Theses and Dissertations   Graduate School

2017

Antidepressant-like Effects of Amisulpride, Ketamine, and Their Enantiomers on Differential-Reinforcement-of-Low-Rate (DRL) Operant Responding in Male C57/BL/6 Mice

Doug Smith
Virginia Commonwealth University

Antidepressant-like Effects of Amisulpride, Ketamine, and Their Enantiomers on Differential-Reinforcement-of-Low-Rate (DRL) Operant Responding in Male C57BL/6 Mice

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science at Virginia Commonwealth University

By: DOUGLAS ANDREW SMITH

Bachelor of Science, Virginia Polytechnic Institute and State University, Virginia, 2014

Director: Joseph H. Porter, PhD

Professor of Psychology

Department of Psychology

Virginia Commonwealth University

Richmond, Virginia

July, 2017

Acknowledgements

Table of Contents

List of Tables

List of Figures

Abstract

Antidepressant-like Effects of Amisulpride, Ketamine, and Their Enantiomers on Differential-Reinforcement-of-Low-Rate (DRL) Operant Responding in Male C57BL/6 Mice

By Douglas A. Smith

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science at Virginia Commonwealth University

Virginia Commonwealth University, 2017

Director: Joseph H. Porter, PhD

Professor of Psychology, Department of Psychology

Major depressive disorder (MDD) is a widespread psychiatric disorder that affects millions of people worldwide and is hypothesized to occur due to impairments in several neurotransmitter systems, including the monoaminergic and glutamatergic neurotransmitter systems. Antidepressant medications targeting multiple monoamine neurotransmitters have been shown to be effective for the treatment of depression. Racemicamisulpride is an atypical antipsychotic that has been used at low doses to treat dysthymia, a mild form of depression, and functions as an antagonist at $DA_{2/3}$, $5\text{-}HT_{2B}$, and $5\text{-}HT_7$ receptors. Recent preclinical studies have suggested that the *S(+)*isomer may be more critical for amisulpride's antidepressant-like effects; however, this interpretation has not been fully characterized in comparison to the *R(-)*isomer. The glutamatergic system also has been shown to play a critical role in alleviating depression. Several studies have demonstrated that the noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist ketamine produces rapid and sustained antidepressant-like effects in clinical trials; however, few studies have examined the degree to which ketamine's isomers contribute to antidepressant-like effects. Fully characterizing these differences in a preclinical model of depression may offer important insight into the role of these neurotransmitter systems on

depression. The present study used a 72-sec differential-reinforcement-of-low-rate (DRL) task to assess the antidepressant-like effects of amisulpride, ketamine, and their isomers in mice. The DRL 72-sec task has shown to be a reliable and sensitive screen for drugs that possess antidepressant-like activity as reflected by an increase in the number of reinforcers, a decrease in the number of responses, and a right-ward shift in the interresponse time distributions (IRTs; i.e. the elapsed time between two successive responses). For comparison, the effects of the tricyclic antidepressant imipramine and the N-methyl-D-aspartate antagonist MK-801 as positive and negative controls, respectively, were determined. Consistent with previous findings, we hypothesized that amisulpride and *S(-)*-amisulpride, but not *R(+)*-amisulpride, would produce antidepressant-like effects, andall formulations of ketamine would produce antidepressant effects. Racemic amisulpride and *S(-)*-amisulpride, but not *R(+)*-amisulpride, produced an antidepressant-like effect, evidenced by a significant increase in the number of reinforcers and a significant decrease in the number of responses. Racemic ketamine and *R(-)*-ketamine significantly increased the number of reinforcers and decreased the number of responses, while *S(+)*-ketamine significantly increased the number of reinforcers, but did not decrease the number of responses (at the doses tested). Overall, these results indicate that the racemic formulations of amisulpride and ketamine, *S(-)*-amisulpride, and both ketamine isomers demonstrate antidepressant-like effects as assessed in the DRL task and may be useful in a clinical context. If either of the ketamine isomers can be shown to produce fewer psychotomimetic effects in humans, then the isomers may offer a significant clinical advantage over the parent compound ketamine. Regarding amisulpride, the present results demonstrate that the *S(-)* isomer, but not the *R(+)*isomer, possess antidepressant-like activity similar to racemic amisulpride.

Introduction

Major depressive disorder (MDD) is a widespread psychiatric disorder that affects

millions of people worldwide. Per a report from the World Health Organization (2014),

depression accounts for nearly 5% of the total worldwide burden of diseases. MDD worsens the

health of patients with other chronic illnesses, such as chronic pain and respiratory diseases, and

is associated with increasing disability over time. In addition to the psychological harm to the

individual, MDD also negatively impacts both economic and occupational public health

(Greenberg et al., 2003; Fostick et al., 2010). For instance, Fostick and colleagues (2010) posited

that the economic costs to US society due to MDD is estimated to be over $83 billion when

factoring in costs due to the pharmacological and behavioral treatments of depression, loss of

work and productivity, and suicide-related mortality costs. Moreover, it has been hypothesized

that treatment-resistant depression (TRD; patients who do not respond to two or more

antidepressant treatments) affects 20-40% of those suffering from MDD (Sackeim, 2001);

however, about half of the patients that do show a reduction of depressive symptoms with their

first treatment have significant residual symptoms. Thus, only about 20-40% of patients are

expected to reach a relative stable asymptotic state after their first treatment.These

epidemiological findings suggest that MDD is a major public health risk that negatively impacts

psychological, economic, and societal factors.

MDD is characterized as a chronic affective disorder comprised of behavioral

abnormalities, including negative affect, insomnia, suicidal ideation, significant impairments to

social and occupational functioning, and manifests in neurobiological abnormalities (Fava, 2003;

Fava and Kendler, 2000; Nestler et al., 2002). Per the National Comorbidity Survey, the lifetime

prevalence of MDD as defined by the American Psychiatric Association's Diagnostic and

Statistical Manual of Mental Disorders V (DSM-V; criteria summarized in Table 1) criteria was estimated at 17%, while 5% of the population reported meeting criteria for MDD in the last 30 days (Fava and Kendler, 2000). While depressive episodes may appear at any age, MDD is most prevalent in adults (18-64 years old) (Kessler, 2012). In addition, females are two-to-three times more likely to be diagnosed with MDD than their male counterparts (Kessler, 2012). While MDD is one of the most common psychiatric disorders in the United States, the exact cause and most efficacious treatment options have remained elusive.

Monoamine and Glutamate Theories of Depression

Monoamine Theory of Depression: Historically, MDD has been hypothesized to be caused by genetic, environment, and psychological factors that eventually manifest into behavioral and neurobiological impairments (Table 1; Belmaker, 2008). Before the early 1950s, the best treatment option for depression was electroconvulsive shock therapy. Later, medication development became widespread after the serendipitous finding that pharmacological compounds improve mental disorders through central nervous system activity, beginning with the discovery of chlorpromazine.

In 1950, the French chemist Paul Charpentier synthesized 4560 RP, now known as chlorpromazine. Within two years, chlorpromazine was shown to produce relaxation in surgical patients and produce decreased manic states in schizophrenic patients (Anton-Stephens, 1954). However, the first documented use of chlorpromazine in psychiatry was in a patient named Jacques Lh, a 24-year-old severely agitated psychotic male. After 20 days of being treated with 50 mg chlorpromazine, along with barbiturates and electroshocks, Hamon and colleagues (1952) discharged Jacques Lh, and he resumed normal life. Taken together, these early findings were

2

the first reports demonstrating that pharmacological agents may be useful for the treatment of

psychosis.

DSM-5 criteria for major depressive disorder and persistent depressive disorder
Five or more of 9 symptoms (including at least 1 of depressed mood and loss of interest or pleasure) in the same 2-week period
Each of these symptoms represents a change from previous functioning
• Depressed mood (subjective or observed)
• Lost of Interest or pleasure
• Change in weight or appetite
• Insomnia or hypersomnia
• Psychomotor retardation or agitation (observed)
• Loss of energy or fatigue
• Worthlessness or guilt
• Impaired concentration or indecisiveness
• Thoughts of death or suicidal ideation or suicide attempt

Table 1. American Psychological Association's Diagnostic and Statistical Manual V criteria for Major Depressive Disorder.

The monoamine hypothesis of depression posits that individuals suffering from

depression have depleted levels of the monoamine neurotransmitters serotonin (5-HT), dopamine

(DA), and norepinephrine (NE) (Bunney and Davis, 1965; Hirschfeld, 2000). Evidence

supporting the monoamine hypothesis began with the discovery that the vesicular monoamine

transporter inhibitor reserpine produced a depletion of serotonin and other monoamines in the

brain, and this depletion led to depressive-like symptoms in humans that could be relieved by

discontinuation of the drug (Shore et al., 1995; Shore et al., 1957). Later, preclinical studies

demonstrated that reserpine produced depressive-like effects in animals (Hirchfield, 2000).

Another supportive finding for the monoamine hypothesis was based on the antidepressant-like effects produced by imipramine, a tricyclic antidepressant (TCA), which acts by blocking the serotonin transporter (SERT) and norepinephrine transporter (NET) resulting in an elevation of synaptic concentrations of these neurotransmitters. This finding spurred further interest in medications that function by monoamine oxidase inhibition (MAOI). The first MAOI that was shown to produce antidepressant effects was iproniazid (Julien, 2013). While originally intended for the treatment of tuberculosis, it was also shown to produce relief from depression. Later, *in vitro* studies demonstrated that iproniazid produced these antidepressant effects through inhibition of monoamine oxidase (West and Dally, 1959). Monoamine oxidase is an enzyme that normally metabolizes and regulates the amount of the biogenic amine transmitters in the presynaptic nerve terminal, namely NE, DA, and 5-HT (Julien, 2013). Thus, administration of iproniazid increases the levels of these neurotransmitters and more transmitter is available for release when stimulated by an action potential reaching the nerve terminals, resulting in robust antidepressant action. Functionally, inhibition of MAO leads to higher concentrations of monoamines in the synaptic cleft, thereby prolonging the activity of these monoamines and producing a reduction in depressive symptoms (Wells and Bjorksten, 1989). Together, the findings that both the tricyclic imipramine and the MAO inhibitor iproniazid increased monoamine neurotransmitter levels at the synapse led to the monoamine hypothesis of depression. Despite these landmark findings of drugs for the treatment of depression, it was soon realized that these first-generation antidepressants had neurotoxic effects (Hindmarch, 2002).

While TCAs exhibited a slow onset of action, limited efficacy across different populations of depressed patients, sexual and metabolic side effects, the MAOIs were found to produce hypertensive issues, especially when taken with certain foods and other medications

(Meyer et al., 2006). For instance, the trace amine tyramine, which also induces release of monoamine neurotransmitters, is found in many foods, including cheese, wine, beans, and liver. When combined with MAOIs, excessive levels of tyramine may cause an elevated rise in blood pressure, resulting in a possible heart attack or rupture of an aneurism or vascular formation (Prus, 2013). Due to these adverse side effects, there was interest in developingnovel, less toxic, antidepressant drugs. Nonetheless, discovery of the antidepressant effects of TCAs and MOAIs was important for the treatment of depression and led to the monoamine hypothesis of depression.

After the negative side effects of these first-generation antidepressants were recognized, a second generation of antidepressants were developed (Julien, 2013). Examples of the second generation of antidepressants included selective serotonin reuptake inhibitors (SSRIs; fluoxetine, citalopram, sertraline, etc.) and selective serotonin and norepinephrine reuptake inhibitors (SSNRIs; e.g. duloxetine). The first second-generation antidepressant drug was introduced to the United States market in the 1980s, when bupropion (a norepinephrine-dopamine reuptake inhibitor) was approved for the treatment of MDD, followed by the SSRI fluoxetine (Julien, 2013). In general, what sets the second generation of antidepressants apart from the first generation is that they more selectively inhibit the reuptake of 5-HT at the presynaptic neuronal membrane. In addition, the second-generation antidepressant drugs have comparable efficacy, comparable or better side effect profiles, and are better tolerated than the first-generation antidepressants (Heninger et al., 1996).

Despite these findings that the monoamine neurotransmitter system is an important factor in MDD (based on the pharmacological mechanisms of the drugs used to treat MDD), this theory has some major flaws. First, there are drugs that can increase brain monoaminergic activity, such

as cocaine or amphetamine, but are not effective clinically as antidepressants (Millan 2004).

Second, not all depressed patients respond equally well to the same antidepressant, suggesting individual differences such as genetics or environment also play a critical role (Elhwuegi, 2004). Third, the changes in the monoamine levels at the synapse take place within minutes or hours after administration of antidepressants, but the therapeutic response requires weeks of repeated administration (Millan, 2004). These flaws in the monoamine hypothesis soon led to a modified hypothesis that posited acute increase in the levels of the monoamines at the synapse may be the initial step which then leads to a cascade of events downstream that result in antidepressant activity. However, this cascade of events considers many other neurotransmitter receptor systems, notably the glutamatergic N-methyl-D-aspartate (NMDA) receptors. This revised theory for the role of monoamines ultimately states that monoamines may play a modulatory role, which led to investigation of other neurotransmitter systems that may play a role in the pathogenesis of MDD.

Glutamate Theory of Depression: As early as the 1990s, inconsistencies in the monoamine hypothesis regarding the pathophysiology of depression led to studies examining the role of the glutamatergic neurotransmitter system. Glutamate is the major excitatory neurotransmitter in the central nervous system (Oreggo and Villanueva, 1993), and the earliest study on the glutamate theory of depression can be traced back to an early study that showed NMDA antagonists produced antidepressant-like action (Trullas and Skolnick, 1990). Sanacora et al. (2012) demonstrated that there are approximately two to three hundred thousand serotonergic neurons out of a hundred billion total neurons in the brain, and about 80% of neurons in the neocortex are excitatory and form 85% of the synapses (Sanacora et al., 2012). These data indicate that

glutamatergic neurons and synapses far outnumber all the other neurotransmitter systems in the brain and suggest that the glutamate system also may play an important role in depression.

Glutamate acts on two types of receptors, ionotropic and metabotropic. Ionotropic glutamatergic receptors include AMPA, kainate, and NMDA receptors. These receptors are ion channels that are permeable to cations and function by allowing sodium (Na) and calcium (Ca) ions to enter the cell, thus causing depolarization and other intracellular changes (Kew and Kemp, 2005). Eight subtypes of metabotropic glutamate receptors (mGluR1-8) are divided into Group I (mGluR1 and mGluR5), Group II (mGluR2 and mGluR3), and Group III (mGluR4,6,7, 8) based upon their homology and function. These glutamatergic receptors are G-protein-coupled receptors (GPCRs) and function by influencing intracellular second messenger formation (Kew and Kemp 2005). NMDA receptors are a specific type of ionotropic glutamate receptors. These receptors are a heteromeric complex that have seven subunits, NR1, NR2A-D, and NR3A-B and functional NMDA receptors must be comprised of an NR1 subunit and at least one NR2 subunit. To be activated, NMDA receptor channels require co-agonist binding at the glycine binding site on the NR1 subunit and at the glutamate binding site on the NR2 subunit (Kew and Kemp, 2005; Marsden 2013). Thus, if one of these co-agonists (glycine or glutamate) is not bound to their respective binding site, the ion channel will not open. Also, the NMDA receptor channels are blocked by magnesium (Mg) ions during the resting state. Depolarization of the neuron is required to dispel the Mg ion from the NMDA receptor channels, which is usually achieved by activation of AMPA receptors. The NMDA receptor ion channel is non-selective and will allow both Na and Ca to enter. The influx of Ca is associated with the induction of various signaling cascades (Marsden, 2013).

Clinical evidence for glutamatergic dysfunction in depression has been demonstrated in plasma, cerebrospinal fluid, and brain tissue of individuals with mood disorders, and antidepressant drug treatment has been found to reduce the serum glutamate (Maes et al., 1998), cerebrospinal fluid (Garakani et al., 2013), and plasma glutamine/glutamate concentrations in MDD patients (Küçükibrahimoğlu et al., 2009). Multiple studies also have reported elevated glutamate levels, and a trend for decreased plasma glutamine/glutamate ratios in the plasma of depressed patients compared to healthy controls (Kim, 1982; Altamura, 1993; Mitani, 2006). Other studies have provided evidence that treatment with antidepressant agents may decrease the plasma glutamate levels in depressed individuals (Altamura et al., 1995, Maes et al., 1998). Furthermore, a postmortem study of the frontal cortex showed a significant increase in tissue glutamate levels in individuals with major depressive and bipolar disorders after controlling for postmortem interval (Hashimoto et al., 2007). Consistent with this finding, an analysis of postmortem dorsolateral prefrontal cortex tissue revealed elevated glutamate levels in bipolar individuals (Lan et al., 2009). Studies also have shown that MDD patients have a greater sensitivity to glutamate as measured by intracellular calcium influx (Berk et al., 2001).

Collectively, these findings suggest that the glutamatergic system plays an important role in the pharmacology of antidepressant-like drugs. Since the discovery of the importance of glutamatergic neurotransmission in MDD, both the monoamine and glutamatergic hypotheses have led to a better understanding of the neurochemical, physiological, and behavioral correlates of MDD.

Preclinical Models of Depression

Preclinical models have been useful for understanding how monoaminergic- and glutamatergic-based medications produce antidepressant-like effects. Different-reinforcement-of-

8

low-rate operant responding (DRL), the forced-swim test (FST), tail suspension test (TST), and learned helplessness paradigms have been widely utilized to measure antidepressant-like effects in animals. However, each of these procedures have positive and negative methodological considerations that impact their applicability as models of depression.

Learned Helplessness in Depression: From a historical perspective, one of the first landmark findings in preclinical models of depression emerged from Martin Seligman's work on learned helplessness. Seligman (1972) described learned helplessness as a condition in which the subject is administered repeated aversive stimuli and is unable to escape. After repeated pairings with an aversive stimulus, the animals eventually will become immobile and will not escape even when escape is presented to them. This finding quickly led to the learned helplessness theory which posits that clinical depression may result from the perceived absence of control over the outcome of a situation. To show evidence for this theory, Seligman and colleagues used an escape avoidance shuttle box in dogs. Here, the dog is administered repeated electrical shock to the floor of one side of the shuttle box. Eventually, the dogs learned to escape to the other side of the shuttle box that did not have an electrical floor. However, after repeated trials, he observed that some dogs would not attempt to escape and would endure the electrical shock. He hypothesized that this maladaptive behavior was the underlying cause of clinical depression in humans. This behavior is defined by a reduced response mechanism and negative cognitive set (difficulty in learning that one's own response will succeed). This behavior has also been translated to other species, including rats, cats, fish, and humans (Seligman, 1972). In addition, Seligman has observed three behaviors from learned helplessness experiments. First, response initiation is compromised, which is the probability the subject will initiate responses to escape is lower because part of the incentive for making such responses is the expectation that they will bring

relief. Second, retardation of learning is evident from the subject learning that responding and shock are independent. Third, the subject learns that trauma is uncontrollable, which may produce emotional stress rather than learning that it is controllable. While this theory provides important insight into the behavioral consequences underlying depression, learned helplessness has been criticized for not distinguishing between cases in which outcomes are uncontrollable for all people and cases in which they are uncontrollable only for some people (i.e. universal vs. personal helplessness), and it does not explain when helplessness is general and when specific, or chronic and acute.

Forced Swim Test: The forced swim test (FST) is one of the most commonly used preclinical animal models to screen antidepressant drugs, developed by Porsolt and colleagues in 1977. In this procedure, rats were placed into a cylinder of water from which they cannot escape and are forced to swim. After an initial intense escape-directed behavior, the rats would eventually become immobile, making only those movements necessary to keep their head above water. Porsolt et al. (1977) hypothesized that this period of immobility was a preclinical proxy of 'behavioral despair' and represented hopelessness in the animal. Subsequent tests with different doses of the TCAs imipramine, desipramine, amitriptyline, and the MAOI nialamide reduced immobility in a biphasic manner, where a certain dose produced a maximal effect and lower/higher doses produced less immobility. They also tested drugs from other drug classes, including anxiolytics, stimulants, and tranquilizers, which did not produce this antidepressant effect. These findings established the FST as a reliable screening tool for antidepressant drugs.

Although there are different methodological manipulations to this procedure, Porsolt et al. (1977) measured immobility in rats during a 15-min pretest session to measure baseline levels of immobility. After 24 hours, the animal was then placed in the FST chamber and immobility

was again measured for a 5-min test session. Typically, drugs are administered before the test session and any changes in immobility are recorded. Nearly forty years later, this procedure is still widely used to assess the effectiveness of antidepressant drugs by pharmaceutical industries and scientific researchers in both rats and mice.

Despite its widespread use and validation as a preclinical assay for measuring the antidepressant-like activity of drugs, the FST has a wide range of positive and negative indications as a scientific procedure (Petit-Demouliere et al., 2005). First, the FST is an effective preclinical assay for measuring the antidepressant-like activity of drugs in that it generally satisfies both the empirical model (experimental conditions in which known antidepressants exert pharmacological effects not shared by other therapeutic classes of drugs) and theoretical model (translatability to reproduce in animals the human symptomatology of a disorder) of depression (Borsini and Meli, 1988). Other major advantages of the FST in comparison to other preclinical models of depression include high interlaboratory reliability, high throughput (i.e. data can be obtained rapidly), data are clearly conveyed and interpretation is straightforward, and it does not require the use of complex equipment, thus reducing costs. Moreover, the validity and translatability of the FST procedure as a preclinical model of depression is not without controversy. For instance, the "behavioral despair" interpretation of immobility has been questioned due to the lack of an established relationship between inescapability and immobility, since rats are less fearful on subsequent immersion than on previous ones, which suggests that behavioral immobility is a consequence of an adaptive response to a stressful situation more than "despair" (Hawkins et al. 1978). Similarly, Borsini and Meli (1988) showed that familiarity with the environment rather than "despair" may induce behavioral immobility. Another disadvantage to the FST as a model of depression is that stimulants, sedatives, and drugs that induce motor

11

impairment can lead to false-negative or false positive results (Slattery and Cryan, 2012). Furthermore, one major side effect of most antidepressants is sedation; however, data derived from the FST do not distinguish sedative effects from antidepressant properties of drugs. These examples are reflective of a major limitation in that many established tests are influenced by drugs that modulate the general activity of the animal being tested. Lastly, a major criticism of the FST is that antidepressant-like effects are interpreted from acute administration of antidepressant drugs, but many of the antidepressant drugs in clinical use take weeks to provide alleviation of depressive symptoms. While this criticism can be applied to other preclinical models of depression, this disconnect between the antidepressant-like effects regarding acute and chronic drug administration illustrates a lack of translatability for the FST as a preclinical model of depression in humans.

Tail Suspension Test: The tail suspension test is another procedure used as a model for assessing antidepressant activity. In this procedure, an animal (most studies have used rodents) is suspended by their tail, causing a short-term stress response (Porsolt et al., 1977). Eventually, the animal will develop an immobile posture, somewhat like the immobile behavior an animal exhibits in the forced swim test. Studies have shown that when antidepressant medications are administered before the TST, they will actively persist in escape-directed behaviors for longer periods of time than after vehicle treatment.

Advantages of this procedure are that the entirety of the procedure is quite short, since the actual session takes about 5 minutes. Other advantages of this procedure are its ability to detect a broad spectrum of antidepressant drug effects irrespective of their underlying mechanism, it is inexpensive, methodologically unsophisticated, and easily amenable to automation.

Like the criticism of the forced swim test, the tail suspension test is a measure of acute drug

effects, even though antidepressant medications take weeks until antidepressant effects are

exhibited. This procedure is also very sensitive to species differences. For instance, some strains

of mice will try to climb their tail to escape, instead of going immobile, so caution must be

exercised in interpreting species differences. Finally, the tail suspension test also produces false

positives and false negatives, similar to the FST (Steru et al., 1985).

Differential Reinforcement of Low Rate (DRL): The differential-reinforcement-of-low-rate

(DRL) task is an operant conditioning method, wherein a subject must withhold an operant

response for a specified amount of time in order to obtain a reinforcer. In preclinical research,

laboratory animals are typically trained to press a lever (operant response), and only responses

that exceed a predetermined interval result in delivery of a reinforcer. Over the course of

training, the animal learns to limit operant responses and eventually produce a frequency

distribution of interresponse times (IRTs) that peaks near the DRL criterion interval (Fowler et

al., 2009), and previous results from our research program have shown that IRTs peak at 36-37

seconds (Hillhouse & Porter, 2014). Responses emitted prior to fulfillment of the DRL criterion

result in no reinforcer and a reset in the IRT requirement. The DRL procedure has been used in

human and laboratory animals to examine 1) timing behavior, 2) impulsivity and attention-

deficit/hyperactivity disorder (Bull et al. 2000; Andrzejewski, 2014), 3) neural substrates of

timing behavior (Chiang, 2015), 4) pharmacological effects on temporal processing (Cho et al.,

2010), and 5)as a preclinical screen for antidepressant-like effects (Fowler et al., 2009). Studies

examining antidepressant medication using the 72-second DRL task have shown that a wide

variety of antidepressant drugs produce an antidepressant-like effect, evidenced byan increase in

reinforcers, a decrease in responses, and a rightward shift in the peak location of the IRT

distribution (O'Donnell et al., 2005). These effects are hypothesized to be indicative of antidepressant-like drug action because 1) antidepressant drugs target serotonin receptor subtypes to alleviate symptoms which is the main neurotransmitter system implicated in depression and antipsychotic medication treatments, 2) since impulsivity is correlated with higher rates of depression, antidepressant drugs may selectively decrease impulsivity in laboratory animals, and 3) temporal discrimination is thought to play a role in depression and impulsivity (O'Donnell et al., 2005).

Studies suggest that longer DRL intervals affect behavior through mediation of serotonergic mechanisms, since administration of most antidepressant and antipsychotic drugs in laboratory animals leads to a decrease in responses and an increase in rewards. The 72-sec DRL task has been utilized extensively as a preclinical drug screening tool for serotonin-selective reuptake-inhibitors, tricyclic antidepressants, typical and atypical antipsychotics, all of which target serotonin systems (O'Donnell et al., 2005). Thus, drugs that exhibit selectivity for inhibition of serotonin uptake, improve performance in rodents trained on a 72-sec DRL interval. Studies have shown that most antidepressant drugs increase reinforcement rate, decrease response rate, and shift the IRT distribution to the right; whereas, other drugs such as caffeine, opioids, barbiturates, ethanol, and anticholinergics have mixed results on DRL behavior depending on species used, age of animal, dose of drug, and different environmental parameters used in different studies (O'Donnell et al., 2005). Furthermore, amphetamine, cocaine, and other monoaminergic-type drugs decrease reinforcement rate, increase response rate, and shift the IRT distribution to the left (O'Donnell et al., 2005), producing an amphetamine-like effect in the DRL task. These findings indicate that the DRL 72-sec IRT is selectively sensitive to antidepressant drug effects relative to most other drug classes.

Considering the positive and negative features of each of these preclinical assays, the DRL 72-sec taskhas two major advantages:first, this procedure has not been shown to produce false positives as seen in the FST and TST assays;second, the DRL 72-sec task is selectively sensitive to antidepressant-like drug effects(O'Donnell et al., 2005).

Ketamine and Amisulpride

Ketamine: Ketamine is a dissociative anesthetic with hallucinogenic properties that is a derivative of phencyclidine (PCP). Originally developed by Dr. Calvin Lee Stevens at Wayne State University for the pharmaceutical company Parke-Davis in 1964, ketamine was shown to produce anesthetic, sedative, and analgesic properties in humans and animals. After being approved by the Food and Drug Administration for use as a short-acting anesthetic in 1970, ketamine became a widely-used treatment for both American soldiers during the Vietnam War (Domino, 2010) and veterinary applications under the trade names Ketalar, Ketaset, and Vetamine. While ketamine continues to be useful in human and veterinary medicine, it was classified as a Schedule III substance in 1999 under the Controlled Substances Act due to recreational use for its hallucinogenic properties at high doses (DEA, 2013). Ketamine, known as "Special K," was shown to elicit hallucinogenic-like dissociative (perceptual distortions) effects and exhibited profound effects on psychological functioning, resulting in feelings of depersonalization (detachment from the 'body and self') and derealization (detachment from the 'environment and reality'). However, ketamine also has been shown to have therapeutic effects for heroin addiction (Krupitsky et al., 2002) and as a rapid-acting antidepressant for MDD (Lapidus et al., 2014). Finally, due to the similarities between the acute pharmacological effects of ketamine and the behavioral impairments observed in schizophrenic patients, researchers have

used ketamine and its analogs to characterize the neurobiological determinants of schizophrenia
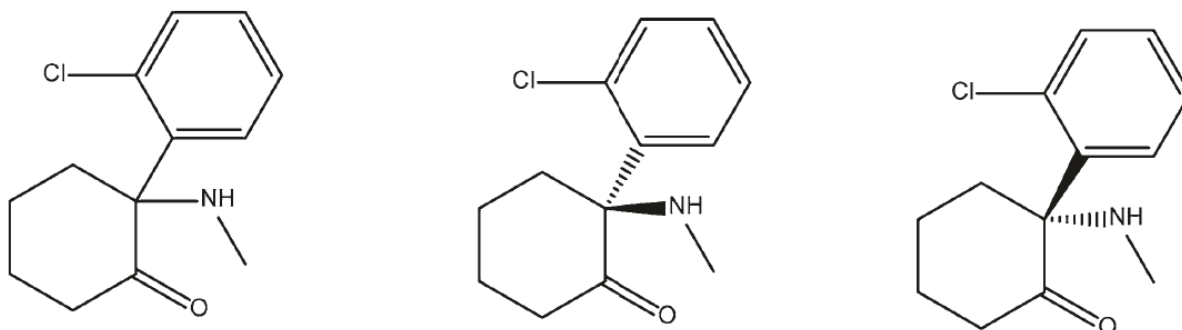
(Lahti et al., 1995).

Figure 1. Chemical structures for racemic ketamine, *S(+)*-ketamine, and *R(-)*-ketamine (chemical structures obtained from Zhang et al., 2014).

Mechanistically, ketamine is an N-methyl-D-aspartate (NMDA) noncompetitive

antagonist (channel blocker) that primarily affects the glutamatergic system by binding to

allosteric sites on NMDA receptors. Allosteric sites are specific sites on the receptor protein

where a molecule acts to inhibit or activate the receptor without affecting the active site (agonist

site) of the receptor for the endogenous neurotransmitter. In this way, ketamine binds to the PCP

site inside the NMDA receptor ion channel and is nonselective for the NR2A-D subunits of the

NMDA receptor channel (Lord et al., 2013; Yamakura et al., 1993; Yamakura et al., 1999).

Studies have shown that ketamine is 12-20-fold more selective for NMDA receptors as

compared to serotonin 5-HT$_{2A}$ and mu opioid receptors (Kapur and Seeman, 2002), has binding

affinity for sigma, muscarinic, and opioid (Kappa and sigma) receptors (Hillhouse et al., 2014),

and DA, NE, and 5-HT receptors (Moghaddam et al., 1997; see Table 2 for binding affinities).

Ketamine is the racemic mixture (optically inactive) of two enantiomers of equal

quantity, the *S(+)* and *R(-)* isomers (molecules with the same formula but different arrangement

of atoms, chemical structure, and rotate light in opposite ways; Figure 1). The *S(+)*-ketamine

enantiomer ("S" spatial structure, light rotated to the right) is approximately two times more

potent than the racemic form and four times more potent than the *R(-)*-ketamine isomer ("R"

spatial structure, light rotated to the left). Interestingly, one preclinical study has shown that the

R(-)-ketamine stereoisomer shows greater potency and longer lasting antidepressant-like effects

than the *S(+)*-stereoisomer on depression-like behavior in juvenile mice after neonatal

dexamethasone exposure in the tail suspension test and the forced swim test (Zhang et al., 2014).

Receptor	Ligand	Racemic ketamine	S(+)-ketamine	R(-)-ketamine
NMDA (1)	[3H]MK-801	0.53 (nM)	0.30 (nM)	1.40 (nM)
Mu opioid (2)	[3H]DAGO	2.5 (nM)	11 (nM)	28 (nM)
Sigma (2)	[3H]DPDPE	1.0 (nM)	130 (nM)	130 (nM)
Kappa (2)	[3H]U69593	4.2 (nM)	24 (nM)	100 (nM)
PCP (2)	[3H]TCP	2.9 (nM)	1.1 (nM)	3.2 (nM)
Omega (2)	[3H]+SKF10,047	0.15 (nM)	131 (nM)	19 (nM)
Muscarinic (2)	[3H]QNB	1.8 (nM)	20 (nM)	37 (nM)

Table 2. NMDA binding affinities for racemic ketamine, *S(+)*-ketamine, and *R(-)*-ketamine. Data from Ebert et al. (1997) (1 – ligand was MK801) in the rat cortex. All others are adapted from Hustveit et al. (1995) (in guinea pig brain homogenate)

Pharmacokinetic studies have shown that ketamine has a low binding affinity to plasma proteins, has extensive distribution due to its high lipid solubility, and is metabolized by the liver, kidneys, the intestine, and lungs (Idvall et al., 1979). Because of N-demethylation, ketamine is mostly metabolized to norketamine (80%), an active metabolite that is itself principally hydroxylized to 6-hydroxy-norketamine (15%), and is excreted in bile and urine. Ketamine elimination clearance is high (1000-1600 ml/min or 12-20 ml/min/kg) and elimination half-life is 2-3 hours in humans. The *S(+)* isomer demethylation is more pronounced to that of the *R(-)* isomer and has a higher distribution volume. Studies have shown that the *R(-)* isomer inhibits *S(+)* isomer demethylation to a proportion of 30%, while *S(+)* isomer in turn inhibits *R(-)* metabolism. However, functional differences in enantiomers are essentially due to pharmacodynamic effects since their cerebral and blood concentrations are similar in humans (White et al., 1985).

Functional magnetic resonance imaging has shown that plasma concentrations of 200 ng/ml of ketamine in humans correlates with reduced pain scores, decreased levels in insular cortex and thalamic activity, and is activated by nociceptive stimulation (Mion et al., 2013). Intravenous *S(+)*-ketamine at doses of 0.05-0.15 mg/kg/h dose dependently decreased pain perception and decreased activation of the secondary somatosensory cortex, insula, and anterior cingulate cortex, which has been linked to the affective pain component that underlies the potency of ketamine in modulating affective pain processing (Arendt-Nielson et al., 1996). Several studiesalso have demonstrated that the medial reticular formation, which processes pain perception, is depressed, as well as the medial thalamic nuclei (Klepstad et al., 1990). Interestingly, it has also been shown that ketamine enhances the descending inhibiting serotonergic pathway (Pekoe, 1982).

Amisulpride: Amisulpride (Amazeo, Amival, Solian) was developed in the 1990s by Sanofi-Aventis as an atypical antipsychotic used to treat psychosis in schizophrenia and mania in bipolar disorder (Rozenweig, 2002) and is also clinically approved for the treatment of schizophrenia and dysthymia, a mild form of depression, in multiple European countries. One previous study showed that amisulpride was as effective as imipramine and fluoxetine in terms of treating depression in humans as measured by the Montgomery and Asberg Depression Rating Scale (MADRS) (Perrault et al., 1997). In another study, amisulpride demonstrated significantly greater improvement in severely depressed patients compared to haloperidol and risperidone (Peuskens et al., 2002).
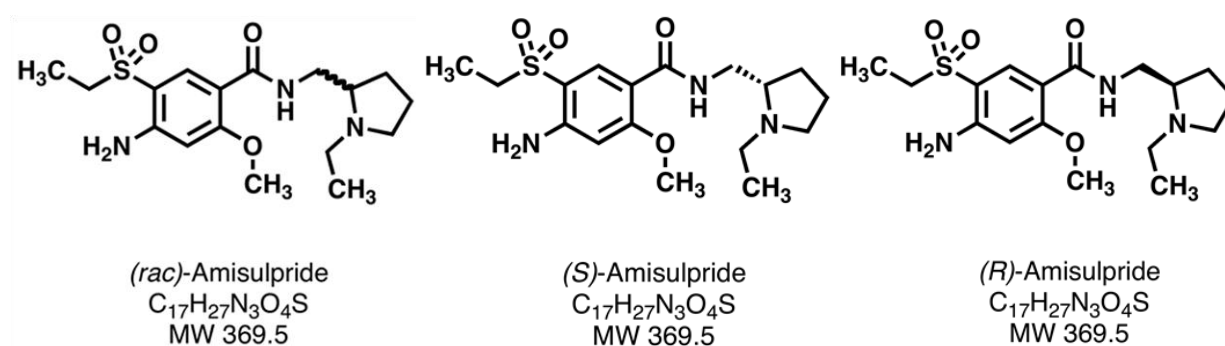
Figure 2. Chemical structures for racemic amisulpride, *S(-)*-amisulpride, and *R(+)*-amisulpride. (Chemical structures drawn by E.O. De Oliveira)

Amisulpride is a benzamide derivative and has been shown to produce both antipsychotic and antidepressant-like effects (Perrault et al., 1997) and has been shown to be a selective antagonist at dopamine D_2 and D_3 receptors and serotonin 5-HT_{2B} and 5-HT_7 receptors (Shoemaker et al., 1996). Amisulpride is a chiral molecule composed of two optical isomers: *(S)*-amisulpride and *(R)*-amisulpride, which in combination form the therapeutic drug, rac-amisulpride (see Figure 2 for chemical structures). *(S)*-amisulpride is more potent than racemic

amisulpride and *(R)*-amisulpride and is hypothesized to be responsible for the antipsychotic and antidepressant-like effects of amisulpride (Shoemaker et al., 1997; Perrault et al., 1997). It has been shown that amisulpride blocks the synthesis and release of dopamine by targeting presynaptic neurons, and at high doses, it has been suggested that the occupancy and antagonism of D_2 receptors is more critical to itsmechanism of action (Schoemaker et al., 1996). However, the antagonismof 5-HT$_{7a}$ receptors is hypothesized to be responsible for amisulpride's antidepressant efficacy (Abbas et al., 2009). In addition, it also has been shown that the *S(+)* stereoisomer is twice as potent at $D_{2/3}$ receptors twice than the racemic form and 30 times more potent than the *R(-)* isomer (Castelli, et al., 2001). In human studies, it has been shown that amisulpride treatment (up to 100 mg/kg) resulted in alleviation of negative symptoms in schizophrenia (Danion et al. 1999). In contrast, it has been shown that lower doses of amisulpride (50 mg/kg) is useful for the treatment of dysthymia (Rocca et al. 2002). These antidepressant effects have been hypothesized to occur by pre-synaptic blockade of DA receptors, which then increases DA release, while the antipsychotic effects are thought to be due to blockade of post-synaptic DA receptors (Rosenzweig et al. 2002; see Table 3 for binding affinities). However, the therapeutic efficacy of each individual enantiomer has not been determined and requires further testing.

Drug	5-HT2A (rat striatum) Ki(nM)+S.E.M.	D2 (rat cortex) Ki(nM)+S.E.M.	Alpha-2 (rat cortex) Ki(nM)+S.E.M.
Rac-amisulpride	>5000	9.8+-0.4	783+-27
S(-)-amisulpride	>5000	5.2+-0.1	1528+-45
R(+)-amisulpride	>5000	244+-12	375+-14

Table 3. Binding affinities for racemic amisulpride, *S(-)*-amisulpride, and *R(+)*-amisulpride. Data

from Marchese et al., (2002). Data represent mean (+-S.E.M) of four independent experiments.

Hypotheses and Rationale

Since the therapeutic efficacy of each individual enantiomer has not been determined for both amisulpride and ketamine, the specific aims of this study were to characterize the antidepressant-like effects of amisulpride, ketamine, and their optical isomers using the DRL 72-sec IRT in mice. The TCA imipramine will be used as a positive control, the NMDA agonist MK-801 as a negative control, and amisulpride and ketamine racemic formulations and their isomers as our test compounds. Imipramine is a tricyclic antidepressant and has been shown to improve performance on longer DRL schedules, while MK-801 is a psychostimulant which has been shown to impair performance on DRL schedules. We hypothesize that imipramine and amisulpride will increase reinforcers, decrease responses, and shift the IRT distribution to the right. We also hypothesize that MK-801 will decrease reinforcers, increase responses, and shift the IRT distribution to the left. Since previous literature indicates that the *S*-stereoisomer for amisulpride and the *R*-stereoisomer for ketamine are responsible for antidepressant-like effects,we hypothesized that both enantiomers will produce antidepressant-like effects. In contrast, we do not expect to observe antidepressant-like activity (or reduced activity) with the *R*-stereoisomer for amisulpride and *S*-stereoisomer for ketamine Thus, these results will add important information about the antidepressant-like behavioral effects of amisulpride and ketamine using a preclinical model in C57BL/6 mice.

Furthermore, we chose to use C57BL/6 mice in this study because all previous studies have only used rats. Using mice would help determine any potency or species differences on the antidepressant-like effects induced by amisulpride, ketamine, and their enantiomers.

Methods

Subjects and apparatus:Twenty-six adult male C57BL/6 mice (Envigo, Indianapolis, IN) weighing 20-28 grams were used as subjects. All mice were acclimated to normal laboratory handling and free feeding weights. After acclimation to laboratory conditions, daily access to food was restricted to maintain the mice at 85-90% of their free feeding body weights, and water was continuously available except during testing and training DRL sessions (approximately 12:00 to 16:00 daily). All animals were housed individually in plastic cages in the temperature-controlled vivarium (22° C) with a 12h/12h light/dark cycle (light on at 7:00 AM) and all behavioral sessions were conducted during the light cycle. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University (IACUC protocol AM10215), and all procedures were conducted in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 2011).

Testing was conducted in six standard computer-interfaced operant conditioning chambers (15 cm L X 11.5 cm D X 17.5 cm H; Model ENV-307A, Med Associates Inc., St. Albans, VT) housed inside sound attenuating cubicles equipped with ventilation fans and a house light. Each operant box was composed of a grid floor constructed of parallel stainless steel rods and two retractable levers were positioned on the front panel of the operant chamber (8cm apart) which extended 0.8 cm into the chamber. A dipper was located between the two levers that delivered 0.03 ml of sweetened milk (Sam's West, Inc. Bentonville, AR).

Drugs:(S)-amisulpride, *(R)*-amisulpride, and racemic amisulpride hydrochloride were donated by Dr. de Oliveira(Department of Chemical & Life Science Engineering, Virginia Commonwealth University, Richmond, VA), andimipramine (positive control), MK-801 (dizocilpine; negative

control), and all formulations of ketaminewere bought from Sigma Aldrich Chemical Company (St. Louis, Missouri). All drugs were distilled in 0.9 saline. All injections were administered subcutaneously (s.c.) and the injection volume was 10 ml/kg body weight.Drug doses and injection times were determined from previously published research (Hillhouse and Porter, 2014; Donahue et al., 2014). The order in which the drugs were tested are shown in figure3.

Procedure: All mice were weighed before each behavioral session and maintained at 85-90% of their free feeding body weight. All mice were first given one session of magazine training during which the house light was on, but the levers were not extended into the chamber. During magazine training, one reinforcer (0.03 ml of sweetened condensed milk) was delivered from a liquid dipper located in between the retractable levers every 5 seconds to acclimate them to the dipper, which remained in the up position for 5 seconds. All responses were then reinforced according to a fixed ratio (FR) 1 schedule of reinforcement for three sessions, in which each lever press resulted in delivery of a reinforcer. The position of the lever associated with the FR 1 schedule was counterbalanced across animals, with half of the mice assigned to the right lever and half assigned to the left lever. Following completion of the FR 1 schedule training, all mice began DRL training.

During DRL training, a response produced a reinforcer only after a specified inter-response interval had elapsed. Responses emitted before the end of the inter-response interval reset the timer and did not produce a reinforcer. The inter-response interval was gradually increased from an initial value of 4.5s to a terminal value of 72s over approximately 30 sessions. Specifically, mice were initially trained on a DRL 4.5s schedule for three sessions. Next, the DRL schedule was increased to 9s for five sessions, 18s for 10 sessions, 36s for 20 sessions, and the final 72s DRL criterion until performance stabilized. DRL performance was considered

stable when the number of responses for each mouse did not vary by more than 10% of the mean for five of six consecutive sessions.

During the testing phase, mice were run 4-5 days per week, consisting of at least two baseline sessions and 1-2 test sessions. If mice baseline data varied by more than 10% of the mean for at least two baseline sessions, they were excluded from testing until baseline performance was stable. The drugs,dose ranges, and pretreatment timeswere as follows: racemic amisulpride (1.0-32.0 mg/kg; 60 min), *R(+)*-amisulpride (10.0-56.0 mg/kg; 60 min), *S(-)*-amisulpride (3.2-10.0 mg/kg; 60 min), racemicketamine (3.2-10 mg/kg; 10 min), *R(-)*-ketamine (5-10 mg/kg; 10 min), *S(+)*-ketamine (2.5-5 mg/kg; 10 min), *(+)*-MK-801 (negative control; 0.01-0.1; 15 min), and imipramine (positive control; 3.2-17.8 mg/kg; 60 min).

Data analysis: The dependent variables include (a) total number of earned reinforcers during each test session, (b) total number of responses during each test session, (c) IRTs for all responses during each test session. All data are expressed as means (+/-SEM) unless otherwise noted. Reinforcer and response data were analyzed using a one-way repeated-measures analysis of variance (ANOVA), followed by Dunnett's post hoc test to compare all drug doses to vehicle. Only main effects for the number of reinforcers, number of responses, and IRT distributions are reported.The IRT distributions were obtained by recording responses in 25 6-sec bins, with the first 6-sec bin representing 'burst responding.' To determine whether there was a shift in the IRT distribution, a peak location analysis was performed, whereby the median of the IRT distribution for each mouse was determined after eliminating burst responses from the total number of responses. Medians were analyzed using one-way repeated-measures ANOVA for dose [for more information on IRT analysis, see Richards et al. (1993)]. For the IRT graphs, the relative frequency for each 6-sec bin was found for each mouse (total number of responses divided by

number of responses for each time bin) and then averages were calculated for each time bin. The criterion for significance was set at the 95% confidence level($P<0.05$), and all data were analyzed using GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, California, USA).

Results

DRL training and baseline performance: All mice met the DRL training criterion in a mean of 27.4 ($\pm$4.6) training sessions. To determine whether the vehicle baselines changed throughout the study, data from the vehicle baselines for imipramine, amisulpride and its stereoisomers, ketamine and its stereoisomers, and MK-801 were analyzed for the number of reinforcers and number of responses with separate one way ANOVAs. For number of reinforcers, there was no statistically significant main effect [$F(7, 83) = 0.705$, $p = 0.67$]. For number of responses, there was no statistically significant main effect [$F(7, 83) = 0.62$, $p = 0.74$]. Thus, vehicle baselines did not significantly change for any of the drugs during the study (see Figure 3).

Imipramine and MK-801: For number of reinforcers (Figure 4, top panel), imipramine produced a significant main effect of dose [$F(3,45)=6.41$,$p< 0.001$], and Dunnett's post hoc test revealed that the number of reinforcers was significantly increased by the 17.8 mg/kg dose of imipramine relative to vehicle ($p < 0.05$). For number of responses (Figure 4, bottom panel), imipramine produced a significant main effect of dose [$F(3,45)=5.30$,$p= 0.003$], and Dunnett's post hoc test revealed that the number of responses was significantly decreased by the 17.8 mg/kg dose of imipramine compared to vehicle ($p <0.01$). A dose of 32 mg/kg induced cataleptic behavior in six mice so further testing was discontinued.

For number of reinforcers, MK-801 (Figure 5, top panel) produced a significant main effect of dose [$F(2,22)= 18.65$,$p< 0.0001$], and Dunnett's post hoc test revealed that the number

25

of reinforcers was significantly decreased by the 0.1 mg/kg dose of MK-801 relative to vehicle

($p < 0.05$). For number of responses (Figure 5, bottom panel), MK-801 produced a significant

main effect of dose [$F_{(2,22)} = 3.48, p = 0.048$], and Dunnett's post hoc test revealed that the

number of responses was significantly increased by the 0.1 mg/kg dose of MK-801 compared to

vehicle ($p < 0.05$). A dose of 0.32 mg/kg was administered to six mice but induced rapid

jumping and hyperactivity so further testing was discontinued.

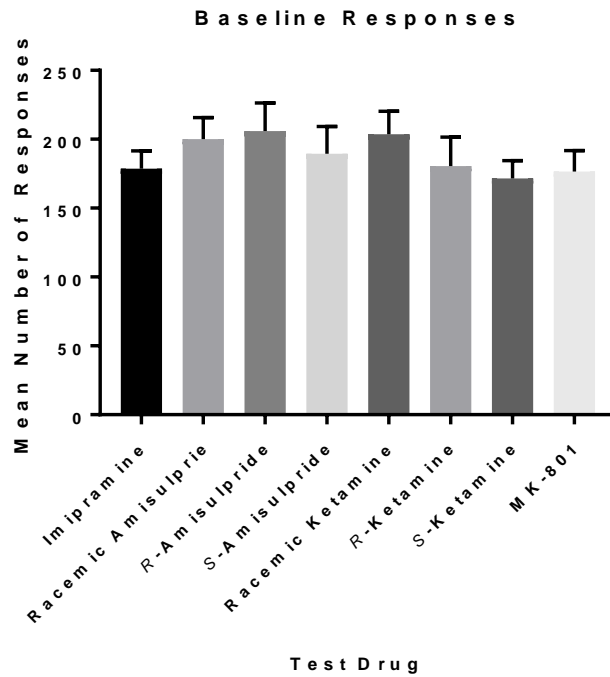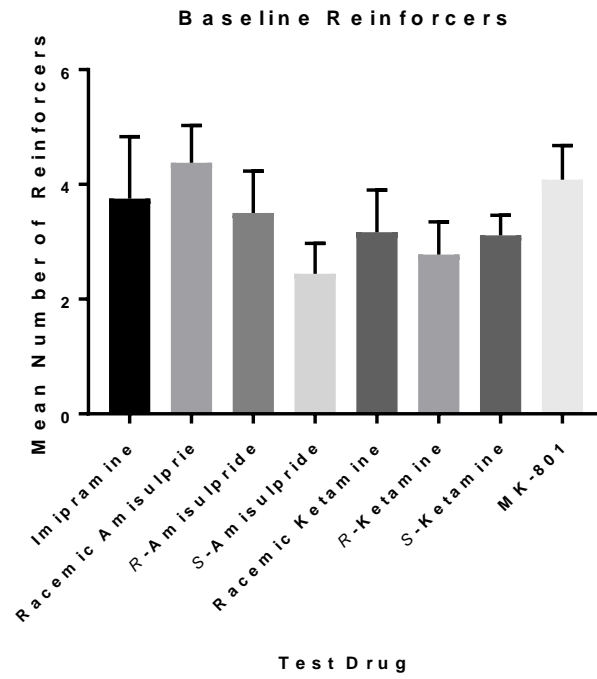**Baseline Reinforcers**

Baseline Responses

Figure 3. Effects of vehicle baselines on mean number of reinforcers (top; n = 26) and number of responses (bottom; n = 26) for all test drugs.
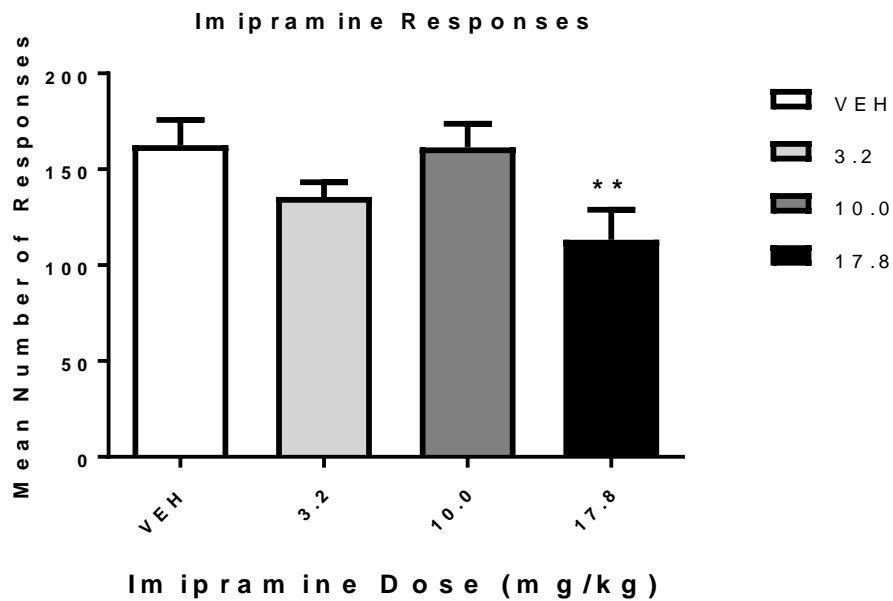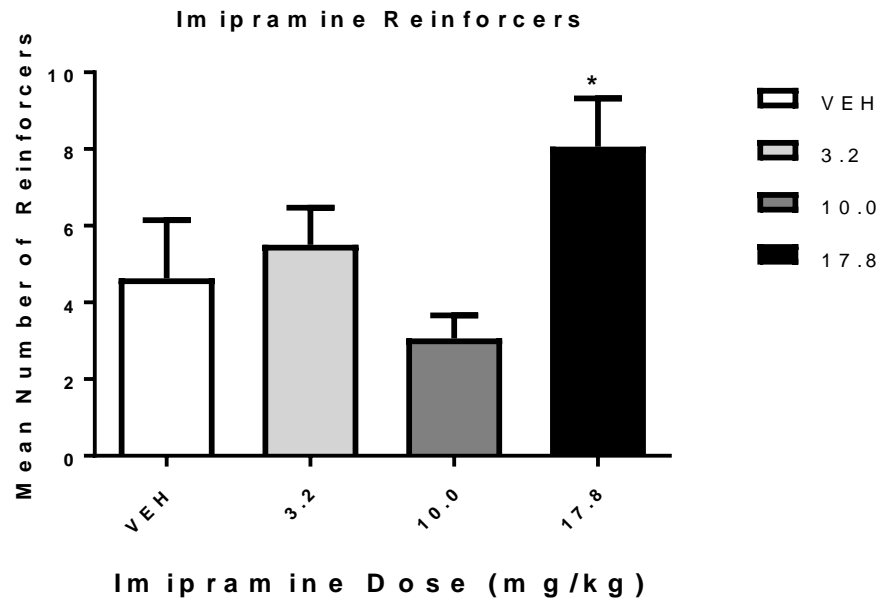
Figure 4. Effects of imipramine on mean number of reinforcers (top; n=16) and number of responses (bottom; n=16). Asterisks represent significant differences from vehicle, and all data are expressed as means +SEM ($p < 0.05$). For all graphs, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

M K - 8 0 1 R e i n f o r c e r s

M K - 8 0 1 D o s e (m g / k g)

M K - 8 0 1 R e s p o n s e s

M K - 8 0 1 D o s e (m g / k g)

Figure 5. Effects of MK-801 on mean ($\pm$SEM) number of reinforcers (top; n=12) and number of responses (bottom; n=12). See Figure 4 for other details.

Racemic amisulpride and its enantiomers

For number of reinforcers (Figure 6, top panel), racemic amisulpride produced a significant main effect for dose [$F_{(6,90)}=7.88$, $p<0.0001$)]. Compared with other doses of racemic amisulpride, Dunnett's post hoc test revealed that the 17.8, 32, and 56 mg/kg doses produced significant increases in the number of reinforcers compared to vehicle. For number of responses (Figure 6, bottom panel), racemic amisulpride produced a significant main effect of dose [$F_{(6, 90)}=2.56$, $p= 0.025$], and Dunnett's post hoc test revealed that the 56.0 mg/kg dose produced a small but significant ($p <0.05$) decrease in responses relative to vehicle.

For number of reinforcers (Figure 7, top panel), *R(+)*-amisulpride did not produce a significant main effect of dose [$F_{(3,21)}= 1.06$, $p = 0.39$]. For number of responses (Figure 7, bottom panel), *R(+)*-amisulpride did not produce a significant main effect of dose [$F_{(3,21)}=1.02$, $p= 0.40$].

For number of reinforcers (Figure 8, top panel), *S(-)*-amisulpride produced a significant main effect of dose [$F_{(3,24)} = 5.94$, $p = 0.003$]. Compared with other doses of *S(-)*-amisulpride, Dunnett's post-hoc test revealed that the 10.0 mg/kg dose was significantly different than vehicle ($p < 0.05$). For number of responses (Figure 8, bottom panel), *S(-)*-amisulpride did not produce a significant main effect of dose [$F_{(3,24)}=0.24$, $p= 0.87$].

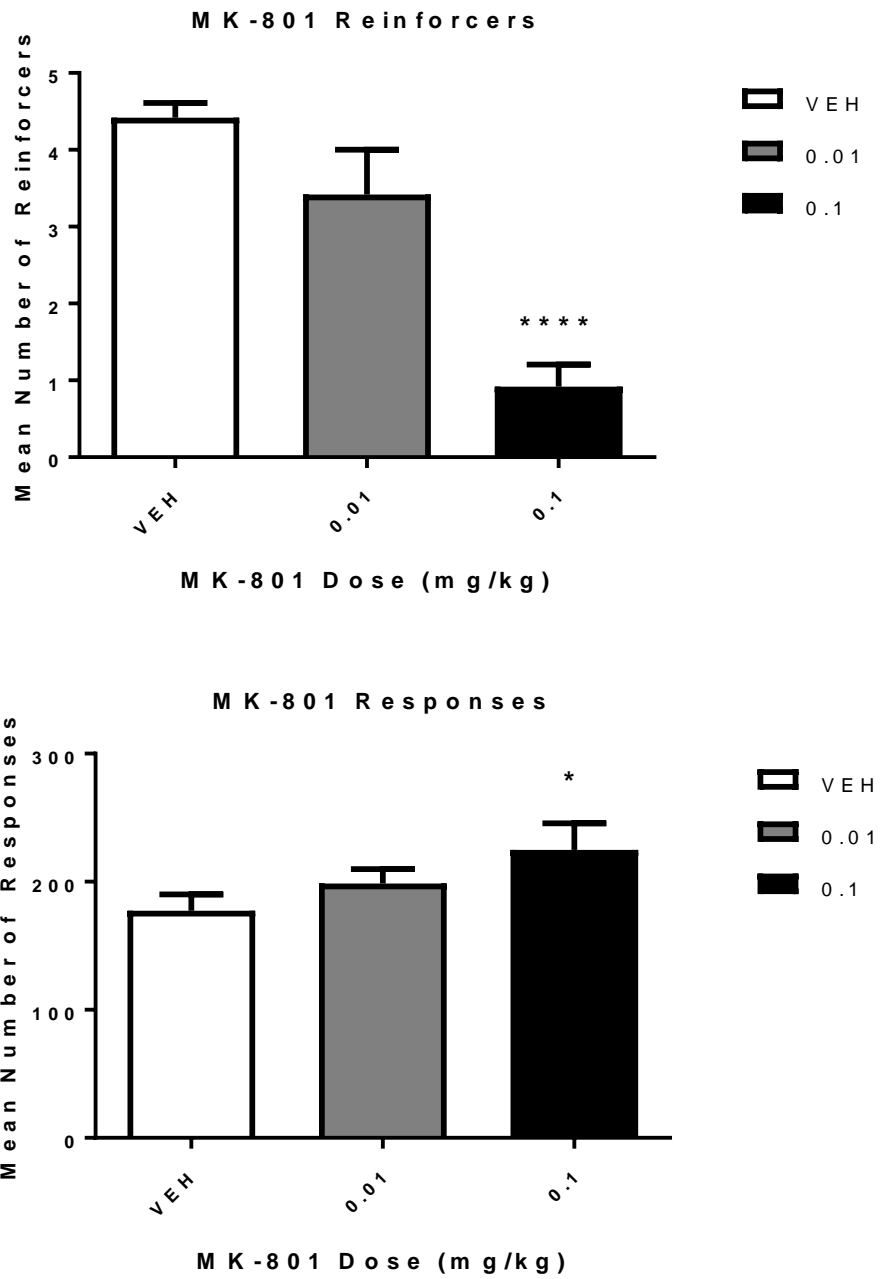Figure 6. Effects of racemic amisulpride on mean (±SEM) number of reinforcers (top; n=16) and number of responses (bottom; n=16). See Figure 4 for other details.

R (+)-a m is u lp rid e R e in fo rce rs

R (+)-a m isu lp rid e R e sp o n se s

Figure 7. Effects of *R(+)*-amisulpride on mean (±SEM) number of reinforcers (top; n=8) and number of responses (bottom; n=8). See Figure 4 for other details.

S (-)-amisulpride Reinforcers

S (-)-amisulpride Responses

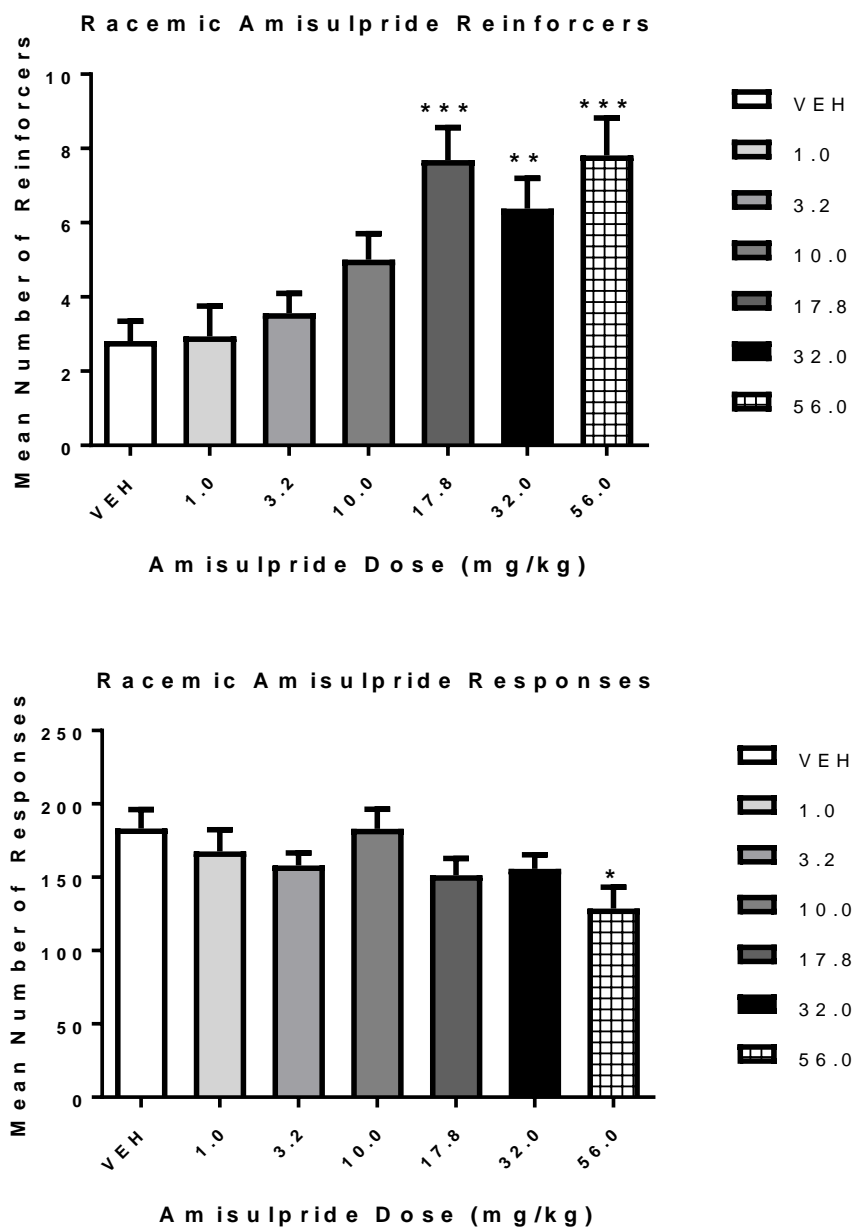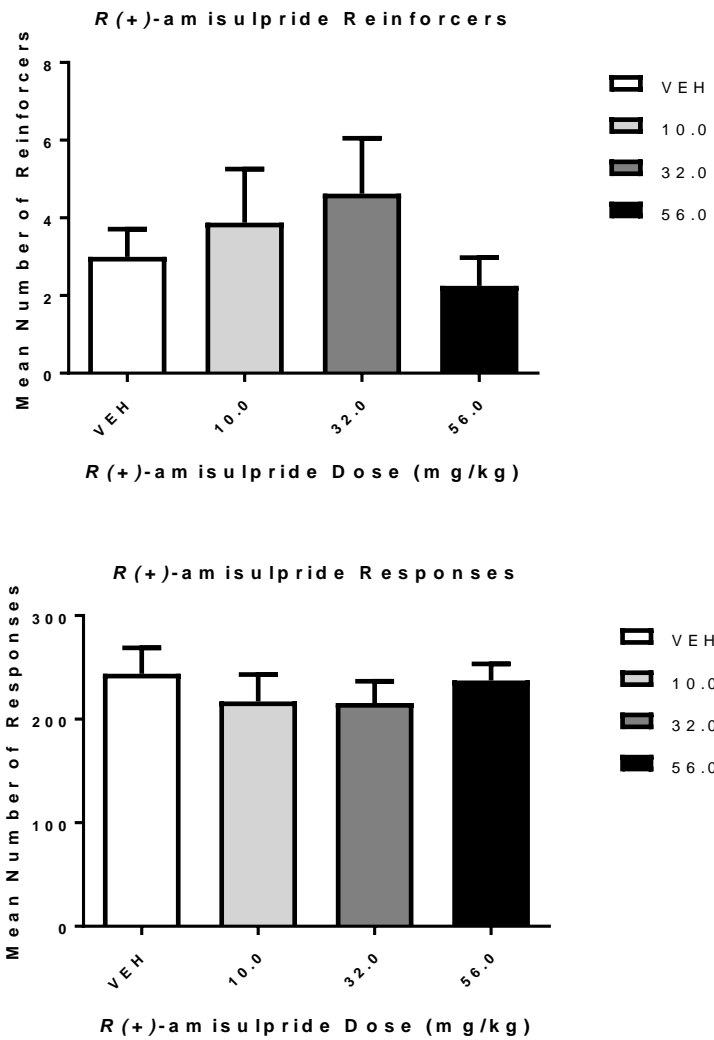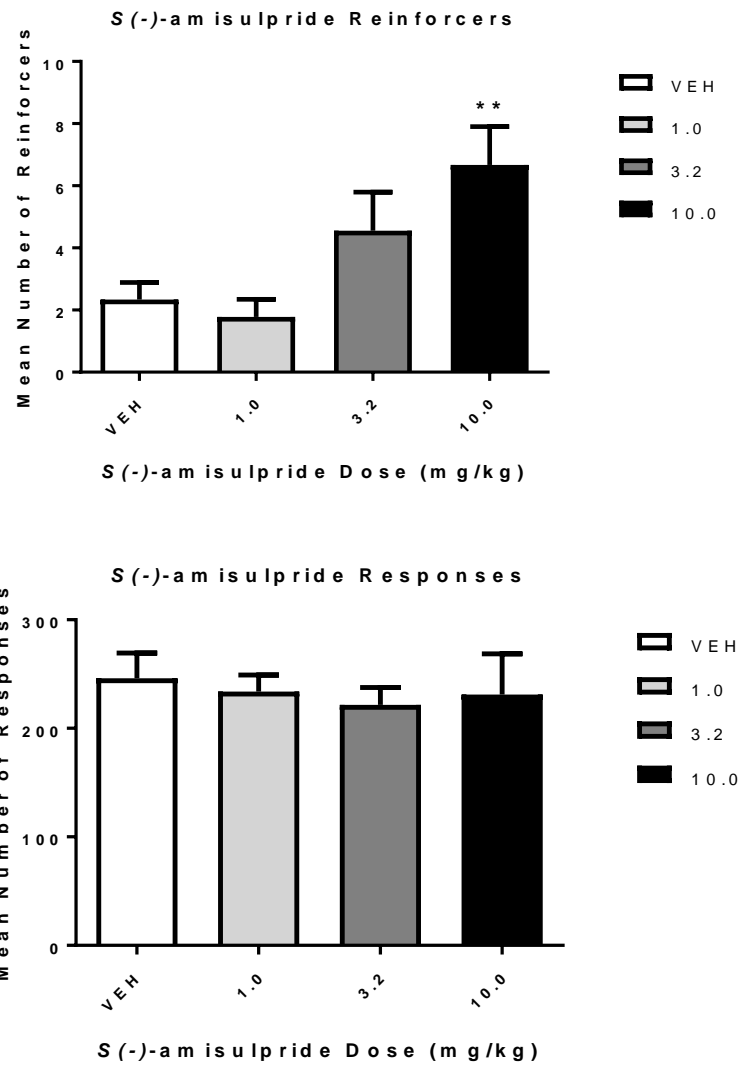Figure 8. Effects of *S(-)*-amisulpride on mean (±SEM) number of reinforcers (top; n=9) and number of responses (bottom; n=9). See Figure 4 for other details.

Racemic ketamine and its enantiomers

For number of reinforcers (Figure 9, top panel), racemic ketamine produced a significant main effect of dose [$F_{(2,22)}=14.82, p< 0.0001$], and compared with other doses of racemic ketamine, Dunnett's post-hoc test revealed that the 10.0 mg/kg dose was significantly different than vehicle ($p < 0.05$). For number of responses (Figure 9, bottom panel), racemic ketamine produced a significant main effect of dose [$F_{(2,22)} = 14.13, p < 0.0001$)]. The Dunnett's post hoc test revealed that both the 3.2 and 10.0 mg/kg dose were significantly different than vehicle ($p < 0.05$).

For number of reinforcers (Figure 10, top panel), *R(-)*-ketamine produced a significant main effect of dose [$F_{(2,16)}=11.88, p< 0.0007$], and Dunnett's post-hoc test revealed that the 10.0 mg/kg dose was significantly different than vehicle ($p < 0.05$). For number of responses (Figure 10, bottom panel), *R(-)*-ketamine produced a significant main effect of dose [$F_{(2,16)}=5.38, p= 0.016$], and Dunnett's post-hoc test revealed that the 10.0 mg/kg dose was significantly different than vehicle ($p < 0.05$).

For number of reinforcers (Figure 11, top panel), *S(+)*-ketamine produced a significant main effect of dose [$F_{(2,16)}=9.83, p< 0.001$]. Compared to other doses of *S(+)*-ketamine, Dunnett's post hoc test showed that the 5.0 mg/kg dose was significantly different than vehicle ($p < 0.05$). For number of responses (Figure 11, bottom panel), *S(+)*-ketamine did not produce a significant main effect of dose [$F_{(2,16)}=2.45, p= 0.06$]. A dose of 10.0 mg/kg of *S(+)*-ketamine was administered to six mice but induced cataleptic behavior so further testing with this dose was discontinued.

R a c e m i c   K e t a m i n e   R e i n f o r c e r s

Mean Number of Reinforcers

8

6

4

2

0

* * * *

VEH 3.2 10.0

VEH
3.2
10.0

R a c e m i c   K e t a m i n e   D o s e   (m g / k g)

R a c e m i c   K e t a m i n e   R e s p o n s e s

Mean Number of Responses

300

200

100

0

*

* * * *

VEH 3.2 10.0

VEH
3.2
10.0

R a c e m i c   K e t a m i n e   D o s e   (m g / k g)

Figure 9. Effects of racemic ketamine on mean ($\pm$SEM) number of reinforcers (top; n=12) and number of responses (bottom; n=12). See Figure 4 for other details.

Figure 10. Effects of *R(-)*-ketamine on mean ($\pm$SEM) number of reinforcers (top; n=9) and number of responses (bottom; n=9). See Figure 4 for other details.
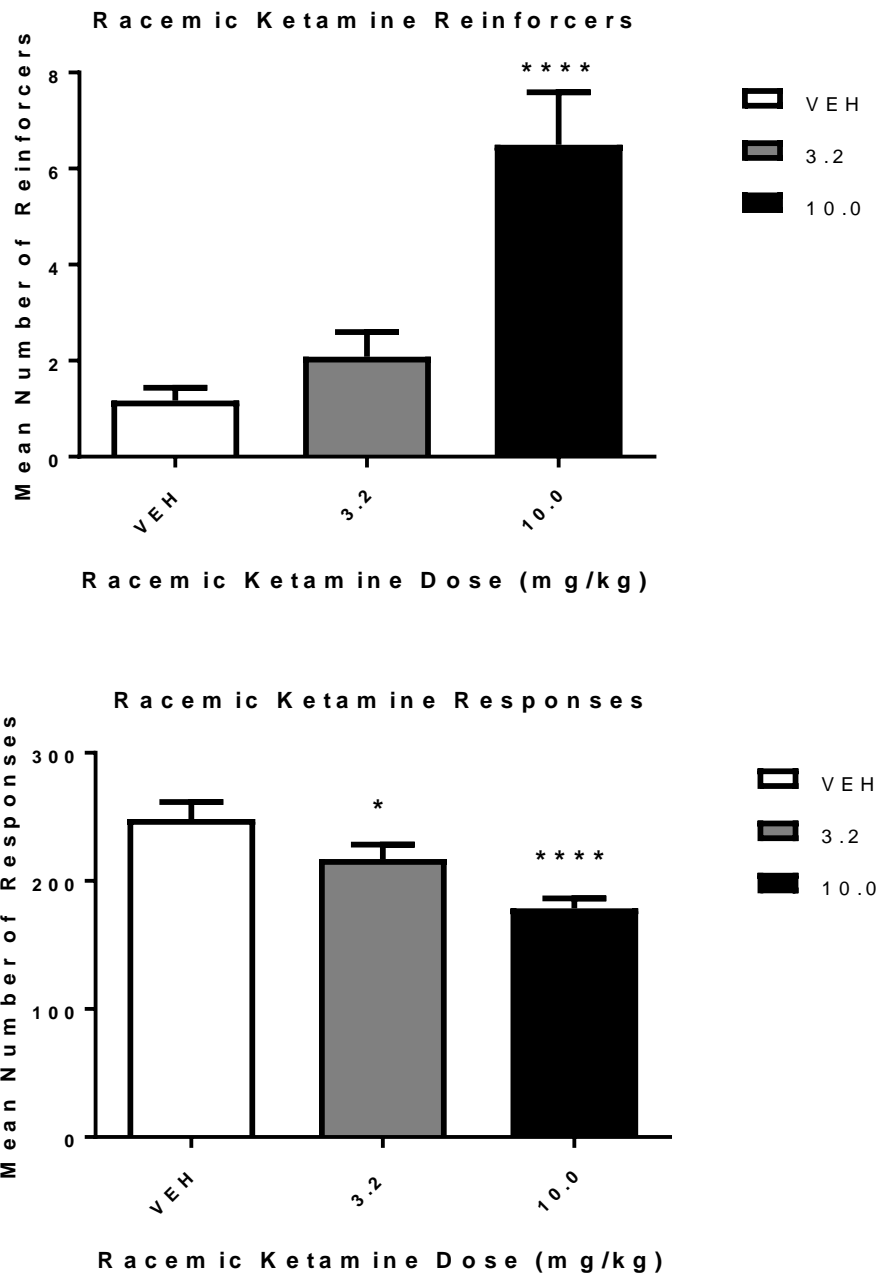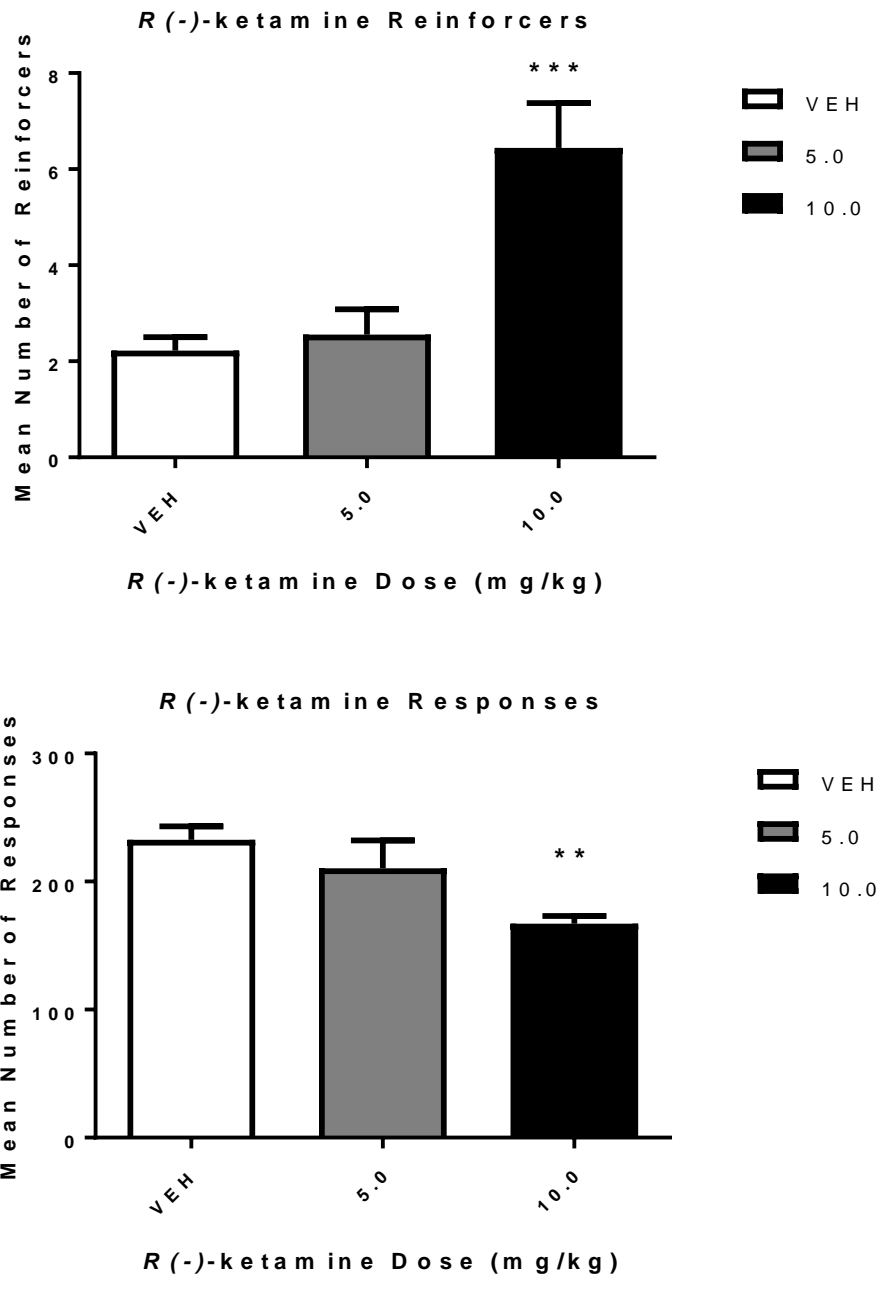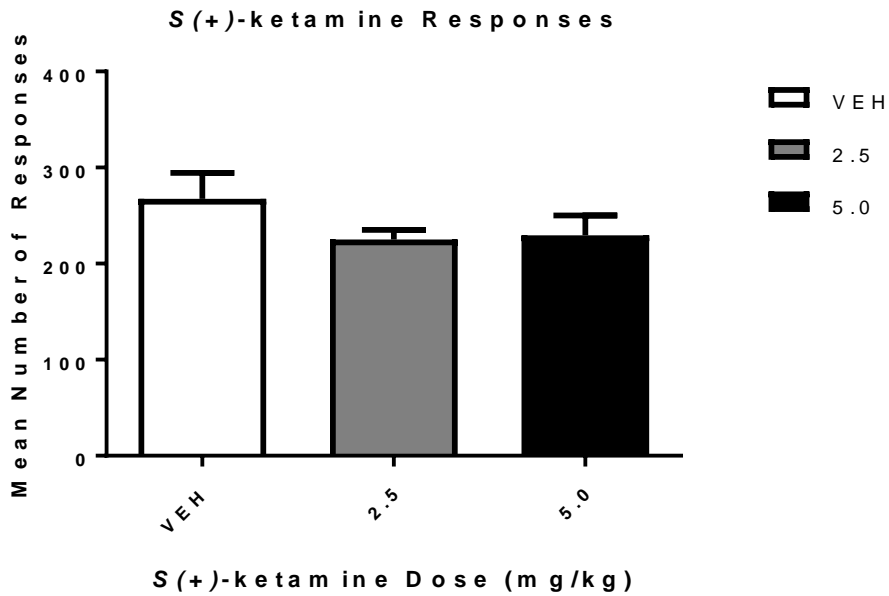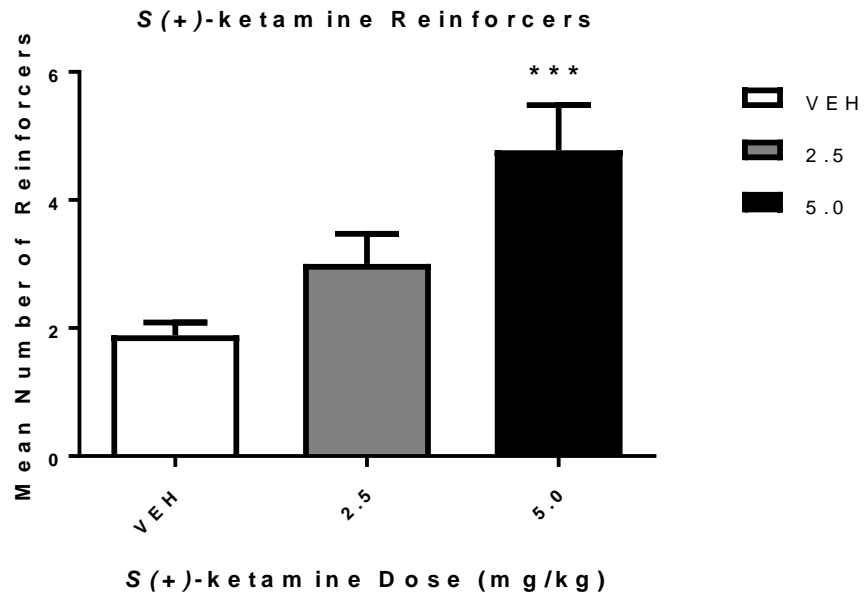
Figure 11. Effects of *S(+)*-ketamine on mean (+SEM) number of reinforcers (top; n=9) and number of responses (bottom; n=9). See Figure 4 for other details.
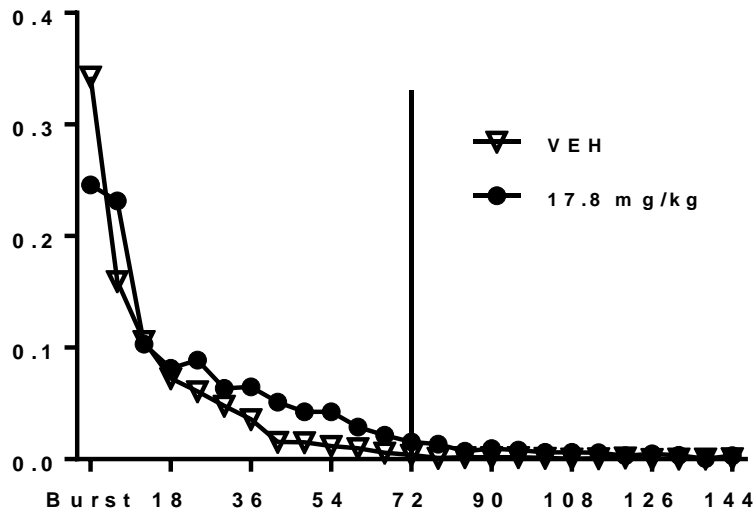
IRT distributions: For IRT distributions, the highest dose for test drugs that produced a significant effect (or change) for reinforcers and responses were compared to vehicle.

Imipramine and MK-801 IRTs: For imipramine (Figure 12, top panel), 17.8 mg/kg did not produce a significant shift in the IRT distribution compared to vehicle ($p > 0.05$) although there was a slight right-ward shift for imipramine. For MK-801(Figure 12, bottom panel), 0.1 mg/kg did not produce a significant shift in the IRT distribution compared to vehicle ($p > 0.05$)although there was a slight left-ward shift for MK-801.

Racemic amisulpride and its enantiomers IRTs: The 56.0 mg/kg dose of racemic amisulpride (figure 13, top panel) did not produce a significant shift in the IRT distribution, although there was a slight right-ward shift in the IRT distribution ($p > 0.05$). *R*(+)-amisulpride (32 mg/kg; figure 13, middle panel) and *S*(-)-amisulpride (10.0 mg/kg; figure 13, bottom panel) also did not produce a significant shift in the IRT distribution ($p > 0.05$).

Racemic ketamine and its enantiomers: The 56.0 mg/kg dose of racemic ketamine (Figure 14, top panel) did not produce a significant shift in the IRT distribution ($p > 0.05$). The enantiomers *R*(-)-ketamine (10.0 mg/kg; figure 14, middle panel) and *S*(+)-ketamine (5.0 mg/kg; Figure 14, bottom panel) also did not produce a significant shift in the IRT distribution ($p > 0.05$).

Im ipram ine IRT Distribution

M K - 8 0 1 IRT Distribution
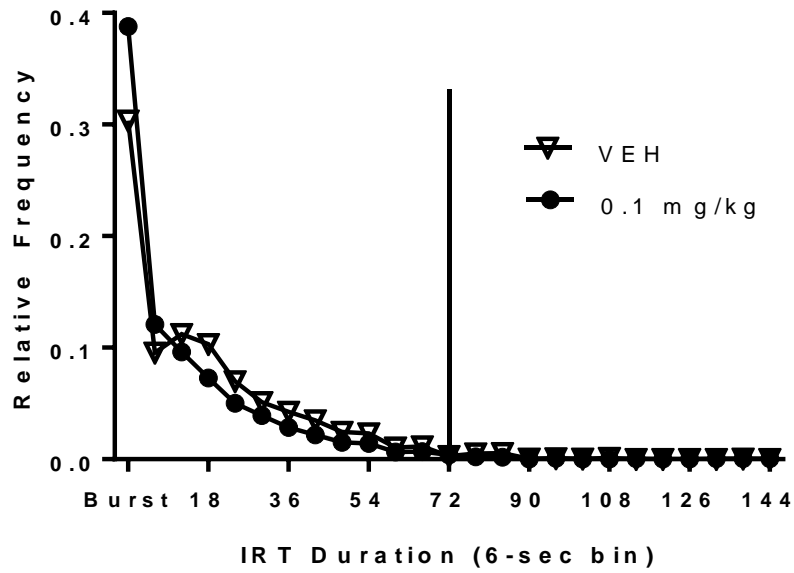

Figure 12. IRT Distributions are shown for imipramine (top panel) and for MK-801 (bottom panel).
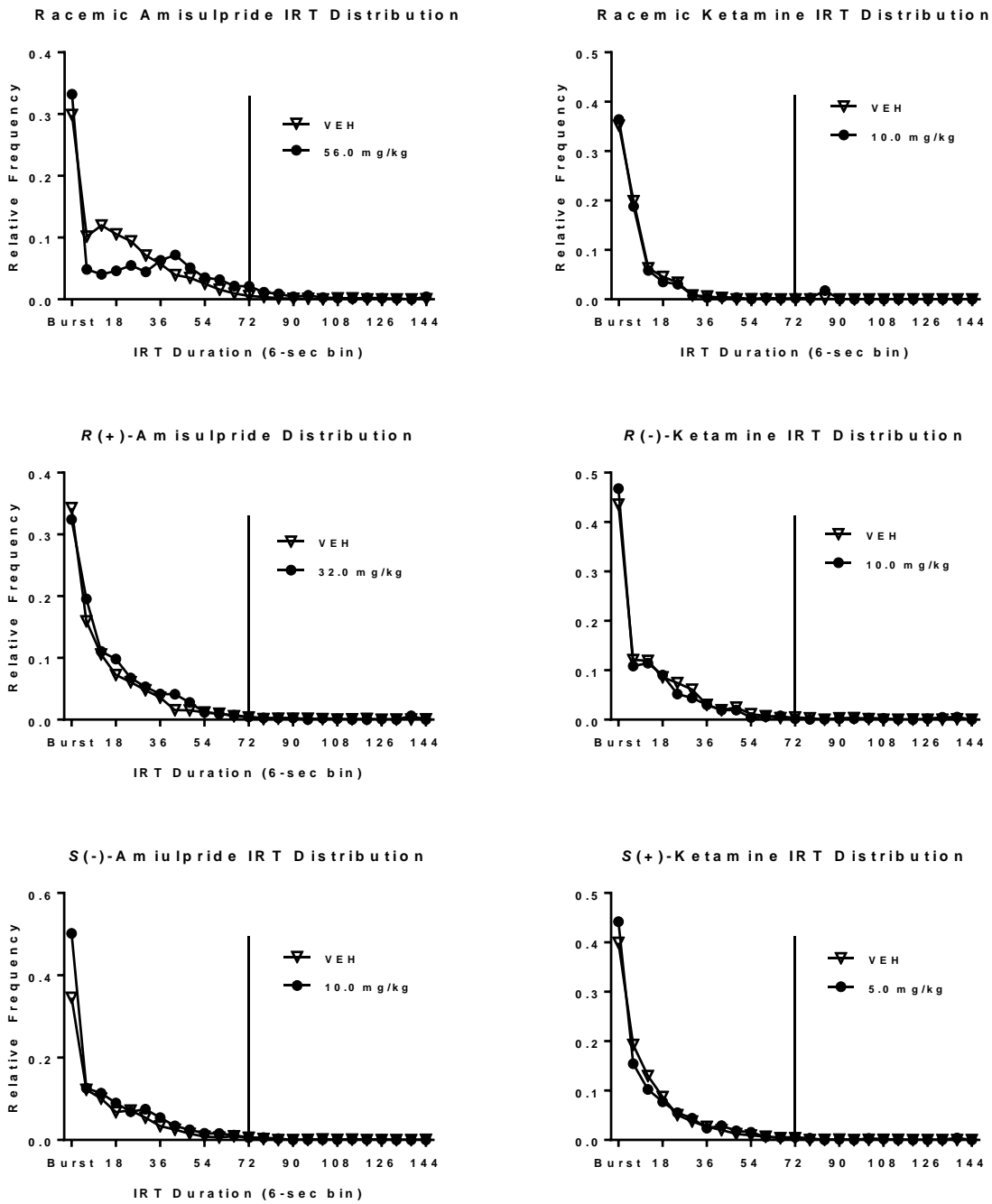
Figure 13. IRT Distributions are shown for racemic amisulpride (left top panel), for *R*(+)-amisulpride (left middle panel), and for *S*(-)-amisulpride (left bottom panel), racemic ketamine (right top panel), *R*(-)-ketamine (right middle panel), and *S*(+)-ketamine (right bottom panel).

Discussion

The aim of the present study was to determine whether the enantiomers for ketamine and amisulpride produced antidepressant-like effects in a manner similar to their parent compounds using the differential-reinforcement-of-low-rate 72-sec task. Consistent with previous findings in the literature, we hypothesized that amisulpride and *S(-)*-amisulpride, but not *R(+)*-amisulpride, would produce antidepressant-like effects, while all formulations of ketamine would produce antidepressant effects. Racemic amisulpride and *S(-)*-amisulpride, but not *R(+)*-amisulpride, produced an antidepressant-like effect, evidenced by a significant increase in the number of reinforcers and a significant decrease in the number of responses. Racemic ketamine and *R(-)*-ketamine significantly increased the number of reinforcers and decreased the number of responses, while *S(+)*-ketamine significantly increased the number of reinforcers, but did not decrease the number of responses (at the doses tested). Overall, these results indicate that racemic amisulpride, *S(-)*-amisulpride, and all formulations of ketamine demonstrate antidepressant-like effects as assessed in the DRL 72-sec task and may be useful in a clinical context. If either of the ketamine isomers can be shown to produce fewer psychotomimetic effects in humans, then the isomers may offer a significant clinical advantage over the parent compound ketamine. Regarding amisulpride, the present results indicate that the *S(+)* isomer is the active component of the parent drug and that the *R(-)* isomer is relatively inactive at the doses tested.

Theantidepressant-like effects observed with racemic ketamine and its isomers in the present study are consistent with previous research that showed the 10.0 mg/kg dose of racemic ketamine and *R(-)*-ketamine produce antidepressant-like effects in several preclinical procedures used to assess antidepressant effects. For instance, Zhang et al. (2014) showed that all

41

formulations of ketamine produce antidepressant-like effects in the tail suspension test, forced swim test, and sucrose preference test.After establishing that neonatal dexamethasone exposure causes depression-like behavior in juvenile mice, Zhang et al. (2014) showed that both isomers for ketamine significantly attenuated the increase in immobility time in the tail suspension test and forced swim test at doses similar to those used in the present study.Another study showed that *R(-)*-ketamine produced greater potency and longer lasting antidepressant-like effects than did *S(+)*-ketamine in the social defeat stress and learned helplessness models of depression in mice (Yang et al., 2015). The present study results are also consistent with previous research in rats, which showed that ketamine, but not MK-801, produced antidepressant-like effects in the DRL 72-sec task; thus, demonstrating cross-species similarities in antidepressant activity for ketamine (Hillhouse & Porter, 2014). The current study results extend these findings in rats to a new species (mice) and to ketamine's stereoisomers. Taken together, these data suggest that all formulations of ketamine produce antidepressant-like effects and may be useful in a clinical context.

The antidepressant-like effects observed with racemic amisulpride are in agreement with previous preclinical studies in male Wistar rats that showed racemic amisulpride (1 and 3 mg/kg) produced antidepressant-like effects in the forced swim test while doses of 5 and 10 mg/kg elicited antidepressant-like effects in the chronic mild stress model (Papp & Wieronska, 2000). However, it is not immediately known why low doses in that study produced an antidepressant-like effect; whereas, our study found that a dose of 56.0 mg/kg of racemic amisulpride produced the strongest antidepressant-like effect. These differences in dose effects may be attributable to pharmacokinetic differences between male C57BL/6 mice and male Wistar rats. Furthermore, to

42

the best of our knowledge, this is the first preclinical study to investigate the antidepressant-like activity of amisulpride's stereoisomers.

A variety of monoaminergic compounds have been assessed in the DRL 72-sec task and have shown mixed results in terms of their antidepressant-like effects. For instance, drugs that function as norepinephrine transporter inhibitors (Marek et al., 1988; Wong et al., 2000; Dekeyne et al., 2002), tricyclic antidepressants (O'Donnell and Seiden, 1983; Marek and Seiden, 1988; Richards and Seiden, 1991; Richards et al., 1993; Cousins and Seiden, 2000; Ardayfio et al., 2008; Hillhouse and Porter, 2014), serotonin transporter inhibitors (Richards et al., 1993; Balcells-Olivero et al., 1998; Sokolowski and Seiden, 1999; Cousins and Seiden, 2000), 5-HT$_{2A}$ receptor antagonists (Marek and Seiden, 1988; Marek et al., 1989, 2005), and 5-HT$_{2C}$ receptor agonists (Martin et al., 1998) have all been shown to produce antidepressant-like effects in the DRL 72-sec task and most of these drugs produce antidepressant effects in humans. Amisulpride is an atypical antipsychotic that functions primarily as a dopamine D$_2$ and D$_3$ receptor antagonist, but has been shown to be a potent 5-HT$_7$ receptor antagonist (Abbas et al., 2000). Abbas et al. (2000) argue that the 5-HT$_7$ receptor antagonist effects of amisulpride are responsible for its antidepressant-like effects, but further research is needed to confirm that hypothesis. Other studies have shown that, at low doses, amisulpride's antidepressant effects may be related to presynaptic antagonism of DA receptors, while its antipsychotic effects are related to postsynaptic antagonism. At high doses (40-80 mg/kg), amisulpride blocks postsynaptic dopaminergic receptors similar to that seen with other antipsychotic medications; however, at low doses (<10 mg/kg) amisulpride increases dopaminergic transmission by blocking autoreceptors on the presynaptic terminals (Möller, 2003). Presynaptic blockade leads to increased dopaminergic transmission (Sanger, Perrault, Schoemaker, & Scatton, 1999;

Schoemaker et al., 1997), and it has been hypothesized that increaseddopamine release via amisulpride's antagonism of presynaptic autoreceptors may beresponsible for its therapeutic effects in alleviating depression. However, in the DRL 72-sec task, antipsychotics have mixed effects (for a review, see O'Donnell et al., 2005 and Marek et al., 2016). Our findings in the present study showing that racemic amisulpride and *S(-)*-amisulpride, but not *R(+)*-amisulpride, produce antidepressant-like effects are likely due to a number of pharmacodynamic differences between the isomers. First, *S*(-)-amisulpride is approximately twice as potent as racemic amisulpride and 20-50 times more potent than *R*(+)-amisulpride at dopamine D_2 and D_3 receptors, while *R*(+)-amisulpride is twice as potent as racemic amisulpride and four times more potent than *S*(-)-amisulpride at alpha2-adrenoreceptors (Marchese et al., 2002). Second, while both racemic amisulpride and *R*(+)-amisulpride have been shown to generalize to the *S*(-)-amisulpride stimulus in a drug discrimination procedure (Donahue et al., 2014), *S*(-)-amisulpride was about three times more potent than racemic amisulpride and ten times more potent than *R*(-)-amisulpride, suggesting that its therapeutic effects may be particularly sensitive to dose-related factors.

In contrast to the wide variety of monoaminergic drugs tested in the DRL 72-sec task, the effects of glutamatergic drugs in this procedure is less understood. Hillhouse and Porter (2014a) showed that ketamine, but not MK-801, produced antidepressant-like effects in rats, as evidenced by an increase in reinforcers, decrease in responses, and a rightward shift in the IRT distribution for ketamine (MK-801 produced opposite results). The lack of antidepressant-like effects for MK-801 were also demonstrated by Ardayfio et al. (2008). Furthermore, Hillhouse et al., (2014b) also showed that phencyclidine (from which ketamine is derived) increased reinforcers and decreased responses without shifting the peak location of the IRT distribution,

44

suggesting phencyclidine produces weaker antidepressant-like effects as compared to ketamine. While the effects of glutamatergic drugs in the DRL 72-sec task are yet to be fully established, a recent review by Marek et al., (2016) proposed that the DRL procedure is not simply an antidepressant screening task. Rather, they posited that the wide range of behavior exhibited in the DRL 72-sec task in response to different drugs is due to an interaction between impulsivity and cognitive functions that may be disturbed in depressed patients, particularly the severely depressed. This hypothesis is in agreement with clinical studies that have shown ketamine is particularly useful in those who are severely depressed, while the antidepressant-like effects of drugs used to treat less severe forms of depression (dysthymia) may not be as apparent in the DRL 72-sec task.

In conclusion, the present study results are in agreement with both the preclinical and clinical literature demonstrating that racemic amisulpride and its *S(-)* isomer and ketamine and its isomers *S(+)* and *R(-)* produce antidepressant-like effects in a mannersimilar to the tricyclic antidepressant imipramine. In contrast to ketamine, the more selective and higher affinity NMDA receptor antagonist MK-801 failed to produce an antidepressant-like effect in the DRL 72-sec task, but rather produced effects more similar to psychostimulant-like effects (also see Hillhouse and Porter 2014). It also should be noted that the IRT distributions for the drugs tested did not show the typical right-ward shifts associated with antidepressant-like drugs that have been tested in rats (see Hillhouse and Porter 2014) These data demonstrate that the antidepressant-like effects of ketamine and its isomers observed in the present study cannot be simply explained by noncompetitive antagonism of NMDA receptors and off-target mechanisms could be responsible for these effects. For instance, MK-801 is about 30-fold more selective for the NET, three-fold more selective for the SERT (Nishimura et al., 1998), and eight-fold more

selective for the kappa opioid receptor (Smith et al., 1987). Specifically, kappa opioid receptor agonists have been shown to produce dysphoria, hallucinations, and dissociation, which has limited their clinical usefulness; thus, the lack of antidepressant-like effects observed by MK-801 in the present study could be due to stronger psychotomimetic effects compared to ketamine. In addition, the present study also established cross-species antidepressant-like effects in the DRL 72-sec task in male C57/BL mice that complements the current literature that has predominantly used rats, suggesting that ketamine has robust antidepressant-like effects in preclinical animal models. Further studies using different glutamatergic and novel monoaminergic drugs with multimodal effects are warranted. Studies examining the differences between male and female subjects would also be pertinent, considering, in female rats, the FST elicited a significant decrease in serotonergic activity in the hypothalamus and a decrease in 5-HT1A mRNA levels, whereas 5-HT1A mRNA levels were increased in male rats (Drossopoulou et al., 2004). Furthermore, Liu and Gershenfeld (2001) demonstrated that female mice had longer durations of immobility across four different strains of mice compared to male mice. This discrepancy between male and female mice on antidepressant-like effects in preclinical models could be further understood by examining sex differences in the DRL 72-sec task. Finally, it must be noted that the current study and many of the previous preclinical studies in the literature have only studied the acute dosing effects of ketamine and other drugs in tasks designed to assess the antidepressant-like effects of drugs. Given that the clinical effects of antidepressants in humans are typically only evident after several weeks of repeated dosing, preclinical studies also need to address this issue. With repeated dosing regimens, it might be possible to show stronger antidepressant-like effects or show effects at lower doses than seen with acute dosing. In the present study there was a trend for the *R(-)*isomer of amisulpride to increase reinforcers up to the

46

32 mg/kg dose tested but it did not reach significance. It is possible that the antidepressant-like effects of amisulpride are only evident at lower doses and that repeated dosing may be required for those effects to be evident in this assay.Interestingly, the dose effect functions between the R and S enantiomers and the racemic form of both amisulpride and ketamine may have important implications for future testing of antidepressant-like effects. First, in agreement with previous literature, the *S*(-) isomer for amisulpride appears to be more critical for eliciting antidepressant-like effects since a dose of 10.0 mg/kg produced a significant increase in reinforcers, while much higher doses of racemic amisulpride produced antidepressant-like effects and *R*(+)-amisulpride produced no antidepressant-like effects at the doses tested. Furthermore, while *R*(-)-ketamine produced a significant increase in reinforcers and a decrease in responses at the 10.0 mg/kg dose, *S*(+)-ketamine produced a significant increase in reinforcers at the 5.0 mg/kg dose. These data indicate that the *R* and *S*ketamine enantiomers may not be equally important for producing antidepressant-like effects. It would be interesting to conduct combination testing with the isomers of ketamine and amisulpride to determine the relative contributions of each isomer to the effects seen with the parent compounds.

References

Abbas, A. I., Hedlund, P. B., Huang, X. P., Tran, T. B., Meltzer, H. Y., & Roth, B. L. (2009). Amisulpride is a potent 5-HT7 antagonist: relevance for antidepressant actions in vivo. *Psychopharmacology*, *205*(1), 119-128.

Altamura, C. A. (1993). Glutamate. *Am J Psychiatry*, *1*(50), 1-1.

Altamura, C., Maes, M., Dai, J., & Meltzer, H. Y. (1995). Plasma concentrations of excitatory amino acids, serine, glycine, taurine and histidine in major depression. *European Neuropsychopharmacology*, *5*, 71-75.

Andrzejewski, M. E., Spencer, R. C., Harris, R. L., Feit, E. C., McKee, B. L., & Berridge, C. W. (2014). The effects of clinically relevant doses of amphetamine and methylphenidate on signal detection and DRL in rats. *Neuropharmacology*, *79*, 634–641.

Anton-Stephens D. 1954. Preliminary observations on the psychiatric uses of chlorpromazine (Largactil). Journal of Mental Science, 100:543–57.

Ardayfio, P. a, Benvenga, M. J., Chaney, S. F., Love, P. L., Catlow, J., Swanson, S. P., & Marek, G. J. (2008). The 5-hydroxytryptamine2A receptor antagonist R-(+)-alpha-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl-4-piperidinemethanol (M100907) attenuates impulsivity after both drug-induced disruption (dizocilpine) and enhancement (antidepressant drugs) of dif. *The Journal of Pharmacology and Experimental Therapeutics*, *327*(3), 891–897.

Arendt-Nielsen, L., Nielsen, J., Petersen-Felix, S., Schnider, T. W., & Zbinden, A. M. (1996). Effect of racemic mixture and the (S+)-isomer of ketamine on temporal and spatial summation of pain. *British journal of anaesthesia*, *77*(5), 625-631.

Balcells-Olivero, M., Cousins, M. S., & Seiden, L. S. (1998). Holtzman and Harlan Sprague-Dawley rats: differences in DRL 72-sec performance and 8-hydroxy-di-propylamino tetralin-induced hypothermia. *Journal of Pharmacology and Experimental Therapeutics*, *286*(2), 742-752.

Ban TA. 2001. Pharmacotherapy of mental illness. A historical analysis. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 25:709–27.

Bayley, P. J., Bentley, G. D., & Dawson, G. R. (1998). The effects of selected antidepressant drugs on timing behaviour in rats. *Psychopharmacology*, *136*(2), 114–22.

Berk, M., Sanders, K. M., Pasco, J. A., Jacka, F. N., Williams, L. J., Hayles, A. L., & Dodd, S. (2007). Vitamin D deficiency may play a role in depression. *Medical hypotheses*, *69*(6), 1316-1319.

Belmaker, R. H., & Agam, G. (2008). Major depressive disorder. *New England Journal of Medicine*, *358*(1), 55-68.

Borsini, F., & Meli, A. (1988). Is the forced swimming test a suitable model for revealing antidepressant activity?. *Psychopharmacology*, *94*(2), 147-160.

Branch, T., & Ground, A. P. (1982). Effects of Anticholinergic Drug L Performance of Rhesus MO, *17*(1978), 85–90.

Bull, E., Reavill, C., Hagan, J. J., Overend, P., & Jones, D. N. (2000). Evaluation of the spontaneously hypertensive rat as a model of attention deficit hyperactivity disorder: acquisition and performance of the DRL-60s test. *Behavioural brain research*, *109*(1), 27-35.

Bunney, W. E., & Davis, J. M. (1965). Norepinephrine in depressive reactions: A review. *Archives of General Psychiatry*, *13*(6), 483-494.

Canon, J. G., & Lippa, A. S. (1977). Use of DRL in differentiating anxiolytic and neuroleptic properties of CNS drugs. *Pharmacol Biochem Behav*, *6*(5), 591–593.

Castelli, M. P., Mocci, I., Sanna, A. M., Gessa, G. L., & Pani, L. (2001). (−) S amisulpride binds with high affinity to cloned dopamine D 3 and D 2 receptors. *European journal of pharmacology*, *432*(2), 143-147.

Chiang, F.-K., Cheng, R.-K., & Liao, R.-M. (2015). Differential effects of dopamine receptor subtype-specific agonists with respect to operant behavior maintained on a differential reinforcement of low-rate responding (DRL) schedule. *Pharmacology Biochemistry and Behavior*, *130*, 67–76. http://doi.org/10.1016/j.pbb.2015.01.002

Cho, Y. H., & Jeantet, Y. (2010). Differential involvement of prefrontal cortex, striatum, and hippocampus in DRL performance in mice. *Neurobiology of Learning and Memory*, *93*(1), 85–91.

Cousins, M. S., & Seiden, L. S. (2000). The serotonin-1A receptor antagonist WAY-100635 modifies fluoxetine's antidepressant-like profile on the differential reinforcement of low rates 72-s schedule in rats. *Psychopharmacology*, *148*(4), 438-442.

Danion, J. M., Rein, W., Fleurot, O., & Amisulpride Study Group. (1999). Improvement of

schizophrenic patients with primary negative symptoms treated with amisulpride. *American Journal of Psychiatry*, *156*(4), 610-616.

Dekeyne, A., Gobert, A., Auclair, A., Girardon, S., & Millan, M. J. (2002). Differential modulation of efficiency in a food-rewarded" differential reinforcement of low-rate" 72-s schedule in rats by norepinephrine and serotonin reuptake inhibitors. *Psychopharmacology*, *162*(2), 156.

Domino, E. F. (2010). Taming the ketamine tiger. *The Journal of the American Society of Anesthesiologists*, *113*(3), 678-684.

Donahue, T. J., Hillhouse, T. M., Webster, K. A., Young, R., De Oliveira, E. O., & Porter, J. H. (2014). (S)-amisulpride as a discriminative stimulus in C57BL/6 mice and its comparison to the stimulus effects of typical and atypical antipsychotics. *European journal of pharmacology*, *734*, 15-22.

Drossopoulou, G., Antoniou, K., Kitraki, E., Papathanasiou, G., Papalexi, E., Dalla, C., & Papadopoulou-Daifoti, Z. (2004). Sex differences in behavioral, neurochemical and neuroendocrine effects induced by the forced swim test in rats. *Neuroscience*, *126*(4), 849-857.

Ebert, B., Mikkelsen, S., Thorkildsen, C., & Borgbjerg, F. M. (1997). Norketamine, the main metabolite of ketamine, is a non-competitive NMDA receptor antagonist in the rat cortex and spinal cord. *European journal of pharmacology*, *333*(1), 99-104.

Elhwuegi, A. S. (2004). Central monoamines and their role in major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *28*(3), 435-451.

Fava, M. (2003). Diagnosis and definition of treatment-resistant depression. *Biological psychiatry*, *53*(8), 649-659.

Fava, M., & Kendler, K. S. (2000). Major depressive disorder. *Neuron*, *28*(2), 335-341.

Fostick, L., Silberman, A., Beckman, M., Spivak, B., & Amital, D. (2010). The economic impact of depression: resistance or severity? *European Neuropsychopharmacology*, *20*(10), 671-675.

Fowler, S. C., Pinkston, J., & Vorontsova, E. (2009). Timing and space usage are disrupted by amphetamine in rats maintained on DRL 24-s and DRL 72-s schedules of reinforcement. *Psychopharmacology*, *204*(2), 213–225.

Garakani, A., Martinez, J. M., Yehuda, R., & Gorman, J. M. (2013). Cerebrospinal fluid levels of glutamate and corticotropin releasing hormone in major depression before and after treatment. *Journal of affective disorders*, *146*(2), 262-265.

Greenberg, P. E., Kessler, R. C., Birnbaum, H. G., Leong, S. A., Lowe, S. W., Berglund, P. A., & Corey-Lisle, P. K. (2003). The economic burden of depression in the United States: how did it change between 1990 and 2000?*Journal of clinical psychiatry*, *64*(12), 1465-1475.

Hamon, J., Paraire, J., & Velluz, J. (1952). Remarques sur l'action du 4560 RP sur l'agitation maniaque. *Ann Med Psychol (Paris)*, *110*, 331-5.

Hashimoto, K., Sawa, A., & Iyo, M. (2007). Increased levels of glutamate in brains from patients with mood disorders. *Biological psychiatry*, *62*(11), 1310-1316.

Hawkins, J., Hicks, R. A., Phillips, N., & Moore, J. D. (1978). Swimming rats and human depression. *Nature*, *274*(5670), 512-513.

Heninger, G. R., Delgado, P. L., & Charney, D. S. (1996). The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. *Pharmacopsychiatry*, *29*(01), 2-11.

Hillhouse, T. M., & Porter, J. H. (2014a). Ketamine, but not MK-801, produces antidepressant-like effects in rats responding on a differential-reinforcement-of-low-rate operant schedule. *Behavioural Pharmacology*, *25*(1), 80–91. http://doi.org/10.1097/FBP.0000000000000014

Hillhouse, T. M., Porter, J. H., & Negus, S. S. (2014b). Comparison of Antidepressant-Like and Abuse-Related Effects of Phencyclidine in Rats. *Drug Development Research*, *75*(8), 479–488. Hillhouse, T. M., Shankland, Z., Matazel, K. S., Keiser, A. A., & Prus, A. J. (2014c). The Quetiapine Active Metabolite N -Desalkylquetiapine and the Neurotensin NTS 1 Receptor Agonist PD149163 Exhibit Antidepressant-Like Effects on Operant Responding in Male Rats, *22*(6), 548–556.

Hindmarch, I. (2002). Beyond the monoamine hypothesis: mechanisms, molecules and methods. *European Psychiatry*, *17*, 294-299.

Hippius H. 2004. From chlorpromazine to modern ("atypical") antipsychotics. The International Journal of Neuropsychopharmacology, 7 (supplement 1):21–2.

Hirschfeld, R. M. (2000). History and evolution of the monoamine hypothesis of depression. *The Journal of clinical psychiatry*, *61*(suppl 6), 4-6.

Hustveit, O., Maurset, A., & Øye, I. (1995). Interaction of the chiral forms of ketamine with opioid, phencyclidine, σ and muscarinic receptors. *Basic & Clinical Pharmacology & Toxicology*, *77*(6), 355-359.

Idvall, J., Ahlgren, I., Aronsen, K. F., & Stenberg, P. (1979). Ketamine infusions: pharmacokinetics and clinical effects. *British Journal of Anaesthesia*, *51*(12), 1167-1173.

Jayarajan, P., Nirogi, R., & Shinde, A. (2013). Effect of olanzapine on scopolamine induced deficits in differential reinforcement of low rate 72 s (DRL-72 s) schedule in rats: Involvement of the serotonergic receptors in restoring the deficits. *European Journal of Pharmacology*, *720*(1-3), 344–354.

Kapur, S., & Seeman, P. (2002). NMDA receptor antagonists ketamine and PCP have direct effects on the dopamine D 2 and serotonin 5-HT 2 receptors—implications for models of schizophrenia. *Molecular psychiatry*, *7*(8), 837-844.

Kessler, R. C. (2012). The costs of depression. *The psychiatric Clinics of north America*, *35*(1), 1.
Kew, J. N., & Kemp, J. A. (2005). Ionotropic and metabotropic glutamate receptor structure and pharmacology. *Psychopharmacology*, *179*(1), 4-29.

Kim, Y. C., Kim, S. R., Markelonis, G. J., & Oh, T. H. (1998). Ginsenosides Rb 1 and Rg 3 protect cultured rat cortical cells from glutamate-induced neurodegeneration. *Journal of neuroscience research*, *53*(4), 426-432.

Klepstad, P., Maurset, A., Moberg, E. R., & Øye, I. (1990). Evidence of a role for NMDA receptors in pain perception. *European journal of pharmacology*, *187*(3), 513-518.

Krupitsky, E., Burakov, A., Romanova, T., Dunaevsky, I., Strassman, R., & Grinenko, A. (2002). Ketamine psychotherapy for heroin addiction: immediate effects and two-year follow-up. *Journal of substance abuse treatment*, *23*(4), 273-283.

Küçükibrahimoğlu, E., Saygın, M. Z., Çalışkan, M., Kaplan, O. K., Ünsal, C., & Gören, M. Z. (2009). The change in plasma GABA, glutamine and glutamate levels in fluoxetine-or S-citalopram-treated female patients with major depression. *European journal of clinical pharmacology*, *65*(6), 571-577.

Lahti, A. C., Koffel, B., LaPorte, D., & Tamminga, C. A. (1995). Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology*, *13*(1), 9-19.

Lan, M. J., McLoughlin, G. A., Griffin, J. L., Tsang, T. M., Huang, J. T. J., Yuan, P., ... & Bahn, S. (2009). Metabonomic analysis identifies molecular changes associated with the pathophysiology and drug treatment of bipolar disorder. *Molecular psychiatry*, *14*(3), 269-279.

Lapidus, K. A., Levitch, C. F., Perez, A. M., Brallier, J. W., Parides, M. K., Soleimani, L., ... & Murrough, J. W. (2014). A randomized controlled trial of intranasal ketamine in major depressive disorder. *Biological psychiatry*, *76*(12), 970-976.

Lehmann HE, Hanrahan GE. 1954. Chlorpromazine, new inhibiting agent for psychomotor exctietemnt and manic states. Archives of Neurology and Psychiatry (Chicago), 71:227–37.

Liu, X., & Gershenfeld, H. K. (2001). Genetic differences in the tail-suspension test and its relationship to imipramine response among 11 inbred strains of mice. *Biological psychiatry*, *49*(7), 575-581.

Lord, B., Wintmolders, C., Langlois, X., Nguyen, L., Lovenberg, T., & Bonaventure, P. (2013). Comparison of the ex vivo receptor occupancy profile of ketamine to several NMDA receptor antagonists in mouse hippocampus. *European journal of pharmacology*, *715*(1), 21-25.

Maes, M., Verkerk, R., Vandoolaeghe, E., Lin, A., & Scharpe, S. (1998). Serum levels of excitatory amino acids, serine, glycine, histidine, threonine, taurine, alanine and arginine in treatment- resistant depression: modulation by treatment with antidepressants and prediction of clinical responsivity. *Acta Psychiatrica Scandinavica*, *97*(4), 302-308.

Marek, G. J. (2012). Activation of adenosine$_1$ receptors induces antidepressant-like, anti-impulsive effects on differential reinforcement of low-rate 72-s behavior in rats. *The Journal of Pharmacology and Experimental Therapeutics*, *341*(2), 564–70. http://doi.org/10.1124/jpet.112.191718

Marek, G. J., Martin-Ruiz, R., Abo, A., & Artigas, F. (2005). The selective 5-HT2A receptor antagonist M100907 enhances antidepressant-like behavioral effects of the SSRI fluoxetine. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *30*(12), 2205–2215.

Marek, G. J., & Seiden, L. S. (1988). Effects of selective 5-hydroxytryptamine-2 and nonselective 5-hydroxytryptamine antagonists on the differential-reinforcement-of-low-rate 72-second schedule. *Journal of Pharmacology and Experimental Therapeutics*, *244*(2), 650-658.

Marek, G. J., Day, M., & Hudzik, T. J. (2016). The utility of impulsive bias and altered decision making as predictors of drug efficacy and target selection: rethinking behavioral screening for

antidepressant drugs. *Journal of Pharmacology and Experimental Therapeutics*, *356*(3), 534-548.

Marsden, W. N. (2013). Synaptic plasticity in depression: molecular, cellular and functional correlates. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *43*, 168-184.

Mcguire, P. S., & Seiden, L. S. (1980). Differential effects of imipramine in rats as a function of DRL schedule value. *Pharmacol Biochem Behav*, *13*, 691–694.

Martin, J. R., Bös, M., Jenck, F., Moreau, J. L., Mutel, V., Sleight, A. J., ... & Ruigt, G. S. F. (1998). 5-HT2C receptor agonists: pharmacological characteristics and therapeutic potential. *Journal of Pharmacology and Experimental Therapeutics*, *286*(2), 913-924.

Meyer, J. H., Ginovart, N., Boovariwala, A., Sagrati, S., Hussey, D., Garcia, A., ... & Houle, S. (2006). Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. *Archives of general psychiatry*, *63*(11), 1209-1216.

Millan, M. J. (2004). The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. *European journal of pharmacology*, *500*(1), 371-384.

Mion, G., & Villevieille, T. (2013). Ketamine pharmacology: an update (pharmacodynamics and molecular aspects, recent findings). *CNS neuroscience & therapeutics*, *19*(6), 370-380.

Mitani, H., Shirayama, Y., Yamada, T., Maeda, K., Ashby, C. R., & Kawahara, R. (2006). Correlation between plasma levels of glutamate, alanine and serine with severity of depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *30*(6), 1155-1158.

Moghaddam, B., Adams, B., Verma, A., & Daly, D. (1997). Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *The Journal of neuroscience*, *17*(8), 2921-2927.

Möller, H. J. (2003). Amisulpride: limbic specificity and the mechanism of antipsychotic atypicality. Prog Neuropsychopharmacol Biol Psychiatry, 27(7), 1101-1111.

Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J., & Monteggia, L. M. (2002). Neurobiology of depression. *Neuron*, *34*(1), 13-25.

Nikiforuk, A., Popik, P., & Drescher, K. (2010). Effects of a positive allosteric modulator of group II metabotropic glutamate receptors, LY487379, on cognitive flexibility and impulsive-

like responding in rats. *of Pharmacology and*, *335*(3), 665–673.

Nishimura, M., Sato, K., Okada, T., Yoshiya, I., Schloss, P., Shimada, S., & Tohyama, M. (1998). Ketamine inhibits monoamine transporters expressed in human embryonic kidney 293 cells. *Anesthesiology: The Journal of the American Society of Anesthesiologists*, *88*(3), 768-774.

Orrego, F & Villanueva, S. (1993). The chemical nature of the main central excitatory transmitter: a critical appraisal based upon release studies and synaptic vesicle localization. *Neuroscience*, *56*(3), 539-555.

O'Donnell, J. M., Marek, G. J., & Seiden, L. S. (2005). Antidepressant effects assessed using behavior maintained under a differential-reinforcement-of-low-rate (DRL) operant schedule. *Neuroscience and Biobehavioral Reviews*, *29*(4-5), 785–798.

O'Donnell, J. M., & Seiden, L. S. (1983). Differential-reinforcement-of-low-rate 72-second schedule: selective effects of antidepressant drugs. *Journal of Pharmacology and Experimental Therapeutics*, *224*(1), 80-88.

Pawar, G. R., Agrawal, R. P., Phadnis, P., Paliwal, A., Vyas, S., & Solanki, P. (2009). Evaluation of antidepressant like property of amisulpride per se and its comparison with fluoxetine and olanzapine using forced swimming test in albino mice. *Acta Poloniae Pharmaceutica - Drug Research*, *66*(3), 327–331.

Pekoe, G. M., & Smith, D. J. (1982). The involvement of opiate and monoaminergic neuronal systems in the analgesic effects of ketamine. *Pain*, *12*(1), 57-73.

Perrault, G. H., Depoortere, R., Morel, E., Sanger, D. J., & Scatton, B. (1997). Psychopharmacological profile of amisulpride: an antipsychotic drug with presynaptic D2/D3 dopamine receptor antagonist activity and limbic selectivity. *Journal of Pharmacology and Experimental Therapeutics*, *280*(1), 73-82.

Petit-Demouliere, B., Chenu, F., & Bourin, M. (2005). Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology*, *177*(3), 245-255.

Peuskens, J., Möller, H. J., & Puech, A. (2002). Amisulpride improves depressive symptoms in acute exacerbations of schizophrenia: comparison with haloperidol and risperidone. *European Neuropsychopharmacology*, *12*(4), 305-310.

Porsolt, R. D., Le Pichon, M., & Jalfre, M. L. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature*, *266*(5604), 730-732.

Rocca, P., Fonzo, V., Ravizza, L., Rocca, G., Scotta, M., Zanalda, E., & Bogetto, F. (2002). A comparison of paroxetine and amisulpride in the treatment of dysthymic disorder. *Journal of affective disorders*, *70*(3), 313-317.

Richards, J. B., & Seiden, L. S. (1991). A quantitative interresponse-time analysis of DRL performance differentiates similar effects of the antidepressant desipramine and the novel anxiolytic gepirone. *Journal of the experimental analysis of behavior*, *56*(2), 173-192.

Rosenzweig, P., Canal, M., Patat, A., Bergougnan, L., Zieleniuk, I., & Bianchetti, G. (2002). A review of the pharmacokinetics, tolerability and pharmacodynamics of amisulpride in healthy volunteers. *Human Psychopharmacology: Clinical and Experimental*, *17*(1), 1-13.

Sackeim, H. A. (2001). The definition and meaning of treatment-resistant depression. *Journal of Clinical Psychiatry*, *62*, 10-17.

Sanacora, G., Treccani, G., & Popoli, M. (2012). Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology*, *62*(1), 63-77.

Sanger, D. J., Perrault, G., Schoemaker, H., & Scatton, B. (1999). Pharmacological findings with amisulpride. Psychopharmakotherapie, 6 (Suppl. 9), 5-8.

Schoemaker, H., Claustre, Y., Fage, D., Rouquier, L., Chergui, K., Curet, O., ... & Scatton, B. (1997). Neurochemical characteristics of amisulpride, an atypical dopamine D2/D3 receptor antagonist with both presynaptic and limbic selectivity. *Journal of Pharmacology and Experimental Therapeutics*, *280*(1), 83-97.

Seligman, M. E. (1972). Learned helplessness. *Annual review of medicine*, *23*(1), 407-412.

Shore, P. A., Silver, S. L., & Brodie, B. B. (1955). Interaction of reserpine, serotonin, and lysergic acid diethylamide in brain. *Science*, *122*(3163), 284-285.

Shore, P. A., Pletscher, A., Tomich, E. G., Carlsson, A., Kuntzman, R., & Brodie, B. B. (1957). Role of brain serotonin in reserpine action. *Annals of the New York Academy of Sciences*, *66*(3), 609-617.

Slattery, D. A., & Cryan, J. F. (2012). Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nature protocols*, *7*(6), 1009-1014.

Sokolowski, J. D., & Seiden, L. S. (1999). The behavioral effects of sertraline, fluoxetine, and paroxetine differ on the differential-reinforcement-of-low-rate 72-second operant schedule in the rat. *Psychopharmacology*, *147*(2), 153-161.

Smith, D. J., Bouchal, R. L., DeSanctis, C. A., Monroe, P. J., Amedro, J. B., Perrotti, J. M., & Crisp, T. (1987). Properties of the interaction between ketamine and opiate binding sites in vivo and in vitro. *Neuropharmacology*, *26*(9), 1253-1260.

Steru, L., Chermat, R., Thierry, B., & Simon, P. (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology*, *85*(3), 367-370.

Trullas, R., & Skolnick, P. (1990). Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *European journal of pharmacology*, *185*(1), 1-10.

Wells, D. G., & Bjorksten, A. R. (1989). Monoamine oxidase inhibitors revisited. *Canadian journal of anaesthesia*, *36*(1), 64-74.

West, E. D., & Dally, P. J. (1959). Effects of iproniazid in depressive syndromes. *British Medical Journal*, *1*(5136), 1491.

White, P. F., Schüttler, J., Shafer, A., Stanski, D. R., Horai, Y., & Trevor, A. J. (1985). Comparative pharmacology of the ketamine isomers Studies in volunteers. *British journal of anaesthesia*, *57*(2), 197-203.

Wong, E. H., Sonders, M. S., Amara, S. G., Tinholt, P. M., Piercey, M. F., Hoffmann, W. P., ... & Carfagna, N. (2000). Reboxetine: a pharmacologically potent, selective, and specific norepinephrine reuptake inhibitor. *Biological psychiatry*, *47*(9), 818-829.

World Health Organization. (2014). *Global status report on alcohol and health*. World Health Organization.

Yamakura, T., Mori, H., Masaki, H., Shimoji, K., & Mishina, M. (1993). Different sensitivities of NMDA receptor channel subtypes to non-competitive antagonists. *Neuroreport*, *4*(6), 687-690.
Yamakura, T., Sakimura, K., & Shimoji, K. (1999). Direct inhibition of the N-methyl-D-aspartate receptor channel by high concentrations of opioids. *The Journal of the American Society of Anesthesiologists*, *91*(4), 1053-1053.

Zhang, J. C., Li, S. X., & Hashimoto, K. (2014). R (−)-ketamine shows greater potency and longer lasting antidepressant effects than S (+)-ketamine. *Pharmacology Biochemistry and Behavior*, *116*, 137-141.

Douglas A. Smith

EDUCATION

BS Psychology *Honors*

Virginia Tech   2014

PUBLICATIONS

Smith DA, Blough BE, Banks ML (2017). Cocaine-like discriminative stimulus effects of amphetamine, cathinone, methamphetamine, and their 3,4-methylenedioxy analogs in male rhesus monkeys.*Psychopharmacology*, 234(1), 117-127.

Smith DA, Negus SS, Poklis JL, Blough BE, Banks ML (2016). Cocaine-like discriminative stimulus effects of alpha-pyrrolidinovalerophenone, methcathinone, and their 3,4-methylenedioxy or 4-methyl analogs in rhesus monkeys. *Addiction Biology*. doi: 10.1111/adb.12399

Banks ML, **Smith DA**, Blough BE (2016). Methamphetamine-like discriminative stimulus effects of bupropion and its two hydroxy metabolites in male rhesus monkeys. *Behavioural Pharmacology*, *27*(2 and 3-Special Issue), 196-203.

Banks, ML, **Smith, DA**, Kisor, DF, Poklis, JL (2016). Relationship between discriminative stimulus effects and plasma methamphetamine and amphetamine levels of intramuscular methamphetamine in male rhesus monkeys. *Pharmacology Biochemistry and Behavior*, *141*, 58-65.

Fantegrossi WE, Gray BW, Bailey JM, **Smith DA**, Hansen M, Kristensen JL (2015). Hallucinogen-like effects of 2-([2-(4-cyano-2, 5-dimethoxyphenyl) ethylamino] methyl) phenol (25CN-NBOH), a novel N-benzylphenethylamine with 100-fold selectivity for 5-HT2A receptors, in mice. *Psychopharmacology*, 232(6), 1039-1047.

Smith DA, Bailey JM, Williams D, Fantegrossi WE (2014). Tolerance and cross-tolerance to head twitch behavior elicited by phenethylamine- and tryptamine-derived hallucinogens in mice. *Journal of Pharmacology and Experimental Therapeutics*, 351(3), 485-491.