX (HAL = 0.0125 mg/kg, IDX = 1.5 mg/kg) are shown in figure 9. Data for percent escape, escape failures and intertrial crosses for HAL+IDX are shown in table 10. Percent avoidance was significant overall ($F(5, 35) = 2.734$, $p<0.05$), however, post-hoc analysis found no significant differences between pairs. Percent escape was significant overall ($F(5, 35) = 2.585$, $p<0.05$), however, post-hoc analysis found no significant differences between pairs. Intertrial crosses did not differ significantly overall ($F(5, 35) = 1.310$, $p=0.2825$).

Raclopride + 8-OH-DPAT

The data for avoidance responding for RAC+8-OH-DAPT (RAC = 0.025 mg/kg, 8-OH-DPAT (DPAT) = 0.04 or 0.08 mg/kg) are shown in figure 10. Data for percent escape, escape failures and intertrial crosses for RAC+DPAT are shown in table 11. Avoidance responding differed significantly across treatments ($F(6, 42) = 75.74$, $p<0.0001$) with RAC+DPAT 0.04 mg/kg producing significantly lower percent

avoidance than SAL+DPAT 0.04 mg/kg and DPAT 0.04 mg/kg ($p<0.001$). RAC+DPAT 0.08 mg/kg produced significantly lower percent avoidance than SAL+DPAT 0.08 mg/kg and DPAT 0.08 mg/kg ($p<0.001$). Percent escape differed significantly overall ($F(6, 42) = 31.57$, $p<0.0001$). RAC+DPAT 0.04 mg/kg produced significantly more escape responses than SAL+DPAT 0.04 mg/kg and DPAT 0.04 mg/kg ($p<0.001$) and RAC+DPAT 0.08 mg/kg produced significantly more escape responses than SAL+DPAT 0.08 mg/kg and DPAT 0.08 mg/kg ($p<0.001$). Escape failures differed significantly overall ($\chi^2(6) = 24.00$, $p<0.001$), however, post-hoc analysis did not find any significant differences between pairs. Intertrial crosses differed significantly overall ($F(6, 42) = 12.63$, $p<0.0001$). RAC+DPAT 0.04 mg/kg produced significantly fewer intertrial crosses than SAL+DPAT 0.04 mg/kg and DPAT 0.04 mg/kg ($p<0.001$). RAC+DPAT 0.08 mg/kg produced significantly fewer intertrial crosses than SAL+DPAT 0.08 mg/kg ($p<0.01$) and DPAT 0.08 mg/kg ($p<0.001$).

Figure 7. Mean percent avoidance responding for raclopride (0.025 mg/kg) + idazoxan (1.5 mg/kg) and saline contol (SAL). RAC+IDX had significantly lower percent avoidance than SAL+SAL (***$p<0.001$) and RAC+SAL (##$p<0.01$).

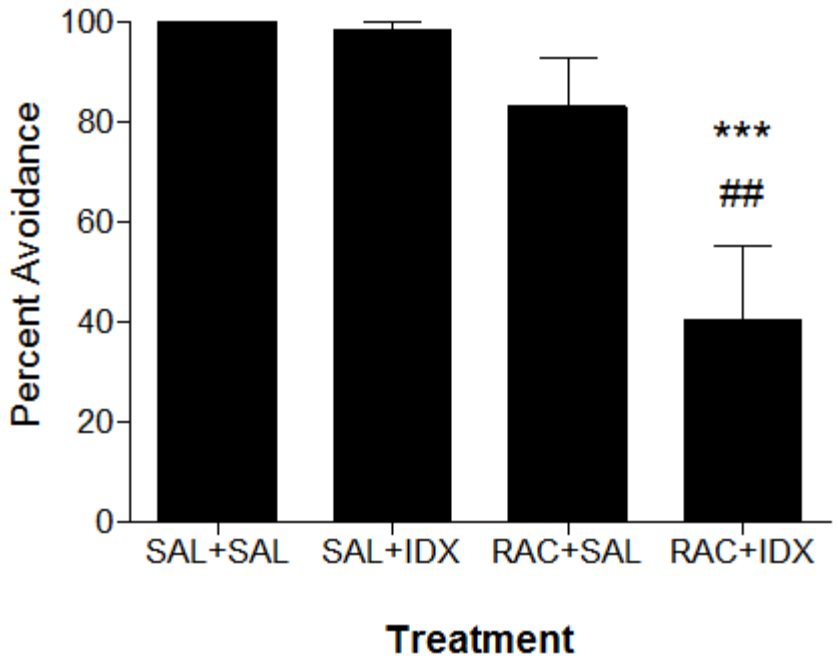

Figure 7. Mean percent avoidance responding (+/- SEM) for raclopride + idazoxan.

Table 8. Mean percent escape responses, number of escape failures and mean number of intertrial crosses for raclopride (0.025 mg/kg) + idazoxan (1.5 mg/kg) (RAC+IDX) and vehicle controls (SAL = saline).

Treatment	*%Escape*	*Escape Failures*	*Intertrial Crosses*
SAL+SAL	0	0	9.5
SAL+IDX	1.5	0	15
RAC+SAL	16.9	0	3
RAC+IDX	56	2	2.6

Table 8. Mean percent escape, number of escape failures and intertrial crosses for raclopride + idazoxan.

Figure 8. Mean percent avoidance responding (+/- SEM) for raclopride (0.025 mg/kg) + yohimbine (1.0 and 2.0 mg/kg) (RAC+YOH) and vehicle controls (SAL = saline, H_20/LAC = water and lactic acid). RAC+YOH 1.0 mg/kg had significantly lower percent avoidance than SAL+H_20/LAC (*$p<0.05$) and SAL+YOH 1.0 mg/kg (#$p<0.05$). RAC+YOH 2.0 mg/kg had significantly lower percent avoidance than SAL+H_20/LAC (***$p<0.001$) and SAL+YOH 2.0 mg/kg (###$p<0.001$).

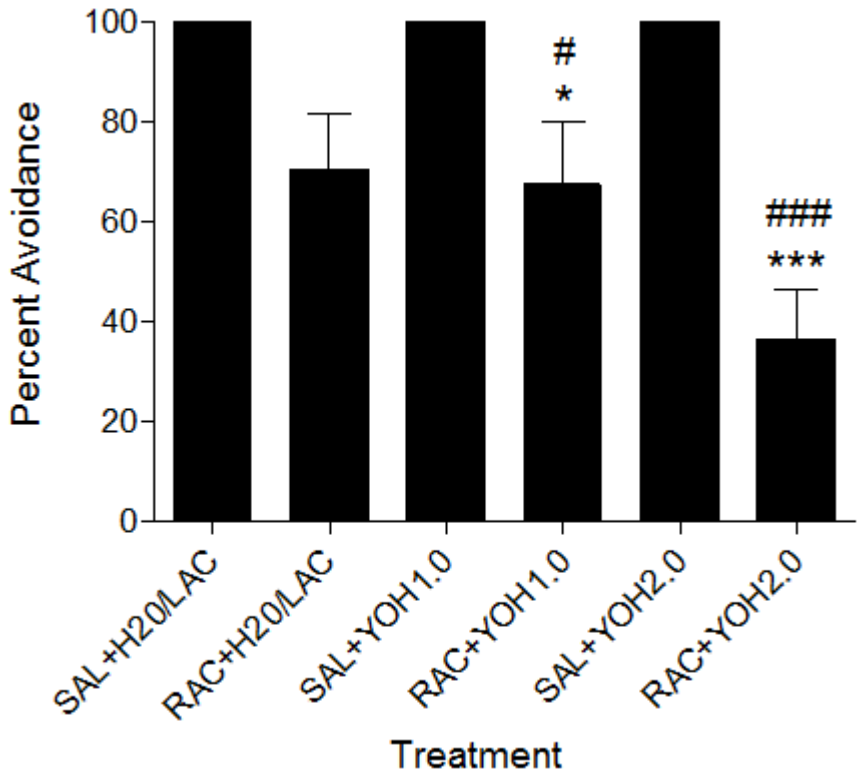

Figure 8. Mean percent avoidance responding (+/- SEM) for raclopride + yohimbine.

Table 9. Mean percent escape responding, number of escape failures and mean number of intertrial crosses for raclopride (0.025 mg/kg) + yohimbine (1.0 and 2.0 mg/kg) and vehicle controls (SAL = saline, H_20/LAC = water with lactic acid).

Treatment	*%Escape*	*Escape Failures*	*Intertrial Crosses*
SAL+H_20/LAC	0	0	11.9
RAC+H_20/LAC	29.7	0	2.5
SAL+YOH 1.0 mg/kg	0	0	13.3
RAC+YOH 1.0 mg/kg	32.6	0	2.8
SAL+YOH 2.0 mg/kg	0	0	16.5
RAC+YOH 2.0 mg/kg	60.5	2	1.6

Table 9. Mean percent escape, number of escape failures and intertrial crosses for raclopride + yohimbine.

Figure 9. Mean percent avoidance (+/- SEM) for haloperidol (0.0125 mg/kg) + idazoxan (1.5 mg/kg) (HAL+IDX) and vehicle controls (SAL = saline, H_20/LAC = water and lactic acid).

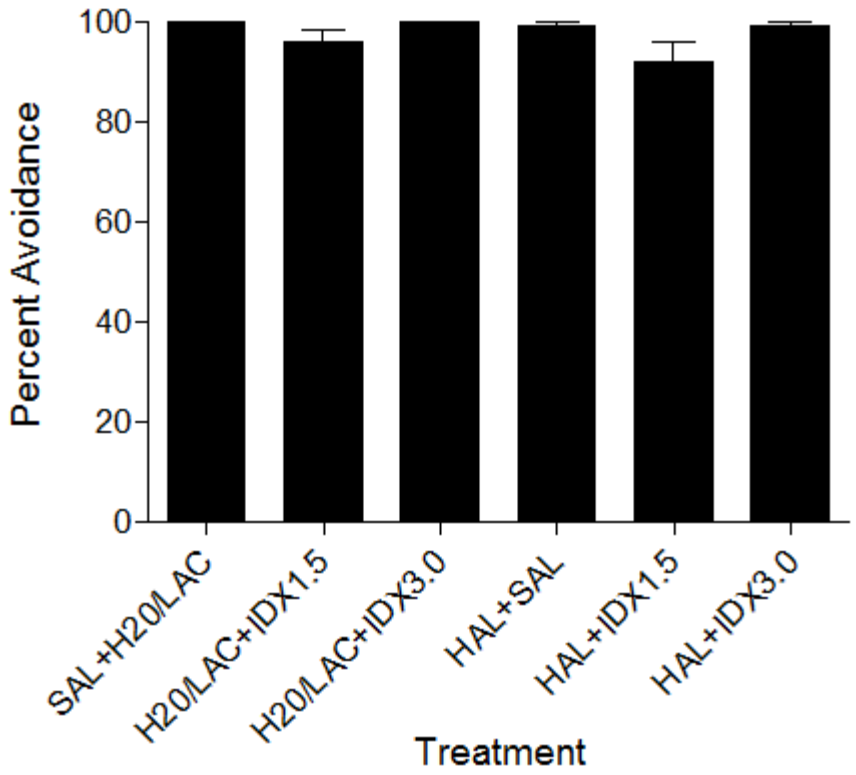

Figure 9. Mean percent avoidance responding (+/- SEM) for haloperidol + idazoxan.

Table 10. Mean percent escape, number of escape failures and mean number of intertrial crosses for haloperidol (0.0125 mg/kg) + idazoxan (1.5 mg/kg) (HAL+IDX) and vehicle controls (SAL = saline, H_20/LAC = water with lactic acid).

Treatment	*%Escape*	*Escape Failures*	*Intertrial Crosses*
SAL+H20/LAC	0	0	11.9
H_20/LAC+IDX 1.5 mg/kg	3.8	0	11.5
H_20/LAC+IDX 3.0 mg/kg	0	0	17.5
HAL+SAL	0.7	0	10.3
HAL+IDX 1.5 mg/kg	8.0	0	11.1
HAL+IDX 3.0 mg/kg	0.8	0	12.3

Table 10. Mean percent escape, number of escape failures and intertrial crosses for haloperidol + idazoxan.

Figure10. Mean percent avoidance (+/- SEM) for raclopride (0.025 mg/kg) + 8-OH-DPAT (0.04 and 0.08 mg/kg) (RAC+DPAT) and vehicle controls (SAL = saline). RAC+DPAT 0.08 mg/kg had significantly lower percent avoidance than SAL+SAL (***$p<0.001$) and SAL+DPAT 0.08 mg/kg (###$p<0.001$). RAC+DPAT 0.04 mg/kg had significantly lower percent avoidance than SAL+SAL (***$p<0.001$) and SAL+DPAT 0.04 mg/kg (###$p<0.001$).

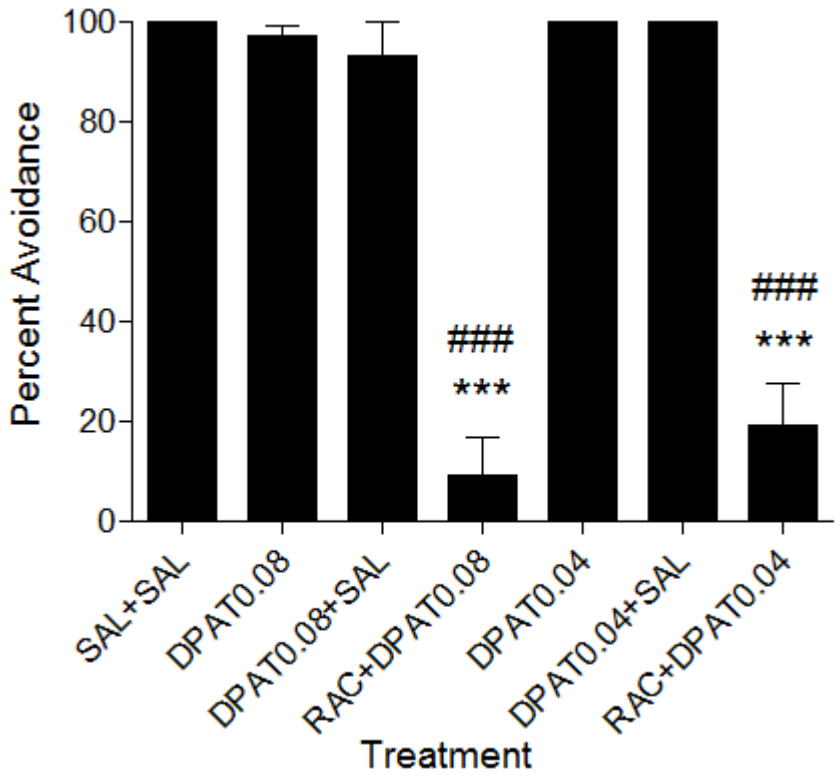

Figure 10. Mean percent avoidance responding (+/- SEM) for raclopride + (+) 8-OH-DPAT.

Table 11. Mean percent escape responding, number of escape failures and mean number of intertrial crosses for raclopride (0.025 mg/kg) + 8-OH-DPAT (0.04 and 0.08 mg/kg) (RAC+DPAT) and vehicle controls (SAL = saline).

Treatment	*%Escape*	*Escape Failures*	*Intertrial Crosses*
SAL+SAL	0	0	12.1
DPAT 0.08 mg/kg	2.8	0	13.9
DPAT 0.08 mg/kg+SAL	6.7	0	12.4
RAC+DPAT 0.08 mg/kg	73.5	4	0.4
DPAT 0.04 mg/kg	0	0	15
DPAT 0.04 mg/kg+SAL	0	0	16.6
RAC+DPAT 0.04 mg/kg	61.3	4	0.3

Table 11. Mean percent escape, number of escape failures and intertrial crosses for raclopride + (+)8-OH-DPAT.

Three Part Combinations

Raclopride + Idazoxan + WAY100635(0.05 mg/kg)

The data for avoidance responding for RAC+IDX+WAY (RAC = 0.025 mg/kg, IDX = 1.5 mg/kg, WAY = 0.05 mg/kg) are shown in figure 11. Data for percent escape, escape failures and intertrial crosses for RAC+IDX+WAY are shown in table 12. Avoidance responses differed significantly overall ($F(5, 35) = 17.98$, $p<0.0001$). RAC+IDX+WAY produced significantly lower avoidance responding than SAL+SAL+WAY ($p<0.001$), RAC+SAL+WAY ($p<0.05$), and SAL+IDX+WAY ($p<0.001$). RAC+SAL+WAY produced significantly lower percent avoidance than SAL+IDX+WAY ($p<0.05$) and SAL+SAL+WAY ($p<0.05$). RAC+IDX produced significantly lower percent avoidance than SAL+SAL+WAY ($p<0.001$) and SAL+IDX+WAY ($p<0.001$).

Escape responses differed significantly overall ($F(5, 35) = 17.08$, $p<0.0001$). RAC+IDX+WAY produced significantly more escape responses SAL+SAL+WAY ($p<0.001$), RAC+SAL+WAY ($p<0.05$), and SAL+IDX+WAY ($p<0.001$), and SAL+SAL+SAL ($p<0.001$). RAC+SAL+WAY produced significantly more escape responses than SAL+SAL+WAY ($p<0.01$), and SAL+IDX+WAY ($p<0.01$). RAC+IDX produced significantly more escape responses than SAL+IDX+WAY ($p<0.001$), and SAL+SAL+WAY ($p<0.001$). Number of escape failures did not differ significantly across treatments ($\chi^2(5) = 10.00$, $p = 0.0752$). Intertrial crosses differed significantly across treatments ($F(5, 35) = 9.249$, $p<0.0001$) with RAC+IDX+WAY producing significantly fewer crosses than SAL+SAL+SAL ($p<0.001$), and SAL+SAL+WAY ($p<0.001$).

Raclopride + Idazoxan + WAY100635(0.2 mg/kg)

The data for avoidance responding for RAC+IDX+WAY (RAC = 0.025 mg/kg, IDX = 1.5 mg/kg, WAY = 0.2 mg/kg) are shown in figure 12. Data for percent escape, escape failures and intertrial crosses for RAC+IDX+WAY are shown in table 13. Avoidance responses differed significantly overall ($F(5, 35) = 17.64$, $p<0.0001$). RAC+IDX+WAY produced significantly fewer avoidance responses than SAL+SAL+SAL ($p<0.001$), SAL+SAL+WAY ($p<0.001$), and SAL+IDX+WAY ($p<0.001$). RAC+SAL+WAY produced significantly fewer avoidance responses than SAL+SAL+SAL ($p<0.001$), SAL+SAL+WAY ($p<0.001$), and SAL+IDX+WAY ($p<0.001$).

Escape responses differed significantly overall ($F(5, 35) = 19.46$, $p<0.0001$). RAC+IDX+WAY produced significantly more escape responses than SAL+SAL+SAL ($p<0.001$), SAL+SAL+WAY ($p<0.001$), and SAL+IDX+WAY ($p<0.001$). RAC+SAL+WAY produced significantly more escape responses than SAL+SAL+SAL ($p<0.001$), SAL+SAL+WAY ($p<0.001$), and SAL+IDX+WAY ($p<0.001$). Escape failures did not differ significantly across treatments ($\chi^2(5) = 10.63$, $p<0.0593$). Intertrial crosses differed significantly overall ($F(5, 35) = 4.769$, $p<0.01$) with RAC+IDX+WAY producing significantly fewer intertrial crosses than SAL+SAL+SAL ($p<0.01$).

Raclopride + Idazoxan + Guanfacine

The data for avoidance responding for RAC+IDX+Guanfacine (RAC = 0.025 mg/kg, IDX = 1.5 mg/kg, Guanfacine (GF) = 0.08 mg/kg) are shown in figure 13. Data for percent escape, escape failures and intertrial crosses for RAC+IDX+GF are

shown in table 14. Avoidance responses differed significantly overall ($F(5, 35) = 4.735$, $p<0.01$). RAC+IDX+GF produced higher percent avoidance than RAC+IDX+SAL ($p<0.05$). Escape responses differed significantly overall ($F(5, 35) = 4.591$, $p<0.01$). RAC+IDX+SAL produced significantly more escape responses than SAL+SAL+SAL ($p<0.05$), SAL+IDX+GF ($p<0.05$), and GF alone ($p<0.05$). Escape failures did not differ significantly across treatments ($\chi^2(5) = 5.000$, $p = 0.4159$). Intertrial crosses differed significantly overall ($F(5, 35) = 4.994$, $p<0.01$), with SAL+IDX+GF differing significantly from GF alone ($p<0.05$), RAC+SAL+GF ($p<0.01$) and RAC+IDX+SAL ($p<0.05$).

Figure 11. Mean percent avoidance (+/- SEM) for raclopride (0.025 mg/kg) + idazoxan (1.5 mg/kg) + WAY100635 (0.05 mg/kg) (RAC+IDX+WAY0.05) and vehicle controls (SAL = saline). RAC+IDX and RAC+IDX+WAY0.05 had significantly lower percent avoidance than SAL+SAL+SAL (***$p<0.001$). RAC+SAL+WAY0.05 had significantly lower percent avoidance than SAL+SAL+SAL (*$p<0.05$).

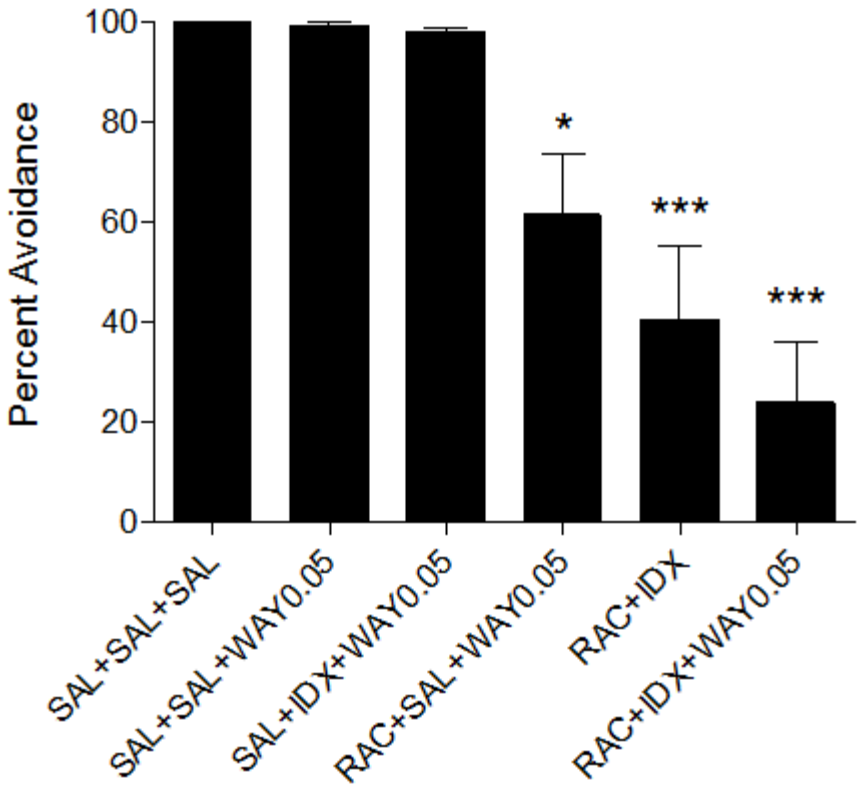

Figure 11. Mean percent avoidance responding (+/- SEM) for raclopride + idazoxan + WAY100635 (0.05 mg/kg).

Table 12. Mean percent escape for raclopride (0.025 mg/kg) + idazoxan (1.5 mg/kg) + WAY100635 (0.05 mg/kg) (RAC+SAL+WAY) and vehicle controls (SAL =saline).

Treatment	*%Escape*	*Escape Failures*	*Intertrial Crosses*
SAL+SAL+SAL	1.4	0	12.4
SAL+SAL+WAY 0.05 mg/kg	0.7	0	12
SAL+IDX+WAY 0.05 mg/kg	2.1	0	6.6
RAC+SAL+WAY 0.05 mg/kg	36.1	1	1.9
RAC+IDX	56	2	2.6
RAC+IDX+WAY 0.05 mg/kg	68	3	1.9

Table 12. Mean percent escape, number of escape failures and intertrial crosses for raclopride + idazoxan + WAY100635 (0.05 mg/kg).

Figure 13. Mean percent avoidance (+/- SEM) for raclopride (0.025 mg/kg) + idazoxan (1.5 mg/kg) + WAY100635 (0.2 mg/kg) (RAC+IDX+WAY0.2) and vehicle controls (SAL =saline). RAC+SAL+WAY0.2, RAC+IDX, and RAC+IDX+WAY0.2 had significantly lower percent avoidance than SAL+SAL+SAL (***$p<0.001$).

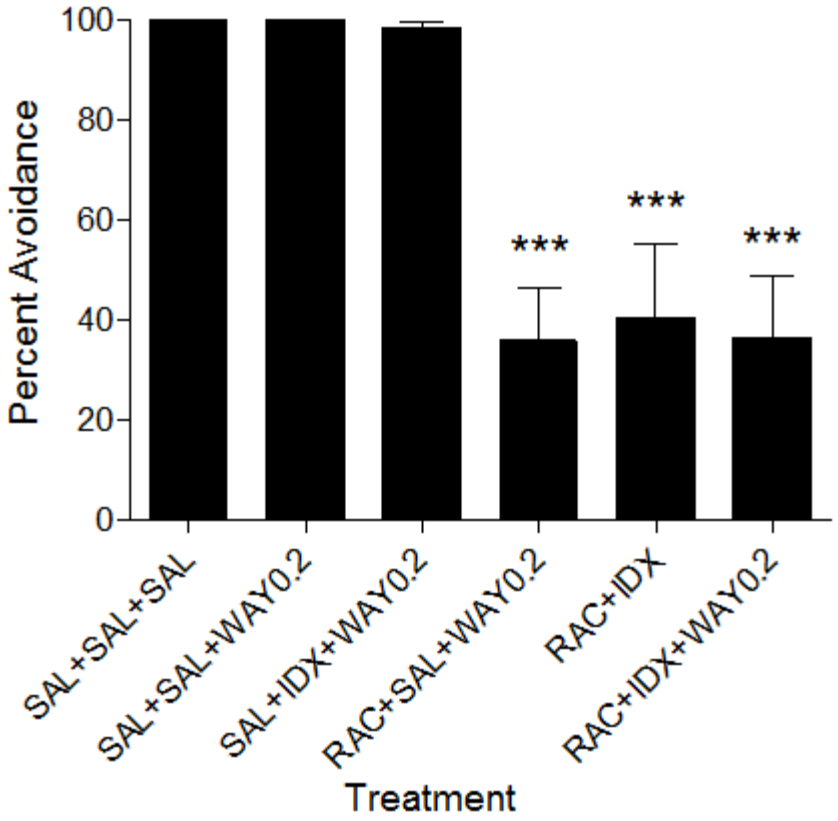

Figure 13. Mean percent avoidance responding (+/- SEM) for raclopride + idazoxan + WAY100635 (0.2 mg/kg).

Table 13. Mean percent escape, number of escape failures and mean number of intertrial crosses for raclopride (0.025 mg/kg) + idazoxan (1.5 mg/kg) + WAY100635 (0.2 mg/kg) (RAC+IDX+WAY) and vehicle controls (SAL = saline).

Treatment	*%Escape*	*Escape Failures*	*Intertrial Crosses*
SAL+SAL+SAL	1.4	0	12.4
SAL+SAL+WAY 0.2 mg/kg	0	0	6.4
SAL+IDX+WAY 0.2 mg/kg	1.4	0	9.9
RAC+SAL+WAY 0.2 mg/kg	58.1	3	0.3
RAC+IDX	56	2	2.6
RAC+IDX+WAY 0.2 mg/kg	45.7	3	0.5

Table 13. Mean percent escape, number of escape failures and intertrial crosses for raclopride + idazoxan + WAY100635 (0.2 mg/kg).

Figure 13. Mean percent avoidance (+/- SEM) for raclopride (0.025 mg/kg) + idazoxan (1.5 mg/kg) + guanfacine (0.8 mg/kg) (RAC+IDX+GF) and vehicle controls (SAL = saline). RAC+IDX+SAL had significantly lower percent avoidance than SAL+SAL+SAL (*$p<0.05$) and RAC+IDX+GF (+$p<0.05$).

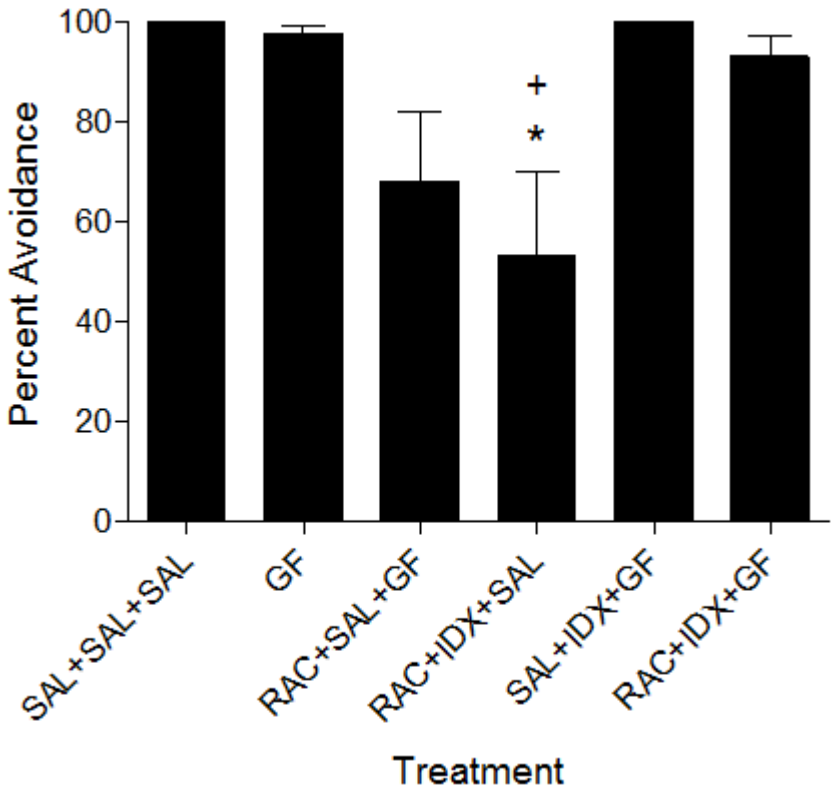

Figure 13. Mean percent avoidance responding (+/- SEM) for raclopride + idazoxan + guanfacine.

Table 14. Mean percent escape, number of escape failures and mean number of intertrial crosses for raclopride (0.025 mg/kg) + idazoxan (1.5 mg/kg) + guanfacine (0.8 mg/kg) (RAC+IDX+GF) and vehicle controls (SAL = saline).

Treatment	*%Escape*	*Escape Failures*	*Intertrial Crosses*
SAL+SAL+SAL	0	0	9.6
GF	2.3	0	5
RAC+SAL+GF	30.5	1	3.6
RAC+IDX+SAL	41.2	2	4.9
SAL+IDX+GF	0	0	14.1
RAC+IDX+GF	6.1	1	10.4

Table 14. Mean percent escape, number of escape failures and intertrial crosses for raclopride + idazoxan + guanfacine.

Discussion

The current study was conducted to elucidate the role of 5-HT_{1A} receptor stimulation in the ability of idazoxan to reduce avoidance responding when paired with an ineffective dose of a typical antipsychotic drug in the conditioned avoidance response task. This was accomplished through the use of compounds selective for dopamine D_2 receptors, 5-HT_{1A} receptors and α_2 adrenoceptors. The α_2 adrenoceptor antagonists, idazoxan and yohimbine, significantly reduced avoidance responding when paired with a low-dose of raclopride. Avoidance responding was also significantly reduced by the combination of low-dose raclopride with the 5-HT_{1A} agonist, 8-OH-DPAT. Deficits in avoidance responding produced by the combination of idazoxan and low-dose raclopride were not restored by the 5-HT_{1A} antagonist, WAY100635. The α_2 adrenoceptor antagonist guanfacine, however, was able to restore the avoidance responding deficits induced by the combination of raclopride and idazoxan.

Dopamine D_2 receptor antagonism is a critical mechanism for producing antipsychotic effects and reliably reduces avoidance in the conditioned avoidance response task. The present study used the typical antipsychotics, raclopride and haloperidol. Both drugs were found to effectively reduce avoidance in the conditioned avoidance response task, which is in agreement with previous studies (Arnt, 1982; Hertel et al., 1999; Taboada et al., 1979; Wadenberg, 2000).

$Alpha_2$ adrenoceptor antagonists have been previously shown to be ineffective in producing antipsychotic effects in the conditioned avoidance response task. The α_2 adrenoceptor antagonists used in these studies, idazoxan and yohimbine, were both

ineffective in reducing avoidance responding, even at very high doses. These findings are in agreement with previous research (Hertel et al., 1999; Wadenberg et al., 2007). Serotonin$_{1A}$ receptor blockade is also known to be ineffective alone at reducing avoidance responding. The current research demonstrated that the 5-HT$_{1A}$ receptor antagonist, WAY100635, does not produce antipsychotic effects. These findings are in agreement with previous research (Wadenberg et al., 2001).

Previous studies by Hertel and colleagues (1999) were successfully replicated, which showed that an ineffective dose of the dopamine D_2 receptor antagonist, raclopride, when paired with the α_2 adrenoceptor antagonist, idazoxan, produces an antipsychotic effect in the conditioned avoidance response task. This finding was replicated by another α_2 adrenoceptor antagonist, yohimbine in the present study. However, the present study failed to replicate research by Wadenberg and colleagues (2007) which showed that the typical antipsychotic drug, haloperidol, reduced avoidance responding when paired with idazoxan. The effects of idazoxan, when paired with raclopride, were blocked by the α_2 adrenoceptor agonist, guanfacine. Because guanfacine, but not WAY, was able to restore avoidance responding, 5-HT$_{1A}$ receptor stimulation may not be responsible for the ability of idazoxan and raclopride to reduce avoidance responding when combined.

The reductions in avoidance responding produced by the combination of raclopride and 8-OH-DPAT in the current study are in support of other research that shows 5-HT$_{1A}$ stimulation contributes to antipsychotic effects. Prinssen and colleagues (1999, 2002) reversed typical antipsychotic-induced catalepsy with 8-OH-DPAT treatment, while preserving pre-clinical measures of therapeutic efficacy.

Increases in prefrontal cortical dopamine are thought to attenuate the cognitive deficits noted in schizophrenia, while striatal dopamine increases are thought to contribute to the development of negative side effects such as movement disorders. Microdialysis studies by Rollema and colleagues (2000) show that 8-OH-DPAT increases antipsychotic drug-induced dopamine release in the prefrontal cortex preferentially over striatal dopamine levels. Stimulation of 5-HT_{1A} receptors by 8-OH-DPAT also reduces antipsychotic-induced increases of dopamine in the nucleus accumbens (Ichikawa & Meltzer, 2000). Therefore, the therapeutic effects of antipsychotic drugs appear to be enhanced by 5-HT_{1A} receptor stimulation, and the negative symptoms and cognitive deficits of schizophrenia may be reduced by the same mechanism.

The inability of WAY100635 (WAY) to restore avoidance responding deficits induced by idazoxan and raclopride may indicate that 5-HT_{1A} receptor stimulation does not mediate the effects of idazoxan in the conditioned avoidance response task. In addition to being ineffective at restoring avoidance deficits, WAY also caused more escape failures and decreased intertrial crosses when given with the combined treatment of raclopride and idazoxan. These data suggest that 5-HT_{1A} receptor blockade may potentiate the cataleptic effects of antipsychotic drugs. Evidence from previous behavioral and *in vivo* research has shown the effects of 5-HT_{1A} receptor stimulation and blockade on typical and atypical antipsychotic drug efficacy. Raclopride- and haloperidol-induced catalepsy is enhanced by co-treatment with WAY (Prinssen et al., 2000; Prinssen et al., 2002). WAY does not, however, interrupt the anti-cataleptic effects of idazoxan (Kleven et al., 2005). WAY alone

increases dopamine levels in motor regions of the brain, whereas 8-OH-DPAT does not, indicating that 5-HT_{1A} receptor blockade may increase motor side effects such as EPS (Ichikawa & Meltzer, 2000).

It is important to note that α_2 adrenoceptor blockade, while sufficient to contribute to antipsychotic effects, may not be necessary. The atypical antipsychotic drugs amisulpiride and aripiprazole have therapeutic efficacy with a low propensity for EPS, but do act as antagonists at α_2 adrenoceptors (Natesan et al., 2008; DeLeon et al., 2004). Because glutamate receptors are found to be abnormal in patients with schizophrenia, and glutamate antagonists induce hallucinations in healthy subjects, glutamate NMDA receptor agonists have been implicated in the treatment of schizophrenia. Specifically, these drugs reduce the occurrence of negative symptoms, and are effective in improving cognitive functioning in patients with schizophrenia (see Goff & Coyle, 2001, for review). The current study therefore supports the hypothesis that the combination of α_2/D_2 receptor blockade produces antipsychotic effects.

Although the present research supports the hypothesis that combined α_2/D_2 receptor blockade produces an antipsychotic effect in the conditioned avoidance response task, it is unclear whether this combination constitutes an atypical antipsychotic drug. As a preclinical tool, the conditioned avoidance response task can only be used to identify antipsychotic effects, and does not differentiate between typical and atypical antipsychotic drugs, as both have the ability to reduce avoidance responding. Antipsychotic atypicality has previously been defined as an antipsychotic with therapeutic efficacy and a low propensity for EPS. In animal

models, this definition of atypicality requires that a drug does not induce catalepsy at doses correlated with those that are effective in preclinical antipsychotic animal models. The conditioned avoidance response task is best used for demonstrating positive symptom efficacy, which may not necessarily include 5-HT_{1A} receptor stimulation. Future research could utilize another behavioral measure, such as the paw test, drug discrimination or prepulse inhibition, to clarify the type of antipsychotic effects produced by the combined blockade of α_2 adrenoceptors and dopamine D_2 receptors. Given the anti-cataleptic effects of α_2 adrenoceptor antagonists such as idazoxan and yohimbine, it may be that the α_2/D_2 hypothesis of antipsychotic atypicality is accurate.

Previous research in this lab and others has sought to evaluate the role of 5-HT_{1A} receptor stimulation in idazoxan's effects on atypicality. Kleven and colleagues (2005) found that idazoxan reverses typical antipsychotic-induced catalepsy using a crossed-leg position bar test. While pretreatment with WAY100635 blocked the effect of idazoxan to attenuate haloperidol-induced catalepsy using the crossed-legged position test, WAY100635 failed to do so for the bar test. These results suggest that 5-HT_{1A} receptor agonism may only play a modest role in mediating the anti-cataleptic effects of idazoxan. Drug discrimination studies in this laboratory have found that partial generalization to idazoxan occurs with 8-OH-DPAT treatment, while WAY100635 partially blocks the idazoxan cue (Zornio, Kopp, Winiarski, Jacobson, Rehberg, et al., 2008). These data support previous findings that idazoxan acts as an agonist at 5-HT_{1A} receptors. Drug discrimination does not, however, identify antipsychotic efficacy. Microdialysis studies in this laboratory have

demonstrated that idazoxan potentiates raclopride-induced increases in prefrontal cortical dopamine. This effect was significantly attenuated by pre-treatment with WAY100635 (Prus, Jacobson, Keusch, Li, Huang et

al., 2007). These data suggest that 5-HT_{1A} receptor stimulation may contribute to drug effects that are not detected in the conditioned avoidance response task.

In conclusion, the inability of WAY100635 to restore avoidance responding deficits induced by the combination of raclopride and idazoxan may reflect the limits of the conditioned avoidance response task in identifying atypical antipsychotic drugs. It is possible that 5-HT_{1A} receptor stimulation, although possibly favorable for improving cognitive deficits and negative symptoms, may not mediate improvements in positive symptoms as measured by the conditioned avoidance response task. The ability of guanfacine to restore deficits in avoidance responding induced by the combined treatment of raclopride and idazoxan indicates that α_2 adrenoceptor blockade may mediate the positive symptom efficacy of an antipsychotic drug treatment. Therefore, hypotheses regarding the atypicality of combined α_2/D_2 receptor stimulation should be explored using a range of pre-clinical evaluations that not only predict efficacy for positive symptoms, but also negative symptoms and cognitive deficits as well.

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APPENDIX A

Conditioned avoidance training program (MedPC)

```
\Conditioned Avoidance Response
\Written by Adam Prus and Sarah Jacobson on December 23, 2008
\15 min session
\---------SHUTTLE BOX------------
\                           FAN
\
\ X                           X
\ X           DOOR            X
\ LEFTKEY       D      RIGHTKEY
\ LEFTNOISE     D    RIGHTNOISE
\ X             D             X
\ X             D             X
\ X-R1----------------------R2-X
\   LEFTSHOCK     RIGHTSHOCK
\            SHOCK
\-------------------------------------------------------------------
-------------------------------------
\Display:  1. Time            2. TrialStart         3. Trial
4. Avoidance     5. Escape
\          6. B4Trial_Crosses 7. Intertrial cross   8. Blank
9. %Avoidance   10. %Escape
\          11.Shock On/Off   12. Blank            13.Blank
14. Av_Avoid_Lat 15. Avg_Escape_Latency
\-------------------------------------------------------------------
-------------------------------------
^Leftkey = 1
^Rightkey = 2
^Shock = 6
^Door = 5
^Leftnoise = 3
^Rightnoise = 4
^Leftshock = 7
^Rightshock = 8
^Fan = 10
```

```
\A=left entries
\B=right entries
\C=Variables
\D=number of trials
\J=Avoidance latency counter
\K=Escape latency counter

LIST X = 20",25",30",35",40"
DIM C = 30 \within session variables for program functionality
DIM E = 45 \avoidance per trial
DIM F = 45 \escape per trial
DIM G = 45 \time to escape per trial (escape latency)
DIM H = 45 \trial where animal failed to escape
DIM I = 45 \number of intertrial crosses based on trial number.
Counts are made after trial is complete.
DIM L = 45 \time to avoid per trial (avoidance latency)
DIM M = 9  \for organizing total session dependent variables

\C(1)=Time till next session counter
\C(2)=Equal to 1 when between trials
\C(3)=Sum of avoidance latencies
\C(4)=Sum of escape latencies
\C(5)=Time till escape
\C(6)=Left warning stimuli
\C(7)=Right warning stimuli
\C(8)=R2 Beam Counter
\C(9)=R2 Beam long enough
\C(10)=R7 Beam Counter
\C(11)=R7 Beam Counter long
\C(12)=Between shock indicator
\C(13)=#R4 or R5 tripped - not a full escape response
\C(14)=
\C(15)=
\
\M(1)=Avoidance counter
\M(2)=Percent avoidance
\M(3)=Avoidance Latency
\M(4)=Escape counter
\M(5)=Percent escape
\M(6)=Escape Latency
\
\Z1=Failure to escape counter
\Z2=Successful escape
\Z3=Before trial crossings
\Z4=Run State Sets for intertrial crosses
\Z5=Intertrial crossings counter
\Z6=Avoidance
\Z7=Escape
\Z8=
\Z9=
\Z10=Countdown to next trial
\Z11=Reset after beam in opposing side broken during avoidance
\Z12=Before trial 1 crossings counter
```

```
\Z13=Indicates when the last trial is finished.  Prevents the
session from ending during the trial.
\Z14=Start avoidance latency counter
\Z15=Stop avoidance latency counter
\Z16=Trial started
\Z17=Start avoidance timer
\Z18=Failure to emit avoidance response
\Z19=Stop avoidance timer (avoidance response emitted)
\Z20=Start intershock interval timer
\Z21=
\Z22=Failure to escape during intershock interval

S.S.1,
S1,
  #Start: ON ^Leftkey,^Rightkey,^Shock,^Door,^Fan;Set D=999; Set
C(2)=1;Set M(1)=999;Set M(4)=999;Z3--->S2
S2,
  30":Set D=1;Show 3,Trial,D;Z16--->S6
S3,
  0.1": RANDI Y = X;Z4;Set C(2)=1;Set C(1)=Y;Set C(9)=0;Set
C(11)=0;Set C(13)=0;Z10;Z13--->S4
S4,
  Y#T:Z13;Z16;Set C(2)=0--->S5
S5,
  1":ADD D;Show 3,Trial,D--->S6
S6,\Where's the rat?
  #R1:ON ^Leftnoise, ^Rightnoise;Add C(6);Z14;Z17--->S7 \left
warning stimulus on
  #R2:ON ^Leftnoise, ^Rightnoise;Add C(7);Z14;Z17--->S10 \right
warning stimulus on
S7, \Left side for failure to avoid or avoidance response
  10":ON ^Leftshock, ^Rightshock;Z1;Z15;Show 11,SHOCK,1--->S8
\failure to avoid WHY NOT 10"?
  #R2: OFF ^Leftnoise, ^Rightnoise;Z6;Z13;Z15--->S3 \avoidance
S8,
  0.5": OFF ^Leftshock, ^Rightshock;Z20;Show 11,OFF,1--->S9
S9, \Shock after 2.5" for left side
  2.5":ON ^Leftshock, ^Rightshock;Set C(13)=0;Z22;Show
11,SHOCK,1--->S8
  #R2:Z2;Z13;Z22;OFF ^LEFTNOISE, ^RIGHTNOISE--->S3 \escape
S10, \Right side
  10":ON ^Leftshock, ^rightshock;Z1;Z15;Show 11,SHOCK,1--->S11
\failure to avoid
  #R1:OFF ^Leftnoise, ^Rightnoise;Z6;Z13;Z15--->S3 \avoidance
response
S11, \Right side
  0.5": OFF ^Leftshock, ^Rightshock;Z20;Show 11,OFF,1--->S12
S12, \Right side
  2.5":ON ^Leftshock, ^Rightshock;Set C(13)=0;Z22;Show
11,SHOCK,1--->S11
  #R1:Z2;Z13;Z22;OFF ^LEFTNOISE, ^RIGHTNOISE--->S3 \escape
response

\-------------------------------------------------------------
```

```
S.S.2, \Failure to escape timer
S1,
  #Z1:--->S2
S2,
  50":OFF ^Leftnoise, ^Rightnoise, ^LeftKey, ^Rightkey,
^Shock;Set H(D)=1--->Stopabortflush
  #Z2: IF M(4)=999 [@True, @False]
       @True:Set M(4)=0;Add M(4);Set F(D)=1;Set
M(5)=(M(4)/D)*100;Show 4,Avoidance,M(1);Show
9,%_Avoidance,M(2);Show 5,Escape,M(4);Show 10,%_Escape,M(5)--->S1
       @False:Add M(4);Set F(D)=1;Set M(5)=(M(4)/D)*100;Show
4,Avoidance,M(1);Show 9,%_Avoidance,M(2);Show 5,Escape,M(4);Show
10,%_Escape,M(5)--->S1
\-------------------------------------------------------------
S.S.3, \Time to escape (escape latency)
S1,
  #Z1:Set K=0--->S2
S2,
  0.1": Set K=K+0.1--->S2
  #Z2:Set G(D)=K;Set C(4)=C(4)+K;Z13--->S1
\-------------------------------------------------
S.S.4, \Intertrial crosses for left to right
S1,
  #Z4:--->S2
S2,
  #R1:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:--->S3
  #Z16:--->S1
S3,
  #R2:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:Z5--->S1
  #Z16:--->S1

S.S.5, \Intertrial crosses for right to left
S1,
  #Z4:--->S2
S2,
  #R2:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:--->S3
  #Z16:--->S1
S3,
  #R1:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:Z5--->S1
  #Z16:--->S1

S.S.6, \Intertrial counts
S1,
  #Z5:Add M(8);Add I(D);Show 7,IT_Crosses,M(8)--->SX
\-------------------------------------------------
S.S.7, \Avoidance counter
```

```
S1,
  #Z6: IF M(1)=999 [@True, @False]
       @True:Set M(1)=0; Add M(1);Set E(D)=1;Set
M(2)=(M(1)/D)*100;Show 4,Avoidance,M(1);Show
9,%_Avoidance,M(2);Show 5,Escape,M(4);Show 10,%_Escape,M(5)--->S1
       @False: Add M(1);Set E(D)=1;Set M(2)=(M(1)/D)*100;Show
4,Avoidance,M(1);Show 9,%_Avoidance,M(2);Show 5,Escape,M(4);Show
10,%_Escape,M(5)--->S1
\-------------------------------------------------
S.S.8, \Session timer
S1,
  #Start:Set T = 900--->S2
S2,
  1":Set T=T-1;Set M(2)=(M(1)/D)*100;Set M(5)=(M(4)/D)*100;Show
1,Seconds,T;Show 4,Avoidance,M(1);Show 9,%_Avoidance,M(2);Show
5,Escape,M(4);Show 10,%_Escape,M(5);IF T=0 [@True,@False] \
          @True:IF C(2)=0 [@True, @False]
                    @True:--->S3
                    @False:OFF ^LeftKey, ^Rightkey, ^Shock;Set
M(3)=C(3)/M(1);Set M(6)= C(4)/M(4);Show
14,Avoid_Latency,M(3);Show 15,Escape_Latency,M(6)---
>Stopabortflush
          @False:--->S2
S3,
  #Z13:OFF ^LeftKey, ^Rightkey, ^Shock;Set M(3)=C(3)/M(1);Set
M(6)=C(4)/M(4);Show 14,Avoid_Latency,M(3);Show
15,Escape_Latency,M(6)--->Stopabortflush
\-------------------------------------------------------------
S.S.9, \Before trial crosses for left to right
S1,
  #Z3:--->S2
S2,
  #R1:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:--->S3
  #Z16:--->S1
S3,
  #R2:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:Z12--->S1
  #Z16:--->S1

S.S.10, \Before trial crosses for right to left
S1,
  #Z3:--->S2
S2,
  #R2:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:--->S3
  #Z16:--->S1
S3,
  #R1:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:Z12--->S1
```

```
  #Z16:--->S1

S.S.11, \Before trial counts
S1,
  #Z12:Add M(7);Show 6,Before_Crosses,M(7)--->S1

\-----------------------------------------------------------------
-
S.S.12, \Time to avoidance (avoidance latency)
S1,
  #Z14:Set J=0--->S2
S2,
  0.1": Set J=J+0.1--->S2
  #Z15:Set L(D)=J; IF J>=10 [@True, @False]
        @True:--->S1
        @False:Set C(3)=C(3)+J--->S1
\-----------------------------------------------------------------
-
S.S.13, \Time until next trial
S1,
  #Z10:--->S2
S2,
  1":Set C(1)=C(1)-100;Show 2,Next_Trial,C(1);IF C(1)=0
[@True,@False]
        @True:--->S1
        @False:--->S2
\-----------------------------------------------------------------
----
S.S.14, \Resets the indicator that the rat failed to exit the
compartment completely during an avoidance response
S1,
  #Z11:--->S2
S2,
  0.02": Set C(13)=0--->S1
\-----------------------------------------------------------------
-----
S.S.15, \10" Avoidance timer
S1,
  #Z17:--->S2
S2,
  10":Z18--->S3
  #Z19:--->S1
S3,
  0.01":Z18--->S3
  #Z19:--->S1
\-----------------------------------------------------------------
-----
S.S.16, \2.5" Intershock interval timer
S1,
  #Z20:--->S2
S2,
  2.5":Z21--->S3
  #Z22:--->S1
S3,
```

```
0.01":Z21--->S3
#Z22:--->S1
```

APPENDIX B

Conditioned avoidance testing program (MedPC)

```
\Conditioned Avoidance Response
\10 min test session
\Written by Adam Prus and Sarah Jacobson on December 23, 2008
\10 min session
\---------SHUTTLE BOX------------
\                           FAN
\
\ X                           X
\ X           DOOR            X
\ LEFTKEY       D      RIGHTKEY
\ LEFTNOISE     D    RIGHTNOISE
\ X             D             X
\ X             D             X
\ X-R1----------------------R2-X
\   LEFTSHOCK     RIGHTSHOCK
\            SHOCK
\-----------------------------------------------------------------
-------------------------------------
\Display:  1. Time            2. TrialStart         3. Trial
4. Avoidance     5. Escape
\          6. B4Trial_Crosses 7. Intertrial cross   8. Blank
9. %Avoidance   10. %Escape
\          11.Shock On/Off   12. Blank            13.Blank
14. Av_Avoid_Lat 15. Avg_Escape_Latency
\-----------------------------------------------------------------
-------------------------------------
^Leftkey = 1
^Rightkey = 2
^Shock = 6
^Door = 5
^Leftnoise = 3
^Rightnoise = 4
^Leftshock = 7
^Rightshock = 8
^Fan = 10
```

```
\A=left entries
\B=right entries
\C=Variables
\D=number of trials
\J=Avoidance latency counter
\K=Escape latency counter

LIST X = 20",25",30",35",40"
DIM C = 30 \within session variables for program functionality
DIM E = 45 \avoidance per trial
DIM F = 45 \escape per trial
DIM G = 45 \time to escape per trial (escape latency)
DIM H = 45 \trial where animal failed to escape
DIM I = 45 \number of intertrial crosses based on trial number.
Counts are made after trial is complete.
DIM L = 45 \time to avoid per trial (avoidance latency)
DIM M = 9  \for organizing total session dependent variables

\C(1)=Time till next session counter
\C(2)=Equal to 1 when between trials
\C(3)=Sum of avoidance latencies
\C(4)=Sum of escape latencies
\C(5)=Time till escape
\C(6)=Left warning stimuli
\C(7)=Right warning stimuli
\C(8)=R2 Beam Counter
\C(9)=R2 Beam long enough
\C(10)=R7 Beam Counter
\C(11)=R7 Beam Counter long
\C(12)=Between shock indicator
\C(13)=#R4 or R5 tripped - not a full escape response
\C(14)=
\C(15)=
\
\M(1)=Avoidance counter
\M(2)=Percent avoidance
\M(3)=Avoidance Latency
\M(4)=Escape counter
\M(5)=Percent escape
\M(6)=Escape Latency
\
\Z1=Failure to escape counter
\Z2=Successful escape
\Z3=Before trial crossings
\Z4=Run State Sets for intertrial crosses
\Z5=Intertrial crossings counter
\Z6=Avoidance
\Z7=Escape
\Z8=
\Z9=
\Z10=Countdown to next trial
\Z11=Reset after beam in opposing side broken during avoidance
\Z12=Before trial 1 crossings counter
```

```
\Z13=Indicates when the last trial is finished.  Prevents the
session from ending during the trial.
\Z14=Start avoidance latency counter
\Z15=Stop avoidance latency counter
\Z16=Trial started
\Z17=Start avoidance timer
\Z18=Failure to emit avoidance response
\Z19=Stop avoidance timer (avoidance response emitted)
\Z20=Start intershock interval timer
\Z21=
\Z22=Failure to escape during intershock interval

S.S.1,
S1,
  #Start: ON ^Leftkey,^Rightkey,^Shock,^Door,^Fan;Set D=999; Set
C(2)=1;Set M(1)=999;Set M(4)=999;Z3--->S2
S2,
  30":Set D=1;Show 3,Trial,D;Z16--->S6
S3,
  0.1": RANDI Y = X;Z4;Set C(2)=1;Set C(1)=Y;Set C(9)=0;Set
C(11)=0;Set C(13)=0;Z10;Z13--->S4
S4,
  Y#T:Z13;Z16;Set C(2)=0--->S5
S5,
  1":ADD D;Show 3,Trial,D--->S6
S6,\Where's the rat?
  #R1:ON ^Leftnoise, ^Rightnoise;Add C(6);Z14;Z17--->S7 \left
warning stimulus on
  #R2:ON ^Leftnoise, ^Rightnoise;Add C(7);Z14;Z17--->S10 \right
warning stimulus on
S7, \Left side for failure to avoid or avoidance response
  10":ON ^Leftshock, ^Rightshock;Z1;Z15;Show 11,SHOCK,1--->S8
\failure to avoid WHY NOT 10"?
  #R2: OFF ^Leftnoise, ^Rightnoise;Z6;Z13;Z15--->S3 \avoidance
S8,
  0.5": OFF ^Leftshock, ^Rightshock;Z20;Show 11,OFF,1--->S9
S9, \Shock after 2.5" for left side
  2.5":ON ^Leftshock, ^Rightshock;Set C(13)=0;Z22;Show
11,SHOCK,1--->S8
  #R2:Z2;Z13;Z22;OFF ^LEFTNOISE, ^RIGHTNOISE--->S3 \escape
S10, \Right side
  10":ON ^Leftshock, ^rightshock;Z1;Z15;Show 11,SHOCK,1--->S11
\failure to avoid
  #R1:OFF ^Leftnoise, ^Rightnoise;Z6;Z13;Z15--->S3 \avoidance
response
S11, \Right side
  0.5": OFF ^Leftshock, ^Rightshock;Z20;Show 11,OFF,1--->S12
S12, \Right side
  2.5":ON ^Leftshock, ^Rightshock;Set C(13)=0;Z22;Show
11,SHOCK,1--->S11
  #R1:Z2;Z13;Z22;OFF ^LEFTNOISE, ^RIGHTNOISE--->S3 \escape
response

\-------------------------------------------------------------
```

```
S.S.2, \Failure to escape timer
S1,
  #Z1:--->S2
S2,
  50":OFF ^Leftnoise, ^Rightnoise, ^LeftKey, ^Rightkey,
^Shock;Set H(D)=1--->Stopabortflush
  #Z2: IF M(4)=999 [@True, @False]
       @True:Set M(4)=0;Add M(4);Set F(D)=1;Set
M(5)=(M(4)/D)*100;Show 4,Avoidance,M(1);Show
9,%_Avoidance,M(2);Show 5,Escape,M(4);Show 10,%_Escape,M(5)--->S1
       @False:Add M(4);Set F(D)=1;Set M(5)=(M(4)/D)*100;Show
4,Avoidance,M(1);Show 9,%_Avoidance,M(2);Show 5,Escape,M(4);Show
10,%_Escape,M(5)--->S1
\-------------------------------------------------------------
S.S.3, \Time to escape (escape latency)
S1,
  #Z1:Set K=0--->S2
S2,
  0.1": Set K=K+0.1--->S2
  #Z2:Set G(D)=K;Set C(4)=C(4)+K;Z13--->S1
\-------------------------------------------------
S.S.4, \Intertrial crosses for left to right
S1,
  #Z4:--->S2
S2,
  #R1:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:--->S3
  #Z16:--->S1
S3,
  #R2:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:Z5--->S1
  #Z16:--->S1

S.S.5, \Intertrial crosses for right to left
S1,
  #Z4:--->S2
S2,
  #R2:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:--->S3
  #Z16:--->S1
S3,
  #R1:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:Z5--->S1
  #Z16:--->S1

S.S.6, \Intertrial counts
S1,
  #Z5:Add M(8);Add I(D);Show 7,IT_Crosses,M(8)--->SX
\-------------------------------------------------
S.S.7, \Avoidance counter
```

```
S1,
  #Z6: IF M(1)=999 [@True, @False]
       @True:Set M(1)=0; Add M(1);Set E(D)=1;Set
M(2)=(M(1)/D)*100;Show 4,Avoidance,M(1);Show
9,%_Avoidance,M(2);Show 5,Escape,M(4);Show 10,%_Escape,M(5)--->S1
       @False: Add M(1);Set E(D)=1;Set M(2)=(M(1)/D)*100;Show
4,Avoidance,M(1);Show 9,%_Avoidance,M(2);Show 5,Escape,M(4);Show
10,%_Escape,M(5)--->S1
\-------------------------------------------------
S.S.8, \Session timer
S1,
  #Start:Set T = 600--->S2
S2,
  1":Set T=T-1;Set M(2)=(M(1)/D)*100;Set M(5)=(M(4)/D)*100;Show
1,Seconds,T;Show 4,Avoidance,M(1);Show 9,%_Avoidance,M(2);Show
5,Escape,M(4);Show 10,%_Escape,M(5);IF T=0 [@True,@False] \
          @True:IF C(2)=0 [@True, @False]
                    @True:--->S3
                    @False:OFF ^LeftKey, ^Rightkey, ^Shock;Set
M(3)=C(3)/M(1);Set M(6)= C(4)/M(4);Show
14,Avoid_Latency,M(3);Show 15,Escape_Latency,M(6)---
>Stopabortflush
          @False:--->S2
S3,
  #Z13:OFF ^LeftKey, ^Rightkey, ^Shock;Set M(3)=C(3)/M(1);Set
M(6)=C(4)/M(4);Show 14,Avoid_Latency,M(3);Show
15,Escape_Latency,M(6)--->Stopabortflush
\-------------------------------------------------------------
S.S.9, \Before trial crosses for left to right
S1,
  #Z3:--->S2
S2,
  #R1:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:--->S3
  #Z16:--->S1
S3,
  #R2:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:Z12--->S1
  #Z16:--->S1

S.S.10, \Before trial crosses for right to left
S1,
  #Z3:--->S2
S2,
  #R2:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:--->S3
  #Z16:--->S1
S3,
  #R1:IF C(2)=0 [@True,@False]
                @True:--->S1
                @False:Z12--->S1
```

```
  #Z16:--->S1

S.S.11, \Before trial counts
S1,
  #Z12:Add M(7);Show 6,Before_Crosses,M(7)--->S1

\-----------------------------------------------------------------
-
S.S.12, \Time to avoidance (avoidance latency)
S1,
  #Z14:Set J=0--->S2
S2,
  0.1": Set J=J+0.1--->S2
  #Z15:Set L(D)=J; IF J>=10 [@True, @False]
        @True:--->S1
        @False:Set C(3)=C(3)+J--->S1
\-----------------------------------------------------------------
-
S.S.13, \Time until next trial
S1,
  #Z10:--->S2
S2,
  1":Set C(1)=C(1)-100;Show 2,Next_Trial,C(1);IF C(1)=0
[@True,@False]
        @True:--->S1
        @False:--->S2
\-----------------------------------------------------------------
----
S.S.14, \Resets the indicator that the rat failed to exit the
compartment completely during an avoidance response
S1,
  #Z11:--->S2
S2,
  0.02": Set C(13)=0--->S1
\-----------------------------------------------------------------
-----
S.S.15, \10" Avoidance timer
S1,
  #Z17:--->S2
S2,
  10":Z18--->S3
  #Z19:--->S1
S3,
  0.01":Z18--->S3
  #Z19:--->S1
\-----------------------------------------------------------------
-----
S.S.16, \2.5" Intershock interval timer
S1,
  #Z20:--->S2
S2,
  2.5":Z21--->S3
  #Z22:--->S1
S3,
```

```
0.01":Z21--->S3
#Z22:--->S1
```

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MEMORANDUM

October 7, 2008

TO: Dr. Adam Prus
Sarah Jacobson
Department of Psychology

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

RE: **Application to use Vertebrate Animals**
Application # IACUC 104
Approval Period: 10/07/2008-05/01/2009

The Institutional Animal Care and Use Committee have approved your modification to IACUC 104 ~~094~~ application to use vertebrate animals in research, "Role of noradrenergic and dopamine receptors in antipsychotic effects".

If you have any questions, please contact me.

kjm

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