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ESTABLISHING THE DISCRIMINATIVE STIMULUS PROPERTIES OF THE
ATYPICAL ANTIPSYCHOTIC AMISULPRIDE IN C57BL/6 MICE

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science at Virginia Commonwealth University

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Table of Contents

	Page
Acknowledgements	ii
List of Tables	v
List of Figures	vi
Abstract	vii
Introduction	1
Synopsis of antipsychotic drugs in the treatment of schizophrenia	1
Schizophrenia	2
Symptoms	3
Disorganized thinking	3
Cognitive impairments	4
Disorganized behavior	6
Negative symptoms	6
Pharmacological treatments for schizophrenia	7
First generation typical antipsychotic medications	8
Second generation atypical antipsychotic medications	11
Haloperidol	13
History	13
Pharmacokinetic properties	14
Pharmacodynamic properties	16
Receptor binding profile	17
Amisulpride	19
History	19
Atypicality defined	20
Receptor binding profile	21
Theories of atypicality	22
Pharmacokinetic properties	24
Pharmacodynamic properties	24
Drug discrimination as a behavioral assay	27
Drug discrimination and antipsychotic drugs	28
History	29
Rationale	32
Methods	34
Subjects	34
Drugs	35
Apparatus	36

	Page
Phase I: autoshaping	36
Phase II: single-lever training	38
Phase III: drug discrimination acquisition training	39
Drug discrimination training criteria	40
Phase IV: drug discrimination	40
Operational definition of dependent variables	40
Data analysis	41
Results	42
(-) <i>S</i> amisulpride acquisition	42
(-) <i>S</i> amisulpride generalization curve	42
(-) <i>S</i> amisulpride time course	45
Haloperidol substitution	47
Clozapine substitution	48
Aripiprazole substitution	49
(+) <i>R</i> amisulpride substitution	50
Racemic (+/-) <i>SR</i> amisulpride substitution	51
Discussion	52
Amisulpride as a discriminative stimulus	52
Comparison of generalization curves for amisulpride	53
Amisulpride time course	55
Haloperidol substitution	56
Clozapine and aripiprazole substitution	57
Future studies	58
Conclusion	59
List of References	60
Appendices	73
(-) <i>S</i> amisulpride (HCl) chemical structure	73
(+) <i>R</i> amisulpride (free base) chemical structure	73
(+/-) <i>SR</i> amisulpride (free base) chemical structure	73
Amisulpride (HCl) chemical structure	73
Sulpiride chemical structure	73
Haloperidol chemical structure	73
Chlorpromazine chemical structure	73
Clozapine chemical structure	74
Aripiprazole chemical structure	74
Vita	75

List of Tables

	Page
Table 1. Pharmacokinetic properties of haloperidol and amisulpride	15
Table 2. Dissociation rate constants for tested drugs	18
Table 3. Summary of the pharmacodynamic profile of amisulpride	26
Table 4. Amisulpride ED ₅₀ values for forms of amisulpride	54

List of Figures

	Page
Figure 1. Chemical Structure of amisulpride	21
Figure 2. (-) <i>S</i> amisulpride Acquisition	43
Figure 3. (-) <i>S</i> amisulpride Generalization Dose Effect Curve	44
Figure 4. (-) <i>S</i> amisulpride Time Course	46
Figure 5. Haloperidol Substitution Curve.....	47
Figure 6. Clozapine Substitution Curve	48
Figure 7. Aripiprazole Substitution Curve	49
Figure 8. (+) <i>R</i> amisulpride Substitution Curve	50
Figure 9. Racemic (+/-) <i>SR</i> amisulpride Substitution Curve	51
Figure 10. Dose-effect Curve for Three Forms of Amisulpride	55

Abstract

ESTABLISHING THE DISCRIMINATIVE STIMULUS PROPERTIES OF THE ATYPICAL ANTIPSYCHOTIC AMISULPRIDE IN C57BL/6 MICE

By Timothy John Donahue

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
at Virginia Commonwealth University

Virginia Commonwealth University, 2012

Major Director: Joseph H. Porter PhD
Professor of Psychology
Department of Psychology

Antipsychotic medications are used to treat schizophrenia. The present study used the drug discrimination paradigm to measure the subjective effects of the atypical antipsychotic amisulpride and to examine the underlying neuropharmacological mechanisms responsible for the discriminative stimulus property of the drug. Male C57BL/6 mice were trained to discriminate 10 mg/kg (-)*S* amisulpride from vehicle in a two-lever drug discrimination task. A dose effect curve for (-)*S* amisulpride yielded an $ED_{50} = 1.77$ mg/kg 95% CI [1.28, 2.45 mg/kg]. Substitution testing was conducted for the isomer (+)*R* amisulpride, racemic (\pm)*SR* amisulpride, the atypical antipsychotics clozapine, aripiprazole and the typical antipsychotic haloperidol. There was partial substitution for (+)*R* amisulpride, and full substitution for (\pm)*SR* amisulpride with a significant rightward shift in the dose effect curves. Clozapine, aripiprazole, and haloperidol failed to fully substitute with significant rate suppression at the higher doses. These results demonstrated that (-)*S* amisulpride has a unique discriminative stimulus that differs from other antipsychotic drugs.

Establishing the Discriminative Stimulus Properties of the Atypical Antipsychotic Amisulpride in C57BL/6 Mice

Synopsis of Antipsychotic Drugs in the Treatment of Schizophrenia

The development of antipsychotic drugs in the 1950s for treating schizophrenia was a monumental milestone in the care of individuals afflicted with this devastating mental illness. The early drugs, known as the first generation or typical antipsychotics such as chlorpromazine and haloperidol, proved effective in treating some of the symptoms of the disorder but were not without their drawbacks, primarily severe extrapyramidal motor side effects (EPS). Additionally the typical antipsychotics proved ineffective in treating negative symptoms of the disorder and a segment of individuals with schizophrenia who proved to be treatment-resistant (J. Kane, G, J, H, & 1988; Meltzer, 1991). Spurred by the hope of more effective antipsychotic medications with less unwanted side effects, researchers developed a second generation of antipsychotic drugs known as atypical antipsychotics such as clozapine, risperidone, and olanzapine. Clozapine introduced in 1971, is known as the prototypical atypical antipsychotic medication, and proved effective in treating a range of symptoms of schizophrenia without triggering EPS. However, clozapine was not without its problems as it was associated with a high incidence of agranulocytosis in certain populations leading to it being withdrawn from the marketplace in 1975 and reintroduced in 1989 when it was approved, with special guidelines, for treatment-resistant individuals (Meltzer, 1997). Pharmacological research continued the development of improved medications such as amisulpride, brought to market in the 1990s. Amisulpride is the focus of this thesis. It is hoped that a fuller comprehension and analysis of the pharmacodynamic properties of these antipsychotic medications will yield still more improved

medications, and continue to elucidate the myriad of factors contributing to this very serious mental disorder.

Schizophrenia

Schizophrenia is a devastating mental disorder that entails major disruptions of perception, cognition, emotion, and behavior, and is difficult to explain from an etiological and pathophysiological perspective. The consequences for the individual and society are tragic as most patients suffer from a lifetime of psychiatric disability, periodic hospitalizations, poor social adjustment and disrupted family relationships. The overall U.S. cost of schizophrenia in 2002 was estimated at \$62.7 billion (Wu et al., 2005), and recent studies of the prevalence of schizophrenia indicate that approximately 0.07 % of the global population suffers from it (Saha, Chant, Welham, & McGrath, 2005). Sadly, schizophrenia has an early onset striking people in late adolescence and the early 20s. Schizophrenia is indiscriminate affecting both male and females and cuts across all economic, social and cultural borders. It is a difficult disorder to endure, with 4.9% of schizophrenics committing suicide during their lifetime, usually near illness onset (Palmer, Pankratz, & Bostwick, 2005). The etiology of schizophrenia is complicated and a number of factors have been implicated. Some studies link schizophrenia with genetic factors (Sullivan, 2005). However, genetic components are not the only factors in the development of schizophrenia, as monozygotic twins have a concordance rate of 50% for schizophrenia implying that other environmental or organic factors play a significant role as well (Owen, Craddock, & O'Donovan, 2005).

The nineteenth century saw modern psychiatry progress from merely observing symptoms to defining symptom clusters as part of an illness associated with an illness group, and patterns of recovery. In 1896 Emil Kraepelin used the term “dementia praecox” (early dementia)

for individuals with symptoms we now associate with schizophrenia (Nicholi, 1988). Kraepelin separated what he called early dementia (striking people in the late teens and early twenties) and classical dementia which manifested itself later in one's life. Kraepelin was the first to develop a list of symptoms commonly associated with the disorder. In 1911, Swiss psychiatrist Eugen Bleuler coined the term "schizophrenia" from the Greek meaning split-mind (Tsuang, Faraone, & Green, 1999). He sought to differentiate the disorder from later onset dementia and noticed differences between the two in onset, duration and possible remission/recovery. Bleuler also distinguished between "positive" and "negative" symptoms of the disorder.

Symptoms. Broadly speaking, schizophrenia is characterized by symptoms affecting five basic areas: disorganized thinking, inappropriate emotional responses, bizarre motor behaviors, hallucinations and language disturbances. These areas are further refined into two main categories, either positive or negative symptoms (Crow, 1980). Positive symptoms refer to manifestations in which the patient produces behaviors that are outside the usual behavioral repertoire of human beings. That is, the individual expresses behaviors that should not be there, such as auditory hallucinations (hearing voices), delusional thoughts (being persecuted) or rambling and incoherent speech (word salad) and disconnected or disorganized thinking. The individual may also exhibit bizarre motor behaviors such as tracing imaginary patterns in the air with his hands or moving his hands or arms in a random pattern.

Disorganized thinking. The signature cognitive symptom of the disorder is disorganized thinking. Thoughts may be loosely connected, appear in random order, and bear little association to relevant situations. The individual may be incapable of expressing thoughts in coherent and meaningful language. Disorganized thinking is commonly seen in the form of delusions which come in many forms and distinguishing among them can be a challenge

(Spitzer, 1990). Delusions are false beliefs not amenable to change by reason or experience even though the person is in a clear state of consciousness (Tsuang et al., 1999). Individuals might also exhibit delusions of grandeur, for example thinking one is Napoleon or grandiose delusions, thinking one to be omnipotent or all-knowing. Delusions can also manifest themselves in the form of control, such as thinking others are controlling one's thoughts, or that the individual can control others' thoughts.

Cognitive Impairments. Closely related to the symptom of disorganized thinking are other cognitive impairments. Traditionally the loss of cognitive ability has been framed as a negative symptom. Due to its unique characteristics it is now seen as a separate category of symptoms. Most schizophrenics have some degree of cognitive deficiency (Meltzer, Thompson, Lee, & Ranjan, 1996). These include: disorganized thoughts, difficulty concentrating and or following instructions, difficulty completing tasks, memory problems, impairments in delayed recall, coordinating visual and motor skills, distractibility, and impairments in delayed recognition, perceptual skills, and compromised intellectual skills and ability (Keefe, 2007). The degree of cognitive impairment is important as it is a major predictor of the individual's functional outcome (Green, Kern, Braff, & Mintz, 2000). The more severe the cognitive deficit, the more difficult it is to treat the patient, and the less favorable is the outcome. The National Institute of Mental Health established the MATRICS™ initiative (Measurement and Treatment Research to Improve Cognition in Schizophrenia) to clarify for researchers how the issue of cognitive deficits should be approached (Green et al., 2004). Hopefully, initiatives such as these will be an impetus for research toward the development of novel therapeutic agents tailored for cognitive deficits. Until cognitive deficits are clearly defined from other symptoms of the disorder, the burden falls on pharmaceutical companies to empirically demonstrate the efficacy

of a drug promoted for the treatment of schizophrenia, or failing that, delineate which symptoms a promised treatment will and will not provide therapeutic relief (Laughren & Levin, 2006).

As a point of emphasis, there is a growing trend among researchers and clinicians to broaden the diagnostic criteria for schizophrenia to include cognitive dysfunction as a critical component of the disorder. The current version of the *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., text rev.; DSM-IV-TR; American Psychiatric Association, 2000) does not identify cognitive dysfunction among its criteria for schizophrenia. While mention is made of disorganized thinking (e.g. thought disorders such as delusions and loose associations), disorganized thinking is not operationally defined in the DSM-IV-TR. Andreasen et. al. make a cogent argument that cognitive abnormalities are the “hallmark” of schizophrenia and have been overlooked by researchers and clinicians alike (Andreasen et al., 1996). This cognitive dysfunction is manifest chiefly as difficulty in processing information, formulating responses, retrieving information, and reacting quickly as well as an inability in expressing responses with facility in words or emotions. Andreasen refers to these difficulties as a kind of cognitive “dysmetria” or an inability to coordinate mental functions in a well-modulated and fine-tuned manner. Her research suggests impairments among prefrontal-thalamic-cerebellar circuitry as primarily responsible for such deficits. Such research suggests that schizophrenia ought not to be viewed as a single disease involving one area of the brain. Instead, it should be viewed as a disease of complex circuits involving a whole host of brain regions. Another challenge is clearly delineating among and between the myriad of cognitive functions and processes which become dysfunctional in schizophrenia. Such a collection would include impairments in: problem solving, working memory (McKenna, 1991), verbal memory (Heinrichs & Awad, 1993) attention (Field, 1997), visual-spatial and motor skills, planning, executive functions

(Weinberger, Berman, & Zec, 1986) parallel processing and many more neuropsychological tasks and abilities (Gallhofer, Bauer, Lis, Krieger, & Gruppe, 1996; Saykin et al., 1994). So profound and prevalent are cognitive deficits in schizophrenia that some researchers propose cognitive impairments are at the very core of the disorder and that any model of or diagnostic criteria for schizophrenia is woefully insufficient if cognitive impairments are not addressed as central to the disorder (Elvevåg & Goldberg, 2000).

Disorganized behavior. Disorganized behavior is another prominent characteristic of schizophrenia. Disorganized behaviors are those that are not in accord with the usual, customary socially acceptable repertoire of behaviors and do not express clear intent and purpose. An example of such behavior is a motor disturbance known as catatonic behavior. At one extreme, catatonic excitation, this may consist of episodes of uncontrolled, agitated, and disorganized behavior, pacing around a ward aimlessly. It can appear repetitive, stereotypical, hyperactive, destructive and even violent. The schizophrenic patient may exhibit mannerisms, habitual movements that usually involve a single body part such as, grimaces, tics, moving lips, soundlessly, fidgeting with fingers, or hand wringing. At the opposite extreme, catatonic stupor may be expressed as a complete absence of motor actions, akinesia, such as sitting rigid and motionless in a chair for hours on end, unresponsive to external stimuli.

Negative symptoms. While positive symptoms are characteristic tell-tale signs of the disorder, negative symptoms are no less troublesome and difficult to treat. Negative symptoms affect cognitive, affective, and motor behaviors in the direction of decreased expressiveness and responsiveness. They include the following: avolition (lack of initiative), blunt, flat or restricted affect (emotionally void), anhedonia (lack of pleasure), alogia (absence or poverty of speech), poor eye contact, decreased spontaneous movements, and diminished emotional responsiveness

as seen in the muted ability to feel intimacy or closeness with others. The extreme example of negative symptoms is the catatonic stupor, a total lack of movement and verbal behavior. The person may appear poorly groomed, unable to persist at a task, and withdrawn from social activities. The social behavior in schizophrenic patients indicates that the disorder results in a marked loss of the basic behavioral components necessary for effective social interaction (Curran & Monti, 1982). Traditionally, negative symptoms have proven to be more difficult to treat than positive symptoms (Möller, 1998).

Each person with schizophrenia displays a unique combination of symptoms. Indeed, only a few of the symptoms need be present for a diagnosis of schizophrenia to be made. The DSM-IV-TR outlines several categories (subtypes) of schizophrenics such as, paranoid, catatonic, undifferentiated, residual and disorganized. The presence of specific symptoms and the continuum of positive or negative symptoms will vary with each individual.

Pharmacological Treatments for Schizophrenia

Throughout history, the treatment of individuals with schizophrenia and related disorders has been nothing short of shameful, ineffective and, in many cases, inhumane. Those afflicted with the disorder were subjected to a wide range of treatments such as beatings, isolation, bloodletting, crude medical procedures, exorcism, and generally confined to prisons or asylums known for their dehumanizing conditions (Alexander & Selesnick, 1966). The French physician Philippe Pinel (1745-1826) became the first advocate for the development of more humane treatment of mental patients. He advocated a medical model of mental illness based on his belief in an organic cause for mental illness (Philippe, 1804). Pinel was one of the early founders of psychiatry through his work at Bicêtre Hospital, Paris, and is remembered as the “father of psychiatry.” Yet, even with care for the mentally ill generally improving, most suffering from

schizophrenia were still confined to institutional care with little in the way of hope for treating the disorder. By 1955, more than half a million psychotic patients in the United States found themselves confined to mental institutions (Julien, Advokat, & Comaty, 2010). Early and mid-twentieth century treatments pursued what was seen at the time as a more advanced medically based treatment regimen. They included treatments such as CO₂ inhalation (Lovenhart, Lorenz, & R.M., 1929), injections of apomorphine or the barbiturate sodium amytal (Thorner, 1935), comas induced by insulin (Sakel, 1937), convulsive treatment induced by injections of camphor and metrazol (von Meduna, 1935), and electroconvulsive shock (Cerletti, 1938; Shorter & Healy, 2007). One could argue that these treatments could be considered the prelude to the first pharmacological treatments for schizophrenia.

First generation typical antipsychotic medications. The history of specific pharmacological treatments for schizophrenia began in the 1940s with the French surgeon Henri Laborit (Hamilton & Timmons, 1994). Suspecting that the patient's own fears about the dangers of surgery were a major attributing factor to many of the deaths associated with surgery, Laborit experimented with various drugs to lessen such fears. Conventional sedatives, which merely blocked the autonomic nervous system, seemed not to be that effective in fear suppression. His search led him to experiment with the more recently developed antihistamine compounds, promethazine and pethidine that were combined as part of a presurgical cocktail and proved to be very effective (Laborit H & R., 1952). When administered prior to surgery, his patients were calm, minimally sedated, and the incidence of deaths due to surgical trauma greatly reduced. This calm, detached and sedated state would come to be known as a "neuroleptic" state and would, in short time, become associated with schizophrenia mirroring prominent schizophrenic behaviors such as emotional flatness, apathy, and a loss of initiative. Drugs

treating this state would be known as “neuroleptics” and what are now known as first-generation (typical) antipsychotic drugs (Julien et al., 2010). These drugs were derived from a class of drugs known as phenothiazines and would become the first category of antipsychotic agents. As often happens in the history of pharmacological agents, proving effective in one area results in the drug being refined and used in other areas. On December 11, 1950, chemist Paul Charpentier, working for the French pharmaceutical company Laboratoires Rhône-Poulenc, produced a series of compounds and synthesized RP4560 or chlorpromazine (Charpentier P & Jacob R, 1952). Chlorpromazine was adopted for use in psychiatric clinics and while it was not effective in the treatment of other disorders such as depression, it had dramatic effects in reducing some major symptoms of schizophrenia. It became available for the treatment of schizophrenia in 1953 under the European trade name Largactil[®]. The drug’s profound effect in the treatment of schizophrenia led to its expanded use throughout Europe and North America. It was approved for use in the United States in 1955, marketed under the trade name Thorazine[®], and sparked what has come to be known as the drug revolution in psychiatry (Lopez-Munoz et al., 2005).

The chief benefit of chlorpromazine rested not in its promise as a cure for schizophrenia but in its power to reduce some of the more debilitating symptoms of the disorder. Many patients were able to engage in many day-to-day activities, their disorganized thinking improved, hallucinations abated and they no longer exhibited bizarre motor behaviors. Patients were freed from straightjackets and hundreds of thousands released from institutional care into the community in a movement known as deinstitutionalization (Fuller, 1997). The impact of chlorpromazine for the treatment of mental health was somewhat analogous to the effect of penicillin for bacterial diseases. Mental patients could now be put on a medication which would,

by and large, manage many of the major symptoms of a devastating disorder that seemed untreatable for hundreds of years and led to the beginning of behavioral pharmacology (Thompson, 1997).

Soon, other medications were developed and marketed in the late 1950s and 1960s. This class of neuroleptics was the butyrophenones developed in Belgium in the mid-1960s. They included haloperidol, benperidol, droperidol, loxapine and molindone (Julien et al., 2010). All of these typical neuroleptics shared similar mechanisms of action in reducing dopamine activity in the brain, chiefly as antagonists at dopamine D₂ or D₂ like receptors (Meltzer, 1991).

While dopamine antagonism has proven essential to the therapeutic effects of the typical medications, it is also responsible for the undesirable extrapyramidal motor side effects and is deficient in its ability to treat the negative symptoms of schizophrenia. The extrapyramidal system is a neural network that is part of the motor system responsible for involuntary reflexes and movement, and modulation of movement (i.e. coordination) mainly found in the reticular formation of the pons and medulla, and target neurons in the spinal cord involved in reflexes, locomotion, complex movements, and postural control. These tracts are modulated by various parts of the central nervous system, including the nigrostriatal pathway, basal ganglia, cerebellum, the vestibular nuclei, and different sensory areas of the cerebral cortex. These regulatory components are constituents of the extrapyramidal system, serving mainly to modulate motor activity without directly innervating motor neurons (Purves et al., 2001). Extrapyramidal effects can be devastating and in some cases, permanent. They include: Parkinsonian like tremors, rigidity, involuntary tics, facial grimaces, tardive dyskinesia, involuntary movements and body restlessness known as akathisia (Jeste & Caliguiri, 1993). As these side effects accompanied normal therapeutic doses for the drugs, they were thought to be

regrettable, but an unavoidable part of necessary treatment (van Rossum, 1966). Yet, the risk of extrapyramidal effects and the inability of this first-generation of neuroleptics to alleviate the negative symptoms of schizophrenia gave rise to the development of a second-generation of drugs termed atypical antipsychotic drugs. Pharmacological developments made it possible to produce medications for the disorder with the same or greater therapeutic efficacy without the unwanted extrapyramidal effects (Julien et al., 2010).

While the discovery and development of the first-generation antipsychotics profoundly advanced the treatment of schizophrenia, shortcomings associated with their use were evident and became increasingly unacceptable to both patients and physicians. Many patients proved to be treatment-resistant to the drugs. Additionally, these drugs were not effective in treating the negative symptoms of the disorder, despite improving positive symptoms. Finally, many patients were unable to tolerate the side effects of the antipsychotics. These serious drawbacks spurred the pharmaceutical industry to develop the second-generation “atypical” antipsychotic drugs.

Second generation atypical antipsychotic medications. The second-generation antipsychotics were introduced into the United States with clozapine in 1989, followed by risperidone (1994), olanzapine (1996), sertindole (withdrawn from U.S. markets in 1998, but available in certain European countries) and quetiapine (1997), ziprasidone (2001), aripiprazole (2002), paliperidone (2006), iloperidone (2009) and asenapine (2009). Amisulpride (Solian®) is available in Australia and Europe but not in the United States (Julien et al., 2010). As the first atypical antipsychotic, clozapine demonstrated its superiority to first-generation antipsychotics. Clozapine (Clozaril®) was developed by the European pharmaceutical company Wander Pharmaceutical Company, later acquired by Novartis in 1958. It is viewed as the prototypical

atypical antipsychotic medication and remains, today, as the “gold standard” medication for treatment resistant patients. (Hippius, 1999). It was the first drug to demonstrate success in treating both positive and negative symptoms of schizophrenia (Meltzer, 1994). Additionally, it did not cause extrapyramidal motor side effects, a major drawback to first generation typical antipsychotics (Arnt & Skarsfeldt, 1998; Ellenbroek, 1993a; J. M. Kane, Honigfeld, Singer, & Meltzer, 1988). Further, clozapine was not linked to tardive dyskinesia, a significant handicap for the typical antipsychotic medications such as haloperidol (Meltzer & Luchins, 1984). Clozapine’s initial success suffered a serious blow in 1975 when it was linked to agranulocytosis during a clinical trial in Finland in which several patients died (Anderman & Griffith, 1977; Idnpn-Heikkil, Alhava, & Olkinuora, 1975; Lahdelma & Appleberg, 2012). Subsequently the drug was pulled from the market and reintroduced in the U.S. in 1989 with the restrictions that it carry a “Black Box Warning” and that the drug be used only for treatment-resistant patients with required mandatory and regular white blood cell tests (Volavka et al., 2002). Treatment-resistant is defined as a patient who has not shown beneficial effects from a typical or atypical antipsychotic medications (Chakos, Lieberman, Hoffman, Bradford, & Sheitman, 2001; J. Kane et al., 1988). Due to the medical risks associated with agranulocytosis, fear of litigation, expenses related to blood cell tests clozapine is today relegated to a very proscribed and limited segment of the population suffering from schizophrenia.

These concerns fueled the research to continue developing other atypical antipsychotics without the negatives associated with clozapine. Additionally, second-generation antipsychotics continued to show promise in treating the negative symptoms of schizophrenia. While they were associated with fewer Parkinson like symptoms, they had unique side-effects, produced in some individuals, such as weight gain, a propensity to produce glucose intolerance (diabetes),

elevation in blood lipids, and cardiac electrographic abnormalities. These are collectively known as metabolic syndromes. As the second-generation antipsychotics grew in use, so did their “off label” use for conditions such as depression, bipolar disorder, dysthymia, dementia, autism spectrum disorders, anxiety disorders, borderline personality disorder, and anger, aggression, and various behavioral control disorders. New terms such as *mood stabilizers* and *neuromodulators* are often used for their expanded use in other psychiatric conditions (Crystal, Olfson, Huang, Pincus, & Gerhard, 2009).

This work will now profile two prominent drugs used for schizophrenia, haloperidol (Haldol[®]), a first-generation typical antipsychotic and amisulpride (Solian[®], Sulpitac[®], Amitrex[®] or Soltus[®]) a second-generation atypical antipsychotic. As these two drugs serve as the comparative agents in this drug discrimination study, further elaboration on them is warranted.

Haloperidol

History. Discovered by Paul Janssen in 1958, Haloperidol was developed by the Belgian company Janssen Pharmaceutica and brought to clinical trials that same year (Granger & Albu, 2005). It was approved by the U.S. Food and Drug Administration (FDA) on April 12, 1967 marketed in the U.S.A and other countries under the brand name Haldol[®] by McNeil Laboratories. It was developed as an offshoot of research with meperidine (pethidine [Demerol]) with the hope of finding a more potent analgesic. Janssen’s molecule, R1625, was tested by psychiatrists in a large psychiatric hospital in Liège, Belgium, on individuals with severe psychotic symptoms and the results were dramatic and very effective in controlling agitated states and managing hallucinations, both key hallmarks of schizophrenia (Healy, 2002). Janssen gave his new drug to physicians and investigators from many different countries including France, Switzerland, Portugal, Denmark, Sweden, Finland, Turkey, Germany and the United

States. Reports from these varied sources all reported the same positive results with the drug in treating schizophrenia. Haloperidol's behavioral pharmacological profile, specifically as a D₁, D₂ and 5-HT₂ antagonist, it was similar to that of chlorpromazine, and research on the drug progressed rapidly (Lehmann & Ban, 1997). It was found that a single dose of 1 to 5 mg delivered intravenously, could control motor agitation (Divry, Bobon, & Collard, 1958). At the first International Congress on Haloperidol, September 5, 1959, Janssen proposed a pharmacodynamic profile for haloperidol's effects. Specifically, it would reduce delusional psychosis, mania, and acute and chronic paranoid psychosis at doses from 2 to 3 mg a day (Janssen, 1995). The pharmacodynamic effects of the drug are that it produces sedation and indifference to external stimuli, while also reducing initiative, anxiety, and activity. It also is effective in reducing delusions and hallucinations (Julien et al., 2010).

Pharmacokinetic properties. The pharmacokinetic properties of haloperidol decanoate in humans are that it reaches peak plasma concentrations in about 6 days (after intramuscular injection) with a half-life of approximately 3 weeks. Via intravenous administration, the bioavailability is 100% with rapid onset of action seen within seconds, while oral administration yields a 60-70% bioavailability. The duration of action is 4-6 hours with plasma levels of 4 to 25 micrograms per liter for therapeutic action. Plasma levels are used to calculate dose adjustments and check compliance especially for long-term use. Concentrations in brain tissue is 20 fold that of body levels (Kornhuber et al., 1999). Haloperidol is slowly eliminated from brain tissue, which may account for the slow disappearance of side-effects when haloperidol medication is stopped (Kornhuber, Wiltfang, Riederer, & Bleich, 2006). Elimination of the drug is accomplished primarily via enzymes in a metabolic pathway in the liver (Gorrod & Fang, 1993). Table 1 illustrates salient pharmacokinetic properties of haloperidol and amisulpride.

Table 1

Pharmacokinetic properties of haloperidol and amisulpride in human studies.

Haloperidol	Route	Dose	t_{1/2} (h)	CL (L/h)	F (%)
Cheng et al. (1987) ¹	IV	1.5-5.0 mg	18.8 ± 4.7	33 ± 7.8	100
	PO	2.0-5.0 mg	18.1 ± 4.5		60 ± 18
Forsman & Ohman (1976) ²	IV	10 mg	14.1 ± 3.2	NR	100
	PO	10mg	24.1 ± 8.9		60 ± 11
Holley et al. (1983) ³	IV	0.125/kg ^a	26.2 ± 8.0	49.2 ± 12	100
	PO	0.503/kg	14.5 ± 3.2		65 ± 14
Magliozzi & Hollister (1985) ⁴	IV	0.125/kg	15.1 ± 2.5	NR	100
	PO	0.500/kg	17.5 ± 8.7		64 ± 23
Kudo & Ishizaki (1999) ⁵	IV	5-10 mg/day	14.1-26.2	23.61	100
	PO	6-5 mg/day	NR	14.89	60-70
Amisulpride					
Caukell et.al. (1996) ⁶	PO	50 mg	12.1	NR	47
Nobel & Benfield (1999) ⁷	IV	50 mg	NR	32.8	NR
	PO	50 mg	12	NR	≈50
Rosenzweig et.al. (2002) ⁸	PO	50 mg	1.3±0.1	31.2-41.6	48-51
Sparshatt et.al. (2009) ⁹	PO	50 mg	12	NR	48

a = mean weight 70.0 ± 7.0 kg

Abbreviations: **IV** = intravenous; **PO** = oral; **t_{1/2}** = elimination half life; **CL** = clearance; **F** = bioavailability; **NR** = not reported

¹ (Cheng et al., 1987)

² (Forsman & Ohman, 1976)

³ (Holley, Magliozzi, Stanski, Lombrozo, & Hollister, 1983)

⁴ (Magliozzi & Hollister, 1985)

⁵ (Kudo & Ishizaki, 1999)

⁶ (Caukell, Spencer, & Benfield, 1996)

⁷ (Nobel & Benfield, 1999)

⁸ (Rosenzweig et al., 2002)

⁹ (Sparshatt, Taylor, Patel, & Kapur, 2009)

Pharmacodynamic properties. Haloperidol belongs to a chemical class of drugs called the butyrophenones. The pharmacodynamic property of this class of drugs (similar to that of phenothiazines) arises from its biochemical and physiological effect of competitively blocking dopamine D₂ receptors in the mesolimbic and nigrostriatal pathways (I Creese, Burt, & Snyder, 1976). Indeed, the clinical potencies of antipsychotic drugs correlate positively with their affinity for D₂ receptors (Philip Seeman & Kapur, 2001). It was Arvid Carlsson, Nobel prize winning (2000) Swedish scientist who first promoted the dopamine hypothesis of schizophrenia (Carlsson & Lindquist, 1963). Seeman proposed five lines of evidence that support the dopamine receptor basis for schizophrenia (Philip Seeman, 1987). First, the clinical potencies of all antipsychotic drugs correlate directly with their ability to block D₂ receptors. Second, brain dopamine receptors are consistently occupied at 60% to 80% at therapeutic doses of antipsychotics. Third, the D₂ receptor densities are elevated in schizophrenia patients when measured with radioactive N- methylspiperone. Fourth, while D₁ receptors modulate activity of the D₂ receptor, such as the D₂ role in inhibiting intracellular adenylyl cyclase activity (Missale, Nash, Robinson, Jaber, & Caron, 1998) , this influence is profoundly reduced or missing in postmortem tissue from psychotic individuals. Fifth, levels of dopamine in the extracellular synaptic space are high in schizophrenic patients (Philip Seeman & Kapur, 2001). Radioligand binding assays clearly demonstrate the binding affinity of neuroleptic drugs at D₂ receptor and other studies demonstrate they are antagonist at these receptors (I. Creese, Burt, & Snyder, 1978; Farde et al., 1992; Nord & Farde, 2011). Unfortunately this antagonism at D₂ receptors is responsible for unwanted motor side effects such as Parkinson like symptoms and other extrapyramidal symptoms. It has a rapid onset, especially when given by injection with a bioavailability of 100%. Recommended doses of oral administration range from 2 to 5 mg a day

with the upper threshold of therapeutic level as that amount that would begin to trigger extrapyramidal symptoms (DaSilva, Hould, & Zipursky, 1996). The presence of extrapyramidal effects in 50% to 90% of patients and more severe motor disturbances such as tardive dyskinesia (15% to 20%) continued to be a source of contention with haloperidol as a treatment as with other typical antipsychotics. The most serious side effect, tardive dyskinesia, was seen to be the “Achilles heel of early antipsychotics” (Healy, 2002) . Additionally, there were inherent therapeutic limitations to these medications. Some 30% to 50% of chronic schizophrenics remained unresponsive or only partially responsive to treatment. The typical antipsychotic drugs also failed to treat negative symptoms effectively and did just as poorly in treating neurocognitive deficits (Strauss & Carpenter, 1977). These concerns would help fuel the search for the second-generation atypical antipsychotic drugs, such as amisulpride.

Receptor binding profile. Haloperidol shows high affinity toward striatal dopamine receptors. Autoradiography studies demonstrate that haloperidol recognizes all human dopamine receptor subtypes, especially at D₂, D₃ and D₄. Haloperidol also exhibits high affinity for α_{1A} and α_{1B} adrenoceptors. Haloperidol shows weak affinity at 5-HT_{1A}, stronger affinity for 5-HT_{2A} and moderate affinity at 5-HT_{1B} (Schoemaker et al., 1997) . As mentioned earlier, it is the blockade of dopamine receptors that is believed to account for haloperidol’s therapeutic efficacy for positive symptoms as well as the unwanted extrapyramidal side effects. Haloperidol’s action at other receptors appears negligible at normal human doses. See Table 2 for the receptor binding profile of haloperidol in comparison to the atypical antipsychotic amisulpride.

Table 2

Dissociation Rate Constants (K_i , nM) for tested drugs at selected neurotransmitter receptor subtypes.

Receptor	5-HT _{1A}	5-HT _{1B}	5-HT _{2A}	5-HT _{2B}	5-HT ₆	5-HT ₇	D ₁	D ₂	D ₃	D ₄	D ₅
Haloperidol	7930 ⁷	165 ²	78 ⁷	1420 ⁸	3666 ¹	378 ¹	83 ¹	2 ¹	2.9 ⁹	15 ¹	147 ¹
Amisulpride	NSB ⁵	1,744 ⁴	2000 ⁵	13 ⁴	4154 ⁴	11.50 ⁴	NSB ^{4,5}	1.3 ⁶	2.4 ⁶	2369 ⁴	NSB ⁴
Clozapine	770 ⁷	398 ¹	12 ⁷	7.15 ⁸	17 ¹	18 ¹	189 ¹	69 ⁹	479 ⁹	39 ¹	235 ¹
Aripiprazole	5.6 ¹	833 ¹	13.3 ^{3,10*}	0.36 ¹⁰	570 ¹⁰	10.3 ¹⁰	1173.5 ^{1,10*}	3.3 ¹⁰	5.35 ^{10*}	514 ¹	2133 ^{1,10*}

Receptor	α_{1A}	α_{1B}	α_{2A}	H ₁	M ₁	M ₅
Haloperidol	12 ¹	8 ¹	1130 ¹	3002 ¹	NSB ¹	657 ¹
Amisulpride	NSB ⁴	NSB ⁴	1114 ⁴	NSB ⁴	NSB ⁴	NSB ⁴
Clozapine	1.6 ¹	7 ¹	142 ¹	2 ¹	14 ¹	29 ¹
Aripiprazole	25.7 ¹⁰	34.8 ¹⁰	74.3 ¹⁰	25.1 ¹⁰	6780 ¹⁰	2330 ¹⁰

5-HT = serotonin receptors; **D** = dopamine receptors; **M** = cholinergic muscarinic receptors; **α** = noradrenergic alpha receptors, **H** = histamine receptors; **K_i** = equilibrium dissociation constant of the competitive inhibitor; **NSB** = no significant binding (>10,000 nM); * = average for binding

¹ K_i determinations were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program (PDSP), Contract #HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. For experimental details please refer to the PDSP web site <http://pdsp.med.unc.edu/>. Human cloned and PDSP Certified unless otherwise indicated; original reference is indicated if not PDSP certified data.

² (Schotte et al., 1996), human cloned

³ NIMH PDSP (Roth, 2011) rat cloned

⁴ (Abbas et al., 2009) human cloned

⁵ (Schoemaker et al., 1997) rat cerebral cortex

⁶ (Sokoloff, Giros, Martres, Bouthenet, & Schwartz, 1990) human clone

⁷ (Bymaster et al., 1996) rat cortex

⁸ (Wainscott et al., 1998) human cloned

⁹ (Sokoloff et al., 1992) human cloned

¹⁰ (Shapiro et al., 2003) human cloned; rat clone for 5-HT_{2A}

Amisulpride

The impetus for discovering the second-generation atypical antipsychotic drugs was the need for a new class of medications that alleviated the positive and negative symptoms of schizophrenia without causing debilitating extrapyramidal motor effects. It was well established that clinically effective drugs share D₂ dopamine receptor antagonist properties (P. Seeman, 1992). As well, receptor occupancy of 50% - 60% of central nervous system D₂ dopamine receptors is necessary to elicit antipsychotic activity, whereas higher receptor occupancy amounts of 70% - 80% are responsible for extrapyramidal effects (Farde et al., 1992). The current version of the dopamine hypothesis proposes that antipsychotic effects are linked to activity at limbic dopamine receptors, and antagonism of dopamine receptors in the striatum is responsible for extrapyramidal effects. The mesolimbic pathway is important for memory and motivating behaviors. Antipsychotic drugs which blockade this pathway reduce the intense emotions associated with schizophrenia. Antipsychotics blocking the mesocortical dopamine pathway reduce symptoms such as hallucinations, disordered thinking and delirium. Antipsychotics that blockade the nigrostriatal dopamine pathway are linked to extrapyramidal motor side effects. Thus, a compound possessing selectivity for limbic and mesocortical structures while exerting minimal antagonism on nigrostriatal receptors may function as an antipsychotic drug with fewer propensities for adverse motor effects (Perrault, Depoortere, Morel, Sanger, & Scatton, 1997). Such a hypothesis was validated by the clinical efficacy of a compound known as sulpiride that preferentially blocked limbic dopamine receptors (Zivkovic, Guidotti, Revuelta, & Costa, 1975).

History. The historical roots of sulpiride date from 1958 with the French company Delagrange. Sulpiride was developed from a range of medicinal compounds, belonging to the

chemical class of benzamides, such as metoclopramide used to treat gut disturbances.

Metoclopramide is a D₂ receptor antagonist and a mixed 5-HT₃ receptor antagonist/ 5-HT₄

receptor partial agonist (Donnerer, 2003). Paul Deniker noticed that some patients in the clinic who were taking metoclopramide exhibited neuroleptic-like extrapyramidal side effect.

Although these effects were rare, Deniker speculated (correctly) that metoclopramide might be a neuroleptic. From this, Delagrangé synthesized a range of benzamides and chose sulpiride for antipsychotic testing. The results on psychotic patients demonstrated clearly that sulpiride had obvious antipsychotic effects and was less likely to produce extrapyramidal effects, nor did it lead to tardive dyskinesia. Additionally, it was effective in treating depression and anxiety. The first clinical data on sulpiride's effectiveness in treating neurotic and dysthymia symptoms was presented in Paris in 1968 at the Académie française. During the 1970s it was widely used in both France and Japan (Healy, 2002).

Atypicality. Research on D₂ receptor subtypes expanded avenues for therapeutic agents possessing more selective antidopaminergic properties that have affinity for limbic localization of the D₃ or D₄ receptors versus the more widespread distribution of the D₂ receptors as seen with haloperidol. Paul Janssen was the first to pursue and announced, in 1984, the development of a drug that simultaneously blocked D₂ and 5HT₂ receptors issuing in a new class of compounds called butyrophenones. From this class would come risperidone, which had the desired property of blocking both serotonin and dopamine receptors. This was the first post-clozapine drug of the serotonin-dopamine antagonists to be developed. This serotonin-dopamine antagonistic effect became the molecular basis for what we now call "atypicality" (Healy, 2002). Atypicality would become the catch phrase for any of the newer drugs designed for schizophrenia that differed from haloperidol and its propensity for causing unwanted extrapyramidal effects. The mystery,

both then and now, is explaining how these atypical medications did not produce the neuroleptic effects (catalepsy, extrapyramidal motor side effects, etc.) when they clearly are D₂/D₃ antagonists, a hallmark mechanism of typical antipsychotics known to cause the very same unwanted side effects. In fact, sulpiride, at the time of its development, was the purest D₂ receptor antagonist yet it produced very few extrapyramidal effects (Healy, 1996). The question remained what accounts for sulpiride's profoundly diminished extrapyramidal effects? From research on sulpiride, the drug amisulpride would be developed.

Receptor binding profile. Amisulpride [(± amino-4-N-(1-ethyl-2 pyrrolidinyl) methylsulphonyl-5-methoxy-2-benzamide)] is a substituted benzamide derivative that has a range of effects on dopaminergic and serotonergic transmission. Figure 1 illustrates the chemical structure and data of amisulpride, in its two isomer forms and racemic mixture; all were utilized in this study.

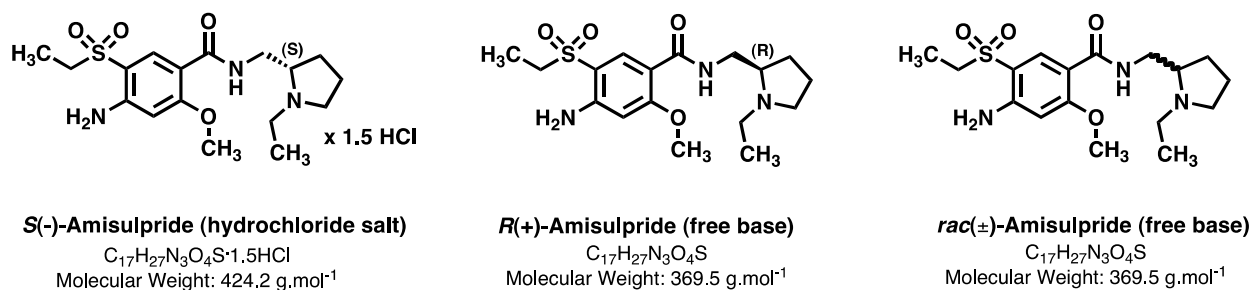


Figure 1. Chemical Structure of amisulpride

Amisulpride was designed to be used as an atypical antipsychotic medication. It is a dopamine antagonist with high selectivity for dopamine D₂ and D₃ receptors as well as antagonistic action at serotonin 5-HT_{2B}/5-HT₇. In high doses, it exhibits dopaminergic blocking activity similar to that of typical antipsychotic medication, whereas in low doses it appears to increase dopaminergic transmission. It was introduced by the French pharmaceutical company Sanofi-Aventis in the mid-90s and sold as Solian[®], Sulpitac[®], Amitrex[®] or Soltus[®]. Merger acquisitions of Sanofi-Aventis delayed amisulpride's introduction and marketing in the United States and the company decided not to pursue market in the United States where numerous atypical medications were already in place. As a result, amisulpride is not approved by the Food and Drug Administration (FDA) for use in the United States, but it is used in Europe (France, Germany, Italy, Switzerland, Russia, United Kingdom, etc.) and Australia to treat psychoses and schizophrenia. While it is mainly used to treat schizophrenia it is also used, off label, to treat depression and dysthymia. As an antipsychotic drug, it shows clinical efficacy for both positive and negative symptoms of schizophrenia at high or low doses with a low incidence of extrapyramidal side effects (Delcker, Schoon, Oczkowski, & Gaertner, 1990). It has been shown that the drug is a relatively selective dopamine receptor antagonist with high and similar affinities for D₂ and D₃ receptor subtypes (Sokoloff et al., 1990). Meltzer (1989) believes that one measure of atypicality is that the ratio binding to 5-HT₂ receptors relative to D₂ receptors is ≥ 1.2 (Meltzer, 1989). Amisulpride fits this criterion, as its ratio of pK_i values of 5-HT₂/D₂ is 29. Table 2 presents the known binding profile of amisulpride.

Theories of atypicality. Questions continue today in the discussion as to why atypical antipsychotic medications, given their binding profile at D₂/D₃, do not produce extrapyramidal

side effects. Two prevalent theories seek to explain this phenomenon. Meltzer (1989) suggested in that the ratio of serotonin to dopamine receptor occupancy provides the answer. Meltzer proposed that atypical antipsychotics can be distinguished from typical antipsychotics on the basis of lower D₂ and higher 5-HT₂ receptor binding affinities and that this ratio be ≥ 1.12 . He holds that this information would be useful in drug screening assays (Meltzer, 1989). An alternative theory is the “fast-off” theory of atypical antipsychotic action proposed by Kapur and Seeman (Kapur & Seeman, 2001). This theory proposes that atypicals have low affinities for the dopamine D₂ receptor, and are loosely bound to, and rapidly released from D₂ receptors. Critical to this theory is the idea that the atypical antipsychotics bind more loosely to D₂ receptors than dopamine itself, while typical antipsychotics bind more tightly than dopamine (Philip Seeman, 2002). Seeman believes this “quick release” from dopamine receptors is the distinguishing characteristic separating atypical antipsychotics from typical antipsychotics and account for the decreased extrapyramidal side effects, as well as explaining the therapeutic effects of the atypicals. Whether the serotonin/dopamine ratio theory or the “fast-off” theory better accounts for the enhanced therapeutic value of the atypicals over the typicals is a question that will continue into the future and fuel additional research. The answer to the question may also be a clue in explaining any potential differences found in the discriminative cue properties of atypical antipsychotics (e.g. amisulpride) versus typical antipsychotics (e.g. haloperidol). Nevertheless, a more complete explanation clarifying why atypicals are more effective than typical antipsychotic medication requires further study, with particular focus on the role of genetic variations and interactions with other neurotransmitters involved in schizophrenia (da Silva Alves, Figuee, Amelsvoort, Veltman, & de Haan, 2008).

Pharmacokinetic properties. The pharmacokinetic properties of a drug address how the drug is handled by the body relative to indices measuring drug absorption, distribution, termination of drug action, time course of the drug distribution, elimination, half-life, tolerance and dependence. Table 1 details the pharmacokinetic properties of amisulpride. Amisulpride has been shown to be well tolerated (Widlöcher, Allilaire, Guérard des Lauriers, & Lecrubier, 1990). In studies done with human volunteers, at doses of 50 mg daily prescribed for dysthymia, the following pharmacokinetic properties have been found. The drug has an oral bioavailability of $\approx 50\%$. Peak plasma concentrations occur at 1 and 3 hours after oral administration, the second peak being the larger of the two. The absorption of amisulpride is significantly reduced by ingestion of a high carbohydrate, mainly fluid meal, but is not affected by a meal high in fat. Protein binding of amisulpride is minimal and the volume of distribution is large (Nobel & Benfield, 1999). The total body clearance is 32.8 hours with renal clearance at 18.7 hours. The terminal elimination half-life is 12 hours with 51 – 71% eliminated in feces and 24 – 47% in urine (Bianchetti, Canal, & Rosenzweig, 1995; Dufour & Desanti, 1988).

Pharmacodynamic properties. The pharmacodynamic properties of a drug account for the biochemical and physiological effects of the drug on the body, particularly at receptor sites. This underscoring the basic principle of pharmacology that any behavioral effects induced by a drug follow from the drug's interaction with receptors (Julien et al., 2010). Amisulpride's mechanism of action is that it binds selectively to dopamine D₂ and D₃ receptors in the limbic system. It is unique in that it is selective, at low doses, for presynaptic autoreceptors that control dopaminergic transmission (Coukell et al., 1996). Amisulpride also preferentially interacts with limbic dopamine D₂-like receptors. The drug does not recognize D₁, D₄ or D₅ receptors, and at low doses (≤ 10 mg/kg) *in vivo* (rodents) it preferentially blocks *presynaptic* D₂ and D₃

dopamine autoreceptors, thereby facilitating both dopamine release and dopaminergic neurotransmission for limbic rather than striatal receptors (Schoemaker et al., 1977). Higher doses block *postsynaptic* receptors, thus inhibiting dopaminergic hyperactivity. Clinical trials and *in vivo* studies have demonstrated that amisulpride has potent 5-HT₇ antagonistic effects, making it useful in depression treatment, specifically dysthymia (Abbas et al., 2009).

Amisulpride has been shown to be effective in treating the positive symptom of schizophrenia as other typical medications (e.g. haloperidol). However, amisulpride is significantly more efficacious in reducing negative symptoms compared to typical antipsychotic drugs. (Möller, 2000). Thus it can be considered for use as a first line treatment for acute and chronic schizophrenia (albeit, its use is restricted geographically). Table 3 summarizes the pharmacodynamic properties of amisulpride.

Table 3.

Summary of the pharmacodynamic profile of amisulpride.

<p>Selectivity for dopamine D₂ and D₃ receptors <i>In vitro</i>: high affinity for human dopamine D₂ and D₃ receptors ($K_i \approx 3\text{nmol/L}$) No affinity for D₁ and D₄ or D₅ receptors Affinity for 5-HT_{2B}, and 5-HT₇ but no significant affinity for other serotonin receptor types and none for histamine H₁, muscarinic or α-adrenergic receptors <i>Ex vivo</i>: higher affinity for D₃ than for D₂ receptors (selectivity ratio = 2)</p>
<p>Selectivity for limbic structures Preferential blockade of dopamine agonist-induced hypermotility vs stereotypies, lack of induction of extrapyramidal motor side effects.</p>
<p>Selectivity for presynaptic D₂ and D₃ autoreceptors at low doses Preferential blockade of apomorphine-induces yawning and hypomotility; potentiation of the incentive value of food in a place preference paradigm</p>
<p>Endocrine effects in humans Mean prolactin level increased from 7.89 (predose baseline) to 36.96 mg/L 5 hours after administration of a single dose of amisulpride 50mg in 21 healthy volunteers; after a further 3 days of amisulpride administration (50mg twice daily), predose and postdose (5 hours) prolactin levels on day 5 were 41.77 and 47.23 mg/L Endocrine adverse events during amisulpride treatment for dysthymia suggest at least some dopamine receptor antagonism at low dosages</p>

K_i = binding constant

Note. Adapted from (Nobel & Benfield, 1999)

Drug Discrimination as a Behavioral Assay

Drug discrimination is a valuable behavioral assay for studying the *in vivo* pharmacology of drugs. As a tool, it involves studying the interoceptive effects of a training drug as a stimulus cue for performing a specific behavioral response (Solinas, Panlilio, Justinova, Yasar, & Goldberg, 2006). In its most basic form the drug discrimination assay is a behavioral procedure whereby an organism must recognize a particular drug state, choose a correct response, and receive reinforcement (Young, 2009). Drug discrimination techniques have been used to study a wide variety of drugs from therapeutic agents to drugs of abuse. In a typical study, an animal such as a mouse is trained via Pavlovian and operant (Skinnerian) principles of learning to associate an interior, subjective state with a particular behavioral response such as lever pressing. The subjects learn to discriminate the internal stimuli associated with a particular drug (called a training drug) from those stimuli of a vehicle state (non-drug agent such as saline). After training in a drug discrimination paradigm, the subjects can recognize the specific interoceptive cues of different drugs. The drugs themselves serve as discriminative stimuli, which is useful in studying the pharmacological profile of the drug (Harris & Balster, 1971; Overton, 1966). After the animal has learned to discriminate the training drug from vehicle, testing can proceed on different doses of the training drug (generalization testing) followed by the introduction of novel agents for the purpose of substitution testing. Substitution testing yields valuable information as to the receptor binding profile of drugs. Drugs in the same pharmacological class tend to substitute for each other. For example, in animals trained to discriminate dihydroetorphine, will substitute and elicit similar behavioral responses to heroin and morphine (Beardsley & L.S, 1997). If a novel drug does not substitute for a training drug, this suggests that the underlying pharmacological mechanisms are dissimilar. As well, drug discrimination is a valuable tool for

assessing a wide variety of factors related to drugs such as: gender, genetic strains, pharmacological history, genetic manipulations (knockout subjects) and other neurobiological factors that can influence the interoceptive property of a chemical agent. Research suggests that there is strong commonality between the discriminative effects of drugs in animals and that of humans (Kamien, Bickel, Hughes, Higgins, & Smith, 1993).

Drug Discrimination and Antipsychotic Drugs

Drug discrimination plays a unique role in the investigation of the biochemical, neurological, and pharmacological properties of antipsychotic medications. It is quite useful as a behavioral assay in the preclinical development of these medications (Goudie & Smith, 1999; Porter & Prus, 2009). Drug discrimination contributes to our understanding of the pharmacological mechanisms that mediate the discriminative stimulus properties between and within antipsychotics and provides a method of analysis for charting the stimulus effects of various doses of a particular drug. Additionally, it provides an assay to measure the extent to which a drug generalizes to various doses of itself, and the degree to which a novel drug may substitute for a training drug. There is a wide assortment of preclinical behavioral tests used in screening drugs in the development of antipsychotic medications (Arnt & Skarsfeldt, 1998; Ellenbroek, 1993b; Geyer & Ellenbroek, 2003). Drug discrimination is unique in that it measures the interoceptive effects of a training drug as a stimulus cue for performing a specific behavioral response. As a behavioral assay and paradigm, it has been used for many years to aid in classifying drugs, identifying the underlying pharmacological mechanisms mediating the stimulus properties of a drug, and providing information on the role of genetics in drug response (Arnt & Skarsfeldt, 1998; Goudie & Smith, 1999; Porter & Prus, 2009).

Procedural variables are an important component in drug discrimination. Some procedures may vary depending on the design of the study, such as: the route of injection (e.g., subcutaneous, intraperitoneal), the pre-injection time (typically 30 to 60 minutes), the reinforcement schedule (fixed ratio 30 or 10 reinforcement schedules), and the reinforcers, most likely food (pellets, a liquid, such as water or sweetened milk). Despite these procedural differences, results from drug discrimination studies tend to be remarkably consistent within the same drug class (Porter & Prus, 2009). Major differences that are found across studies are often directly related to the dose of the training drug and the species utilized.

History. A brief historical sketch of early drug discrimination with antipsychotic drugs studies follows. The first drug discrimination study of antipsychotics was done in 1962 when Stewart trained rats to discriminate 4.0 mg/kg (i.p.) chlorpromazine from saline in a shock-avoidance task using a three-compartment test chamber (Stewart, 1962). Substitution tests revealed that the phenothiazines acepromazine, perphenazine, and prothipendyl fully substituted for chlorpromazine, but that the phenothiazines prochlorperazine and the tricyclic antidepressant imipramine did not substitute. The study found a dose-dependent curve generalization curve for chlorpromazine with appropriate responding ranging from 28.9% (2.0 mg/kg chlorpromazine) to a maximum of 94.3% at the 4.0 mg/kg training dose. In 1966 Overton tried, unsuccessfully, to establish discrimination with a 5.0 mg/kg (i.p.) dose of chlorpromazine in a T-maze (shock avoidance) procedure. He reported that no discrimination could be established (Overton, 1966). Other early drug discrimination studies followed testing chlorpromazine with a two-lever operant conditioning procedure (Harris & Balster, 1971).

In 1974 the first drug discrimination study on chlorpromazine in a two-lever operant task with rats as subjects was conducted by Barry et al. (1974); a study that also included the first

testing of the discriminative stimulus properties metabolites, in this case metabolites of chlorpromazine: 3,7-dihydroxy-CPZ, 7,8-dihydroxy-CPZ and 7-hydroxy-CPZ. Barry et al. were able to train rats to discriminate chlorpromazine (1mg/kg) from saline. Substitution testing using chlorpromazine (2mg/kg) as the training dose, showed that of the three metabolites, only 7-hydroxy-CPZ elicited similar behavioral responses to the drug.

Colpaert et al., in 1976, were the first to test haloperidol as the training drug in a two-lever operant discrimination (food reward). Rats were trained to discriminate 0.02 mg/kg (s.c.) haloperidol from saline. The authors reported that this was a difficult task requiring over 80 training sessions (Colpaert, Niemegeers, & Janssen, 1976). The first drug discrimination study with the atypical antipsychotic clozapine with rats was conducted by Goas and Boston in 1978. Substitution testing showed that haloperidol, clozapine, and the muscarinic antagonist benztropine mesylate produced full substitution for chlorpromazine; however, in the clozapine-trained rats, none of the tested drugs (chlorpromazine, haloperidol, chlordiazepoxide and atropine) substituted for clozapine (Goas & Boston, 1978). Utilizing a T-maze, Overton in 1982 found that clozapine (20 mg/kg, i.p.) and haloperidol (2.5 mg/kg, i.p.) could be established as training drugs; however, no drug discrimination could be established with the antipsychotic drugs chlorpromazine, fluphenazine, haloperidol, or thioridazine (Overton, 1982).

In the years following these early studies, a growing body of research has been published on the discriminative stimulus properties of other antipsychotic drugs. Continued research is revealing more information about the pharmacological mechanisms that underlie the discriminative stimulus properties of antipsychotic drugs. Much of the drug discrimination research has centered on the drug clozapine, a dibenzodiazepine, which continues to be the “gold standard” and “prototypical” atypical antipsychotic medication against which all antipsychotic

drugs are compared. The binding profile of clozapine shows that it binds with a low affinity to dopamine D₂ receptors but has a high affinity for D₁ receptors. Clozapine demonstrates high binding affinity for many other neurotransmitter receptors including dopaminergic D₄, serotonergic 5-HT_{2A/2C}, 5-HT₆, 5-HT₇, cholinergic M₁, M₂, M₃, M₄, adrenergic α₁, α₂, and histaminergic H₁ receptors (Arnt & Skarsfeldt, 1998; Bymaster et al., 1996; Richelson, 1999; Schotte et al., 1996). In contrast to clozapine's antagonistic activity at the aforementioned receptors, clozapine displays a weak partial agonist activity at M₁ receptors (Davies, Compton-Toth, Hufeisen, H.Y., & Roth, 2004) and agonistic activity at M₄ and 5-HT_{1A} receptors (Arnt & Skarsfeldt, 1998; Newman-Tancredi et al., 2005; Zeng, Le, & Richelson, 1997). Such agonistic properties of clozapine may explain some of the unique therapeutic properties of the drug (Porter & Prus, 2009). Many believe that agonistic activity at 5-HT_{1A} receptors contributes to the treatment of negative and cognitive symptoms, mood enhancement, and the reduction of extrapyramidal motor side effects (Millan, 2000). Nielsen contends that muscarinic cholinergic antagonism (in rats) is key to clozapine's discriminative stimulus properties (Nielsen, 1988) a finding reinforced by Kelley and Porter (Kelley & Porter, 1997) and other studies (Goudie, Smith, Taylor, Taylor, & Tricklebank, 1998). Nicotinic cholinergic receptors also have not been shown to be important in the discriminative stimulus properties of clozapine (Prus, Philibin, Pehrson, & Porter, 2006; Villanueva, Arezo, & Rosecrans, 1992). Clozapine's lower affinity for dopamine receptors suggests that the drug's antagonistic effect at these receptors does not seem to be significant as a mediating factor for clozapine's discriminative stimulus properties. Quite the opposite, antagonism of D₂ receptors is thought to inhibit the ability of some antipsychotic drugs to substitute for clozapine (Carey & Bergman, 1997; Cole, Field, Sumnall, & Goudie, 2007). The evidence is clear that clozapine, as do other atypical antipsychotic drugs, has a

diverse and multifaceted binding profile and a compound discriminative stimulus that is not fully understood (Goudie & Smith, 1999; Porter & Prus, 2009).

Currently, three other clinical atypical antipsychotic drugs have been utilized as the training drug in drug discrimination studies: olanzapine, quetiapine, and ziprasidone. Each of these compounds exhibit a greater affinity for 5-HT_{2A} receptors over D₂ receptors; however, like clozapine, they have diverse binding profiles for other receptors as well (Schotte et al., 1996). Olanzapine (a thienobenzodiazepine derivative) has a receptor-binding profile resembling clozapine, but has a much higher affinity for D₁ and D₂ dopamine receptors. Ziprasidone has a higher affinity for 5-HT_{1A} and 5-HT₇ receptors. All three of these drugs have strong affinities for α_1 -adrenoceptors, while only olanzapine exhibits a strong affinity for muscarinic receptors (Millan, 2000; Richelson, 1999; Schotte et al., 1996).

Rationale

Amisulpride is an atypical antipsychotic medication developed in the 1990s. It has a unique binding and clinical profile, possessing a high affinity for dopamine D₂ and D₃ receptors, serotonin 5-HT_{2B}, and 5-HT₇ with a preferential activity in the limbic region of the brain. It has a dual dopamine antagonistic effect. At high doses it blocks postsynaptic D₂/D₃ receptors and at low doses it selectively blocks D₂/D₃ presynaptic autoreceptors amplifying dopaminergic transmission (Coukell et al., 1996; Cudennec, Fage, Benavides, & Scatton, 1997; Schoemaker et al., 1997). Amisulpride is chiral and has two isomers: (+)R and (-)S amisulpride. The racemic form, (+/-)SR Amisulpride, is a 50/50 mixture of the two enantiomers. (-)S amisulpride is the more active enantiomer insofar as its ability to bind to dopamine D₂ and D₃ receptors is twice as potent as the racemic form and 20 to 40 times more potent than (+)R amisulpride in displacing

radioligands from dopamine D₂ and D₃ receptors (Castelli, Mocci, Sanna, Gessa, & Pani, 2001). There is ample evidence showing its efficacy in treating both positive and negative symptoms and the value of its clinical use in the treatment of schizophrenia (Möller, 2000). It is generally well tolerated with the incidence of extrapyramidal motor symptoms (especially for low doses) similar to that of placebo (Nobel & Benfield, 1999). By comparison, the antipsychotic drug haloperidol is also a dopamine antagonist at D₁ receptors and selective antagonism with high affinity for the dopamine D₂ and D₃ receptors producing extrapyramidal motor side effects. What accounts for this discrepancy? Is it that amisulpride is much more selective at D₂ and D₃ receptors? Is it related to the speed with which atypicals bind to and release from receptors (Kapur & Seeman, 2001)? It is the ratio of dopamine-serotonin receptor activity (Meltzer, 1989)? Is it the action of amisulpride at autoreceptors or the particular dopamine pathway in the brain affected? This is a riddle yet to be solved.

Drug discrimination is a powerful *in vivo* assay for determining the subjective effects of drugs and for studying the *in vivo* receptor mechanisms that mediate a drug's discriminative stimulus and perhaps therapeutic effects. The drug discrimination procedure used in the present study allows a direct comparison between the atypical antipsychotic amisulpride and the typical antipsychotic haloperidol.

The present study used the drug discrimination paradigm as a behavioral assay to examine the ability of male C57BL/6 mice to discriminate the atypical antipsychotic drug (-)-S amisulpride from vehicle. C57BL/6 mice were chosen for this study as they have been demonstrated to be an excellent model for preclinical studies of medications used for schizophrenia (Laurent & Podhorna, 2004; Powell, Zhou, & Geyer, 2009; Xu, Yang, McConomy, Browning, & Li, 2009). To date, there are no published drug discrimination studies

of amisulpride as the training drug with mice or rats. As such, this research is an original preclinical study in the effort to investigate the discriminative stimulus properties of amisulpride.

There are four objectives of this study: First, to establish (-)*S* amisulpride, the active enantiomeric form of amisulpride, as a discriminative stimulus in a standard two-lever drug discrimination procedure in C57BL/6 mice; Second, to determine if (-)*S* amisulpride and haloperidol share similar discriminative stimulus properties; Third, to test the enantiomer (+)*R* amisulpride and (+/-) *SR* racemic amisulpride to see if they share discriminative stimuli properties with (-)*S* amisulpride. Fourth, to test the atypical antipsychotic drugs, clozapine and aripiprazole to see if they share discriminative stimuli properties with (-)*S* amisulpride in substitution tests. The major goal of the present study was to determine whether (-)*S* amisulpride, the pharmacologically more salient optical isomer of the atypical antipsychotic (+/-)*SR* amisulpride, could serve as a discriminative stimulus in mice and to evaluate the effects of various chemical and pharmacologically (typical and atypical antipsychotics) related drugs.

Methods

Subjects

Twenty-eight experimentally naïve, adult male C57/BL6 inbred mice (20-25g) obtained from Harlan Laboratories (Indianapolis, IN) were housed individually in clear plastic cages (18 X 29 X 13 cm) with slotted plastic fitted tops and wood chip bedding (sanichips, Teklad, Madison, WI). Mice were transported daily (5-7 days per week) from the vivarium (12 hour light-dark cycle, lights on at 6 a.m.) to the laboratory where experimental training and testing sessions occurred. The vivarium temperature remained between 22 and 24 degrees Celsius. After one week of acclimation, the subjects were food deprived to 85-90% of their free feeding body

weights and were maintained on a food restricted diet of standard rodent chow (Harlan Teklad Lab Diets, Teklad LM-485). Water was available *ad libitum* in the home cages. The *Guide for Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Academy Press, 1996) was followed and the Institutional Animal Care and Use Committee at Virginia Commonwealth University (VCU) approved the procedures that were used in the present study (IACUC Protocol AM10284).

Drugs

S(-)-Amisulpride hydrochloride salt (gift from Drug Discovery Program, Georgetown University, Washington, D.C.), haloperidol (Sigma Chemical Co., St. Louis, Mo.), clozapine (gift from Novartis, East Hanover, N.J.), aripiprazole (National Institute of Mental Health Chemical Synthesis and Drug Supply Program), were dissolved in distilled water with and a small quantity (approximately two drops per 50 ml) of 85% lactic acid. Sodium hydroxide was used as a buffer to insure a pH level of approximately 7.0. (+/-)SR racemic amisulpride free base (gift from Drug Discovery Program, Georgetown University, Washington, D.C.), and the enantiomer (+)R amisulpride (gift from Drug Discovery Program, Georgetown University, Washington, D.C.) were in free base form and dissolved in distilled water. Doses for (-)S amisulpride refer to the salt (HCl) form of the drug. Doses for the (+/-) SR racemic amisulpride, (+)R amisulpride, clozapine, aripiprazole and haloperidol refer to the freebase form of the drugs. Vehicle consisted of 2 drops of lactic acid per 50 ml DH₂O and then buffered with sodium hydroxide for the salt form of the drugs. Vehicle for (+/-) RS amisulpride and (+)R amisulpride was distilled water. All drugs and vehicle were administered subcutaneously (s.c.) at a volume of 10 ml/kg with a 60 minute pre-session injection time for amisulpride drugs

(Perrault et al., 1997) and a 30 minute pre-session injection time for haloperidol (McElroy, Stimmel, & O'Donnell, 1989), clozapine and aripiprazole (Philibin et al., 2009).

Apparatus

Testing was conducted in six standard computer-interfaced operant conditioning chambers (Model ENV-307A, Med Associates Inc., St. Albans, VT) each containing two retractable levers in the left and right positions (8 cm apart) on the front panel of the operant chamber. The levers extended 0.8 cm into the chamber and were positioned 2.5 cm above a grid floor constructed of parallel stainless steel rods. Centered between them was a recessed food trough into which a liquid dipper delivered 0.02 ml of sweetened-milk (by volume: 150 ml powdered milk, 150 ml sugar, and 500 ml water). The inner test chambers consisted of a 15 cm L X 11.5 cm D X 17.5 cm H area surrounded by an aluminum framed box with a single Plexiglas side door. Test chambers were housed in sound attenuating chambers equipped with ventilation fans. MED-PC software (Version 4.2, Med Associates Inc.) was used to control the operant sessions and record data.

Training Procedures

Phase I: Autoshaping. The mice consisted of two cohort groups, 15 mice in cohort group I and 13 mice in cohort group II. Cohort I mice were trained to lever press using a combination of Pavlovian and instrumental conditioning. Pavlovian conditioning is a form of associative learning in which one stimulus, the conditioned stimulus, comes to signal the occurrence of a second stimulus, the unconditioned stimulus. Pavlovian conditioning was first developed by the Nobel Prize (1905) winning physiologist Ivan Petrovich Pavlov (1849-1936). It a traditional learning paradigm used extensively in laboratory research (Domjan, 2005).

Operant conditioning is a form of learning in which the behavior of an organism is modified by its consequences. First developed and termed instrumental conditioning by Edward L. Thorndike (1874–1949) it was reformulated and refined as operant conditioning by B.F. Skinner (1904–1990). Operant conditioning is widely used in preclinical studies of schizophrenia (Gainetdinov, Mohn, & Caron, 2001).

The mice were put into the operant chambers for a period of 2 hours per session with one lever extended. On a variable-time schedule of 45 seconds (range 4 seconds to 132 seconds), a tone and light over the lever came on for a period of 6 seconds. If the animals pressed the lever during this 6 second tone/light period, a contingent reinforcer was delivered and the tone and light were turned off. If the animal did not press the lever, the tone and light turned off at the end of the 6 second tone/light period and a reinforcer was delivered. If lever press responses were made at any time other than during the 6 second interval, they were recorded as non-contingent responses and a reinforcer was not delivered. The reinforcer consisted of a single presentation of sweetened milk delivered by raising the dipper cup and holding in the up position for 4 seconds before being retracted. This Pavlovian condition continued for approximately 7 sessions (days). Due to low response rates, the Pavlovian aspect of the training was dropped; that is, no longer would a reinforcer be paired simply with the tone and light. The animal now had to produce an operant behavioral response of lever pressing, during the 6 second period, in order to obtain the sweetened milk reinforcer. Light and tone were presented similarly to Pavlovian/Instrumental conditioning; however, if the mice did not lever press during the 6 second light/tone presentation no reinforcer was given. This Instrumental conditioning was continued until all animals had two consecutive days of 50 contingent reinforcers per 2 hour period. In an average of 7.3 days (range of 14 days) of instrumental training, all animals reached

50 contingent reinforcers.

Autoshaping for the 14 animals in cohort II initially utilized a nose poke response. The first day combined Pavlovian and Instrumental conditioning. During nose poke training, no lever was presented. A tone and light came on for 6 seconds on a 45 second variable time schedule (range 4 seconds to 132 seconds). If the mice stuck their noses into the dipper well during this period a reinforcer was delivered. If they did not nose-poke, a reinforcer was delivered at the end of the 6 second period. Days 2-5 were Instrumental conditioning. A light and tone came on (for a 6 second period) and if the animal nose poked a reinforcer was delivered. If the mice nose-poked at any other time no reinforcer was delivered and it was recorded as a non-contingent response. Days 6-9 were extinction testing. The light and tone were presented (for a 6 second period of time) on the variable time 45 second schedule. If the mice nose poked during this 6 second period, a reinforcer was delivered but the response was recorded as a contingent response. Responses made at any other time also were not reinforced and were recorded as non-contingent responses. Data collected during this nose poke training were designed to observe how both contingent and non-contingent responses increased over the 4 days of Instrumental training, how the responses decreased during extinction training and how cohort II's nose-poke response rates compared to cohort I's lever pressing response rates. Upon completion of autoshaping, training was suspended for 4 days, and then the autoshaping procedure was repeated and was identical to the nose-poke autoshaping except a lever press response was required instead of a nose-poke response.

Phase II: single-lever training. Single-lever training began upon completion of autoshaping training. A single lever (the vehicle-paired lever) was extended inside the chamber. Each subject was placed in the operant chamber for a 15 minute session and trained to press the

levers for 0.02 ml of sweetened milk on a fixed ratio one (FR1) schedule of reinforcement, in which the reinforcer was delivered after every lever press (dipper was available for 3 sec.). Subjects were trained to lever press on a single lever (i.e. the vehicle-paired lever) until drug administration began. The position of the drug-associated lever (left vs. right) was counterbalanced between each group of subjects to control for olfactory cues (Extance & Goudie, 1981). The value of the FR was gradually increased over the next 7-8 sessions until FR10 was obtained. After response rates were consistently higher than 10 responses per minute, two-lever drug discrimination training began.

Phase III: Drug discrimination acquisition training. Fourteen of the mice were randomly selected to be in the present study for drug discrimination training with amisulpride (the other 14 mice were used in another study). The mice then began single-lever training (errorless training). Subjects were injected daily with vehicle 60 minutes prior to each training session. The vehicle-associated lever was extended in the test chamber and responding was reinforced according to the FR 10 schedule. This vehicle training continued for 25 sessions (days). After response rates were consistently above 10 responses per minute (RPM), the mice were administered 10 mg/kg (-)S amisulpride injections 60 minutes prior to training sessions and were only presented with the (-)S amisulpride-associated lever (opposite of the vehicle-associated lever). Once response rates stabilized at over 10 responses per minute, two-lever drug discrimination training began. During two-lever training sessions both levers were extended into the operant chamber. The subjects were administered amisulpride and vehicle injections according to a double alternation sequence (i.e., DDVVDDVV). On days when the drug was administered, only responding on the drug-associated lever was reinforced. On days when vehicle was administered, only responding on the vehicle associated lever was reinforced.

Responses on the incorrect lever reset the ratio requirement on the correct lever to 10. Subjects received two-lever drug discrimination training until the training criteria were passed during 5 of 6 consecutive sessions.

Drug discrimination training criteria. Successful discrimination training was evaluated and assessed according to three criteria: (1) the first completed fixed ratio (FFR) of the FR10 schedule was executed on the appropriate lever, (2) 80% or greater of total responses made during the session occurred on the appropriate lever, and (3) response rate for the session was equal to or exceeded 10 RPM. Control tests with vehicle and amisulpride were administered and had to be passed prior to generalization testing with all drugs. During control test sessions, responses on both levers were reinforced according to the FR10 schedule and the FR requirement was reset when switching between levers occurred. The three training criteria also had to be met during the two consecutive training sessions immediately prior to all test sessions.

Phase IV: drug discrimination. Once the two lever discrimination training criteria were passed, both vehicle and amisulpride or control tests had to be completed prior to generalization testing. Generalization testing occurred with a minimum of 2 training days between drug tests and the mice were required to pass both a vehicle and amisulpride training session in the two training sessions prior to generalization testing to assure that the test subject was under stimulus control. After successful completion of vehicle and amisulpride control tests, an (-)S amisulpride generalization dose effect curve was determined (0.1563 – 40 mg/kg for 10.0 mg/kg training dose). Next, the substitution testing with the typical antipsychotic drug haloperidol was conducted. Then, (+)R amisulpride and racemic (+/-)RS amisulpride, clozapine and aripiprazole were tested to determine if they would substitute for (-)S amisulpride.

Operational definitions of dependent variables. One measure of stimulus control was

the first fixed ratio (FFR). This was defined as the subject's first set of 10 continuous and uninterrupted responses on either of the two levers. If a subject begins responding on one lever, and then switched to the opposite lever without completing 10 consecutive responses on the initial lever, the counter was reset to 0 and did not record a first fixed ratio until 10 uninterrupted responses were completed on one lever. Another measure of behavior was the percent of drug lever responding (%DLR). This was calculated by counting the number of responses on the appropriate drug lever in a 15 minute session and dividing the quotient by the total number of responses made on both levers, then multiplying that number by 100 to convert the decimal to a percentage. Test drugs that achieve response percentages at 80% or higher were considered full substitution. Response rate was calculated as responses per minute (RPM) for each 15 minute session.

Data analysis. ED₅₀ values [with 95% confidence intervals] were calculated for %DLR data using the least squares method of linear regression with the linear portion of the dose effect curve. For all test drugs that fully substituted for (-)S amisulpride, ED₅₀ values were calculated. Full substitution equaled 80% or greater drug-appropriate lever responding; partial substitution equaled ≥ 60 to < 80 %DLR. Repeated-measures analysis of variance (ANOVA) comparing responses per minute were calculated for each drug (GB-STAT software; Dynamic Microsystems, Inc., Silver Spring, MD.). Significant ANOVAS were followed by Dunnett's post hoc tests ($p < 0.05$).

Results

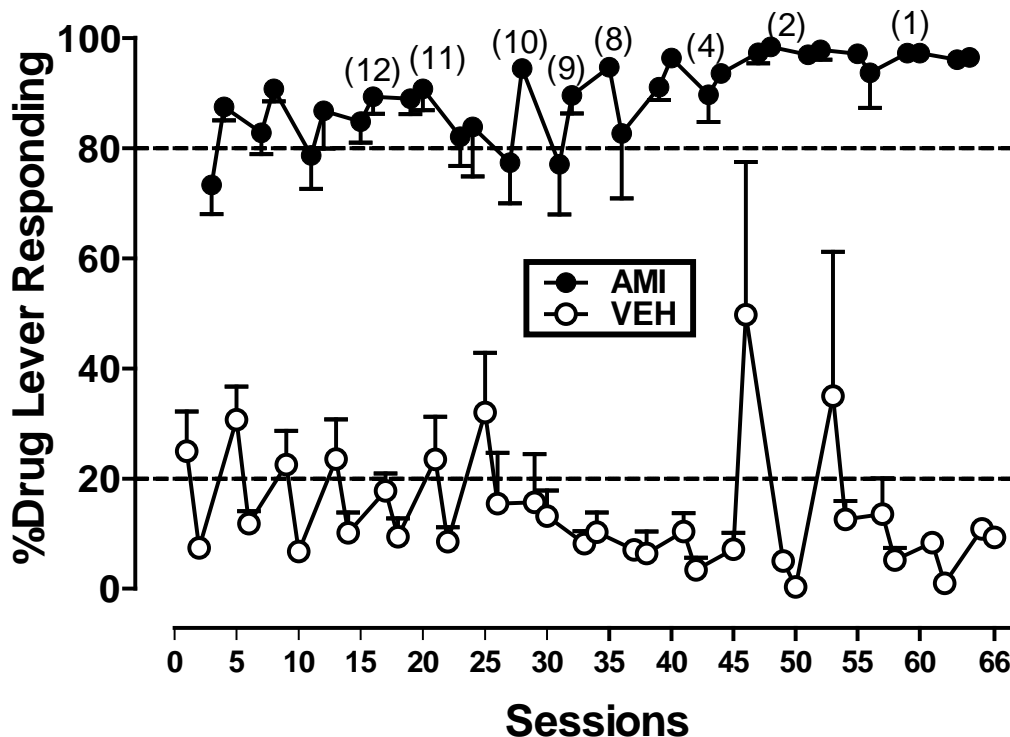
(-)*S* amisulpride Acquisition

The results of the acquisition training for the mice successfully trained to discriminate 10 mg/kg (-)*S* amisulpride from vehicle are shown in Figure 2. Thirteen of the fourteen mice reached training criteria in an average of 43.5 sessions (SEM \pm 4.4) with a range of 11-74 sessions. One mouse failed to acquire the (-)*S* amisulpride discriminative cue and was removed from the study.

(-)*S* amisulpride Generalization Curve

Mean percent drug lever responding (\pm SEM) and mean responses per minute (\pm SEM) for the (-)*S* amisulpride generalization curve (10 mg/kg training dose) are shown in Figure 3. Generalization testing yielded an ED₅₀ = 1.77 mg/kg 95% CI [1.28, 2.45 mg/kg]. Full generalization to the (-)*S* amisulpride discriminative cue was attained at 5.0 mg/kg (87.2% DLR), 10.0 mg/kg (96.31% DLR), 20.0 mg/kg (90.5% DLR) and 40.0 mg/kg (94.05% DLR). A one-way repeated measures ANOVA revealed that there was a significant effect of doses on response rate, $F(9,90) = 3.37, p < .05$. However, a Dunnett's post hoc test failed to reveal any significant differences between any of the drug doses and vehicle.

Acquisition of 10.0 mg/kg (-)S amisulpride Discrimination (N=13)



Mean # of sessions = 43.5 (Range = 11-74)

Figure 2. (-)S amisulpride Acquisition Discrimination

Acquisition of two-lever discrimination is shown for the 10 mg/kg (-)S amisulpride (AMI) training dose. Mean percentage drug lever responses (\pm SEM) are presented separately for drug injections (*closed circles*) and vehicle (VEH) injections (*open circles*). The *dashed line* at 80% indicates drug-appropriate responding and the *dashed line* at 20% indicates vehicle-appropriate responding. As the mice met the training criteria, they were removed from the curves. The numbers in parenthesis indicate the number of remaining mice who had not yet met acquisition criteria.

(-)S Amisulpride (n=11)

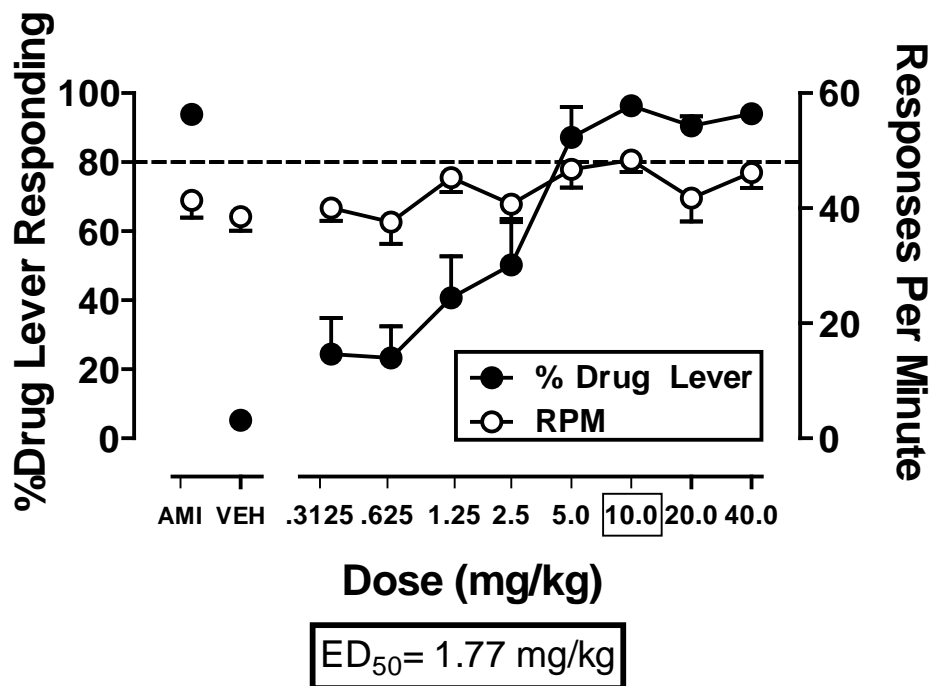


Figure 3. (-)S amisulpride Generalization Dose Effect Curve

Mean percent drug lever responding (\pm SEM) and mean responses per minute (\pm SEM) are shown for the atypical antipsychotic (-)S amisulpride enantiomer generalization curve (10 mg/kg (-)S amisulpride training dose) in a two-lever drug discrimination procedure. The dashed line at 80% indicates drug-appropriate responding indicating full generalization to the training drug. Prior to generalization testing, control test sessions were conducted with both (-)S amisulpride (10mg/kg) and vehicle. For response rate data, significant differences from vehicle are indicated by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

(-)S amisulpride Time Course

Time course data shown in Figure 4 demonstrated that the 10 mg/kg training dose of (-)S amisulpride produced full responding on the drug-paired lever only at the 60 minute s.c. injection time point (average drug lever responding = 95.06%). Partial substitution was seen at 30 minutes post s.c. injection time point (average drug lever responding dropped to 70.28%). A one-way repeated measures ANOVA for drug-lever responding was significant, $F(6,30) = 10.80$, $p < .001$. A Dunnett's multiple comparison post hoc test was used to determine which time points were significantly different from the training pre-injection time (60 minutes). Compared to the 60 minute time point, 0 minutes ($p < .001$), 15 minutes ($p < .001$), 120 minutes ($p < .05$), 240 minutes ($p < .01$) and 480 minutes ($p < .001$) produced significantly less drug-lever responding. The 30 minute and 60 minute time points were not significantly different ($p > .05$). A one-way repeated measures ANOVA for rates of responding showed there was a significant effect of pre-injection time, $F(7,35) = 3.20$, $p < .01$. A Dunnett's post hoc test was used to determine which response rates were significantly different from vehicle rates of responding. The Dunnett's post hoc test revealed that response rates at the 15 minute pre-injection time was significantly increased as compared to vehicle response rates ($p < .01$).

Time Course (n=6)

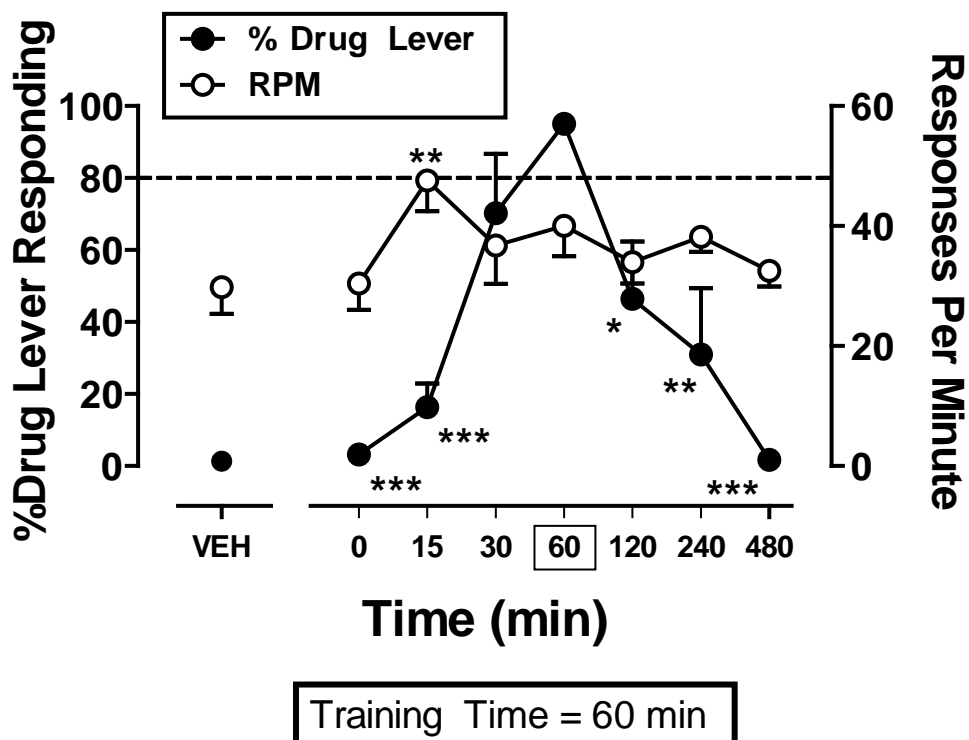


Figure 4. (-)S amisulpride Time Course

Time course data are shown for 0, 15, 30, 60, 120, 240 and 480 minute pre-session s.c. injection times for the 10 mg/kg training dose of (-)S amisulpride. For percent drug lever responding, significant differences from the pre-session injection time (60 min) are indicated by asterisks (* $p < .05$, ** $p < .01$, *** $p < .001$). For responses per minute, significant differences are from the vehicle control.

Haloperidol Substitution

The typical antipsychotic haloperidol did not substitute for (-)*S* amisulpride, as shown in Figure 5, at any of the tested doses (.0078 - .10 mg/kg). Maximum %DLR was seen at .10 mg/kg dose (45.42% DLR). Response rates at the dose .10 mg/kg dose was significantly suppressed, $F(9, 63) = 6.12, p < .01$.

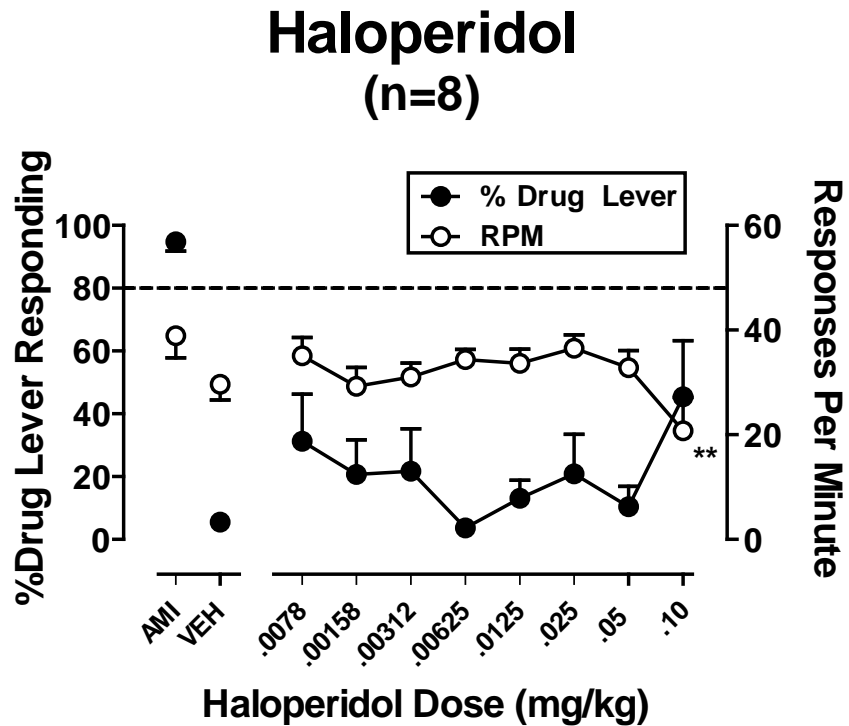


Figure 5. Haloperidol Substitution Curve

Mean percent drug lever responding (\pm SEM) and mean responses per minute (\pm SEM) are shown for the typical antipsychotic haloperidol substitution curve. All other details are the same as Figure 2.

Clozapine Substitution

The atypical antipsychotic clozapine did not fully substitute for (-)S amisulpride at any of the tested doses (Figure 6). There was partial substitution at 3.54 mg/kg (64.73% DLR). Response rates were significantly suppressed at 3.54 mg/kg $F(4,36) = 7.36, p < .001$. Three mice were tested at 5.0 mg/kg clozapine but response rates were completely suppressed, so no other mice were tested at this dose.

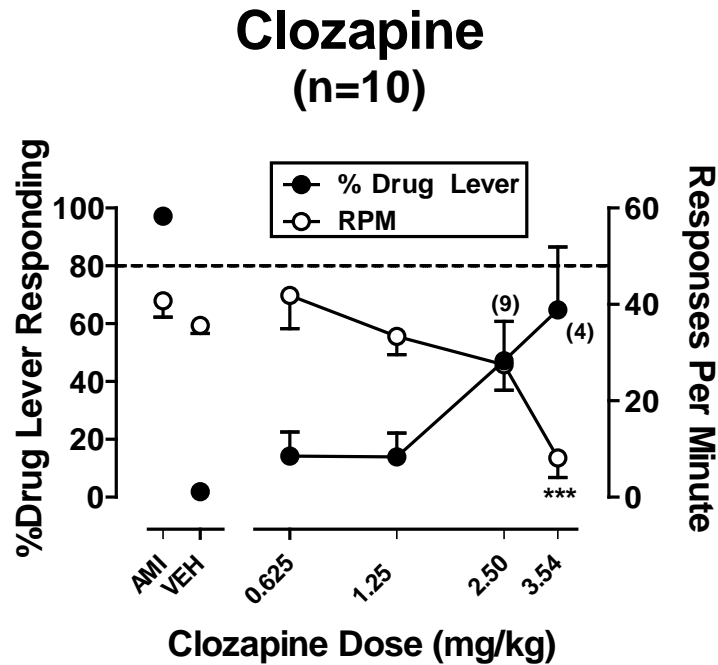


Figure 6. Clozapine Substitution Curve

Mean percent drug lever responding (\pm SEM) and mean responses per minute (\pm SEM) are shown for the atypical antipsychotic clozapine substitution curve. All other details are the same as Figure 2.

Aripiprazole Substitution

The atypical antipsychotic aripiprazole did not substitute for (-)-S amisulpride at any of the tested doses (Figure 7). Response rates were significantly suppressed at the highest dose of 0.625 mg/kg, $F(5, 45) = 7.23, p < .001$.

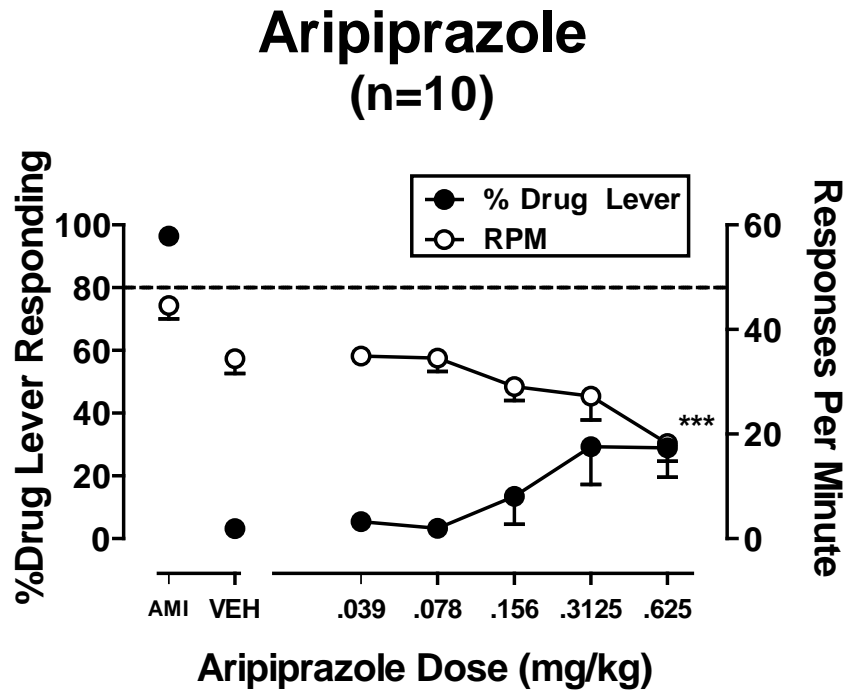


Figure 7. Aripiprazole Substitution Curve

Mean percent drug lever responding (\pm SEM) and mean responses per minute (\pm SEM) are shown for the atypical antipsychotic aripiprazole substitution curve. All other details are the same as Figure 3.

(+)R amisulpride Substitution

The isomer (+)R amisulpride produced high partial substitution at 80.0 mg/kg (75% DLR), see Figure 8. Substitution testing revealed an ED₅₀ 22.36 mg/kg 95% CI [6.37, 78.49 mg/kg]. (+)R amisulpride did not produce any rate suppression at the tested doses.

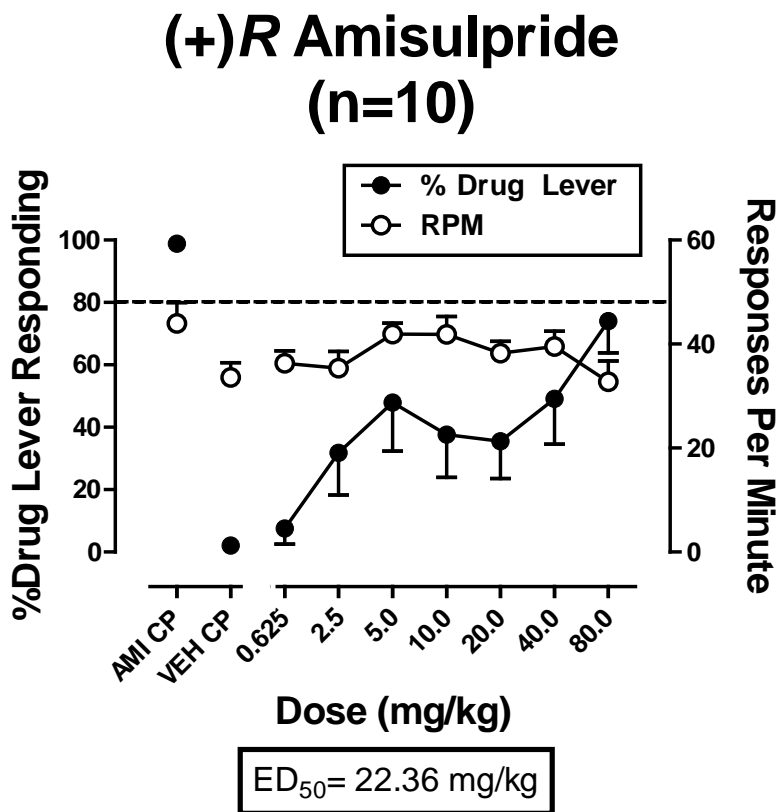


Figure 8. (+)R amisulpride Substitution Curve

Mean percent drug lever responding (\pm SEM) and mean responses per minute (\pm SEM) are shown for the isomer (+)R amisulpride substitution curve. All other details are the same as Figure 3.

Racemic (+/-)SR amisulpride Substitution

The atypical antipsychotic racemic (+/-)SR amisulpride (Figure 9) produced full substitution at 20.0 mg/kg (93.57% DLR), 40 mg/kg (82.50% DLR), and 80 mg/kg (85.71% DLR). Partial substitution was found at 5.0 mg/kg (60.03 %DLR) and 10 mg/kg (70.92% DLR). Generalization testing revealed an $ED_{50} = 4.78$ mg/kg CI [3.37, 6.80 mg/kg]. There were no significant changes in response rates.

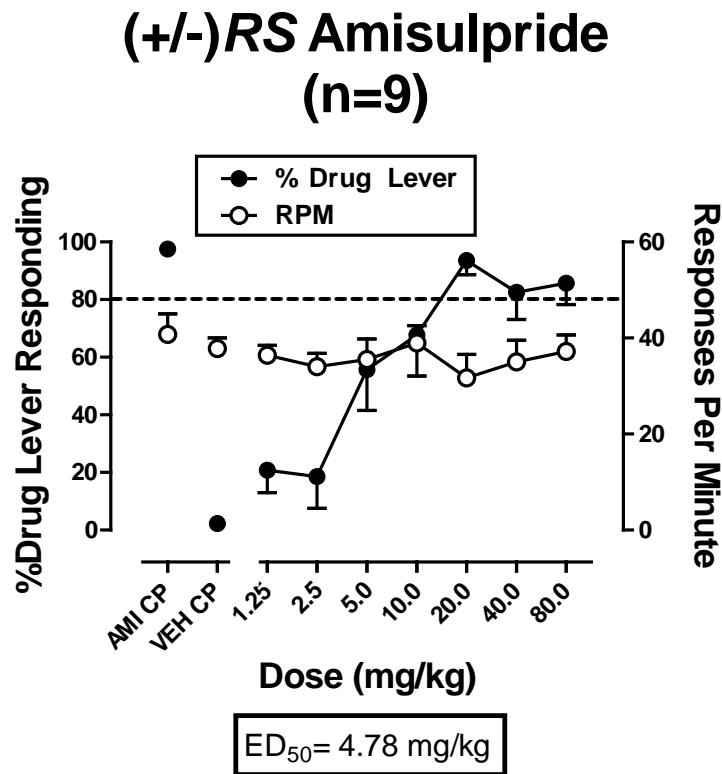


Figure 9. (+/-)SR amisulpride Substitution Curve

Mean percent drug lever responding (\pm SEM) and mean responses per minute (\pm SEM) are shown for the atypical antipsychotic racemic (+/-)SR amisulpride substitution curve. All other details are the same as Figure 3.

Discussion

Amisulpride as a discriminative stimulus. The results of the present study demonstrated that the atypical antipsychotic amisulpride can exert reliable discriminative stimulus control in male C57BL/6 mice at doses that do not significantly suppress rates of responding. This finding is original as, to date; there are no published studies on the discriminative properties of amisulpride with any species. This study utilized the drug discrimination paradigm as a behavioral assay to examine the discriminative stimulus properties of (-)*S* amisulpride and compared it to the enantiomer (+)*R* amisulpride and the (+/-)*SR* racemic mixture. Further, the study compared the stimulus properties of the atypical antipsychotic amisulpride to the typical antipsychotic haloperidol, as well as to the atypical antipsychotics clozapine, and aripiprazole.

This study utilized the (-)*S* amisulpride isomer as the training drug as it has been shown that this enantiomer is twice as potent at D₂ and D₃ dopaminergic receptors as the racemic mixture and 20 to 40 times more potent than (+)*R* amisulpride isomer (Castelli et al., 2001). The affinity of amisulpride for the D₂ and D₃ receptors is suggested as a critical factor for its therapeutic efficacy in treating both the positive and negative symptoms of schizophrenia with fewer propensities than typical antipsychotics to induce extrapyramidal motor side effects.

Acquisition of discrimination with (-)*S* amisulpride was established with thirteen mice in a mean of 43.5 days (SEM ± 4.4) with a range of 11-74 days. While there are no other drug discrimination studies utilizing (-)*S* amisulpride with any species, a comparison can be made to studies of related atypical antipsychotic drugs such as clozapine. Philibin et.al. (2005) trained 17 C57BL/6 mice to discriminate 2.5 mg/kg dose of clozapine from vehicle in an average of 35.6 sessions (SEM±2.84; range = 15-52 sessions) (Philibin, Prus, Pehrson, & Porter, 2005). Thus, establishing the (-)*S* amisulpride discriminative cue in C57BL/6 mice took slightly longer than

establishing clozapine as a discriminative cue. This small difference might be due to the fact that clozapine has a more diverse binding profile including 5-HT, muscarinic, histaminergic, and adrenergic receptors (Schotte et al., 1996) as compared to amisulpride, which is highly selective for dopaminergic D₂/D₃, serotonin 5-HT_{2B}/5-HT₇ receptors (Coukell et al., 1996; Cudennec et al., 1997; Schoemaker et al., 1997). Additionally, the difference in acquisition rates between amisulpride and clozapine might be due to the training dose and pre-injection times for each drug. Clozapine, as a discriminative stimulus, has a history of optimal dose and pre-injection time for a wide variety of species including rats (e.g. Goas and Boston 1978; Wiley and Porter 1992), monkeys (Carey and Bergman 1997), pigeons (Hoenicke et al. 1992), squirrel monkeys (Carey and Bergman 1997) and mice (Philibin et al 2005). In the absence of established drug discrimination studies with amisulpride, the present study selected a training dose (10 mg/kg) and pre-injection time (60 minutes) based on other research done to investigating the pharmacological profile of amisulpride in mice and rats in other behavioral tests (Perrault et al., 1997). The acquisition rates found in the present study might be shorter had we used a dose higher than 10 mg/kg as the training dose, as we found full substitution to occur at both 20 mg/kg (90.5%DRL) and 40 mg/kg (94.5%DRL) doses with no rate suppression.

Comparison of curves. A comparison of the generalization curves for the three isomeric forms of amisulpride tested yielded interesting information regarding percent drug lever responding and respective ED₅₀ values (see Table 4). The (-)S amisulpride isomer at the training dose of 10 mg/kg yielded an ED₅₀ = 1.77 mg/kg 95% CI [1.28, 2.45] with full substitution attained at doses 5.0 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg. The (+/-) SR racemic amisulpride yielded an ED₅₀ = 4.78 mg/kg 95% CI [3.37, 6.80] with partial substitution at 5 mg/kg, 10 mg/kg and full substitution at 20 mg/kg, 40 mg/kg and 80 mg/kg. The (+)R

amisulpride isomer had an $ED_{50} = 22.36$ mg/kg 95% CI [6.37, 78.49] with only partial substitution at the highest dose 80 mg/kg. As seen in Table 4, all three ED_{50} values are significantly different from each other with a distinctive rightward shift (see Figure 10) in the dose-effect curve for these forms of amisulpride tested. This rightward shift is most likely due to differences in binding for each form of amisulpride as it has been demonstrated that the (-)*S* amisulpride isomer is twice as potent as the racemic form and 20 to 40 times more potent than the (+)*R* enantiomer in displacing radioligands from dopamine D_2 and D_3 receptors (Castelli et al., 2001). However, since the potent dopamine D_2/D_3 antagonist haloperidol did not substitute for (-)*S* amisulpride (see Figure 4), this suggests that affinity for D_2/D_3 receptors is not responsible for this difference. Information about the potency of the racemic and isomer forms of amisulpride at serotonin 5-HT- $2B$ and 5-HT- 7 receptors is not currently available, but it may be possible that differences at these receptors might account for the difference in ED_{50} values.

Table 4.

Comparison of ED_{50} values for three forms of amisulpride based on free base weights of the drugs.

Drug Form	ED_{50} Value	95 % Confidence Interval
(-) <i>S</i> amisulpride*	$ED_{50} = 1.54$ mg/kg	1.11 - 2.13 mg/kg
(+/-) <i>RS</i> amisulpride	$ED_{50} = 4.78$ mg/kg	3.37 - 6.80 mg/kg
(+) <i>R</i> amisulpride	$ED_{50} = 22.36$ mg/kg	6.37 - 78.49 mg/kg

*This study tested (-)*S* amisulpride in its hydrochloride (HCl) form which yielded an $ED_{50} = 1.77$ mg/kg 95% CI [1.28, 2.45] but used the free base form of racemic (+/-) *RS* amisulpride and (+)*R* amisulpride. This table shows the adjusted dosage of (-)*S* amisulpride HCl to the free base.

Amisulpride Dose Effect Curves for (-)S and (+)R isomers and racemate

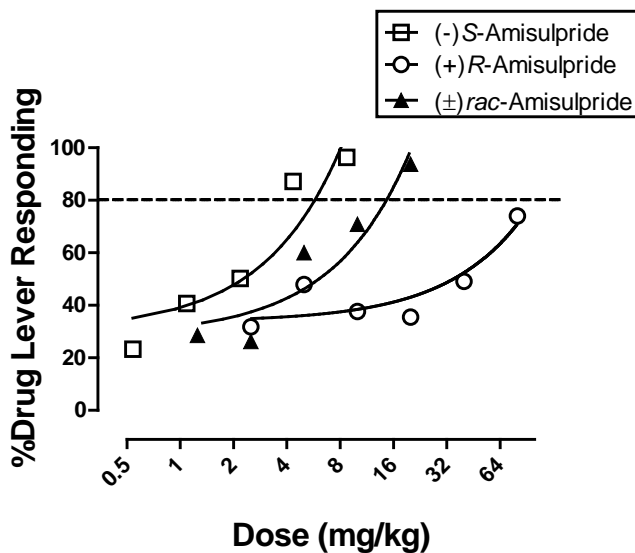


Figure 10.

The % drug lever responding data for racemic amisulpride and its isomers are redrawn on a log base 10 scale with least squares regression lines to illustrate the significant ($p < 0.05$) rightward shift in the dose-effect curves.

Amisulpride time course. Time course data (see Figure 4) demonstrated that the 10 mg/kg training dose of (-)S amisulpride produced partial substitution at 30 minutes (70.28% DLR) and full substitution at 60 minutes (95.06% DLR) with a significant decline in %DLR at 0 minutes (3.20%DLR), 15 minutes (16.38% DLR), 120 minutes (46.46% DLR), 240 minutes (30.97% DLR), and 480 minutes (1.70% DLR). The finding that full substitution was achieved only at the 60 minute time point is consistent with existing research utilizing the same post injection time period to achieve maximum behavioral effects in mice and rats (Manzaneque & Navarro, 1999; Perrault et al., 1997; Scatton, Perrault, & D.J, 1994) and measures of prolactin

levels in rats (Marchese et al., 2002). The steepness of the curve in this study for full substitution may be due to the absorption rates, and/or the slower elimination rates, although specific half-life rates for amisulpride in C57BL/6 mice have not been established. The elimination rate appears consistent with expected normal half-life elimination. If a 90 minute injection time had been tested, this may have resulted in partial substitution based on the rate of elimination in the last three time periods: 120 minutes (46.46 % DLR), 240 minutes (30.97 % DLR) and 480 minutes (1.70 % DLR). As well, time course for full substitution effect may be drug and species dependent. For example, a drug discrimination study in which C57BL/6 mice were trained to discriminate 2.5 mg/kg clozapine from vehicle yielded a time course in which full substitution ($\geq 80\%$ DLR) was achieved at 15-30 minutes after s.c. injection and maintained full substitution at both 30-45 minute and 60-75 minute period (Philibin et al., 2005).

Haloperidol substitution. The present study demonstrated that the typical antipsychotic haloperidol did not substitute for (-)S amisulpride at any of the tested doses (0.0078 - .10 mg/kg) and response rates at 0.10 mg/kg dose were significantly suppressed. Haloperidol has proven to be a burdensome and difficult drug to establish as a discriminative stimulus. Colpaert et al (1976) was successful in training 4 rats to discriminate 0.02 mg/kg (s.c.) of haloperidol from saline in a two-lever drug discrimination paradigm. This proved to be an onerous task requiring over 80 training session with no substitution testing. McElroy et al (1989) trained rats (N=9) to discriminate 0.05 mg/kg (i.p.) from vehicle and was successful in demonstrating that chlorpromazine substituted for haloperidol (McElroy et al., 1989). While haloperidol has been utilized in drug discrimination studies with drugs such as amphetamine (Haenlein, Caul, & Barrett, 1985) and nicotine (Barrett, Caul, & Smith, 2004) there are no studies in which haloperidol has substituted for any atypical antipsychotic drug. Thus, it is not surprising that the

present study found that haloperidol did not substitute for amisulpride. This suggests that the difference in binding profiles between amisulpride and haloperidol most likely accounts for the failure of haloperidol to substitute for amisulpride. Haloperidol has strong binding affinity at dopaminergic D₂, D₃, D₄ and at adrenergic α_{1A} and α_{1B} receptors while (-)S amisulpride binds selectively to dopaminergic D₂, D₃ and to serotonin 5-HT_{2B} and 5-HT₇ receptors. This suggests that the discriminative cue for (-)S amisulpride may be due to its activity at serotonin 5-HT_{2B} and 5-HT₇ receptors or that amisulpride's binding ratio of dopamine to serotonin receptors is implicated as to why haloperidol did not substitute for amisulpride. This dopamine/serotonin ratio is also suggested as a reason why atypical antipsychotics are more effective and better tolerated than typical antipsychotics (Meltzer & Massey, 2011), although the ratio for amisulpride is greater binding to dopamine than serotonin, which is the opposite of what Meltzer had proposed for "atypicality". Another clue as to why haloperidol did not substitute for (-)S amisulpride may be to the "fast-off-D₂" theory. As a typical antipsychotic, haloperidol binds more tightly to D₂ than endogenous dopamine itself whereas amisulpride (and clozapine) dissociate from dopaminergic receptors rapidly in less than 60 seconds. Clinical brain imaging findings demonstrate that haloperidol remains bound to D₂ receptors for as long as 24 hours (Philip Seeman, 2002). Perhaps amisulpride's quick dissociation from D₂ receptors explain why haloperidol did not substitute.

Clozapine and aripiprazole substitution. The present study found that neither of the two atypical antipsychotics tested, clozapine and aripiprazole, fully substituted for 10 mg/kg (-)S amisulpride. Clozapine possesses a complex cue, which appears responsible for its discriminative stimulus properties (Goudie et al., 1998; Porter, Varvel, Vann, Philibin, & Wise, 2000). Clozapine does not have as strong an affinity for D₂/D₃ receptors as does amisulpride.

As well, clozapine is antagonist at multiple receptors: serotonin 5-HT_{2A}, 5-HT_{2B}, 5-HT₆, 5-HT₇, adrenergic α_{1A} , α_{1B} , histaminic H₁ and muscarinic M₁ and M₂. Amisulpride shares antagonism with clozapine only at 5-HT_{2B}, and 5-HT₇ receptors. These differences may explain why clozapine failed to substitute at any of the tested doses (0.0625 mg/kg – 3.54 mg/kg) and only partially substituted at the highest dose 3.54 mg/kg. Aripiprazole also failed to substitute at any dose tested (.039 mg/kg - .625 mg/kg). This may be due to the unique and robust binding profile of aripiprazole as a partial agonist at D₂/ D₄ receptors and its affinity for and intrinsic efficacy at, the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT₇ as well as α_{1A} , α_{2B} adrenergic and H₁ histamine receptors (Shapiro et al., 2003). Aripiprazole possesses a binding profile quite different from amisulpride which may explain why aripiprazole failed to substitute at all for amisulpride.

Future studies. Future studies using mice in amisulpride drug discrimination will test selective ligands for generalization or their ability to block the amisulpride discriminative stimulus in order to determine the underlying pharmacological basis of its cue. There are a number of selective ligands we might test. The selective ligands BW723C86, a 5-HT_{2B} agonist, and the 5-HT_{2B} antagonist RS127445 would provide important data on the role of the 5-HT_{2B} receptor as the discriminative stimulus for amisulpride. Testing the selective ligands LP44, a 5-HT₇ agonist and the 5-HT₇ antagonist SB258719 would yield data on the role of the 5-HT₇ receptor as the discriminative stimulus for amisulpride. Testing the selective ligands pramipexole, a D₃ agonist and SB277011, a D₃ antagonist would provide information on the role of the D₃ receptor, and sulpiride, a D₂ antagonist or quinpirole, a D₂ agonist would help to clarify the role of the D₂ receptor.

Additionally, future tests could utilize knockout mice (KO) in an effort to determine genetic influences on various receptors suspected in the discriminative stimulus cue of

amisulpride. Although the use of transgenic or knockout mice in drug discrimination studies is relatively new, there is published research involving cocaine and D₄ receptors (Chausmer et al., 2002), LSD and serotonin transporters (Krall, Richards, R.A., & Winter, 2008), ethanol and serotonin 5-HT₃ receptors (Shelton, Dukat, & Allan, 2004), and nicotine α 7 receptors (Stolerman, Chamberlain, Bizarro, Fernandes, & Schalkwyk, 2004). In light of the findings of this present study, it would be interesting to utilize a knock out mouse with an inactivated gene for serotonin 5-HT_{2B} or 5-HT₇ receptors; key receptors this study suggested relevant to amisulpride's discriminative stimulus cue of amisulpride.

Conclusion. The use of amisulpride drug discrimination in the present study provided important information about the differences between typical and atypical antipsychotic drugs as well as difference among various atypical drugs. Amisulpride has demonstrated that it has very unique (and robust) discriminative stimulus properties, which may or may not be related to its ability to treat positive and negative symptoms of schizophrenia without initiating extrapyramidal side effects. The exact pharmacological properties that are the basis for the discriminative stimulus properties of amisulpride remain to be determined and relating the discriminative cue properties of amisulpride to its therapeutic efficacy also has yet to be determined. This study provides evidence that (-)S amisulpride can serve as a discriminative stimulus for mice. The (-)S amisulpride is dose-dependent, time-dependent and stereoselective.

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APPENDICES

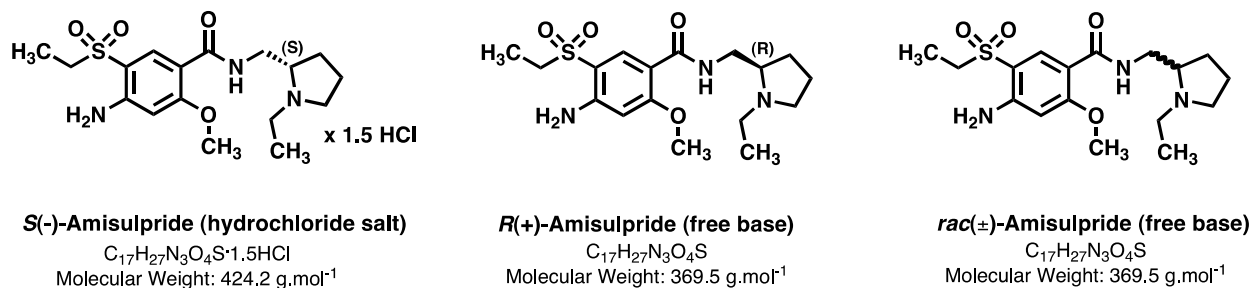


Figure 1: Amisulpride enantiomers *S* and *R*, and its racemate (*rac*). The most active isomer *S* was tested as a hydrochloride salt.

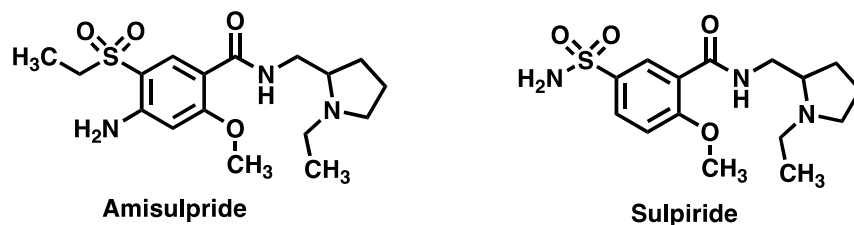


Figure 2: Chemical structures of the two dopaminergic benzamides amisulpride and sulpiride.

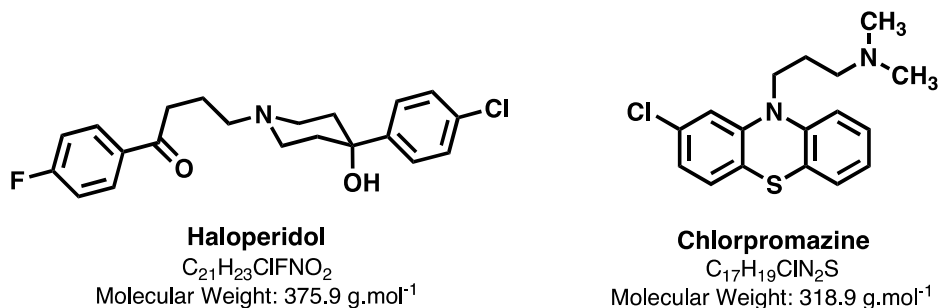
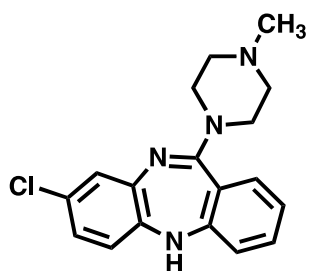
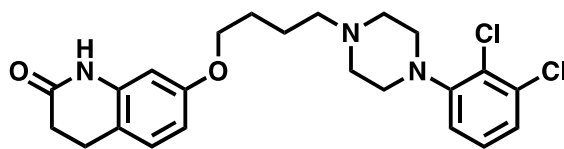


Figure 3: First generation (typical) antipsychotic agents haloperidol and chlorpromazine.



Clozapine
C₁₈H₁₉ClN₄
Molecular Weight: 326.8 g.mol⁻¹



Aripiprazole
C₂₃H₂₇Cl₂N₃O₂
Molecular Weight: 448.4 g.mol⁻¹

Figure 4: Second generation (atypical) antipsychotic agents clozapine and aripiprazole.

VITAE

Timothy J. Donahue was born December 30, 1950 on Guam (U.S. Territory) in the Mariana Islands and is an American citizen. His father was a career officer with the U.S. Army and raised his family in many locations in the U.S.A. and abroad before retiring in Northern Virginia. Tim graduated from Annandale High School in 1969, and went to St. Mary's Seminary & University in Baltimore, Maryland, where he received his Bachelor of Arts, *cum laude*, in Humanities: Behavioral Sciences Concentration (1973). He received a Master of Education from Virginia Commonwealth University (1976), and a Master of Humanities from the University of Richmond (1984). He began a career in teaching in 1973 at Our Lady of Lourdes Elementary School in Richmond Virginia where he taught for three years, and then at Hermitage High School (Henrico County Public Schools) for thirty-four years where he taught AP Psychology. He retired from public school teaching in 2010 to pursue graduate studies in biopsychology at V.C.U. Tim is an adjunct faculty member for the V.C.U. Psychology department and also teaches a college dual-enrollment course in biological psychology at the Maggie L. Walker Governor's School in Richmond.

He is a recipient of the *R.E.B. Award for Teaching Excellence*, sponsored by the R.E.B. Foundation of Richmond which awarded him a generous grant to travel and interview acclaimed neuroscientists. He was awarded a *Neuroscientist-Teacher Travel Award* by the Society of Neuroscience given to teachers who have established effective relationships with SfN neuroscientists to help them teach neuroscience in the classroom. He also received *The Hollins University Teaching Award*, supported by an endowment established by Mary Bernhardt Decker '58 and James DeWitt Becker, honoring secondary school teachers who have devoted their lives to preparing students to achieve and excel in a higher education setting.

Tim is married to Marilee R. Donahue of Youngwood, Pennsylvania and has three children, Kevin, Caitlin and Eric.