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Dissociable antidepressant-like and abuse-related effects of the noncompetitive NMDA receptor antagonists ketamine and MK-801 in rats.

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DISSOCIABLE ANTIDEPRESSANT-LIKE AND ABUSE-RELATED EFFECTS OF THE NONCOMPETITIVE NMDA RECEPTOR ANTAGONISTS KETAMINE AND MK-801 IN RATS

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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Abstract

DISSOCIABLE ANTIDEPRESSANT-LIKE AND ABUSE-RELATED EFFECTS OF THE NONCOMPETITIVE NMDA RECEPTOR ANTAGONISTS KETAMINE AND MK-801 IN RATS

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The noncompetitive NMDA receptor antagonist ketamine produces rapid and sustained antidepressant effects in patients suffering from major depressive disorder. However, abuse liability is a concern. To further evaluate the relationship between antidepressant-like and abuse-related effects of NMDA receptor antagonists, this study evaluated the effects of ketamine, MK-801, and phencyclidine in male Sprague-Dawley rats responding under two procedures that have been used to assess antidepressant-like effects [differential-reinforcement-of-low-rate (DRL) 72 s schedule of food reinforcement] and abuse-related drug effects [intracranial self-stimulation (ICSS)]. Under DRL 72 s, ketamine produced an antidepressant-like effect by increasing reinforcers, decreasing responses, and producing a rightward shift in the peak location of the interresponse time (IRT) distributions. Phencyclidine produced a modest antidepressant-like effect by increasing reinforcers and decreasing responses, but did not shift the IRT distributions. In contrast, MK-801 produced a psychostimulant-like effect by decreasing reinforcers, increasing

responses, and producing a leftward shift in the peak location of the IRT distributions. The antidepressant-like effects of ketamine on the DRL 72 s procedure do not appear to be mediated by inhibiting the reuptake of serotonin via serotonin transporters or antagonism of 5-HT_2 receptors. Additionally, the dissociable effects of ketamine and MK-801 in the DRL 72 s procedure may be mediated by 5-HT_2 receptors. Following acute administration, ketamine produced only dose- and time-dependent depression of ICSS and failed to produce an abuse-related facilitation of ICSS at any dose or pretreatment time. Repeated dosing with ketamine produced dose-dependent tolerance to the rate-decreasing effects of ketamine but failed to unmask expression of ICSS facilitation. Termination of ketamine treatment failed to produce withdrawal-associated decreases in ICSS. In contrast, MK-801 and phencyclidine effects produced dose- and time-dependent facilitation of ICSS by MK-801. Taken together, our findings provide further evidence that expression of these antidepressant-like and abuse-related effects of ketamine, phencyclidine, and MK-801 may be related to NMDA receptor affinity.

Dissociable antidepressant-like and abuse-related effects of the noncompetitive NMDA receptor antagonists ketamine and MK-801 in rats

Major Depressive Disorder

Major depressive disorder (MDD) is the most common mood disorder in the United States with a lifetime and a 12-month prevalence of 14.4% and 7.1%, respectively (Kessler, Petukhova, Sampson, Zaslavsky & Wittchen, 2012). MDD is a chronic, recurring, and debilitating mental disorder that significantly impairs occupational and/or social functioning. Most individuals suffering from MDD have recurring depressive episodes (10.3%) rather than a single lifetime episode (4.1%) (Kessler et al., 2012). A patient is diagnosed with chronic depression when said patient continuously meets the DSM criteria for two or more years, and have fewer than eight weeks of remission during that time period (American Psychiatric Association, 2013). It is important to differentiate MDD from major depressive episode (MDE), which includes individuals with bipolar disorder. Because of the inclusion of bipolar disorder, MDE (16.6%) typically has higher prevalence rates compared to MDD (14.4%) (Kessler et al., 2012). MDD also has been found to have comorbidity with other DSM disorders such as anxiety disorder, substance abuse and impulse control disorder (Kessler et al., 2003).

Table 1 shows the diagnostic criteria for MDD. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM) 5th edition (American Psychiatric Association, 2013), an individual is required to exhibit a minimum of five depressive symptoms every day for a period of at least two weeks, which are newly presented or clearly worsened prior to the onset of the depressive episode, in order to be diagnosed with MDD (see table 1 for details). One of these five symptoms must include depressed mood (Criterion A1), which is often describe mood as being depressed or loss of interest or pleasure in hobbies or activities that were considered pleasurable (Criterion A2). In addition to one of these two symptoms, an individual must have

four other depressive symptoms which may include significant changes in appetite or weight (Criterion A3), sleep (Criterion A4), psychomotor activity (Criterion A5); loss of energy or fatigue (Criterion A6); feelings of worthlessness (Criterion A7); diminished ability to think or concentrate (Criterion A8); or suicidal ideation (Criterion A9). All of these symptoms, with the exception weight lose/gain and suicidal ideation, need to be present every day for the two week period to meet the DSM-V criterion for MDD. Furthermore, depressive episodes must significantly impair social or occupational functioning (Criterion B). Lastly, episodes must not be attributed to substance abuse (Criterion C) or better explained by other psychological disorders (Criterion D and E) such as schizophrenia, bipolar, etc.

Depressive episodes may appear at any age; however, MDD is most prevalent in adults (18-64 years) with a median age of onset in the 20s. For example, adults are twice as likely to be diagnosed with MDD compared to both adolescents (13-17 years) and older adults (65+ years) (Kessler et al., 2003; Kessler et al., 2012). This decline of diagnosis in older adults may be attributed to an increase in diagnosis in adults, failure to report previous episodes, memory loss of past episodes, or sampling bias. Females are two-to-three times more likely to be diagnosed with MDD compared to their male counterparts regardless of age group (Kessler et al., 2012).

MDD has significant economic and social consequences, which are driven by prevalence, medical treatment, and employment rates. For example, 36.7% of individuals suffering from MDD are either unemployed or out of labor force and only 59.8% of the MDD population in the workforce is receiving medical treatment for MDD (Greenberg et al., 2003). In 2000, the total economic impact of MDD was $83.1 billion for the United States. The total direct medical treatment costs (which include inpatient, outpatient, and pharmaceutical costs) reached $26.1

Table 1.

Diagnostic criteria for major depressive disorder

Diagnostic Criteria for Major Depressive Disorder	
A.	Five (or more) of the following symptoms that have been present during the same two week period and represent a change from previous function. Must include at least one of the following main symptoms: (1) Depressed mood most of the day, nearly every day, which is indicated by either subjective reports or observations made by others. (2) Diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day. Must include at least four of the following symptoms: (3) Significant weight loss when not dieting or weight gain, or decrease or increase in appetite nearly every day. (4) Insomnia or hypersomnia nearly every day (5) Psychomotor agitation or retardation nearly every day (observable by others). (6) Fatigue or loss of energy nearly every day. (7) Feelings of worthlessness or excessive or inappropriate guilt, nearly every day. (8) Diminished ability to think or concentrate nearly every day (subjective or observable by others). (9) Recurrent thoughts of death, suicidal ideation, or suicide attempts.
B.	These symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.
C.	The episode is not attributable to the physiological effects of substance or to another medical condition.
D.	The occurrence of the major depressive episodes is not better explained by other disorders such as schizoaffective disorder, schizophrenia, delusional disorder, etc.
E.	There has never been a manic episode or hypomanic episode.

Adapted from *Diagnostic and statistical manual of mental disorders* (5th ed.) (American Psychiatric Association, 2013)

billion, and suicide-related costs (i.e. estimated lost lifetime earnings) exceeded $5.4 billion. Interestingly, workplace-related costs (e.g. missed worked days, reduced productivity, and health care expenses) were $51.5 billion (Greenberg et al., 2003). Individuals with severe MDD cost their employers approximately double in health care expenses, are more likely to file for disability and/or be unemployed, and missed approximately 13.7 MORE hours per month as compared to healthy individuals (Birnbaum et al., 2010).

Monoamine Hypothesis and Monoamine Based Pharmacological Treatments

It has been almost 50 years since the monoamine hypothesis of depression was articulated. The monoamine hypothesis proposes that patients with depression have depleted concentrations of serotonin, norepinephrine, and dopamine (Bunney & Davis, 1965; Delgado, 2000; Hirschfeld, 2000; Schildkraut, 1965). There are two primary lines of evidence that led to the development of this monoamine hypothesis: 1) the effects of reserpine on serotonin; and 2) the pharmacological mechanisms of action of antidepressant drugs. Reserpine, an alkaloid extracted from the *Rauwolfia serpentina*, was utilized as a treatment for hypertensive vascular disease in the 1950s; however, reserpine was found to precipitate depression in some patients. The depression produced by reserpine was reversed after the treatment was terminated, and following either rest or electric shock therapy (Muller, Pryor, Gibbons, & Orgain, 1955). Additionally, reserpine was found to produce depressive-like effects in animals (Hirschfeld, 2000). Reserpine was found to depleted brain serotonin (Shore, Pletscher, Tomich, Carlsson, Kuntzman, & Brodie, 1957; Shore, Silver, & Brodie, 1955), which provided evidence for the role of serotonin in depression. The second line of evidence centered on the underlying pharmacological mechanisms of action of antidepressant drugs. For example, antidepressant treatments primarily target the monoamine neurotransmitters (i.e. serotonin, norepinephrine, and

dopamine) in an attempt to enhance the presence of monoamine neurotransmitters in the synaptic space to activate postsynaptic receptors (see below for the mechanism of action for each antidepressant drug class).

Much like the discovery of the first antipsychotic drug chlorpromazine, the first pharmacological treatment for depression was discovered by serendipity. Chemists at Hoffmann-La Roche Ltd USA developed isonicotinyl hydrazide (isoniazid) for the treatment of tuberculosis. Isoniazid was a successful antitubercular compound as the mortality rate of tuberculosis significantly decreased after being on the market for only one year (Lopez-Munoz, Alamo, Juckel, & Assion, 2007; Pletscher, 1991). While developing new antitubercular compounds, Fox and Gibas (1953) synthesized isopropyl-isonicotinyl hydrazide (iproniazid), a monoalkyl derivative of isoniazid, which would later serve as a catalyst for pharmacological treatment of MDD. Clinical observations reported marked "side effects" of iproniazid in patients being treated for tuberculosis, which included euphoria, psychostimulation, increased appetite, and improved sleep. The "side effects" produced by iproniazid were not originally identified as therapeutic effects because these side effects were not consistent across studies (Lopez-Munoz et al., 2007; Pletscher, 1991). Loomer, Saunders, and Kline (1958) conducted a systematic clinical study on patients with depression, in which these patients were treated with iproniazid for several weeks. Loomer and colleagues reported significant improvements in 70% of these patients (Loomer et al., 1958). In 1958, iproniazid was marketed as an antitubercular compound under the trade name Marsilid®; however, iproniazid was used off-label to treat patients suffering from MDD.

Thus, iproniazid served as the first pharmacological treatment for depression and is classified as a monoamine oxidase (MAO) inhibitor. MAO is an enzyme that produces oxidative

disamination (or break down) of biogenic amines (e.g. serotonin, dopamine, epinephrine, and norepinephrine) and sympathomimetic amines (e.g. tyramine, benzylamine, etc). There are two isoenzymes, MAO_A and MAO_B, and the distribution of these isoenzymes varies throughout the body. MAO_A is primarily responsible for the enzyme activity in the peripheral tissue and for the deaminate of serotonin, melatonin, noradrenaline, and adrenaline (Billett, 2004; Shulman, Herrman, & Walker, 2013). In contrast, MAO_B is responsible for enzyme activity in the brain and for the breakdown of phenethylamine, and benzylamine (Campbell, Marangos, Parma, Garrick, & Murphy, 1982; Shulman et al., 2013). Interestingly, both isoenzymes deaminate dopamine, tyramine, and tryptamine. MAOs responsible for the breakdown of biogenic amines are located in the presynaptic terminal. A result of inhibiting MAO is that monoamine neurotransmitters concentrations increase in the presynaptic terminal and are readily available for release when action potential reaches the nerve terminal. Iproniazid is a non-selective irreversible MAO inhibitor, which led to safety concerns (e.g. hypertensive crises), and ultimately led to the removal of iproniazid from the US market. One example of the safety concern was termed the "cheese reaction". The combination of foods containing high amounts of tyramine, such as cheese or dairy products, and MAO inhibitors, which increase concentrations of tyramine and norepinephrine in the sympathetic nervous system, would lead to increased heart rate, hypertension and sweating. It was later determined that inhibiting the MAO_A was functionally involved in the antidepressant effects of MAO inhibitors (Lopez-Munoz et al., 2007; Shulman et al., 2013). In an attempt to improve the safety of MAO inhibitors, drug development focused on reversible and selective MAO_A inhibitors (e.g. brofaromine and moclobemide).

As a result of the discovery and success of chlorpromazine for the treatment of schizophrenia, the search for more potent antipsychotic drugs intensified. Many of these novel

compounds used the classic antihistamine structure, and were molecularly modified. One compound in particular, G22355 (imipramine), was derived from promethazine by substituting the sulfur bridge of the phenothiazine ring with an ethylene bridge, and shared the same side chain as chlorpromazine (Domino, 1999; Fangmann, Assion, Juckel, Gonzalez, & Lopez-Munoz, 2008). The Geigy Chemical Corp supplied imipramine, which was synthesized by Hafliger and Schinder, to Dr. Roland Kuhn to test in psychiatric patients. Although imipramine did not exhibit antipsychotic properties in patients with schizophrenia, Kuhn found that imipramine produced marked improvements in patients suffering from severe depression. In an essay written by Kuhn (1958), he states "They commence some activity of their own, again seeking contact with other people, they begin to entertain themselves, take part in the games, become more cheerful and are once again able to laugh… The patients express themselves as feeling much better, fatigue disappears, the feeling of heaviness in the limbs vanish, and the sense of oppression in the chest gives way to a feeling of relief" (p. 459). Kuhn also stated that no serious side effects were recorded in the 500 patients treated with imipramine, which was a vast improvement over MAO inhibitors.

Imipramine was approved in 1959 by the Food and Drug Administration (FDA) for the treatment of MDD, which established the class of drugs called tricyclic antidepressants. Tricyclic antidepressants are classified based on the three benzene ring molecular core, in part, because the mechanism of action was unknown at the time of discovery. Thus, the classification of tricyclic antidepressant differs that of other classes of antidepressant drugs, which are classified based on their mechanism of action. Tricyclic antidepressants have a diverse pharmacological profile with significant pharmacological action at five transporter/receptor proteins: inhibiting presynaptic norepinephrine reuptake transporters; inhibiting presynaptic serotonin reuptake transporters;

blocking postsynaptic adrenergic α_1 and α_2 receptors; blocking postsynaptic muscarinic receptors; and blocking postsynaptic histamine H_1 receptors (Cusack, Nelson, & Richelson, 1994; Owens, Morgan, Plott, & Nemeroff, 1997; Sánchez & Hyttel, 1999: Vaischnavi et al., 2004). The inhibitions of norepinephrine and serotonin reuptake at the transport proteins are thought to be responsible for the therapeutic effects of tricyclic antidepressants, and result in increased concentrations of norepinephrine and serotonin in the synaptic cleft, respectively. The selectivity for norepinephrine or serotonin transporters depends on the compounds; however, most tricyclic antidepressants are more selective for the norepinephrine transporter over the serotonin transporter (Owens et al., 1997; Sánchez & Hyttel, 1999; Thomas, Nelson, & Johnson, 1987). The antagonism of histaminergic, adrenergic, and muscarinic receptors contribute primarily to the side effects (e.g. drowsiness, memory impairments, and dizziness, respectively) of tricyclic antidepressants.

In the late 1960s evidence began to emerge suggesting a significant role of serotonin in MDD. For example, a postmortem study revealed decreased concentrations of serotonin in depressive suicides (Shaw, Eccleston, & Camps, 1967). As a result, the pharmaceutical company Eli Lilly began developing ligands that would selectively inhibit the reuptake of serotonin at the serotonin transporter and as a result would increase serotonin concentrations within the synaptic cleft to further stimulate postsynaptic serotonin receptors. In 1974, the first report on the selective serotonin reuptake inhibitor (SSRI) fluoxetine was published and in that publication the authors suggested that fluoxetine would be an antidepressant drug (Wong, Bymaster, & Engleman, 1995; Wong, Perry, & Bymaster, 2005). The following year Wong and colleagues demonstrated that fluoxetine was a potent and selective serotonin reuptake inhibitor with relatively weak affinity for the norepinephrine transporter (Wong, Bymaster, Horng, & Molloy,

1975). Fluoxetine was approved by the FDA in December of 1987 and was launched to the market in January 1988 under the trade name Prozac®. Since the introduction of fluoxetine to the market, several other SSRIs have been approved by the FDA (e.g. sertraline, citalopram, and paroxetine).

SSRIs are 20-1500 fold more selective for inhibiting serotonin over norepinephrine at their respective transporter proteins and have no significant (or weak) binding affinity for other receptors such adrenergic α_1, α_2, and β, histamine H_1, muscarinic, or dopamine D_2 receptors (Owens et al., 1997; Thomas et al., 1987). SSRIs (e.g. fluoxetine and citalopram) do not stimulate the release of serotonin or norepinephrine (Rothman et al., 2001) and have weak or no pharmacological action at postsynaptic serotonin receptors (e.g. 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2c}) (Owens et al., 1997; Sánchez & Hyttel, 1999; Thomas et al., 1987). Therefore, the increase in activity at the postsynaptic serotonin receptors produced by SSRIs is a result of increased concentrations of serotonin in the synaptic cleft via reuptake inhibition rather than direct binding at the postsynaptic receptor. The most common side effects associated with SSRIs are nausea, insomnia, and sexual dysfunction (Papakostas, 2008).

Several "atypical" antidepressant drugs have been developed in the past couple of decades; however, for the most part, the mechanisms of action for these drugs are similar to established antidepressant drugs. For example, serotonin-norepinephrine reuptake inhibitors (SNRI) are similar to tricyclic antidepressants in that SNRIs inhibit the reuptake of serotonin and norepinephrine at the serotonin and norepinephrine transporters, respectively. Unlike tricyclic antidepressants, SNRIs (e.g. duloxetine, venlafaxine, and milnacipran) do not have significant pharmacological action at adrenergic α_1, α_2, and β, histamine H_1, muscarinic, dopamine, or postsynaptic serotonin receptors (Bymaster et al., 2001; Millan et al., 2001; Owens et al., 1997;

Sánchez & Hyttel, 1999; Vaischnavi et al., 2004). While SNRIs have greater receptor selectivity as compared to tricyclic antidepressant drugs, the clinical efficacy and tolerability of SNRIs are similar to SSRIs (Stahl, Grady, Moret, & Briley, 2005).

Bupropion is an "atypical" antidepressant drugs with a unique binding profile as compared to other antidepressant drugs (e.g. tricyclic, SSRI, SNRI). For example, bupropion is primarily a dopamine reuptake inhibitor, although the binding affinity of bupropion for dopamine transporters is modest (Bymaster et al., 2002; Letchworth et al., 2000; Pristupa, Wilson, Hoffman, Kish, & Niznik, 1994). Bupropion is at least two-fold more selective for dopamine transporters as compared to norepinephrine and serotonin transporters and does not display significant binding affinity for other receptors (Bymaster et al., 2002; Cusack et al., 1994; Sánchez & Hyttel, 1999). A review of the clinical research shows that bupropion is at least as tolerable and efficacious as other antidepressant drugs (Moreira, 2011).

Nonpharmacological Treatments for Major Depressive Disorder

There are several nonpharmacological treatment therapies for MDD. For example, cognitive behavioral analysis system of psychotherapy (CBASP) and cognitive behavioral therapy (CBT) focus on resolving current problems and understanding the connection between behavior and consequences (McCullough, 2000). Both CBASP and CBT has been shown to significantly decrease scores on several depressive scales, such as Hamilton Rating Score for Depression and Beck Depression Inventory, with an efficacy similar to pharmacological antidepressant (Keller et al., 2000; Swan et al., 2014; Zu et al., 2014). A large scale clinical study compared the effects of pharmacological therapy (nefazodone, a potent serotonin 5-HT_{2A} receptor antagonist), CBASP, and the combination of nefazodone and CBASP over a 12-week study and found that all three groups showed a significant improvement according to the

Hamilton Rating Score for Depression-24 scale. However, the combined treatment group (nefazodone + CBASP) showed significantly greater improvement as compared to the nefazodone alone and CBASP alone groups. The combination treatment group achieved 42% remission; whereas, the nefazodone alone and CBASP alone group achieved 22% and 24% remission, respectively (Keller et al., 2000). However, the effectiveness of the CBT and antidepressant combination treatment have been mixed with some clinical studies showing increased efficacy (Ma, Zhang, Zhang, & Li, 2014) and other clinical studies failing to find an improvement over antidepressant treatment alone (Goodyer et al., 2007; Zu et al., 2014).

Treatment-Resistant Depression

Although there are several treatment options (both pharmacological and nonpharmacological) for MDD, 34-46% of MDD patients do not adequately response to treatment (Fava & Davidson, 1996). These patients are categorized as having treatment-resistant depression, which typically is defined as an inadequate response (i.e. fail to achieve full remission) to one or more antidepressant treatment following adequate duration and dose (Fava & Davidson, 1996; Fava, 2003). Treatment-resistant depression is well documented in the literature and is accepted as a subtype of depression; however, there is not a unified definition. For this dissertation, the definition above (i.e. full remission) will be used because the goal for treatment should be to return the patient to the premorbid state of social and occupational functioning. An additional serious issue for treatment of MDD is that even for the patients that do respond to current antidepressant drugs, there is a delayed onset of 4-12 weeks before adequate symptom remission is achieved (Schulberg, Katon, Simon, & Rush, 1998; Uher et al., 2011).

Although limited, some progress has been made in both preclinical and clinical research to address the issue of treatment-resistant depression. For example, preclinical animal models, such as chronic mild stress and social defeat model, have been developed in order to mimic treatment-resistant depression (Samuels, 2011). In clinical research, one group has developed a strategic plan for treatment-resistant depression called Sequenced Treatment Alternatives to Relieve Depression (STAR*D). STAR*D provides a four step treatment plan, in which a patient proceeds to the next treatment step if they do not achieve full remission under the current treatment step (Fava et al., 2003). SSRIs are the first treatment step and as patients progress through the treatment steps they will be introduced to new antidepressant drugs with different mechanism of actions (e.g. SNRIs, bupropion, and tricyclics). Patients that achieve full remission and tolerate treatment at a specific step are then placed on long term treatment with that drug. Although the STAR*D provides clinicians with guidelines and a strategic plan for treating treatment-resistant depression, the results from a large-scale long-term study showed that patients who progress through more treatment steps had higher relapse rates as compared to patients that achieved remission in the first treatment step (Rush et al., 2006).

Experimental approaches such as deep brain stimulation (Bewernick et al., 2010; Bewernick, Kayser, Sturm, & Schlaepfer, 2012; Cook, Espinoza, & Leuchter, 2014; Mayberg et al., 2005; Schlaepfer, Bewernick, Kayser, Mädler, & Coenen, 2013; Williams & Okun, 2013) and electroconvulsive therapy (Eschweiler et al., 2007; Kellner et al., 2012; Khalid et al., 2008), have shown promised as a treatment for treatment-resistant patients. Clinical research also has suggested that the use of atypical antipsychotic drugs as adjunctive treatments with antidepressant drugs may be effective for treatment-resistant depression (Chen, & Tzeng, 2013; Li, Xing, Yu, Chen, & Wu, 2013; Šagud, Mihaljević-Peleš, Mück-Šeler, Jakovljević, & Pivac,

2006; Tohen et al., 2010; Wright, Eiland, & Lorenz, 2013). More recently, research has focused on finding novel, non-monoaminergic based, receptor targets for treatment-resistant depression. In particular, the glutamatergic system has become a focal point for drug development research.

Glutamatergic Systems and Major Depressive Disorder

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS), and is present at over 60% of synapses. The fact that glutamate acts as a rapid excitatory transmitter, combined with its prevalence, suggests that glutamate may be involved in abnormal circuitries, and may facilitate or even cause disease symptoms. The glutamate system has an integrated tripartite synapse that consists of 1) a presynaptic neuron, 2) a postsynaptic neuron, and 3) an astrocyte. The presynaptic neuron releases glutamate in response to action potentials. The released glutamate then binds to various pre- and postsynaptic receptors, as well as receptors on the surrounding astrocytes. Synaptic glutamate reuptake is performed primarily by astrocytes, specifically, the excitatory amino acid transporter 2 (EAAT2). Within the astrocyte, glutamate is converted to glutamine (glutamate/glutamine cycle) via glutamate-ammonia ligase (glutamine synthetase) and then resupplied to the presynaptic neuron where it is used for synthesis of glutamate (Kew & Kemp, 2005; Machado-Vieira, Manji, & Zarate, 2009; Mathews, Henter, & Zarate, 2012).

Like many neurotransmitters, glutamate acts on two types of receptors, ionotropic and metabotropic. Ionotropic glutamatergic receptors include α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and N-methyl-$_D$-aspartate (NMDA) receptors. These receptors are ion channels that are permeable to cations and function by allowing sodium (Na^+) and calcium (Ca^{2+}) ions to enter the cell, thus causing depolarization and other intracellular changes (Kew & 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, mGluR6, mGluR7, and mGluR8) based upon their homology and function. These receptors are G-protein-coupled receptors (GPCRs) and function by either influencing intracellular second messenger formation, and/or releasing G protein, subunits which influence ion channel activity (Kew & Kemp, 2005; Pin & Duvoisin, 1995). Assessing all glutamate receptors and their respective implications in major depressive disorder would be beyond the scope of this dissertation. Therefore, this dissertation will primarily focus on NMDA receptors (for a review on the role of mGluR in pathophysiology and treatment of MDD please see Holly, LaCrosse, & Hillhouse, In Press).

NMDA receptors are a heteromeric complex that has seven subunits, NR1, NR2A-D, and NR3A-B and functional NMDA receptors must be comprised of a NR1 subunit and at least one NR2 subunit. Unlike other ligand-gated ion channels, NMDA receptors require two distinct mechanisms in order to be activated. First, 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 & Kemp, 2005; Marsden, 2011). Thus, if one of these co-agonists (glycine or glutamate) is not bound to their respective binding site, the ion channel will not open. Second, the NMDA receptor channels are blocked by magnesium (Mg^{2+}) ions during the resting state. Depolarization of the neuron is required to dispel the Mg^{2+} ion from 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^{2+} to enter. The influx of Ca^{2+} is associated with the induction of various signaling cascades (Kew & Kemp, 2005; Marsden, 2011).

The glutamatergic system has emerged as a potential target to improve clinical efficacy and onset of remission for the treatment of MDD. Both clinical and postmortem research has

provided evidence of glutamatergic dysfunction in patients diagnosed with MDD. For example, elevated concentrations of glutamate, glutamine, glycine, and serine have been found in patients with MDD using indirect measures such as plasma, serum, or cerebrospinal fluid (Altamura et al., 1993; Kim, Schmid-Burgk, Claus, & Kornhuber, 1982; Küçükibrahimoğlu et al., 2009; Levine et al., 2000; Mauri et al., 1998; Mitani et al., 2006; Sumiyoshi et al., 2004). Additionally, MDD patients have shown a greater sensitivity to glutamate as measured by intracellular calcium influx (Berk, Plein, & Ferreira, 2001). Furthermore, antidepressant drug treatment has been found to reduce the serum glutamate (Maes, Verkerk, Vandoolaeghe, Lin, & Scharpé, 1998), cerebrospinal fluid glutamine (Garakani, Martinez, Yehuda, & Gorman, 2013), and plasma glutamate and glutamine concentrations in MDD patients (Küçükibrahimoğlu et al., 2009). However, there is evidence that has shown decreased concentrations of glutamate and glycine in cerebrospinal fluid (Frye et al., 2006), as well as decreased concentrations of glutamine and glycine in blood plasma (Altamura et al., 1993; Altamura et al., 1995).

These indirect measures of glutamate in CSF, plasma, and serum are difficult to interpret, as these disruptions may be due to medication, metabolic effects, or the inability of these methods to distinguish between central and peripheral glutamate (see table 2). As such, proton magnetic resonance spectroscopy (^{1}H-MRS) has been used to more directly measure neurotransmitter concentration, specifically glutamate/glutamine (Glx), in adults with severe depression (see table 3). ^{1}H-MRS studies have shown reduced Glx in the anterior cingulate cortex (Auer et al., 2000; Pfleiderer et al., 2003), hippocampus (Block et al., 2009), left dorsolateral prefrontal cortex (Michael et al., 2003), dorsomedial and ventromedial prefrontal cortex (Hasler et al., 2007) of adult MDD patients. Moreover, a 19% reduction in glutamate and glutamine has been observed in adolescent patients with severe MDD (Mirza et al., 2004).

Following ECT treatment, the anterior cingulate cortex and left dorsolateral prefrontal cortex Glx concentrations were significantly increased and recovered back to health control Glx concentrations (Michael et al., 2003; Pfleiderer et al., 2003). However, one study has shown increased glutamate concentrations in the occipital cortex (Sanacora et al., 2004). Furthermore, Hashimoto and colleagues found elevated concentrations of glutamate in the frontal cortex of postmortem tissue from patients with MDD (Hashimoto, Sawa, & Iyo, 2007). Additionally, the high affinity glial glutamate transporter (EAAT2), the main source the glutamate reuptake, has been shown to be down-regulated in postmortem brain tissue in MDD patients (Bernard et al., 2001; Choudary et al., 2005).

In combination with significant alterations in glutamate and glutamine, concentrations of NMDA receptor subunit expression are altered in patients with MDD (see table 4). For example, the NR2A and NR2B subunits have been shown to be reduced in the perirhinal and prefrontal cortices in postmortem tissue from MDD patients (Beneyto, Kristiansen, Oni-Orisan, McCullumsmith, & Meador-Woodruff, 2007; Feyissa, Chandran, Stockmeier, & Karolewicz, 2009). Additionally, Nudmamud-Thanoi and Reynolds (2004) found decreased levels of the NR1 subunit in the superior temporal cortex. In contrast, the NR2A and NR2C subunits have been found to be elevated in the lateral amygdala (Karolewicz et al., 2009) and locus coeruleus (Karolewicz, Stockmeier, & Ordway, 2005), respectively.

Taken together, these results suggest a bi-directional glutamatergic dysfunction in MDD, with disruptions both in glutamate/glutamine cycling and glutamatergic receptor expression. Although the role of glutamatergic system has yet to be fully elucidated, a "proof of concept" clinical study reported that the noncompetitive the NMDA antagonist ketamine produces rapid and prolonged antidepressant effects in patients suffering from MDD (Berman et al., 2000).

Table 2.

Glutamatergic dysfunction in major depressive disorder using indirect measures for analysis

Interest	Region of Analysis	Change	Reference
Glutamate	Serum	Increased	Kim et al., 1982
		Decreased	Maes et al., 1998[a]
	Plasma	Increased	Altamura et al., 1993
			Mauri et al., 1998
			Mitani et al., 2006
			Küçükibrahimoğlu et al., 2009
	Cerebrospinal fluid	Decreased	Frye et al., 2007
	Platelet-rich plasma[c]	Supersensitivity to glutamate	Berk et al., 2001
Glutamine	Serum	Increased	Maes et al., 1998[a]
	Plasma	Decreased	Altamura et al., 1993
	Cerebrospinal fluid	Increased	Levine et al., 2000
		Decreased	Garakani et al., 2013[b]
		Decreased	Frye et al., 2007
Glycine	Plasma	Increased	Mitani et al., 2006
		Decreased	Altamura et al., 1995

[a] decrease after 5-week antidepressant; however, no difference prior to treatment
[b] decrease after 8-week antidepressant; however, no difference prior to treatment
[c] platelet intracellular calcium response to glutamate stimulation

Table 3.

Glutamatergic levels measured by proton magnetic resonance spectroscopy (^{1}H-MRS)

Interest	Region of Analysis	Change	Reference
Glutamate/ Glutamine (Glx)	Occipital cortex	Increased	Sanacora et al., 2004
	Anterior cingulate cortex	Decreased	Auer et al., 2000
			Pfleiderer et al., 2003[a]
	Hippocampus	Decreased	Block et al., 2009[b]
	Dorsolateral prefrontal cortex	Decreased	Michael et al., 2003[a]
	Dorsomedial and ventromedial prefrontal cortex	Decreased	Hasler et al., 2007

[a] Glx major depressive disorder levels recovered to healthy control following ECT treatment
[b] no change in Glx levels following 8-weeks of citalopram treatment

Table 4.

Alterations in NMDA receptor subunit expression

Receptor Subunit/site	**Region of Analysis**	**Molecular Assay**	**Change**	**Reference**
NR1	Superior temporal cortex	Western blot	Decreased	Nudmamud-Thanoi et al., 2004
	Prefrontal cortex	Western blot	No change	Feyissa et al., 2009
NR2A	Perirhinal cortex	In situ hybridization	Decreased	Beneyto et al., 2007
	Lateral amygdala	Western blot	Increased	Karolewicz et al., 2009
	Prefrontal cortex	Western blot	Decreased	Feyissa et al., 2009
NR2B	Perirhinal cortex	In situ hybridization	Decreased	Beneyto et al., 2007
	Prefrontal cortex	Western blot	Decreased	Feyissa et al., 2009
NR2C	Locus coeruleus	Western blot	Increased	Karolewicz et al., 2005
Glutamate Binding site	Hippocampus	Autoradiography	Decreased binding	Beneyto et al., 2007

Ketamine: Antidepressant and Drug of Abuse

Ketamine, a dissociative anesthetic with hallucinogen properties, is a derivative of phencyclidine and was developed in the 1960s by Dr. Calvin Lee Stevens of Wayne State University for the pharmaceutical company Parke-Davis. In 1964, ketamine was experimentally administered to human subjects to test the safety and general anesthetic properties of ketamine. The subjects reported low side effects and described feelings of floating or having no feeling in their limbs. Ketamine was approved by the FDA in 1970 for use as a short-acting anesthetic in humans and animals, and was given to injured American soldiers during the Vietnam War (Domino, 2010). In 1999, ketamine became a Schedule III non-narcotic substance under the Controlled Substances Act (Drug Enforcement Administration, 2013). Ketamine, which is distributed under the trade names Ketalar®, Ketaset®, Vetamine®, is still widely used in human and veterinary medicine with approximately 16,000 dispensed ketamine prescriptions in 2012 (Domino, 2010; Drug Enforcement Administration, 2013).

Ketamine is a noncompetitive NMDA receptor antagonist (aka channel blocker) that binds to the phencyclidine site inside the ion channel of the NMDA receptor, similarly to Mg^{2+}, and is non-selective for the NR2A-D subunits of the NMDA receptor channel (Lord et al., 2013; Yamakura, Mori, Masaki, Shimoji, & Mishina, 1993; Yamakura & Shimoji, 1999). While many have categorized ketamine as a high affinity NMDA receptor antagonists, *in vivo* autoradiography studies suggest that ketamine has relatively low affinity for the NMDA receptor (Ki = 659-1190 nM) (Bresink, Danysz, Parsons, & Mutschler, 1995; Roth et al., 2013). Additionally, selectivity of ketamine for the NMDA receptor appears to be weak compared to other receptors (see table 5). For example, ketamine has been shown to have higher receptor affinity for *in vitro* human cloned dopamine D_2 receptors (Ki = 55 nM) compared to NMDA

receptors (Seeman, Ko, & Tallerico, 2005); however, a follow up study found that ketamine did not exert functional activity (i.e. as a full agonist, partial agonist, or antagonists) at *in vitro* dopamine D_2 receptors (Jordan et al., 2006). Furthermore, ketamine is approximately 12 to 20-fold more selective for NMDA receptors as compared to serotonin 5-HT_{2A} and mu opioid receptors (Kapur & Seeman, 2002; Smith et al., 1987). Ketamine also has considerable binding affinity for sigma (Mendelsohn, Kalra, Johnson, & Kerchner, 1985), muscarinic, and opioid (κ and δ) receptors (Hirota, Hashimoto, & Lambert, 2002), as well as dopamine, norepinephrine, and serotonin transporters (Nishimura et al., 1998). Moreover, several behavioral procedures, such as forced swim test, drug discrimination, novelty-suppressed feeding, tail suspension and hypothermia, have shown the involvement of serotonin mechanisms in the behavioral effects of ketamine (Fahim, Ismail, & Osman, 1973; Fukumoto, Iijima, & Chaki, 2014; Gigliucci et al., 2013; Kos et al., 2006; Yoshizawa et al., 2013)

Ketamine, also known as "Special K," became popular as a recreational drug in the mid-1990s. Although the illicit used of ketamine appears to be relatively low in the United States with less than 2% of the population using hallucinogens (Substance Abuse and Mental Health Services Administration, 2013), which also include lysergic acid diethylamide, phencyclidine, peyote, mescaline, psilocybin mushrooms, and "ecstasy," the popularity of ketamine continues to grow in other countries. For example, ketamine (23.2%) is the second most abused substance in Hong Kong with heroin (61.7%) being the most abused substance (Shek, 2007). The use of ketamine has been on a rise over the past decade, specifically among "dance" or "club" drug users in Europe, which may be in part to the decline of Lysergic acid diethylamide usage (McCambridge, Winstock, Hunt, & Mitcheson, 2007, 2007; Shek, 2007; Winstock, Mitcheson, Gillatt, & Cottrell, 2012). At subanesthetic or emergence from anesthetic doses, ketamine

Table 5.

Receptor binding affinities for ketamine.

N/A, not applicable; NMDA, N-methyl-D-aspartate; PCP, phencyclidine.

Receptor/region	**Ketamine K_i (μM)**	**Citation**
NMDA (PCP site)		
Cortex	1.190 ± 0.237	Bresink et al, 1995
Cerebellum	2.507 ± 1.900	Bresink et al, 1995
Striatum	3.100 ± 0.300	Seeman et al, 2005
3-D model	0.420	Tikhonva et al, 2004
Dopamine D_2	0.055 ± 0.012[a]	Seeman et al, 2005
Norepinephrine Transporter (NET)	66.8 ± 25.9[b]	Nishimura et al, 1998
Dopamine Transporter (DAT)	62.9 ± 2.3[c]	Nishimura et al, 1998
Serotonin Transporter (SERT)	161.7 ± 28.3[d]	Nishimura et al, 1998
Serotonin		
5-HT_2	15.0 ± 5.0	Kapur et al, 2002
Opiate receptor		
Mu (μ)	26.8 ± 2.7	Smith et al, 1987
Delta (δ)	101 ± 9.0	Smith et al, 1987
Kappa (κ)	85.2 ± 26.0	Smith et al, 1987
Sigma (σ)	66.0 ± 10.0	Smith et al, 1987
Muscarinic		
M_1	45.0[e] (SEM n/a)	Hirota et al., 2002

[a] Chinese hamster ovary (CHO) cells (human cloned dopamine D2long receptors).
[b] Transfected human embryonic kidney (HEK 293) cells (human NET).
[c] Transfected human embryonic kidney (HEK 293) cells (rat DAT).
[d] Transfected human embryonic kidney (HEK 293) cells (rat SERT).
[e] Chinese hamster ovary (CHO) cells (human cloned Muscarinic M1 receptors).

produces hallucinations (i.e. distorts perceptions of sight and sounds), mood and body image changes, and make the user feel disconnected (or dissociated) from their body/reality. The hallucinogenic effects of ketamine have been termed the "K-hole" by users, and the duration of these effects are relatively short, 30 to 60 min, compared to phencyclidine (Drug Enforcement Administration, 2013).

In 2000, the noncompetitive NMDA receptor antagonist ketamine was first used in a "proof of concept" randomized, double-blinded study to assess the effects of ketamine on MDD patients. A single, subanesthetic dose (0.5 mg/kg) of ketamine was intravenously infused over 40 minutes, and the antidepressant effects of ketamine were assessed using the Hamilton Depression Rating Scale and Beck Depression Inventor. In comparison, an anesthetic dose for ketamine in humans ranges from 1.0 mg/kg to 4.5 mg/kg intravenous and from 6.5 mg/kg to 13.0 mg/kg intramuscular. In this study, ketamine produced rapid, within 4 hours, and prolonged antidepressant effects that lasted up to 72 hours as compared to placebo control (Berman et al., 2000). This rapid antidepressant effect of ketamine is far superior to the 4-12 week delay with current antidepressant drugs (Schulberg et al., 1998; Uher et al., 2011). The hallucinogenic effects of ketamine subsided prior to the onset of the antidepressant effects as measured by the Visual Analog Scales for intoxication "high" and Brief Psychiatric Rating Scale (Berman et al., 2000). This was the first clinical study to demonstrate that selective glutamatergic drugs may be effective for the treatment of MDD.

One limitation of the Berman et al. (2000) study was the limited three day follow-up period. In that study, the ketamine treatment group did not return baseline depression scores during the follow-up period, which makes it difficult to determine the duration of the antidepressant effects of ketamine. Thus, Zarate and colleagues conducted a clinical study to

assess the antidepressant effects of ketamine in treatment-resistant depression and to determine a better understanding of the duration of the antidepressant effects. Following a single 0.5 mg/kg intravenous infusion of ketamine, treatment-resistant patients showed a significant reduction in depression scores at 110 min that lasted up to 7 days (Zarate et al., 2006). Specifically, 71% of the patients achieved response criteria one day after the fusion, while 29% achieved full remission. Additionally, 35% maintained response criteria on day seven (Zarate et al., 2006). Again, the hallucinogenic (or euphoric) effects diminished before the onset of the antidepressant effects of ketamine. This study confirmed the finding in the Berman et al. (2000) study that ketamine produces rapid and prolonged antidepressant effects in the treatment of depression.

Two studies have evaluated the effects of repeated ketamine infusion in patients suffering from treatment-resistant depression to determine if repeating dosing could extend the antidepressant effects of ketamine beyond seven days. In both studies, patients received up to six ketamine infusions over approximately 12 days. One study found that the average relapse rate was 19 days after the sixth and final ketamine infusion, with one patient remaining depression free for more than three months (ann het Rot et al., 2010). In the second study, ketamine infusions produced an antidepressant effect in 70.8% of the patients and these antidepressant effects lasted for a median of 18 days (four participants did not relapse by the last follow up day [83 days]) (Murrough et al., 2013). Both of these studies reported safety and tolerability similar to that seem with the single ketamine infusion studies.

A multi-site large scale clinical study compared the effects of a single low dose of ketamine (0.5 mg/kg, i.v.) to an active placebo, the anesthetic benzodiazepine midazolam (0.045 mg/kg, i.v.), in treatment-resistant patients. Following drug infusion, the ketamine group showed a 64% response rate at 24 hours and 46% response rate on day seven; whereas, the midazolam

group showed a response rate of 28% at 24 hours and 18% on day seven (Murrough et al., 2013). These results suggested that the antidepressant effects of ketamine are not a result of its anesthetic properties. A second study compared the effects of ketamine and ECT in patients suffering from MDD. This study found that both ketamine and ECT produced antidepressant effects; however, ketamine produced superior antidepressant effects in terms of response onset. For example, ketamine produced rapid antidepressant effects starting at 24 hours; whereas, the antidepressant effects of ECT were not expressed until day two. By day three, both ketamine and ECT were equally efficacious (Ghasemi et al., 2014). These results suggest that ketamine is as efficacious, if not more efficacious, as ECT for treating MDD.

There are several stages to the drug development process, and safety and ethical concerns limit human research early in the drug development process. Animal (or preclinical) research plays an important role in the drug development process and allows the research to have greater control over the experimental conditions. Researchers have worked to develop preclinical assays, both behavioral and molecular, to screen novel drugs for their clinical implications. Most behavioral assays used to screen antidepressant drugs have face, predictive, and/or construct validity, and some behavioral models will have more than one type of validity. For example, the chronic unpredictable stress is a procedure in which animals are exposed to mild stressors (e.g. food deprivation, dirty cages, temperature changes, etc.) for 21 or more days and several behavioral measures are assessed (e.g. latency to feed, sucrose preference, etc.). Chronic unpredictable stress is a preclinical procedure that has high face validity because the animals are exposed to chronic stressors and show a decrease in behaviors that would be considered pleasurable (e.g. sucrose consumption, etc.). Additionally, this procedure has predictive validity because it takes chronic (not acute) administration of antidepressant drugs to restore behavior,

which is similar to findings in humans with depression. Most researchers will use a variety of preclinical procedures as tools for assessing the effects and clinical implications of novel drugs.

Ketamine has been shown to produce antidepressant-like effects in several preclinical models of depression: forced swim test, tail suspension test, learned helplessness, and chronic unpredictable stress. For example, ketamine reduces immobility time in the forced swim test (Engin, Treit, & Dickson, 2009; Gigliucci et al., 2013). Additionally, ketamine reverses corticosterone-induced changes in the forced swim test, which is used to model treatment-resistant depression (Koike, Iijima, & Chaki, 2013). Furthermore, acute and chronic ketamine treatment has been shown to reverse physiological-induced changes in body weight, adrenal gland weight, and corticosterone and adrenocorticotropic hormone concentrations following 40 days of chronic mild stress (Garcia et al., 2009). Also consistent with clinical data, ketamine produces rapid antidepressant-like effects following repeated shocks in the learned helplessness paradigm after a single injection, while repeated dosing with monoaminergic antidepressant drugs is required to product antidepressant-like effects (Maeng et al., 2007; Koike et al., 2011). Furthermore, these antidepressant-like effects of ketamine have been shown to last 7-14 days in the forced swim test (Maeng et al., 2007; Autry et al., 2011; Tizabi et al., 2012), chronic unpredictable stress paradigm (Li et al., 2011), and tail suspension test (Koike et al., 2011).

Differential-Reinforcement-of-low-Rate 72 s Operant Procedure

The different-reinforcement-of-low-rate (DRL) 72 sec operant procedure has been used since the 1980s to selectively screen novel and established antidepressant ligands including tricyclic, SSRIs, SNRI, and MAO inhibitors (McGuire and Seiden, 1980; O'Donnell, Marek, & Seiden, 2005; O'Donnell and Seiden, 1982; O'Donnell and Seiden, 1983). Under the DRL schedule, animals are trained to wait a specific interresponse time (e.g. 72 sec) between lever

responses in order to receive a reinforcer and drugs with clinical efficacy as antidepressants have been shown to reliably increase the number of reinforcers received, usually decrease the number of responses emitted, and produce a rightward shift in the peak location of the interresponse time (IRT) distribution. McGuire and Seiden (1980) found dissociable effects of the tricyclic antidepressant imipramine in rats responding on a DRL 9 s schedule as compared to rats responding on a DRL 72 s schedule. Imipramine did not exert an effect on reinforcement rate or response rate under the DRL 9 s schedule; whereas, it produced a significant increase in reinforcement rate and decreased response rate under the DRL 72 s schedule. In a follow up study, the MAO inhibitors isocarboxazid, iproniazid, and phenelzine increased reinforcement rate and decreased response rate; whereas, alcohol only decreased response rate and morphine exerted no effects on DRL responding (O'Donnell & Seiden, 1982). Additionally, SSRIs produce the same behavioral effects as tricyclics and MAO inhibitors by increasing reinforcement rate and decreasing response rate (Sokolowski & Seiden, 1999).

The underlying factors responsible for antidepressant efficacy in the DRL task are unknown; however, O'Donnell et al. (2005) suggest three hypotheses. The first is that the effects of antidepressant drugs on DRL performance may be related to the animals' ability to make a temporal discrimination. The second hypothesis is that antidepressant drugs may selectively decrease 'impulsiveness' in the animals. The third hypothesis is that animals are trained and tested while under food or water deprivation (which is often used as an environmental stressor) and antidepressant drugs may improve response efficacy by reducing interactions between temporal discrimination or impulsivity and the stress of the behavioral contingencies in the DRL procedure.

In contrast, the pharmacology of the DRL 72 sec operant procedure is better understood. The DRL 72 sec procedure has high predictive validity for screening antidepressant drugs, as other drug classes (e.g. opioids, benzodiazepines, and amphetamines) do not produce the same behavioral profile as antidepressant drugs (for review see O'Donnell et al., 2005). For example, amphetamine and caffeine decrease reinforcement rate and increase response rate under the DRL 72 s procedure (Richard, Sabol, & Seiden, 1993; Marek et al., 1993; van Hest, van Drimmelen, & Olivier, 1992); whereas, alcohol and pentobarbital decrease response rate and reinforcement rate, respectively (O'Donnell & Seiden, 1982). Furthermore, the DRL 72 sec procedure is not vulnerable to the false positives of psychostimulants, which is an issue with the forced swim test. For example, caffeine has been shown to produce increased locomotor activity (Paterson et al., 2010), but it also produces antidepressant-like effects in the forced swim test (Sunal et al., 1994). However, caffeine does not produce antidepressant-like effects in the DRL 72 s procedure (Marek et al., 1993). Furthermore, the DRL 72 s has some face validity because the animals are single housed (or isolated) for the duration of training and testing. Also, the animals are food restricted, and food restriction is stressor commonly used in the chronic unpredictable stress procedure.

Table 6.

Effects of antidepressant, glutamatergic, and abused drugs in the DRL 72 s procedure

Drug Pharmacology	Strain/Sex	Reinforcement rate	Responses rate	Interresponse Time (IRT)	Reference
Tricyclic antidepressants (Imipramine, Desipramine, Nortriptyline)	S-D/males	↑	↓	→, NC	Dekeyne et al. 2002; Marek et al. 1988; O'Donnell et al. 1983; Paterson et al. 2010*; Richards et al. 1993;
MAO inhibitors (Iproniazid, Isocarboxazid)	S-D/males	↑	↓	→	O'Donnell et al. 1982
SSRIs (Fluoxetine, Sertraline, Paroxetine, Fluvoxamine)	S-D/males	↑	↓, NC	→, NC	Dekeyne et al. 2002*; Sokolowski et al. 1999*; Paterson et al. 2010; Marek et al. 1989a; Seiden et al. 1985
Atypical Antidepressants Bupropion	S-D/males	↓, NC	↑, NC	←, NC	Dekeyne et al. 2002*;Paterson et al. 2010; Seiden et al. 1985
Glutamatergic drugs					
MK-801	S-D/males	↓	↑	←	Ardayfio et al. 2008
LY-341495 (mGluR2/3 antagonist)	S-D/males	NC	NC	Not Analyzed	Bespalov et al. 2008
MTEP (mGluR5 antagonist)	S-D/males	↑	↓	Not Analyzed	Molina-Hernandez et al., 2006
Other Drugs					
Alcohol	S-D/males	NC	↓	Not Analyzed	O'Donnell et al. 1982
Morphine	S-D/males	NC	NC	Not Analyzed	O'Donnell et al. 1982
Pentobarbital	S-D/males	↓	NC	Not Analyzed	O'Donnell et al. 1982
Caffeine	S-D/males	↓	↑	←	Marek et al. 1993
Amphetamine	S-D/males	↓	↑	←	Van Hest et al. 1992; Richards et al. 1993

S-D, Sprague-Dawley; ↑, increase; ↓, decrease; →, rightward shift; ←, leftward shift; NC, No Change

* indicate articles that produced no change (NC)

Table 6.

Effects of glutamatergic drugs on ICSS

Drug Pharmacology	Strain/Sex	ICSS Procedure	Effect	References
Noncompetitive NMDA antagonist				
Ketamine	L-H/male	VI-10s	Mixed ↑↓	Herberg et al. 1989
MK-801	L-H/male	VI-10s	Mixed ↑↓	Herberg et al. 1989
	S-D/male	Rate	↑ rate	Olds, 1996
	S-D/male	Frequency-rate/VI-3s	↓T-LR, -Max rate	Sundstrom et al. 2002
	Wistar/male	Discrete Trial	↓CIT, ↑L	Bespalov et al. 2006; Kenny et al. 2003
Phencyclidine	L-E/male	Frequency-rate	↓T-LR,θ-0 ↓Max rate	Carlezon et al. 1993
	S-D/male	FI-60s	-rate	Schaefer et al. 1990
	Fisher/male	Discrete Trial	↓CIT	Kornetsky et al. 1979
Competitive NMDA antagonist				
D-CPPene	Wistar/male	Discrete Trial	↑CIT, ↑L	Bespalov et al. 2006
AMPA antagonist				
NBQX	Wistar/male	Discrete Trial	-CIT, -L	Kenny et al. 2003
GYKI-53655	Wistar/male	Discrete Trial	-CIT, -L	Bespalov et al. 2006
Glycine site antagonist				
L-701,324	Wistar/male	Discrete Trial	-CIT, ↑L	Bespalov et al. 2006
mGluR5 antagonist				
MPEP	Wistar/male	Discrete Trial	↑CIT, -L	Kenny et al. 2003
	Wistar/male	Discrete Trial	-CIT, ↑L	Bespalov et al. 2006
mGluR2 agonist				
LY314582	Wistar/male	Discrete Trial	-CIT, -L	Kenny et al. 2003
mGluR2 antagonist				
LY341495	Wistar/male	Discrete Trial	-CIT, -L	Kenny et al. 2003

S-D, Sprague-Dawley; L-H, Lister Hooded; L-E, Long Evans; VI, Variable Interval; FI, Fixed Interval; ↑, increase; ↓, decrease; T-LR, Threshold-Locus of Rise; CIT, Current Intensity Threshold; θ, Theta; L, Latency

Intracranial Self-Stimulation Operant Procedure

The abuse related effects of ketamine have been evaluated in a number of preclinical procedures including self-administration, drug discrimination, and conditioned place preference. Ketamine has been shown to maintain self-administration in rhesus monkeys (Broadbear, Winger, & Woods, 2004; Moreton, Meisch, Stark, & Thompson, 1977; Young & Woods, 1981) and rats (de la Peña et al., 2012; Rocha, Ward, Lytle, & Emmett-Oglesby, 1996). Moreover, ketamine has been shown to produce cross generalization to other noncompetitive NMDA antagonists, such as PCP and MK-801, in drug discrimination studies (DeVry & Jentzsch, 2003; Grant, Colombo, Grant, & Rogawski, 1996; Killinger, Peet, & Baker, 2010; Overton, Shen, Ke, & Gazdick, 1989; Rocha et al., 1996) and produces a place preference in the conditioned place preference model (de la Peña et al., 2012; Suzuki et al., 2000). It is important to note that some rats have failed to self-administer ketamine or to acquire the discriminative stimulus of ketamine (Rocha et al., 1996). There is very limited research on the abuse-related effects of ketamine as assessed using intracranial self-stimulation (ICSS) (Herberg & Rose, 1989).

Olds and Milner (1954) were the first to report the rewarding properties of ICSS in which rats with electrodes implanted in the septal area of the brain would press a lever for electrical stimulation. Intracranial self-stimulation (ICSS) is one approach used to assess and compare abuse liability of drugs (Carlezon and Chartoff, 2007; Kornetsky & Esposito, McLean, & Jacobson, 1979; Wise, 1996). For ICSS in general, animals are trained to lever press for brain stimulation delivered via electrodes implanted in brain regions such as the medial forebrain bundle. Electrical stimulation of the medial forebrain bundle depolarizes descending myelinated axonal fibers that project to and synapse on dopaminergic neurons in the ventral tegmental area. In turn, the ventral tegmental area activates the ascending mesolimbic pathway, specifically the

nucleus accumbens (Wise, 1996). Over the years, a number of ICSS procedures have been established to assess the abuse related effects of drugs such as rate, discrete trail current intensity, and frequency rate procedures.

In "frequency-rate" ICSS procedures, different frequencies of brain stimulation are used to engender frequency-dependent increases in ICSS rates and thereby establish a wide range of baseline ICSS rates. This type of procedure is useful in part because of its sensitivity to detect drug effects on both low and high rates of operant responding. Many drugs of abuse, and especially stimulants like cocaine and amphetamine, increase low ICSS rates maintained by low brain-stimulation frequencies, and this "facilitation" of ICSS is often interpreted as an abuse-related effect (Vlachou and Markou, 2011; Bauer et al., 2013b). For example, amphetamine, methamphetamine, cocaine, and methcathinone (an active and common constituent in "bath salts") exclusively increase low ICSS rates in the frequency-rate ICSS procedures (Bauer, Banks, Blough, & Negus, 2013a; Bauer, Banks, Negus, 2014; Bonano, Glennon, De Felice, Banks, & Negus, 2014). Most antidepressant drugs decrease high rates of behavior maintained at high frequencies of brain stimulation in the frequency-rate ICSS procedure (Rosenberg, Carroll, & Negus, 2013). For example, the tricyclic antidepressant drugs clomipramine and nortriptyline decreased ICSS across a board range of high and intermediate stimulation frequencies, while the SSRI citalopram decreased ICSS rates only at high frequencies (Rosenberg et al., 2013).

The noncompetitive NMDA receptor antagonists phencyclidine and MK-801 have been evaluated under a number of ICSS parameters including frequency-rate ICSS. For example, phencyclidine and MK-801 increase low ICSS rates that are maintained at low brain-stimulation frequencies with minimal effects exerted on high ICSS rates maintained at high brain-stimulation frequencies (Carlezon and Wise, 1993; Corbett, 1989; Sundstrom et al., 2002; Wise, Bauco, &

Carlezon, 1992). The effects of other glutamatergic compounds also have been evaluated on ICSS; however, none of these drugs have been shown to produce abuse-related effects (see table 6). The only study that has examined the effects of ketamine on ICSS used a fixed frequency and intensity of brain stimulation under a variable interval 10 s schedule and rates were assessed in 10-min bins for 60 min (Herberg and Rose, 1989). That study found that 3.0 mg/kg ketamine increased mean ICSS rates in the 10-20 and 40-50 min time bins, but did not effect ICSS rates during the other time bins. Thus, these rate increases were small and inconsistent across the test session. Additionally, higher ketamine doses (10-100 mg/kg) decreased ICSS. Ketamine effects on ICSS have yet to be fully elucidated and have not been reported in subjects responding under a frequency-rate ICSS procedure.

Rationale for Experiment 1: Effects of Ketamine on Differential-Reinforcement-of-low-Rate 72 s Responding in Rats

Experiment 1 sought to extend the preclinical evidence of the antidepressant-like effects of ketamine using the DRL 72 s operant procedure and to determine if the DRL 72 s operant procedure is suitable for evaluating the antidepressant effects of ketamine. In addition to testing ketamine in the DRL 72 sec procedure, the present study tested the more selective and higher affinity noncompetitive NMDA receptor antagonist MK-801, which is often used as a comparator in other preclinical studies (Autry et al., 2011; Engin et al., 2009; Maeng et al., 2008) and the NMDA receptor agonist NMDA for comparison. Finally, the selective serotonin reuptake inhibitor (SSRI) fluoxetine and the tricyclic imipramine served as positive controls and the psychostimulant d-amphetamine served as a negative control.

Rationale for Experiment 2: Effects of Acute and Repeated Ketamine on Intracranial Self-Stimulation in Rats

To address the issue of limited data on the abuse-related effects of ketamine using the ICSS assay, Experiment 2 compared the effects of ketamine, MK-801, and phencyclidine on ICSS using a frequency-rate procedure that has been used previously to evaluate the effects of opioids (Altarifi and Negus, 2011; Altarifi, Rice, & Negus, 2013; Negus, Bilsky, Carmo, & Stevenson, 2010; Negus et al., 2012), monoamine releasers and uptake inhibitors (Bauer et al., 2013a,b; Bonano et al., 2013; Negus, O'Connell, Morrissey, Cheng, & Rice, 2012; Rosenberg et al., 2013), and cannabinoids (Kwilasz and Negus, 2012). The present study evaluated the dose-effect and time-course of effects of ketamine, MK-801, and phencyclidine. Additional, studies were conducted to examine the effects of repeated ketamine dosing using a testing strategy that has been shown to produce tolerance to ICSS rate-decreasing effects and to enhance expression of ICSS rate-increasing effects with mu opioid receptor agonists (Altarifi and Negus, 2011; Altarifi et al., 2013).

Rationale for Experiment 3: Glutamatergic Antagonists and Involvement of Serotonin in the Antidepressant-like Effects of Ketamine in the Differential-Reinforcement-of-low-Rate 72 s Operant Procedure

Experiment 3 was designed to evaluate the effects of other glutamatergic antagonists (i.e NMDA and AMPA) and to determine the possible role of serotonin in the antidepressant-like effects of ketamine using the DRL 72 s operant procedure. First, the NMDA antagonists phencyclidine and memantine were tested because of their receptor binding profile. For example, phencyclidine, from which ketamine is derived from, has a considerably higher binding affinity for the NMDA receptor channel (Ki = 42.2 nM) as compared to ketamine (Ki = 1,190 nM), but phencyclidine does not appear to be particularly selective for the NMDA receptor channel over other receptors (Bresink et al., 1995; Roth et al., 2013). Similarly to ketamine, memantine has a

relatively weak receptor binding affinity for the NMDA receptor channel (Ki = 690); however, memantine is selective for NMDA receptor channels as it has no significant affinity for other receptors (Bresink et al., 1995; National Institute of Mental Health's Psychoactive Drug Screening Program, 2014). Additionally, the AMPA receptor antagonist NBQX was tested to determine if NMDA receptor antagonism was responsible for the antidepressant-like effects of ketamine, rather than non-specific blockade of glutamatergic activity.

Drug combination studies then were conducted to determine the role of serotonin transporters and of serotonin 5-HT_2 receptors in the antidepressant-like effects of ketamine in the DRL 72 s procedure. Several studies have shown the involvement of serotonin in the behavioral effects of ketamine (Fahim et al., 1973; Fukumoto et al., 2014; Yoshizawa et al., 2013). For example, serotonergic mechanisms have been shown to mediate the antidepressant-like effects of ketamine in both the forced swim test (Gigliucci et al., 2013) and the tail suspension test (Kos et al., 2006). Most clinically available antidepressant drugs (e.g. tricyclics, SSRIs, and SNRIs) share a common pharmacological mechanism, which is inhibiting serotonin transporters. Ketamine has virtually no affinity for serotonin transporters (Ki = 161,700 nM) compared to NMDA receptors (Ki = 1,190 nM); however, Martin et al. (1982) demonstrated that high doses of ketamine inhibit the serotonin transporter in rats. The goal of experiment 3 was to determine if the antidepressant-like effects ketamine in the DRL 72 s procedure are produced by inhibition of serotonin transporters. The SSRI fluoxetine was used in combination with ketamine to evaluate the role of inhibiting serotonin transporters in the antidepressant-like effects of ketamine.

The serotonin 5-HT_{2A} receptor is an excitatory G-protein coupled receptor that is widely distributed in both peripheral and central tissues and activation of 5-HT_{2A} receptors stimulates the release of the stress hormone adrenocorticotropic (ACTH) (Hoyer, Hannon, & Martin, 2002;

Green, 2006). Additionally, 5-HT_{2A} receptors act in opposition to postsynaptic 5-HT_{1A} receptors, which are inhibitory G-couple protein receptors that are located in cortical and hippocampal areas and also regulate ACTH. Thus, antagonism of 5-HT_{2A} receptors enhances activity of 5-HT_{1A} receptors (Hoyer et al., 2002; Lakoski & Aghajanian, 1985). Recently, 5-HT_{1A} receptor agonists have shown promise as potential antidepressant targets (Pehrson and Sanchez, 2014). Ketamine has less binding at serotonin 5-HT_2 receptors (Ki = 15,000 nM) as compared to NMDA receptors (Ki = 1,190 nM); however, the functionality of ketamine at 5-HT_2 receptors is unknown (Kapur & Seeman, 2002). Additionally, ketamine treatment reverses corticosterone and adrenocorticotropic hormone concentrations increases following 40 days of chronic mild stress in rats (Garcia et al., 2009). Furthermore, atypical antipsychotic drugs, which primarily act as serotonin 5-HT_2 receptor antagonists at low doses, have been shown to be efficacious as adjunctive treatment and display rapid effects for the remission of depressive symptoms as compared to antidepressant drug treatment alone (Ostroff & Nelson, 1999; Tohen et al., 2010; Wright et al., 2013). Lastly, the serotonin 5-HT_{2A} antagonist M1009067 attenuates the psychostimulant-like effects of MK-801 in the DRL 72 s procedure (Marek, Li, & Seiden, 1989b), indicating that the psychostimulant-like effects of MK-801 may be mediated through 5-HT_{2A} agonism. Ketamine and MK-801 produce opposing behavioral effects in the DRL 72 s procedure (see Experiment 1 results), and thus, it is possible that the receptor actions of ketamine and MK-801 are different at the 5-HT_{2A} receptors. The goal of experiment 3 was to determine if the antidepressant-like effects ketamine in the DRL 72 s procedure are mediated by 5-HT_{2A} antagonism. The serotonin 5-HT_2 receptor antagonist ritanserin and serotonin 5-HT_2 receptor agonist quipazine were used to determine the role of serotonin 5-HT_2 receptors in the antidepressant-like effects of ketamine.

Experiment 1: Effects of Ketamine on Differential-Reinforcement-of-low-Rat 72 s Responding in Rats

Rationale

Experiment 1 sought to extend the preclinical evidence of the antidepressant-like effects of ketamine using the DRL 72 s operant procedure. In addition to testing ketamine in the DRL 72 s procedure, the present study tested the more selective and higher affinity noncompetitive NMDA receptor antagonist MK-801 and the NMDA receptor agonist NMDA for comparison. Finally, the selective serotonin reuptake inhibitor (SSRI) fluoxetine and the tricyclic imipramine served as positive controls and the psychostimulant d-amphetamine served as a negative control.

Experiment 1 Methods

Subjects

Twelve adult male Sprague-Dawley rats (Harlan Laboratories Inc, Frederick, MD) weighing between 300 and 350 grams at the start of the experiment were used in this study. All animals were housed individually in plastic cages (43.8 x 22.2 x 22.2 cm) in the vivarium with a 12-hr/12-hr light/dark cycle (lights on at 0600 hr) and all training and testing sessions were conducted during the light portion of the cycle (0900 to 1600 hr). After one week of acclimation to the vivarium, daily access to food (Teklad Lab Animal Diets, Harlan Laboratories Inc.) was restricted in order to maintain the rats at 85% of their free feeding body weights; however, water was freely available in the home cages. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University (IACUC Protocol AM10215) and conducted in accordance with National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animals Resources, 2011).

Apparatus

Four operant conditioning chambers (29.2 x 30.5 x 24.1 cm) enclosed in sound attenuating cubicles were used (Model ENV-008-VP, Med-Associates, St. Albans, VT). Each chamber was equipped with a house light, ventilation fan, two retractable levers, and a food trough. The house light was mounted on the middle panel of the back wall. On the front wall, two retractable levers (4.5 cm wide, extended 2.0 cm through the center of one wall, 3 cm off the floor) were mounted on either side of a food trough. The reinforcers were 45 mg purified diet pellets (Product #F0021, Bio-Serv, Frenchtown, NJ). The fans mounted in the cubicles provided background white noise. Programming of behavioral sessions and data collection were computer controlled by Med-State software (Med PC, Version 4.1, Med-Associates) running on a Windows XP operating system.

Drugs

The noncompetitive NMDA receptor antagonist (±) ketamine HCl (Sigma Aldrich, St. Louis, MO), the NMDA receptor agonist N-Methyl-D-aspartic acid (gift from H Lundbeck A/S, Copenhagen, Valby, Denmark), the tricyclic imipramine HCl (gift from A.H. Robins Pharmaceuticals, Richmond, VA), the SSRI (±) fluoxetine HCl (NIMH Drug Repository, Bethesda, MD, USA), the dopamine receptor agonist d-amphetamine sulfate salt (Sigma Aldrich), and the noncompetitive NMDA receptor antagonist (+) MK-801 maleate (dizocilpine) (Sigma Aldrich) were dissolved in 0.9% physiological saline. All drugs were administered *intraperitoneally* at a volume of 1.0 ml/kg and doses represent the salt forms of the drugs. Testing of each drug was completed before initiating testing with another drug. Doses and pretreatment times were based on preliminary studies and previous studies in the literature (O'Donnell and Seiden, 1983; Richards et al., 1993; Sokolowski and Seiden, 1999; Ardayfio et al., 2008; Páleníček et al., 2011; Vunck et al., 2011). Drugs, doses, and pretreatment times were

as follows: ketamine (1.0-10.0 mg/kg; 5 min), NMDA (3.0-30.0 mg/kg; 10 min), imipramine (1-10.0 mg/kg; 60 min), fluoxetine (2.5-10 mg/kg; 60 min), d-amphetamine (0.5-2.0 mg/kg; 20 min), and MK-801 (0.0125-0.1 mg/kg; 15 min). For the combination experiment, NMDA was administered 5 min prior to ketamine. Ketamine and NMDA were administered in ascending doses in order to determine the doses that had rate suppressant effects. The doses for imipramine, fluoxetine, d-amphetamine, MK-801 and for the combination experiments with ketamine and NMDA were administered using a Latin-square design.

Training Procedures

Magazine training. Drug-naïve rats were placed in the operant chamber in which the house light was on, but the levers were not extended into the chamber. During the single magazine training session, one reinforcer (i.e. food pellet) was delivered noncontingently according to a fixed-time 60 s schedule for 30 min.

Lever press training. For the remaining training and test sessions the house light was on and one lever was extended from the wall. During the next three sessions, rats were trained to press either the left or right lever under a fixed-ratio 1 (FR 1) food-reinforcement schedule in which each lever press resulted in delivery of a reinforcer. Sessions ended after delivery of 50 reinforcers or 60 min, whichever occurred first. The position of the lever associated with the FR 1 schedule was counter balanced with half of the rats assigned the right lever and half assigned to the left lever. Following the completion of lever press training, all rats started DRL training.

DRL training. Under the DRL schedule, a response produced a reinforcer only after a specified interresponse interval had elapsed. Responses emitted before the end of the interresponse interval reset the timer and did not produce a reinforcer. The interresponse interval was gradually increased from an initial value of 4.5 s to a terminal value of 72 s over 28 sessions.

Rats were initially trained on a DRL 4.5 s schedule for 3 sessions. Next, the DRL schedule was increased to 9 s for five sessions. The DRL schedule was then increased to 18 s for 10 sessions and then to 36 s for 10 sessions. Finally, the DRL schedule was increased to the final schedule value of 72 s until performance stabilized. DRL performance was considered stable when the number of responses for each rat did not vary more than 10% of the mean for 5 of 6 consecutive sessions. All DRL training and testing sessions were 60 min in length.

Testing Procedures

DRL testing. Once stable performance under the DRL 72 s schedule was established, test sessions occurred twice weekly (typically Tuesday and Friday) with a minimum of one training sessions prior to each test session. Testing of each drug was completed before initiating testing with another drug, and a saline baseline was determined for each drug tested. Testing continued until each drug had been tested in groups of 8-12 rats.

Statistical Analysis

The dependent variables included 1) total number of earned reinforcers during each test session, 2) total number of responses during each test session, and 3) inter-response times (IRT) for all responses during each test session. All data were expressed as means (+/- standard error of the mean [S.E.M.]). Data during the test sessions were recorded in three 20 min time bins in order to determine if drug treatment effects changed during the test sessions. Response and reinforcer data were analyzed using a two-factor repeated measures analysis of variance (ANOVA) with both drug dose and time bin treated as within-subjects factors. Only main effects for the number of reinforcers, number of responses, and IRT distributions are reported for drugs that failed to produce an interaction effect. Additionally, combination test data for ketamine and NMDA were analyzed using one-way repeated measures ANOVA with drug dose as the factor.

The IRT distributions were obtained by recording responses in 25 6-s bins, with the first 6 s bin representing "burst responding". To determine if there was a shift in the IRT distribution, a peak location analysis was performed. Specifically, the median of the IRT distribution for each individual rat was determined after eliminating burst responses from the total number of responses. Medians (peak location) 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 s bin was found for each rat (total number of responses divided by number of responses for each time bin) and averages were calculated for each bin. When interactions were significant, IRT distributions are shown for the 20 min time bins. Planned multiple comparisons using Tukey post hoc tests were conducted after all significant ANOVAs, as appropriate. The criterion for significance was $p < 0.05$. Data were analyzed using GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, CA, USA).

Experiment 1 Results

DRL training and baseline performance. All 12 rats met the DRL training criterion in a mean of 30.3 (± 3.2 SEM) training sessions. In order to determine if the saline baselines changed during the study, data from the saline baselines for ketamine, MK-801, NMDA, imipramine, fluoxetine and d-amphetamine were analyzed for the number of reinforcers and number of responses with separate two-factor ANOVA's (i.e. drug vehicle x time bin). These analyses did not reveal any significant differences ($p > 0.05$), indicating that the saline baselines did not significantly change for any of the drugs during the study.

Ketamine. For number of reinforcers (Figure 1a), the noncompetitive NMDA receptor antagonist ketamine produced significant main effects for dose ($F[4,44] = 25.68$, $p < 0.001$) and time bin ($F[2, 22] = 5.00$, $p = 0.016$), but the interaction was not significant ($F[8,88] = 1.73$, $p =$

0.102). Compared to all other doses, 10.0 mg/kg ketamine significantly increased the number of reinforcers during all three time bins ($p < 0.001$). Tukey post hoc tests also revealed that significantly more reinforcers were obtained in the first 20-min time bin as compared to the third 20-min time bin ($p < 0.05$).

For number of responses (Figure 1b), ketamine produced a significant main effect of dose ($F[4,44] = 10.55$, $p < 0.001$) and a significant interaction ($F[8,88] = 6.63$, $p < 0.001$). The main effect for time bin was not significant ($F[2,22] = 0.27$, $p = 0.763$). As compared to all other doses, 10.0 mg/kg ketamine significantly reduced the number of responses during both the first ($p < 0.001$) and the second ($p < 0.01$) 20-min time bins, but not in the third, with the largest decrease in responses occurring in the first 20-min time bin.

The top 3 panels in Figure 2 show the IRT distributions for ketamine for each 20-min time bin. As compared to all other doses, 10.0 mg/kg ketamine produced a significant rightward shift in the peak location of the IRT distribution in the first time bin ($F[4,44] = 10.38$, $p < 0.001$; Figure 2a). The 10.0 mg/kg ketamine dose produced a significant rightward shift in the peak location of the IRT distribution in the second time bin as compared to saline ($F[4,44] = 5.65$, $p < 0.001$; Figure 2b), but not in the third ($F[4,44] = 1.71$, $p = 0.165$; Figure 2c).

MK-801. For number of reinforcers (Figure 1c), the noncompetitive NMDA receptor antagonist MK-801 produced significant main effects for dose ($F[3,21] = 21.02$, $p < 0.001$) and time bin ($F[2,14] = 6.95$, $p = 0.008$), but the interaction was not significant ($F[6,42] = 1.78$, $p = 0.126$). Compared to saline, 0.05 and 0.1 mg/kg MK-801 produced significant decreases in the number of reinforcers obtained throughout the test session ($p < 0.001$). For time bin, Tukey post hoc tests revealed that significantly fewer reinforcers were obtained during the third time bin as compared to the first 20-min time bin ($p < 0.01$).

For number of responses (Figure 1d), MK-801 produced a significant main effect for dose (F[3,21] = 14.83, $p < 0.001$) and a significant interaction (F[6,42] = 2.86, $p = 0.02$); the main effect for time bin was not significant (F[2,14] = 1.71, $p = 0.217$). Compared to all doses, 0.1 mg/kg MK-801 produced a significant increase in the number of responses in all 3 time bins, with the greatest increase found in the second time bin ($p < 0.01$).

The middle 3 panels in Figure 2 show the IRT distributions for MK-801 for the 20-min time bins. Compared to all other doses, 0.1 mg/kg MK-801 produced a significant leftward shift in the peak location of IRT distribution in the first (F[3,21] = 12.09, $p < 0.001$; Figure 2d) and second time bin (F[3,21] = 18.69, $p < 0.001$; Figure 2e). The 0.1 mg/kg MK-801 dose also produced a significant leftward shift in the peak location of the IRT distribution in the third time bin (F[3,21] = 11.97, $p < 0.001$; Figure 2f) as compared to saline.

NMDA. For number of reinforcers (Figure 1e), NMDA produced a significant main effect for dose (F[3,30]=7.54, $p < 0.001$) and a significant interaction (F[6,60]=2.50, $p = 0.031$); the main effect for time bin was not significant (F[2,20] = 2.73, $p = 0.089$). Compared to saline, 30.0 mg/kg NMDA produced a significant increase in the number of reinforcers obtained during all 3 time bins, with the greatest increase found in the first time bin ($p < 0.001$).

For number of responses (Figure 1f), NMDA produced a significant main effect for dose (F[3,30] = 9.85, $p < 0.001$) and a significant interaction (F[6,60] = 4.29, $p < 0.001$); the main effect for time bin was not significant (F[2,20] = 1.87, $p = 0.179$). Compared to saline, 30.0 mg/kg NMDA produced a significant decrease in number of responses during both the first ($p < 0.001$) and third ($p < 0.05$) time bins, but not in the second time bin. The 30.0 mg/kg dose of NMDA produced the greatest decrease in number of responses in the first time bin compared to

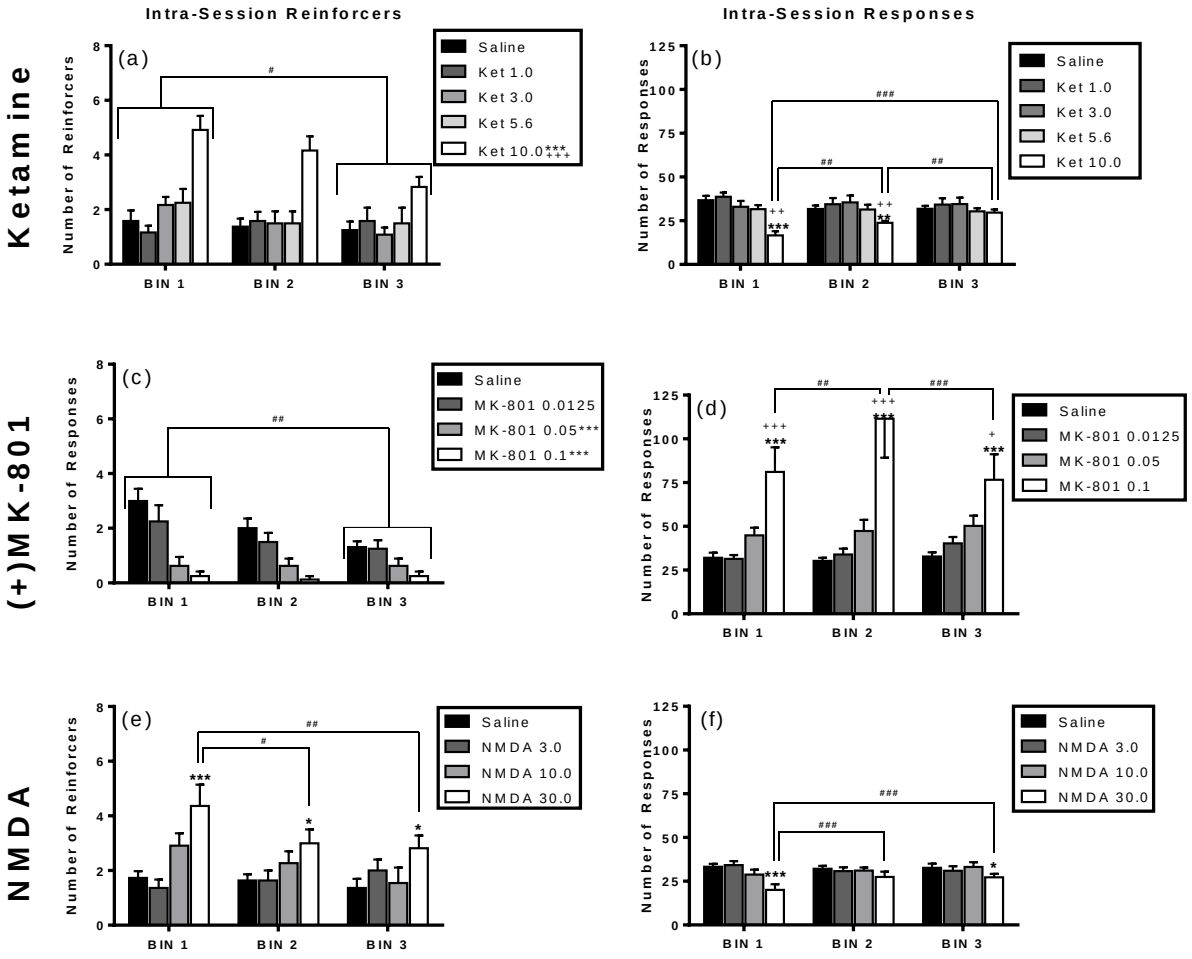

Figure 1. Effects of ketamine (top; n = 12), MK-801 (center; n = 8), and NMDA (bottom; n = 11) on DRL 72 s performance. Left panels (a, c, e) show drug effects on number of reinforcers earned. Abscissae: Time bin (20 min). Ordinates: Number of reinforcers. Right panels (b, d, f) show drug effects on number of responses emitted. Abscissae: Time bin (20 min). Ordinates: Number of responses. Drug doses are indicated in figure legend. Asterisks and plus signs directly over the bars represent significant differences within the time bins. The lines show significant differences across time bins. Main effects of dose have symbols on the figure legend. All data were expressed as means ± S.E.M for 8-12 rats (ketamine, n = 12; MK-801, n = 8; NMDA, n = 11).*$p < 0.05$, ***$p < 0.001$ versus saline; +$p < 0.05$, +++$p < 0.001$ versus all doses; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ versus time bins.

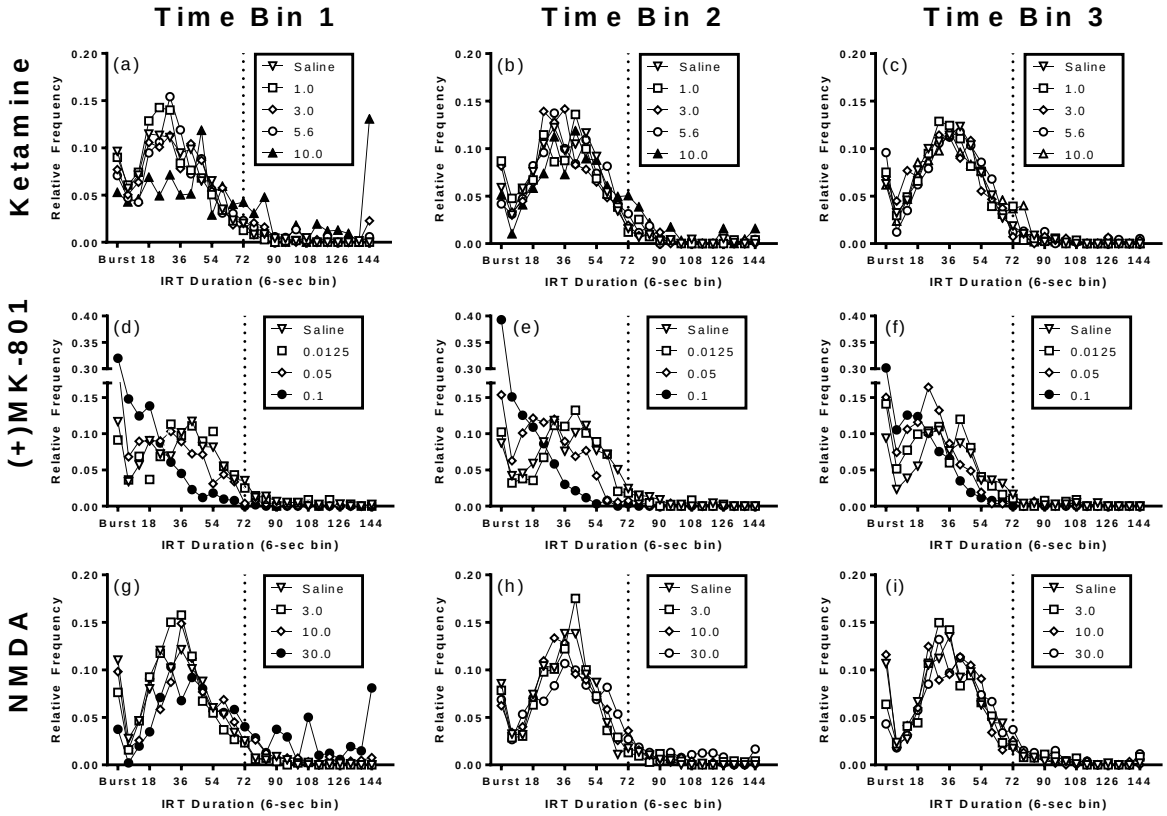

Figure 2. Effects of ketamine (top; n = 12), MK-801 (middle; n = 8), and NMDA (bottom; n = 11) on interresponse time (IRT) distribution for each of the three 20-min time bins. All panels show drug effects on interresponse time (IRT) distributions. Abscissae: Interresponse time. Ordinates: Relative frequency of responses. Drug doses are indicated in figure legend. Filled points represent significant shifts in peak location after drug treatment as determined by peak location analysis followed by a Tukey post hoc test, $p < 0.05$. All data were expressed as means for 8-12 rats (ketamine, n = 12; MK-801, n = 8; NMDA, n = 11).

the second and third time bins ($p < 0.001$), while the second time bin was not significantly different from the third time bin.

The bottom 3 panels in Figure 2 show the IRT distributions for NMDA for each 20-min time bin. Compared to saline, 30.0 mg/kg NMDA produced a significant rightward shift in the peak location of the IRT distribution in the first time bin ($F[3,30] = 5.18$, $p = 0.005$; Figure 2g). NMDA did not produce any significant changes to the peak location during the second ($F[3,30] = 2.70$, $p = 0.063$; Figure 2h) or third ($F[3,30] = 0.96$, $p = 0.424$; Figure 2i) time bins.

The following drugs failed to produce a significant interaction effect for reinforcers, responses, and IRT time bins; therefore, figure 3 presents only the main effects produced by drug dose.

Imipramine. For number of reinforcers (Figure 3a), the tricyclic antidepressant imipramine produced a significant main effect for dose ($F[3,30] = 13.42$, $p < 0.001$); however, the main effect for time bin ($F[2,20] = 0.03$, $p = 0.974$) and the interaction were not significant ($F[6,60] = 1.26$, $p > 0.288$). Imipramine dose-dependently increased the number of reinforcers obtained throughout the test session. As compared to all doses, 10.0 mg/kg imipramine produced a significant increase in the number of reinforcers obtained throughout the test session ($p < 0.01$). Additionally, 3.0 mg/kg imipramine significantly increased the numbers of reinforcers throughout the test session relative to saline ($p < 0.05$).

For number of responses (Figure 3b), imipramine produced significant main effects for dose ($F[3,30] = 13.03$, $p < 0.001$) and time bin ($F[2,20] = 9.80$, $p = 0.001$); the interaction was not significant ($F[6,60] = 0.70$, $p = 0.647$). As compared to all doses, 10.0 mg/kg imipramine produced a significant decrease in the number of responses ($p < 0.001$). Additionally, 3.0 mg/kg imipramine produced a significant decrease in the number of responses compared to saline ($p <$

0.05). For time bin, Tukey post hoc tests revealed that significantly more responses were made in the first (33.74 +/- 1.85) time bin compared to the second (29.86 +/- 1.42) and the third (29.75 +/- 1.82) time bins ($p < 0.01$). For IRT distributions (Figure 3c), 10.0 mg/kg imipramine produced a significant rightward shift in the peak location as compared to saline ($F[3,30] = 8.19$, $p < 0.001$).

Fluoxetine. For number of reinforcers (Figure 3d), the SSRI antidepressant fluoxetine produced a significant main effect for dose ($F[3,30] = 8.26$, $p < 0.001$); the main effect for time bin ($F[2,20] = 1.01$, $p = 0.381$) and the interaction were not significant ($F[6,60] = 2.15$, $p = 0.06$). As compared to saline, 10.0 mg/kg fluoxetine significantly increased the number of reinforcers throughout the entire test session ($p < 0.001$).

For number of responses (Figure 3e), fluoxetine produced significant main effects for both dose ($F[3,30] = 5.76$, $p = 0.003$) and time bin ($F[2,20] = 4.57$, $p = 0.023$); the interaction was not significant ($F[6,60] = 1.40$, $p = 0.228$). Compared to saline, 10.0 mg/kg fluoxetine significantly decreased the number of responses across the entire session ($p < 0.01$). For time bin, Tukey post hoc tests revealed that significantly more responses were made in the first (32.92 +/- 01.76) time bin compared to the second (29.22 +/- 1.52) time bin ($p < 0.05$). Fluoxetine (2.5, 5.0, and 10.0 mg/kg) produced a significant rightward shift in the peak location of the IRT distribution (Figure 3f) as compared to saline ($F[3,30] = 6.63$, $p = 0.001$).

d-amphetamine. For number of reinforcers (Figure 3h), the indirect dopamine agonist d-amphetamine did not produce a significant main effect of dose ($F[3,30] = 2.89$, $p = 0.052$) or time bin ($F[2,20] = 2.83$, $p = 0.083$), and the interaction was not significant ($F[6,60] = 1.73$, $p = 0.129$). For number of responses (Figure 3j), d-amphetamine produced a significant main effect of dose ($F[3,30] = 12.49$, $p < 0.001$); the main effect of time bin ($F[2,20] = 2.27$, $p = 0.129$) and

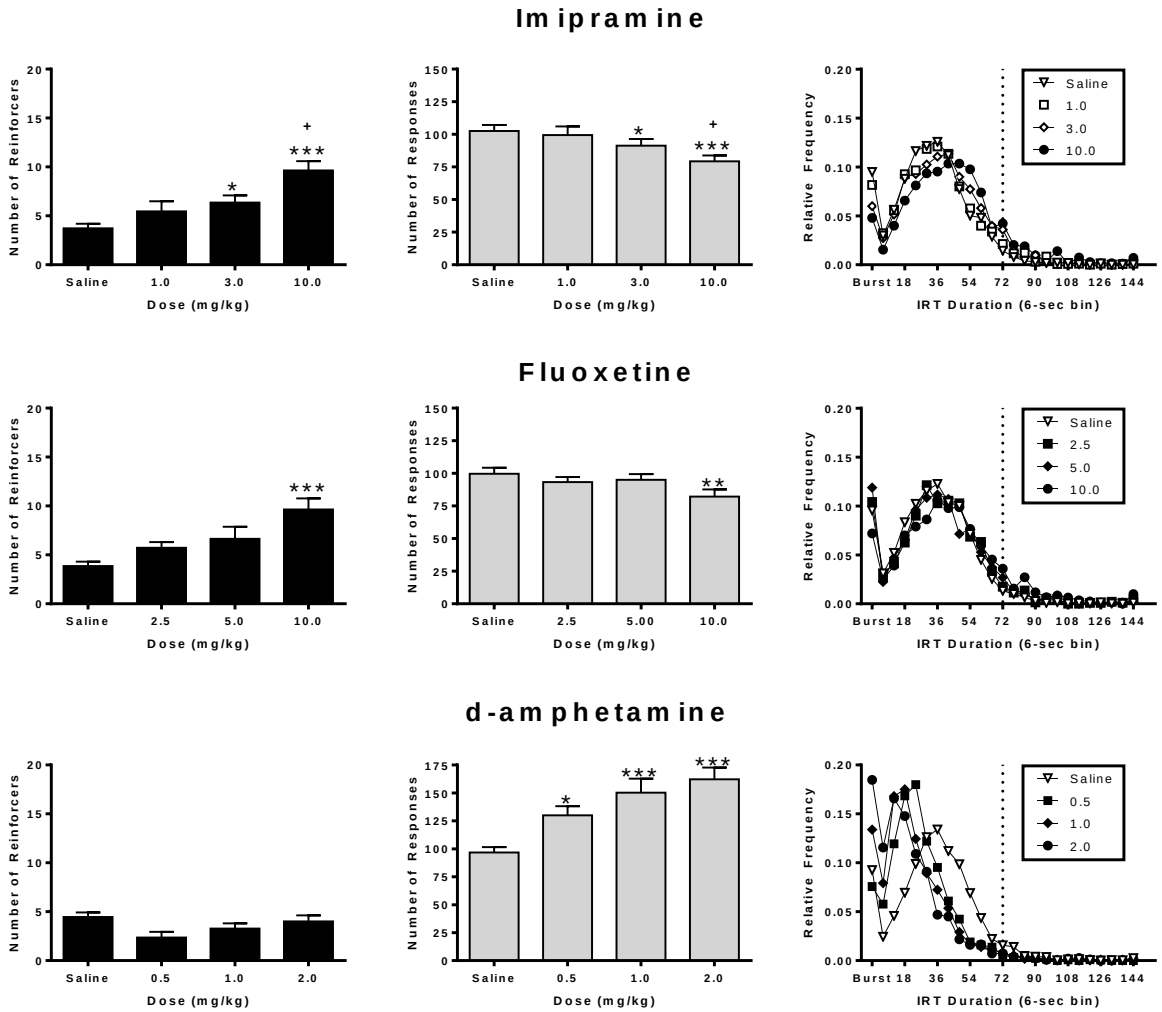

Figure 3. Effects of imipramine, fluoxetine, and d-amphetamine on DRL 72 s performance. Only main effects are shown as these drugs failed to produce any significant interaction effects. Left panels (a, d, h) show drug effects on number of reinforcers earned. Abscissae: Drug dose. Ordinates: Number of reinforcers. Center panels (b, e, i) show drug effects on number of responses emitted. Abscissae: Drug dose. Ordinates: Number of responses. Right panels (c, f, j) show drug effects on interresponse time (IRT) distributions. Abscissae: Interresponse time. Ordinates: Relative frequency of responses. Filled points represent significant shifts in peak location after drug treatment as determined by peak location analysis followed by a Tukey post hoc test, $p < 0.05$. Reinforcers and responses are expressed as means ± S.E.M for 11 rats. IRT data are expressed as means. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus saline; +$p < 0.05$ versus all doses.

the interaction were not significant (F[6,60] = 1.74, p = 0.126). All doses of d-amphetamine significantly increased the number of responses across the entire test session ($p < 0.05$). D-amphetamine produced a significant leftward shift in the peak location of the IRT distribution as compared to saline (F[3,30] = 19.53, $p < 0.001$; Figure 3i).

Ketamine and NMDA combination testing. The dose-effect data revealed that both the NMDA receptor antagonist ketamine and agonist NMDA produced similar antidepressant-like effect in the DRL 72 sec operant procedure. To further examine this relationship, combination tests were conducted to determine how these drugs may interact. In order to determine if NMDA would potentiate (or antagonize) ketamine's antidepressant-like effects, 10.0 and 30.0 mg/kg doses of NMDA were co-administered with the 10.0 mg/kg dose of ketamine. As can be seen in Figure 4 (a), the significant increase (F[3,24] = 6.57, p = 0.002) in the number of reinforcers produced by 10.0 mg/kg ketamine was not potentiated or antagonized by either 10.0 or 30.0 mg/kg NMDA. Similarly, the significant decrease in the number of responses (F[3,24] = 13.37, $p < 0.001$; Figure 4b) and the significant rightward shift in the peak location of the IRT distributions (F[3,24] = 11.01, $p < 0.001$; Figure 4c) produced by 10.0 mg/kg ketamine were not potentiated or antagonized by either dose of NMDA.

In order to determine if ketamine could potentiate (or antagonize) NMDA's antidepressant-like effects, the sub-effective dose of 3.0 mg/kg ketamine was co-administered with 30.0 mg/kg NMDA (Figure 5). Interestingly, the 3.0 mg/kg dose of ketamine significantly ($p < 0.05$) antagonized NMDA's increase in the number of reinforcers (F[2,16] = 9.17, $p < 0.001$; Figure 5a) and was no longer significantly different form saline. However, the significant rightward shift (F[2,16] = 8.32, p = 0.003) in the peak location of the IRT distributions (Figure

5c) was not blocked. There were no significant changes in the number of responses (F[2,16] = 2.76, p = 0.093; Figure 5b).

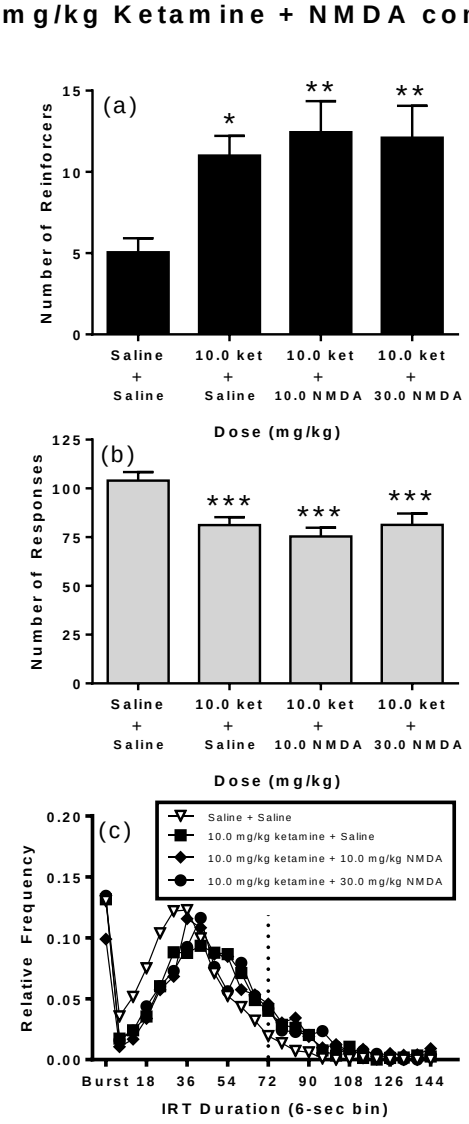

Figure 4. Effects of 10.0 mg/kg ketamine + NMDA combination DRL 72 s performance. Top panel (a) show drug effects on number of reinforcers earned. Center panel (b) show drug effects on number of responses emitted. Bottom panel (c) show drug effects on interresponse time (IRT) distributions. Filled points represent significant shifts in peak location after drug treatment as determined by peak location analysis followed by a Tukey post hoc test, $p < 0.05$. See figure 3 for further details. Reinforcers and responses were expressed as means ± S.E.M for nine rats. IRT data expressed as means. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus saline + saline.

30.0 mg/kg NMDA + Ketamine combination

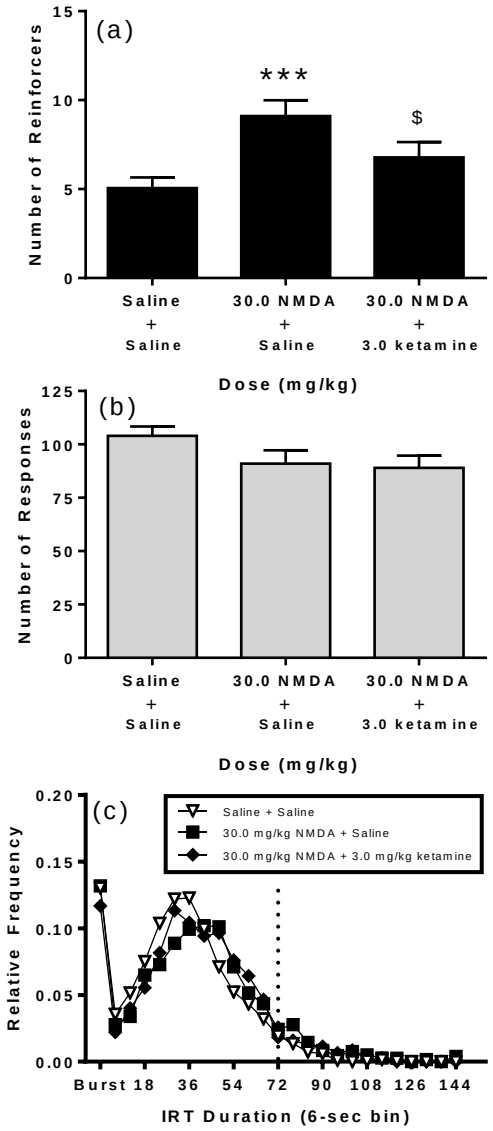

Figure 5. Effects of 30.0 mg/kg NMDA + ketamine combination DRL 72 s performance. Top panel (a) show drug effects on number of reinforcers earned. Center panel (b) show drug effects on number of responses emitted. Bottom panel (c) show drug effects on interresponse time (IRT) distributions. Filled points represent significant shifts in peak location after drug treatment as determined by peak location analysis followed by a Tukey post hoc test, $p < 0.05$. See figure 3 for further details. Reinforcers and responses were expressed as means ± S.E.M for nine rats. IRT data expressed as means. ***$p < 0.001$ versus saline + saline. \$$p < 0.05$ versus NMDA (30.0 mg/kg) + saline.

Experiment 1 Discussion

To our knowledge this is the first study to examine the antidepressant-like effects of the noncompetitive NMDA receptor antagonist ketamine in the DRL 72 sec procedure. Ketamine, NMDA, imipramine, and fluoxetine all produced acute antidepressant-like effects by significantly increasing the number of reinforcers, by decreasing the number of responses, and by producing a rightward shift in the peak location of the IRT distribution. Conversely, d-amphetamine and MK-801 produced a significant increase in the number of responses, either decreased (MK-801) the number of reinforcers, or produced no change (d-amphetamine), and produced a leftward shift in the peak location of the IRT distribution. Moreover, ketamine was found to attenuate the antidepressant-like effects of NMDA. None of the drugs tested produced antidepressant-like effects 24 h post-injection (data not shown).

These results confirm that a relatively low (i.e. subanesthetic) dose of ketamine produces antidepressant-like effects in the DRL-72 sec procedure and to a similar magnitude to that of the clinically efficacious antidepressant drugs, imipramine and fluoxetine. Additionally, these results support previous preclinical studies evaluating the antidepressant-like effects of ketamine. Most current behavioral procedures used to screen antidepressant drugs (e.g. forced swim, tail suspension, and DRL 72 sec) use acute dosing, such that animals are given a single injection typically within one hour of the test session. In contrast, there are behavioral procedures (e.g. chronic unpredictable stress and learned helplessness) that required repeated dosing of the antidepressant drug before anhedonia-like behaviors are reversed. In the present study, ketamine produced acute antidepressant-like effects, but failed to produce a prolonged antidepressant-like effect in the DRL 72 sec procedure. A number of studies have failed to demonstrate prolonged antidepressant-like effects with ketamine in the forced swim and tail suspension tests (Bechtholt-

Gompf et al., 2011; Lindholm et al., 2011; Popik et al., 2008) or have only assessed the acute antidepressant-effects of ketamine (Engin et al., 2009; Li et al., 2010; Yang et al., 2012). In contrast, studies have shown that a single administration of ketamine can produce rapid and prolonged effects in preclinical models. For example, Li et al. (2011) found that the same dose of ketamine (10.0 mg/kg) used in the present study produced rapid reversal of deficits in anhedonia-like behaviors following 21 days of chronic unpredictable stress in rats, while chronic treatment is usually required for clinically efficacious antidepressant drugs. These ketamine effects persisted for 7 days post-injection. Moreover, a number of studies have shown that the antidepressant-like effects of ketamine can persist for up to a week in the forced swim test (Maeng et al., 2008; Autry et al., 2011; Reus et al., 2011), tail suspension test (Koike et al., 2011) and learned helplessness procedure (Maeng et al, 2008). It is important to note that there several dose, species, and procedural differences across these studies that either found or failed to find prolonged antidepressant-like effects with ketamine treatment and these differences may account for the discrepant results.

Glutamatergic ligands have shown mixed results in the DRL procedure. For example, the high-affinity noncompetitive NMDA receptor antagonist MK-801 produced psychostimulant-like effects (Ardayfio et al., 2008; and in the present study), the mGluR2/3 antagonist LY-341495 had no effect on responding (Bespalov et al., 2008), and the mGluR5 antagonist MTEP produced antidepressant-like effects (Molina-Hernandez et al., 2006). In the present study, all of the glutamatergic drugs produced effects that changed during the test sessions. Ketamine and NMDA produced similar effects by increasing reinforcers throughout the test sessions, while the reduction in responses diminished over time. Interestingly, an ineffective dose of ketamine (3.0 mg/kg) significantly attenuated the antidepressant-like effects of NMDA (see Figure 5), rather

than produce an additive effect as might have been predicted. Furthermore, NMDA (10.0 and 30.0 mg/kg) failed to enhance (or block) the antidepressant-like effects of ketamine (see Figure 4). One possible explanation for these findings is the effects of these drugs at the NMDA receptor, specifically related to pharmacological action (agonist versus antagonist) and receptor binding affinities/site location. For example, ketamine's (K_i = 1,190 nM) binding affinity for the NMDA receptor is almost 3-fold higher compared to NMDA (K_i = 3,900 nM) (Bresink et al., 1995; Sakai et al., 2001), which would account for the ability for ketamine to block the behavioral effects of NMDA and the inability of NMDA to block the behavioral effects of ketamine. Furthermore, ketamine is a channel blocker that binds to the phencyclidine (PCP) site inside the ion channel of the NMDA receptor (Bresink et al., 1995); whereas, NMDA binds to the competitive glutamate site on the NMDA receptor (Sakai et al., 2001). Therefore, both of these drugs can bind to the NMDA receptor simultaneously as they are not competing for the same receptor site and, as a result, the pharmacological effects of ketamine could attenuate the effects of NMDA by blocking ions from entering the cell through the ion channel of the NMDA receptor.

Ketamine has been shown to produce a significant increase in glutamate release in the prefrontal cortex at doses (10.0-30.0 mg/kg) that produce an antidepressant-like effect in a variety of preclinical behavioral models, including the current DRL study (Moghaddam et al., 1997; Lorrain et al., 2003;). Additionally, the glutamate dysfunction associated with MDD appears to be bidirectional with evidence suggesting that there is a reduction of glutamatergic activity. For example, researchers have found significantly lower concentrations of glutamate and glycine in cerebral spinal fluid (Frye et al., 2007) and decreased concentrations of glutamine and glycine in plasma (Altamura et al., 1993; Altamura et al., 1995). Moreover, the anterior

cingulate cortex has been shown to have reduced glutamate and glutamine in adolescents (Mirza et al., 2004) and adults (Auer et al., 2000) with severe depression. Preclinical data have shown that the glycine-site partial agonist GLYX-13 produces antidepressant-like effects in the forced swim test, novelty-induced hypophagia, and learned helplessness tests in rats (Burgdorf et al., 2013). Furthermore, during clinical trials GLYX-13 produced rapid (~24 hours) and extended (~7 days) antidepressant effect in treatment-resistant patients suffering from MDD (Moskal et al., 2014). GLYX-13 is currently undergoing Phase II clinical trials (see clinicaltrials.gov at http://www.clinicaltrials.gov/ct2/show/NCT01684163). Both clinical and preclinical findings, suggest that some depressed patients may benefit from increasing glutamatergic activity.

Interestingly, the two NMDA receptor antagonists, ketamine and MK-801, produced dissociable effects in the DRL 72 sec procedure with ketamine producing an antidepressant-like effect and MK-801 producing a psychostimulant-like effect. The 10.0 mg/kg dose of ketamine was found to increase reinforcers across all time bins, while responses were decreased in the first two time bins, which corresponded with a rightward shift in the IRT distributions. Conversely, the more selective NMDA receptor antagonist MK-801 (0.05 and 0.1 mg/kg) produced a decrease in reinforcers across all time bins. Additionally, the 0.1 mg/kg MK-801 dose increased responses and produced a leftward shift in the peak location IRT distribution across all time bins. This finding is consistent with a previous report that found that MK-801 disrupted DRL 72 sec performance (Ardayfio et al., 2008). The present study is not the first to show behavioral differences between the NMDA receptor antagonists ketamine and MK-801. For example, at the doses tested in the DRL procedure MK-801 produces hyperactivity in the open field locomotor behavioral assay (Dravid et al., 2007; Engin et al., 2009; Wegener et al., 2011), while ketamine does not alter locomotor activity (Engin et al., 2009; Koike et al., 2011; Réus et al., 2011; Yang

et al., 2012). Furthermore, Autry et al. (2011) found that both ketamine and MK-801 produced antidepressant-like effects in the forced swim test; however, the antidepressant-like effect of ketamine lasted for one week, while the antidepressant-like effect of MK-801 lasted only 3 hours. Also, the mice treated with MK-801 in that study had nearly no time spent immobile - an effect that may be attributed to MK-801's hyperactivity effects and should be considered a false positive response in the forced swim test.

The behavioral differences between ketamine and MK-801 may be a consequence of differences in these two drugs' receptor binding affinities and receptor selectivity (see Table 5). Compared to MK-801, ketamine is 260 to 500-fold less selective for the PCP binding site on NMDA ion channels depending on the brain region (Bresink et al., 1995; Tikhonova et al., 2004). Seeman et al. (2005) found similar results (183 fold difference) when comparing binding affinities in human cloned dopamine D_2 receptors with ketamine having a significantly lower binding affinity as compared to MK-801. Furthermore, ketamine has modest selectivity for the NMDA receptor over other receptors. For example, ketamine has receptor affinities for norepinephrine, dopamine, and serotonin transporters (Nishimura et al., 1998), opiate receptors (i.e. mu, delta, and kappa), sigma receptors (Smith et al., 1987), and muscarinic receptors (Hirota et al. 2002). This modest receptor selectivity and dissociable behavioral difference compared to the higher affinity and more selective NMDA receptor antagonists MK-801 suggests that ketamine is producing pharmacological effects on other receptors at doses that affect NMDA receptors (see table 5 for binding affinities). While many studies (for example, Autry et al., 2011; Li et al., 2010; Li et al., 2011) are evaluating the glutamatergic (e.g. AMPA and NMDA) or downstream (e.g. the mammalian target of rapamycin) mechanisms underlying ketamine's antidepressant effects, it is also important to evaluate non-glutamatergic mechanisms. For

example, Yang et al. (2012) found that the weak mu opiate receptor agonist tramadol increased the antidepressant-like effects of ketamine in the forced swim test, which may indicate the role of mu receptor agonism in the antidepressant effects of ketamine.

In conclusion, the results from the experiment 1 add to the growing body of literature that ketamine produces antidepressant-like effects preclinically. Further, our findings indicate that the underlying mechanisms responsible for the clinical antidepressant effects of ketamine may not be driven solely through NMDA receptor antagonism. The more selective and higher affinity NMDA receptor antagonist MK-801 failed to produce an antidepressant-like profile in the DRL 72 sec procedure, suggesting that the diverse pharmacological profile of ketamine may play a significant role in its antidepressant effects. Additionally, we have shown that the DRL 72 sec model is appropriate for assessing the antidepressant-like effects of ketamine and can be used to assess the underlying receptor mechanisms responsible for its antidepressant effects. Further studies are needed to evaluate specific glutamatergic and non-glutamatergic mechanisms responsible for these effects using the DRL 72 sec assay, as it appears to be more sensitive to the false positives of psychostimulants seen in the force swim test (i.e. MK-801).

Experiment 2: Effects of Acute and Repeated Ketamine on Intracranial Self-Stimulation in Rats

Rationale

Experiment 2 evaluated the dose-effect and time-course of effects of ketamine, MK-801, and phencyclidine using on ICSS using a frequency-rate procedure. Additionally, follow-up studies were conducted to examine effects of repeated ketamine dosing using a testing strategy that produced tolerance to ICSS rate-decreasing effects and enhanced expression of ICSS rate-

increasing effects with mu opioid receptor agonists (Altarifi and Negus, 2011; Altarifi et al. 2013).

Experiment 2 Methods

Subjects

Eleven adult male Sprague-Dawley rats (Harlan Laboratories Inc, Frederick, MD) weighing between 300 and 350 grams at the start of the experiment were used in this study. All animals were housed individually in plastic cages (32.4 x 19.7 x 19.7 cm) in the vivarium with a 12-hr/12-hr light/dark cycle (lights on at 0600 hr) and all training and testing sessions were conducted during the light portion of the cycle (0900 to 1600 hr). All rats in the ICSS experiments had free access to food and water expect during experimental sessions. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University (IACUC Protocol AD2002) and conducted in accordance with National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animals Resources, 2011).

Surgery

Surgeries were only performed on rats in the ICSS experiments. Rats were anesthetized with isoflurane gas (2.5–3% in oxygen; Webster Veterinary, Phoenix, Arizona, USA) for the implantation of stainless-steel electrodes. The cathode of each bipolar electrode (Plastics One, Roanoke, Virginia, USA) was 0.25 mm in diameter and covered with polyamide insulation, except at the flattened tip. The anode was 0.124 mm in diameter and uninsulated. The cathode was implanted in the left medial forebrain bundle at the level of the lateral hypothalamus (2.8 mm posterior and 1.7 mm lateral from the bregma, and 8.8 mm below the skull). The anode was wrapped around one of three skull screws to serve as the ground, and the skull screws and

electrode assembly were secured with orthodontic resin. Rats received ketoprofen (5 mg/kg i.p. for 2 days) as a postoperative analgesic and were allowed to recover for at least 7 days before commencing ICSS training.

Apparatus

ICSS experiments were conducted in twelve identical sound-attenuating chambers that contained operant conditioning chambers (29.2 x 30.5 x 24.1 cm; Model ENV-007-VP-CT, Med Associates) equipped with a single response lever (4.5 cm wide, extended 2.0 cm through the center of one wall, 3 cm off the floor), stimulus lights (three lights colored red, yellow, and green positioned 7.6 cm directly above the lever), a house light, and an ICSS stimulator (Model SG-510; Med Associates). Electrodes were connected to the stimulator through bipolar cables and a commutator (Model SL2C, Plastics One). Programming of behavioral sessions and data collection were computer controlled by Med-State software (Med PC, Version 4.1, Med-Associates) running on a Windows XP operating system.

Drugs

The NMDA receptor antagonists (±) ketamine HCl (Sigma Aldrich), (+) MK-801 hydrogen maleate (dizocilpine) (Sigma Aldrich) and phencyclidine, and the monoamine reuptake inhibitor cocaine HCl (provided by National Institute on Drug Abuse, National Institute of Health, Bethesda, MD) were dissolved in 0.9% physiological saline. All drugs were administered intraperitoneally at a volume of 1.0 ml/kg. Testing of each drug was completed before initiating testing with another drug. Doses and pretreatment times were based on preliminary studies and previous studies in the literature (Carlezon and Wise, 1993; Corbett, 1989; Herberg and Rose, 1989; Páleníček et al., 2011). Drugs, doses, and pretreatment times were as follows: ketamine (3.2-18.0 mg/kg; 10 min), MK-801 (0.032-0.32 mg/kg; 15 min), phencyclidine (0.32-10.0

mg/kg; 30 min), cocaine (10.0 mg/kg; 10 min). Dose order across rats was counterbalanced using a Latin-square design.

Training Procedures

ICSS training. Rats were trained to press a lever under a FR 1 schedule of brain stimulation using procedures similar to those described previously to study effects of mu/kappa/delta opioid receptor agonists (Altarifi and Negus, 2011; Altarifi et al., 2013; Negus et al., 2010), dopamine/norepinephrine/serotonin releasers and uptake inhibitors (Bauer et al., 2013a,b; Bonano et al., 2013; Rosenberg et al., 2013) and cannabinoids (Kwilasz and Negus, 2012). During initial training sessions, which lasted 30-60 min, the house light was illuminated, and each lever press produced electrical stimulation (0.5 s train of 0.1 ms square-wave cathodal pulses) and illumination for 0.5 s of the colored stimulus lights over the lever. Responses during the 0.5 s stimulation period did not produce additional stimulation. Initially, the frequency of stimulation was held constant at 158 Hz, and the stimulation intensity for each rat was adjusted gradually to the lowest value that would sustain a high rate of ICSS (≥30 stimulations/min). Frequency manipulations were then introduced, and the terminal schedule consisted of sequential 10-min components. During each component, a descending series of 10 brain-stimulation frequencies was presented, with a 60 s trial at each frequency. The frequency range extended from 158 to 56 Hz in 0.05-log increments, and stimulation intensity was individually determined during training and remained constant for each rat (range: 100-260 μA). Each frequency trial began with a 10 s timeout, during which the house light was off and responding had no scheduled consequences. During the last 5 s of this timeout, five noncontingent stimulations were delivered once per second at the frequency available during that trial, and the lever lights were illuminated during each stimulation. This noncontingent stimulation was then followed by a

50 s “response” period, during which the house light was illuminated, and each lever press produced electrical stimulation (0.5 s train of 0.1 ms square-wave cathodal pulses) and illumination for 0.5 s of the colored stimulus lights over the lever. Training continued until rats reliably responded at rates ≥50% maximum control rates (see Data Analysis) for at least three and no more than six trials of all components for at least three consecutive days. Additionally, rats were habituated to saline injections until these injections had significant no effect on ICSS frequency-rate curves as determined by two-way analysis of variance (see Data Analysis).

Testing Procedures

Dose-effect experiments. Drug-naïve rats at the start of the experiment were used for dose effect and time course studies. ICSS test sessions for dose-effect testing consisted of five sequential components. The first component of each test session was considered an acclimation component, and data from this component were discarded. Data from the second and third “baseline” components were used to calculate control parameters of frequency-rate curves for that test session in that rat (see Data Analysis). Immediately after completion of the baseline components, a dose of test drug was administered intraperitoneally (i.p.), and after the designated pretreatment time, ICSS was evaluated during two test components (10 min each, 20 min total).

Time course experiments. Additional studies also were conducted to investigate the time course of effects. For these studies, baseline components were conducted as above. Rats were then immediately injected with a dose of test drug, and pairs of ICSS test components was initiated 10, 30, 100, and 300 min after the injection. Testing was conducted twice per week (typically Tuesday and Friday). For both dose-effect and time-course experiments, testing was initiated with different drugs in different rats and dose order was counterbalanced using a Latin-square design. If ICSS performance remained stable in a given rat after completion of testing

with the initial drug, then the rat was advanced to testing with other drugs or time course. Testing continued until each drug had been tested in groups of 5-7 rats.

Repeated ketamine experiment. Initial dose effect and time course studies with acute ketamine failed to reveal ketamine-induced increases in rates of ICSS maintained by any brain stimulation frequency at any time. Consequently, follow-up studies were conducted in a drug-naïve group of 6 rats to examine effects of repeated dosing using a testing strategy that produced tolerance to ICSS rate-decreasing effects and enhanced expression of ICSS rate-increasing effects with mu opioid receptor agonists (Altarifi and Negus, 2011; Altarifi et al., 2013). Training was conducted as described above, and once training and habituation to saline injections were completed, "pre-drug baseline" sessions were conducted over a period of three consecutive days to establish baseline ICSS performance before administration of ketamine. Each pre-drug baseline session consisted of 3 components as described above. Testing proceeded over a period of 26 days, with ICSS assessment beginning at 1600 hr each day (Figure 6). On Days 0, 7, 14 and 21, ketamine was administered using a cumulative dosing regimen. Specifically, test sessions consisted of three "baseline" ICSS components as described above, followed by cumulative administration of ketamine at 30 min intervals, such that each sequential ketamine dose increased the total cumulative dose by 0.25 log units. A pair of ICSS test components was initiated 10 min after each sequential ketamine dose. The ketamine dose range was 3.2-10 mg/kg on Day 0 and 3.2-18 mg/kg on Days 7, 14, and 21. Ketamine was also administered on intervening days as follows: Days 1-6, 3.2 mg/kg/day; Days 8-13, 10 mg/kg/day; Days 15-20, 10 mg/kg twice per day (0900 hr and 1600 hr). On these intervening days, three baseline ICSS components were conducted, the daily ketamine dose was administered, and two test ICSS components were conducted beginning 10 min after ketamine.

On Days 15-20, when 10 mg/kg ketamine was administered twice per day, one ketamine dose was administered at 0900 hr without ICSS, and the second ketamine dose was administered at 1600 hr in the context of ICSS. On day 22, ketamine was not administered in the morning, and the time course of effects produced by 18 mg/kg/day ketamine was determined at 0900 hr using the time-course testing procedure described above. Ketamine treatment and ICSS testing were omitted on days 23 and 24. On day 25, effects of 10 mg/kg cocaine were tested as a positive control for drug-induced increases in ICSS. Specifically, three baseline components were conducted as described above, followed first by i.p. administration of 10 mg/kg cocaine and then 10 min later by two ICSS test components.

Statistical Analysis

The primary dependent variable was the reinforcement rate in stimulations/trial during each frequency trial. To normalize these raw data, reinforcement rates from each trial were converted into the percent maximum control rate (% MCR). For dose-effect and time course testing, the MCR was determined during the baseline components of each daily test session and was defined as the mean of the maximal simulation rates observed in any frequency trial during the second and third baseline components. Thus, % MCR for each trial was calculated as (reinforcement rate during a frequency trial / MCR) x 100. Normalized data from the frequency trials of consecutive test components were then averaged across rats for display and for statistical analysis using two-way repeated measures analysis of variance (ANOVA), with drug dose or time as one factor and ICSS frequency as the other factor. A significant ANOVA was followed by a Holm–Sidak post-hoc test, and the criterion for significance was set at $p < 0.05$. To provide an additional summary of ICSS performance, the total number of stimulations delivered across all 10 frequency trials was determined for each component. The average number of total

stimulations per test component was expressed as a percentage of the average number of total stimulations per component during the second and third baseline components (% baseline).

Data from the study of repeated ketamine experiment were analyzed using a similar approach, with the exception that baseline MCR and total stimulations were calculated from the three-day pre-drug baseline components conducted before any ketamine administration (six total pre-drug baseline components). Frequency-rate curves for each cumulative dose-effect were examined by two-way repeated measures ANOVA with dose and frequency as the two factors. In addition, baseline frequency-rate curves collected on Day 0 before initiation of ketamine dosing were compared to baseline frequency-rate curves from Days 7, 14 and 21 to assess effects of repeated ketamine exposure and ketamine withdrawal. Specifically, baseline ICSS determinations on Days 7 and 14 represented 24 h withdrawal periods from the most recent dose of six-day treatment with 3.2, and 10 mg/kg/day ketamine, respectively; baseline ICSS determination on Day 21 represented a 7 h withdrawal period from the most recent dose of six-day treatment with 20 mg/kg/day.

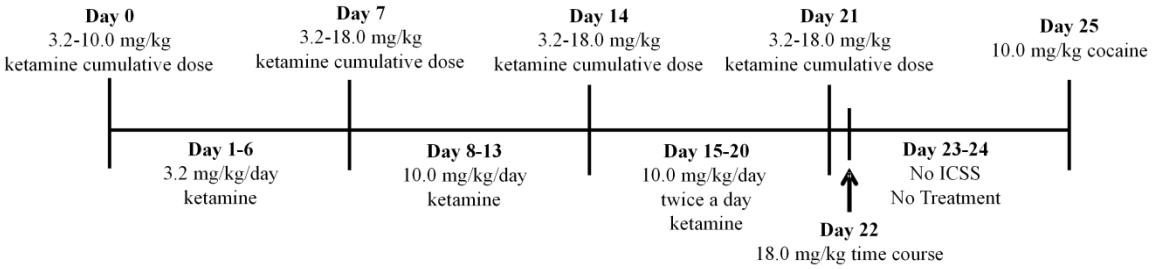

Figure 6. Shows the timeline of events for the repeated ketamine experiment. On days 0, 7, 14, and 21 ketamine was administered using a cumulative dosing regimen. For cumulative dosing, three baseline ICSS components were conducted, followed by cumulative administration of ketamine (0.25 log unit increases) and a pair of ICSS test components were conducted 10 min after each sequential ketamine dose. Ketamine was also administered on intervening days in the same manner as dose effect test days. On day 22, the time course of effects for ketamine 18.0 mg/kg was determined. On day 23 and 24, ketamine treatment and ICSS testing were omitted. On day 25, the effects of 10.0 mg/kg cocaine was determined as a positive control.

Experiment 2 Results

Baseline performance. For the 11 rats used in dose-effect and time-course studies, the mean ± SEM maximum control rate (MCR) was 56.64 ± 1.92 stimulations per trial, and the mean total stimulations per component delivered across all frequencies was 253.45 ± 14.55.

Ketamine. Figure 7 shows the effects of ketamine, MK-801, and phencyclidine on ICSS. After saline treatment, increasing frequencies of brain stimulation maintained increasing rates of ICSS. Ketamine dose-dependently decreased ICSS. Ketamine produced a significant main effect of frequency ($F[9,54] = 75.06$, $p < 0.001$), dose ($F[3,18] = 15.40$, $p < 0.001$) and significant interaction ($F[27,162] = 3.88$, $p < 0.001$). The lowest dose of ketamine, 3.2 mg/kg, exerted no effect on ICSS, but 5.6 mg/kg ketamine decreased ICSS at three intermediate frequencies (112-141 Hz), and 10.0 mg/kg ketamine significantly decreased ICSS at the six highest frequencies (79-158 Hz) (Figure 7a,b).

MK-801. MK-801 produced mixed rate-increasing and rate-decreasing effects that depended on dose and brain stimulation frequency. MK-801 produced a significant main effect of frequency ($F[9,45] = 39.45$, $p < 0.001$), dose ($F[4,20] = 19.78$, $p < 0.001$) and significant interaction ($F[36,180] = 7.58$, $p < 0.001$). Thus, 0.032 mg/kg MK-801 did not alter ICSS, 0.1 mg/kg MK-801 increased ICSS at one intermediate frequency (89 Hz), and 0.18 mg/kg MK-801 produced a biphasic effect, increasing ICSS at low and intermediate frequencies (63-100 Hz) but decreasing ICSS at the highest frequencies (141-158 Hz). The high dose of MK-801, 0.32 mg/kg, significantly depressed ICSS at the five highest frequencies (100-158 Hz) (Figure 7c,d).

Phencyclidine. Phencyclidine produced both rate-increasing and rate-decreasing effects that depended on dose, time, and brain stimulation frequency. Phencyclidine produced a significant main effect of frequency ($F[9,45] = 71.67$, $p < 0.001$), dose ($F[3,15] = 24.91$, $p <$

0.001) and significant interaction (F[27,135] = 6.34, $p < 0.001$). Doses of 1.0 and 5.6 mg/kg phencyclidine exerted no effect on ICSS in the dose-effect study. The intermediate dose of phencyclidine, 3.2 mg/kg, significantly increased ICSS at one frequency (100 Hz); whereas, the high dose of phencyclidine, 10.0 mg/kg, significantly decreased ICSS at the five highest frequencies (100-158 Hz) (Figure 7e,f).

Ketamine time course. Figure 8 shows the time course of effects produced by 5.6 and 10 mg/kg ketamine. Ketamine (5.6 mg/kg) produced a significant main effect of frequency (F[9,45] = 42.43, $p < 0.001$), time (F[4,20] = 5.15, $p = 0.005$) and no significant interaction (F[36,180] = 1.28, $p = 0.15$). Treatment with 5.6 mg/kg ketamine had modest effects manifested as small but significant decreases in ICSS after 10 min at 71 Hz, 30 min at 71 and 126 Hz, and 100 min at 71-79 Hz (Figure 8a,b). Ketamine (10.0 mg/kg) produced a significant main effect of frequency (F[9,54] = 97.14, $p < 0.001$), time (F[4,24] = 11.44, $p < 0.001$) and significant interaction (F[36,216] = 3.27, $p < 0.001$). Treatment with 10.0 mg/kg ketamine produced greater decreases in ICSS across a broader range of frequencies at 10, 30 and 100 min (Figure 8c,d). Neither dose produced significant effects on ICSS after 300 min. ICSS was not increased at any frequency or any time for either ketamine dose.

MK-801 time course. Figure 9 shows the time course of effects produced by 0.18 and 0.32 mg/kg MK-801. MK-801 (0.18 mg/kg) produced a significant main effect of frequency (F[9,36] = 38.33, $p < 0.001$), time (F[4,16] = 11.50, $p < 0.001$) and no significant interaction (F[36,144] = 1.64, $p = 0.023$). Treatment with 0.18 mg/kg MK-801 significantly increased reinforcement rates at 10, 30, and 100 min across a broad range of brain stimulation frequencies (63-126 Hz) (Figure 9a,b); this MK-801 dose did not produce rate-decreasing effects at any time in the time-course study. MK-801 (0.32 mg/kg) produced a significant main effect of frequency

(F[9,45] = 17.47, p < 0.001), time (F[4,20] = 3.90, p = 0.016) and significant interaction (F[36,180] = 7.20, p < 0.001). Conversely, a higher dose of 0.32 mg/kg MK-801 depressed ICSS after 10 and 30 min at high frequencies (100-156 Hz). At 100 min, 0.32 mg/kg MK-801 produced a biphasic effect, increasing reinforcement rats at 63 Hz, but decreasing reinforcement rate at the highest frequencies (126-158). At 300 min, ICSS was still significantly depressed at 124-141 Hz (Figure 9c,d).

Phencyclidine time course. Figure 10 shows the time course of effects produced by 10.0 mg/kg phencyclidine. Phencyclidine (10.0 mg/kg) produced a significant main effect of frequency (F[9,45] = 54.25, p < 0.001), time (F[4,20] = 12.20, p < 0.001) and a significant interaction (F[36,180] = 9.83, p < 0.001). Treatment with 10.0 mg/kg phencyclidine produced a significant decrease on ICSS rates at 10 and 30 min across a range of intermediate and high frequencies (89-158 Hz); however, 10.0 mg/kg phencyclidine produced a significant increase on ICSS rates at 100 (56-71 and 100 Hz) and 300 (63 Hz) min (Figure 10a,b).

Repeated ketamine on cumulative dose-effect curves. For the six rats used in the repeated dosing ketamine experiment, the mean ± SEM maximum control rate (MCR) during pre-drug baseline sessions was 57.00 ± 3.33 stimulations per trial, and the mean total stimulations per component delivered across all frequencies was 276.75 ± 14.59. Under pre-drug baseline conditions (i.e. before any ketamine administration), brain stimulation maintained a frequency-dependent increase in ICSS rates. Figure 11 shows mean frequency-rate ICSS curves for the pre-drug baseline determination before initiation of repeated ketamine treatment and before ketamine testing on Days 0, 7, 14, and 21 of repeated ketamine treatment. Daily baseline ICSS frequency-rate curves were not significantly affected by exposure to and withdrawal from repeated ketamine: Significant main effect of frequency (F[9,45] = 75.73, p < 0.001), no

significant effect of day (F[4,20] = 1.76, p = 0.176) and no significant interaction (F[36,180] = 1.29, p = 0.144).

Figure 12 shows the effects of cumulative ketamine on ICSS on Days 0, 7, 14, and 21 of repeated daily ketamine treatment. On Day 0, before initiation of repeated ketamine, cumulative ketamine (3.2-10.0 mg/kg) produced a dose-dependent decrease in ICSS (Figure 12a,b). Specifically, 3.2 mg/kg ketamine did not alter ICSS, 5.6 mg/kg ketamine decreased ICSS at frequencies of 89, 100 and 126 Hz, and 10.0 mg/kg ketamine decreased ICSS at 89-141 Hz. Following repeated 3.2 mg/kg/day ketamine, cumulative doses of 5.6 and 10 mg/kg ketamine decreased ICSS at fewer frequencies than initially, and a higher dose of 18 mg/kg ketamine was introduced, which decreased ICSS at 100-158 Hz (Figure 12c,d). Following repeated 10 mg/kg/day ketamine (Figure 12e,f) and 20 mg/kg/day ketamine (Figure 12g,h), ICSS was not altered by any cumulative dose of ketamine (3.2-18 mg/kg). Thus, during repeated ketamine treatment, tolerance developed to the rate-decreasing effects of ketamine, but ICSS was not increased by any ketamine dose at any frequency at any time. Statistical results for repeated ketamine are as follows: Drug Naïve (Day 0): Significant main effect of frequency (F[9,45] = 70.40, p < 0.001), dose (F[3,15] = 3.41, p = 0.045) and no significant interaction (F[27,135] = 1.36, p = 0.127). Repeated 3.2 mg/kg/day (Day 7): Significant main effect of frequency (F[9,45] = 52.31, p < 0.001), dose (F[4,20] = 12.51, p < 0.001) and significant interaction (F[36,180] = 2.38, p < 0.001). Repeated 10.0 mg/kg/day (Day 14): Significant main effect of frequency (F[9,45] = 72.65, p < 0.001), no significant effect of dose (F[4,20] = 2.31, p = 0.093) and no significant interaction (F[36,180] = 1.25, p = 0.173). Repeated 10.0 mg/kg/dayx2 (Day 21): Significant main effect of frequency (F[9,45] = 106.30, p < 0.001), no significant effect of dose (F[4,20] = 0.79, p = 0.546) and no significant interaction (F[36,180] = 0.85, p = 0.717).

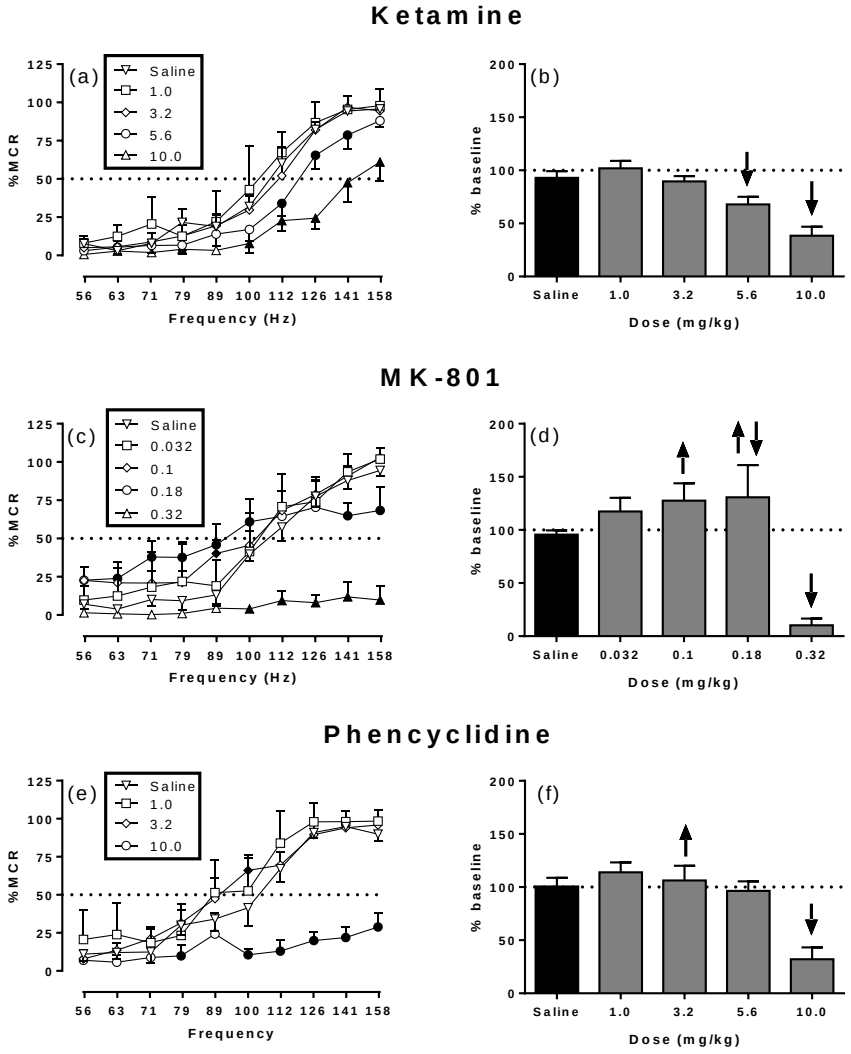

Figure 7. Dose-dependent effect of ketamine, MK-801, and phencyclidine on ICSS. Left panels (a, c, e) show drug effects on full ICSS frequency-rate curves. Abscissae: Frequency of electrical brain stimulation (Hz) (log scale). Ordinates: Percent maximum control reinforcement rate (%MCR). Drug name and doses are indicated in legends. Filled points represent frequencies at which ICSS rates after drug treatment were significantly different from vehicle rates as determined by a two-way ANOVA followed by a Holm-Sidak post hoc test, $p < 0.05$. Right panels (b, d, f) show summary ICSS data expressed as percent baseline total stimulations delivered across all frequencies of brain stimulation per test component. Abscissae: Drug dose (mg/kg). Ordinates: Percent baseline stimulations per test component. Upward/downward arrows indicate significant drug-induced increase/decrease in ICSS relative to vehicle for at least one brain stimulation frequency as determined by analysis of full frequency-rate curves in the left panels. All data show mean ± SEM for six to seven rats (ketamine, n = 7; MK-801, n = 6; phencyclidine, n = 6), except for 1.0 mg/kg ketamine data shown for five rats.

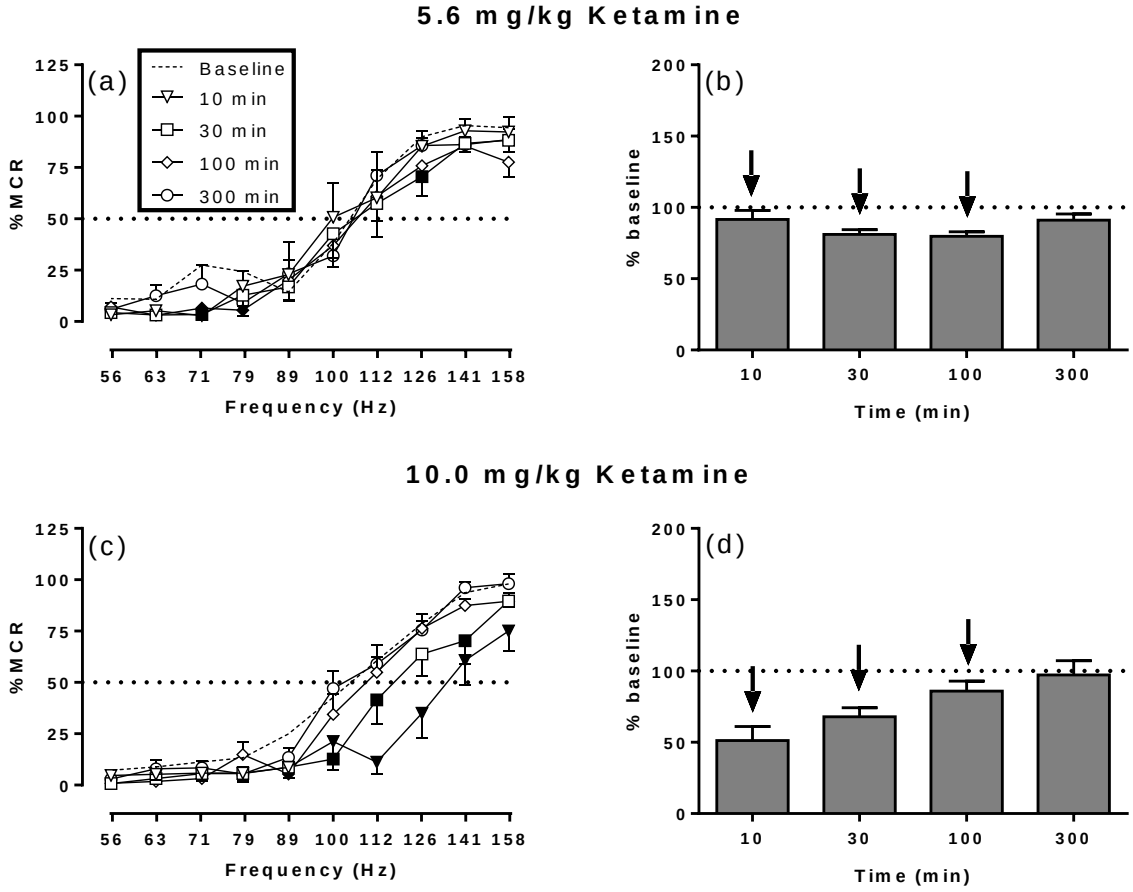

Figure 8. Time course of effects produced by 5.6 and 10.0 mg/kg ketamine. Left panels (a, c) show drug effects on full ICSS frequency-rate curves. Filled points represent frequencies at which ICSS rates after drug treatment were significantly different from baseline rates as determined by a two-way ANOVA followed by a Holm-Sidak post hoc test, $p < 0.05$. Right panels (b, d) show summary ICSS data expressed as percent baseline total stimulations delivered across all frequencies of brain stimulation per test component. Abscissae: Time (min). Ordinates: Percent baseline stimulations per test component. Other details as in Figure 7. All data show mean ± SEM for six to seven rats (5.6 mg/kg, n = 6; 10.0 mg/kg, n = 7).

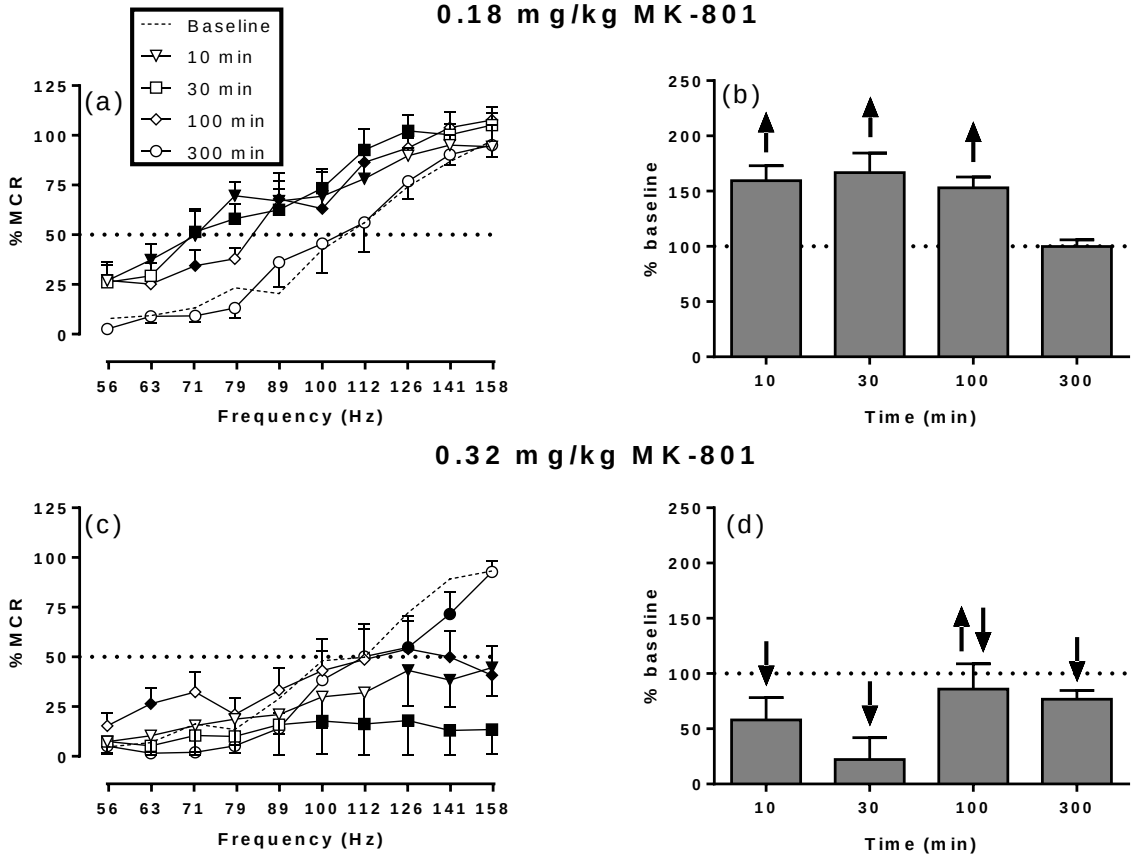

Figure 9. Time course of effects produced by 0.18 and 0.32 mg/kg MK-801. Left panels (a, c) show drug effects on full ICSS frequency-rate curves. Filled points represent frequencies at which ICSS rates after drug treatment were significantly different from baseline rates as determined by a two-way ANOVA followed by a Holm-Sidak post hoc test, $p < 0.05$. Right panels (b, d) show summary ICSS data expressed as percent baseline total stimulations delivered across all frequencies of brain stimulation per test component. Other details as in Figures 7 and 8. All data show mean ± SEM for five to six rats (0.18 mg/kg, n = 5; 0.32 mg/kg, n = 6).

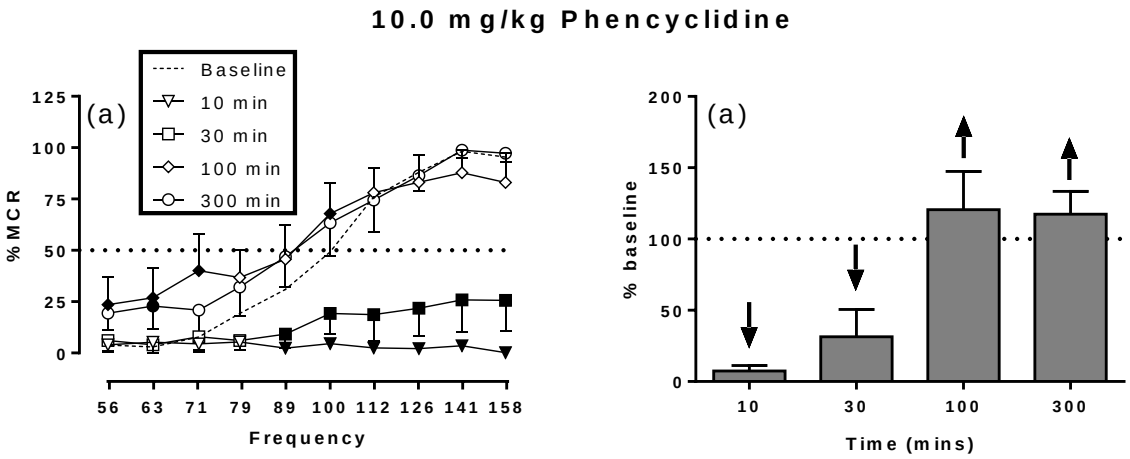

Figure 10. Time course of effects produced by 10.0 mg/kg phencyclidine. Left panels (a) show drug effects on full ICSS frequency-rate curves. Filled points represent frequencies at which ICSS rates after drug treatment were significantly different from baseline rates as determined by a two-way ANOVA followed by a Holm-Sidak post hoc test, $p < 0.05$. Right panels (b) show summary ICSS data expressed as percent baseline total stimulations delivered across all frequencies of brain stimulation per test component. Other details as in Figures 7 and 8. All data show mean ± SEM for five to six rats.

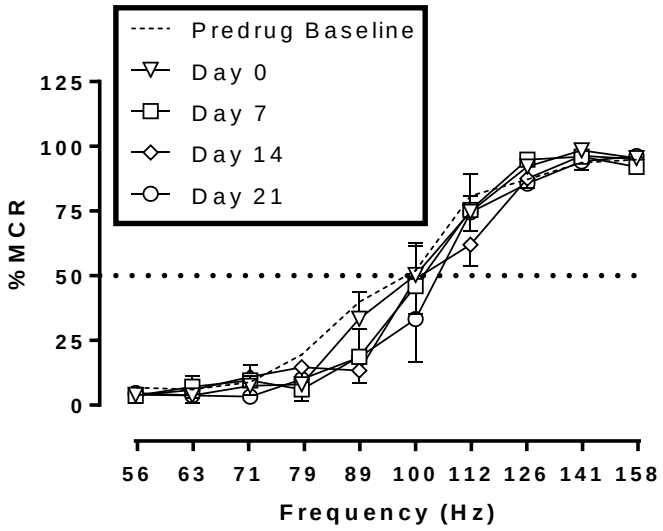

Figure 11. Baseline ICSS performance before initiation of repeated ketamine (Pre-drug Baseline) and on Days 0, 7, 14, and 21 of repeated ketamine treatment. Data for Days 7 and 14 were determined 23 h after the most recent ketamine dose. Data for Day 21 were collected 7 h after the most recent ketamine dose. Filled points represent frequencies at which ICSS rates after drug treatment were significantly different from baseline rates as determined by a two-way ANOVA followed by a Holm-Sidak post hoc test, $p < 0.05$. Other details as in Figure 7. All data show mean ± SEM for six rats.

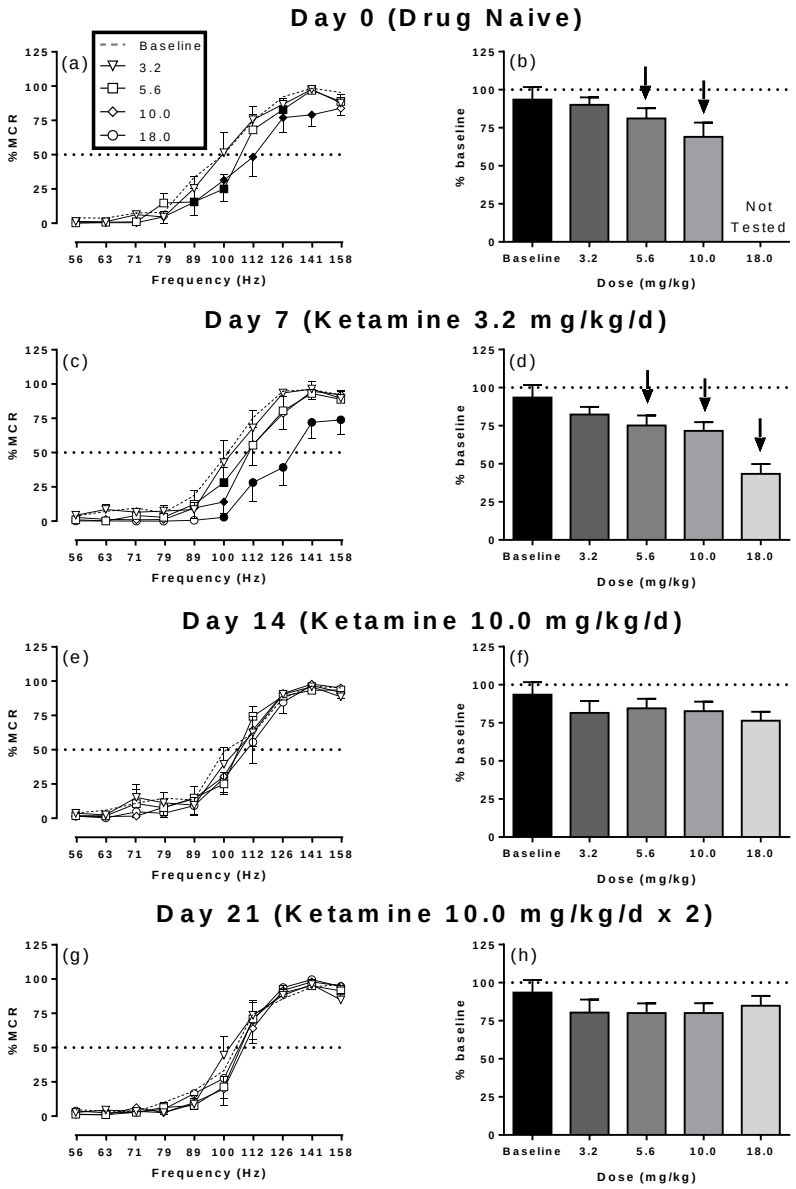

Figure 12. Effects of repeated ketamine on cumulative ketamine dose-effect curves. Cumulative ketamine dose-effect curves were determined before repeated daily ketamine administration (a, b), following six-days of 3.2 mg/kg/day ketamine (c, d), following six days of 10.0 mg/kg/day (e, f), and following six-days of 10.0 mg/kg/day x 2 (twice a day, totaling 20.0 mg/kg/day) (g, h). Left panels (a, c, e, g) show drug effects on full ICSS frequency-rate curves. Filled points represent frequencies at which ICSS rates after drug treatment were significantly different from baseline rates as determined by a two-way ANOVA followed by a Holm-Sidak post hoc test, $p < 0.05$. Right panels (b, d, f, h) show summary ICSS data expressed as percent baseline total stimulations delivered across all frequencies of brain stimulation per test component. Other details as in Figure 7. All data show mean ± SEM for six rats.

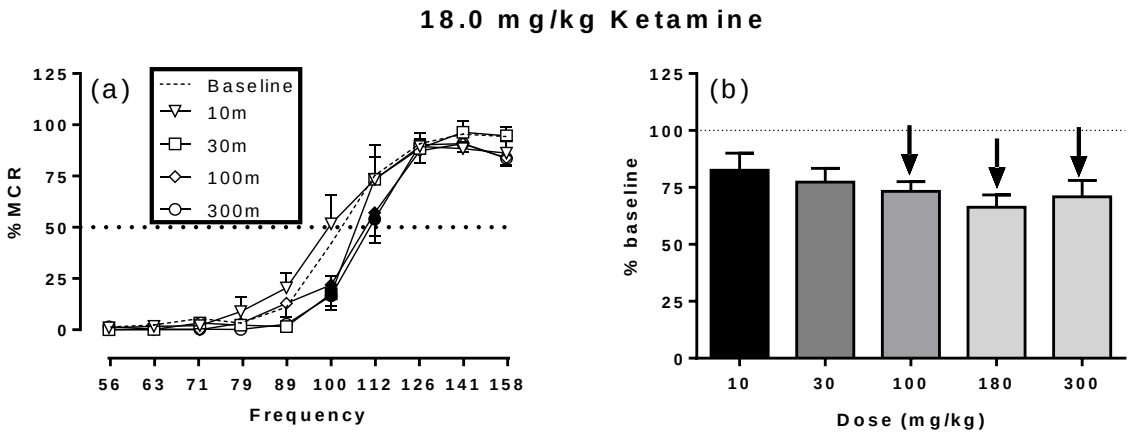

Figure 13. Time course of effects produced by 18.0 mg/kg ketamine during repeated ketamine administration on day 22. Left panels (a) show drug effects on full ICSS frequency-rate curves. Filled points represent frequencies at which ICSS rates after drug treatment were significantly different from baseline rates as determined by a two-way ANOVA followed by a Holm-Sidak post hoc test, $p < 0.05$. Right panels (b) show summary ICSS data expressed as percent baseline total stimulations delivered across all frequencies of brain stimulation per test component. Other details as in Figures 7 and 8. All data show mean ± SEM for five to six rats.

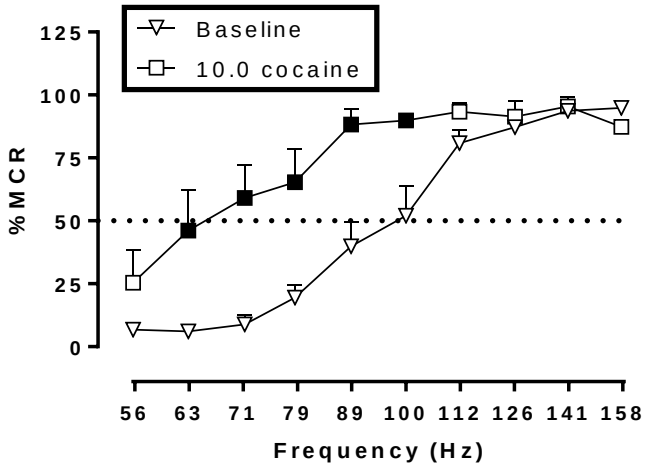

Figure 14. Effects of 10.0 mg/kg cocaine following the termination of repeated ketamine on Day 25. The figure shows drug effects on full ICSS frequency-rate curves. Filled points represent frequencies at which ICSS rates after drug treatment were significantly different from baseline rates as determined by a two-way ANOVA followed by a Holm-Sidak post hoc test, $p < 0.05$. Other details as in Figures 7. All data show mean ± SEM for six rats.

On day 22, the time-course of effects produced by a bolus dose of 18.0 mg/kg ketamine was determined. Treatment with 18.0 ketamine produced a significant main effect of frequency (F[9,45] = 98.32, $p < 0.001$), dose (F[4,20] = 3.97, $p = 0.016$) and significant interaction (F[36,180] = 2.39, $p < 0.001$). Significant decreases in ICSS were observed at 100-112 Hz from 30 to 300 min, but ICSS was not increased at any frequency at any time (Figure 13a,b). On Day 25, the effects of 10 mg/kg cocaine were examined as a positive control, and cocaine produced a significant main effect of frequency (F[9,45] = 48.69, $p < 0.001$), dose (F[1,5] = 15.59, $p = 0.011$) and interaction (F[9,45] = 4.00, $p < 0.001$). Specifically, cocaine significantly facilitated ICSS at brain stimulation frequencies of 63-100 Hz (Figure 14).

Experiment 2 Discussion

Experiment 2 used a frequency-rate ICSS procedure to compare abuse-related effects of the noncompetitive NMDA antagonists ketamine and MK-801. There were two main findings. First, the three compounds produced dissociable behavioral effects. Specifically, ketamine produced only rate-decreasing effects, whereas, MK-801 and phencyclidine produced a mixed profile of both rate-increasing and rate-decreasing effects. Second, repeated ketamine treatment produced tolerance to the rate-decreasing effects of ketamine but failed to unmask abuse-related facilitation of ICSS. Taken together, these findings suggest that effects of ketamine in ICSS may be mediated by mechanisms other than or in addition to NMDA receptor antagonism. These results also suggest that ketamine may be less likely than MK-801 and phencyclidine to produce a stimulant-like profile of abuse-related effects, although failure of ketamine to facilitate ICSS contrasts with other evidence for abuse liability of ketamine.

The present results are consistent with previous studies showing that MK-801 facilitated ICSS in rats across a variety of reinforcement schedules and testing procedures. For example,

MK-801 increased rates of ICSS maintained by fixed brain-stimulation frequencies and intensities under FR 1 and variable-interval 10 sec schedules (Herberg and Rose, 1989; Olds, 1996). MK-801 also decreased brain-stimulation thresholds required to maintain ICSS in procedures that manipulated either frequency of stimulation (Carlezon and Wise, 1993; Corbett, 1989; Sundstrom et al., 2002) or intensity of stimulation (Kenny et al., 2003; Bespalov et al., 2006). The present study extends these earlier results by showing that MK-801 facilitated low ICSS rates maintained by low brain-stimulation frequencies only at doses similar to or just below those that also decreased higher ICSS rates maintained by higher brain-stimulation frequencies. This mixed profile of rate-increasing and rate-decreasing effects distinguishes MK-801 from effects of some other drugs, such as cocaine or amphetamine, that exclusively facilitate ICSS across a broad dose range (Bauer et al., 2013b; Negus et al., 2012).

The ICSS results from the present experiment are consistent with previous studies showing that phencyclidine increased rates and/or decreased threshold of ICSS maintained under FR 1 schedule of reinforcement in ICSS procedures that manipulate frequency of brain stimulation or intensity (Carlezon & Wise, 1993; Wise et al., 1992) or brain stimulation (Kornestsky et al., 1979; Spielewoy & Markou, 2003). The present study extended previous findings by testing a 10-fold dose range of phencyclidine and providing the time-course of phencyclidine effects on ICSS. Specifically, an intermediate phencyclidine dose (3.2 mg/kg) produced weak but exclusive facilitation of ICSS in the dose-effect study. The higher phencyclidine dose initially depressed ICSS consistent with reduced response rates in the DRL 72 s procedure; however, ICSS facilitation was apparent at later time points (100-320 min) after the behavioral disruption subsided. These phencyclidine effects are similar to the dose- and time-

dependent facilitation of ICSS by MK-801, but contrast with the finding that ketamine only depressed ICSS.

In contrast to MK-801 and phencyclidine, ketamine only depressed ICSS. This agrees with a previous study that found more robust rate-increasing effects with MK-801 than with ketamine in rats trained to respond for a fixed frequency and intensity of brain stimulation under a variable interval 10 sec schedule (Herberg and Rose, 1989). That study evaluated ICSS rates in 10-min bins for 60 min. Rates were stable after vehicle treatment and 3.0 mg/kg ketamine increased mean ICSS rates in the 10-20 and 40-50 min time bins. However, these increases were small relative to effects of MK-801, ICSS rates were not affected at other time points by 3.0 mg/kg ketamine, and higher ketamine doses (10-100 mg/kg) only depressed ICSS as in the present study. Additionally, the decrease of ICSS produced by ketamine is similar to the decrease produced by tricyclic and SSRI antidepressant drugs (Rosenberg et al., 2013). Taken together with the present results, these findings provide little evidence for facilitation of ICSS by acute treatment with ketamine.

The effects of acute ketamine in the present study were superficially similar to effects of acute treatment with mu opioid receptor agonists like morphine in opioid-naïve rats (Altarifi and Negus, 2011; Altarifi et al., 2013). Thus, mu agonists also produced primarily ICSS depression at early times after their administration. However, as initial rate-decreasing effects of mu agonists dissipated in time-course studies, rate-increasing effects often emerged and predominated at later times. Moreover, repeated morphine administration produced tolerance to rate-decreasing effects of mu agonists and enhanced expression of rate-increasing effects. This dependence of mu agonist ICSS effects on parameters of acute or repeated mu agonist exposure suggested that ketamine effects may also depend on parameters of acute or repeated exposure.

However, in contrast to morphine, ketamine failed to facilitate ICSS at any time after its acute administration in the present study. Repeated ketamine produced tolerance to ketamine's rate-decreasing effects, but again unlike morphine, repeated ketamine failed to unmask rate-increasing effects of ketamine, although cocaine did facilitate ICSS in these rats. Overall, then, ketamine failed to facilitate ICSS under conditions that were sensitive to ICSS facilitation by other drugs of abuse.

The frequency-rate ICSS procedure used in experiment 2 engendered a wide range of baseline behavioral rates maintained by a wide range of brain-stimulation frequencies. Similarly broad ranges of baseline behavioral rates can be maintained by food or other reinforcers by using fixed-interval (FI) schedules of reinforcement, and one application of FI schedules in behavioral pharmacology has been to study the degree to which drug effects on rates of operant responding correlate with baseline response rates (Kelleher and Morse, 1968; Sanger and Blackman, 1976; Branch, 1984). Given the potential role of rate-dependency as a determinant of drug effects, it is notable that effects of MK-801, phencyclidine, and ketamine on varying rates of ICSS in the present study are consistent with their effects on varying response rates maintained by other reinforcers under FI schedules. For example, in rats responding for food under a multiple FR 30 FI 300 sec schedule, MK-801 increased low rates of responding maintained during early segments of the fixed intervals, but decreased high rates of responding maintained during later segments (McMillan et al., 1992). Furthermore, low and intermediate doses of phencyclidine produced a modest increase of low rates in rats responding under a FI 90 s schedule, while high doses of phencyclidine depressed responding (Wagner, Masters, & Tomie, 1984). These effects are analogous to MK-801 and phencyclidine facilitation of low ICSS rates maintained by low brain-stimulation frequencies, but depression of high rates maintained by high frequencies in the

present study. In contrast to MK-801, McMillan et al. (1992) also found that ketamine only decreased responding across all segments of the FI, similarly to ketamine's exclusive rate-decreasing effects on ICSS in the present study. Ketamine also produced exclusively rate-decreasing effects in squirrel monkeys responding under a FI 8 min schedule of shock presentation (Byrd, 1982).

Another operant conditioning schedule that engenders low rates of behavior is the differential-reinforcement-of-low-rates (DRL) of responding procedure, which requires animals to wait a specific time between operant responses to receive a reinforcer. MK-801 increases low rates of behavior in rats responding under DRL 15 s and 72 s operant schedules (Ardayfio et al., 2008; Hillhouse and Porter, 2014; Sanger, 1992; Stephens and Cole, 1996) and phencyclidine increases response rates in rats maintained under a DRL 10 s and DRL 15 s (Hudzik & Slifer, 1992; Sanger, 1992; Sanger & Jackson, 1989); whereas, ketamine decreases rates of behavior in rats responding on DRL 72 s schedule operant (Hillhouse and Porter, 2014). Taken together, these studies demonstrate that low rates of operant responding are increased by MK-801 and phencyclidine, but decreased by ketamine across a broad range of conditions. Similar results have been obtained in procedures that measure locomotor activity. For example, at doses used in this study, MK-801 and phencyclidine significantly increase relatively low rates of locomotor activity in rats, whereas ketamine does not (Engin et al., 2009; Gilmour et al., 2009; Koike et al., 2011; Réus et al., 2011; Wegner et al., 2011).

Ketamine and MK-801 have long been considered to share a common mechanism of action as noncompetitive NMDA antagonists that vary primarily in terms of their potency. Drug discrimination procedures have provided one source of behavioral data supportive of a shared mechanism of action, and ketamine produces cross substitution with MK-801 and the other

noncompetitive NMDA antagonist phencyclidine in drug discrimination procedures (DeVry and Jentzsch, 2003; Grant et al., 2006; Killinger et al., 2010; Overton et al., 1989; Rocha et al., 1996). However, MK-801 and ketamine, which produced the most dissociable effects on ICSS, have remarkably different affinities at the noncompetitive NMDA binding site. For example, one study found that MK-801 (K_i = 2.5 nM) was nearly 500-fold more potent for the noncompetitive NMDA receptor site than ketamine (K_i = 1,190 nM) (Bresink et al., 1995). Moreover, ketamine's selectivity for NMDA receptors over other receptor types is modest (Hirota et al., 2002; Nishimura et al., 1998; Seeman et al., 2005; Smith et al., 1987), suggesting that ketamine may produce effects at targets other than NMDA receptors. The differential behavioral effects of MK-801 and ketamine in the present study are consistent with different mechanisms of action of these drugs to alter low rates of responding maintained by low frequencies of brain stimulation in ICSS procedures or by other reinforcers in FI or DRL schedules of reinforcement. The identity of these distinct mechanisms remains to be determined.

ICSS is often described as a behavioral procedure for preclinical assessment of abuse liability and drug-induced facilitation of ICSS is often interpreted as an abuse-related effect (Bauer et al., 2013b; Carlezon and Chartoff, 2007; Kornetsky et al., 1979; Vlachou and Markou, 2011; Wise, 1996). The effectiveness of MK-801 to facilitate ICSS in this and other studies is consistent with its effectiveness to produce abuse-related effects in other preclinical procedures, such as drug self-administration in nonhuman primates (Beardsley, Hayes, Balster, 1990; Koek et al., 1988) and rats (Carlezon and Wise, 1996). Moreover, MK-801 produced a profile of mixed rate-increasing and rate-decreasing effects, and we have suggested previously that such a profile may be indicative of lower reinforcing effects relative to drugs like cocaine or amphetamine, which produce exclusive facilitation of ICSS across a broad dose range (Bauer et

al., 2013b; Negus et al., 2012a). In agreement with this possibility, drug self-administration data suggest that NMDA antagonists may have weaker or less reliable reinforcing effects than stimulants like cocaine (Beardsley et al., 1990; French, 1994; Marquis and Moreton, 1987). However, the failure of ketamine to facilitate ICSS in the present study is not consistent with the efficacy of ketamine to maintain self-administration in rats (de la Peña et al., 2012; Rocha et al., 1996) and rhesus monkeys (Broadbear et al., 2004; Moreton et al., 1977; Young and Woods, 1981) or with epidemiological evidence for ketamine abuse cited in the introduction. Ketamine sometimes fails to maintain self-administration in animals (De Luca and Badiani, 2011; Rocha et al., 1996; Woolverton et al., 2001) and its abuse by humans appears to be confined to a small fraction of drug users (Substance Abuse and Mental Health Services Administration, 2013) and a narrow range of contexts (McCambridge et al., 2007; Winstock et al., 2012). Nonetheless, of the more than 60 drugs from multiple drug classes that we have studied in this frequency-rate ICSS procedure, ketamine is comparable only to Δ9-tetrahydrocannabinol and other cannabinoid-1 receptor agonists in its failure to facilitate ICSS despite evidence for reinforcing effects in assays of drug self-administration (Kwilasz and Negus, 2013). It is possible that ketamine could facilitate ICSS under other circumstances that have not yet been identified. For example, Δ9-tetrahydrocannabinol effects on ICSS were reported to be strain- (Lepore et al., 1996) and dose- (Katsidoni et al., 2013) dependent in rats. However, the present results with ketamine illustrate the need for caution in extrapolating from ICSS studies to other preclinical assays of abuse liability or to abuse potential in humans.

Experiment 3: Glutamatergic Antagonists and Involvement of Serotonin in the Antidepressant-like Effects of Ketamine in the Differential-Reinforcement-of-low-Rate 72 s Operant Procedure

Rationale

Experiment 3 sought to evaluate the effects of other glutamatergic antagonists and to determine the role of serotonergic mechanisms in the antidepressant-like effects of ketamine using the DRL 72 s operant procedure. In the first study, the NMDA antagonists phencyclidine and memantine and the AMPA antagonist NBQX were tested as comparators. Drug combination studies then were conducted to determine the role of serotonin transporter and of serotonin 5-HT_2 receptors in the antidepressant-like effects of ketamine in the DRL 72 s procedure. Most clinically available antidepressant drugs (e.g. tricyclics, SSRIs, and SNRIs) share a common pharmacological mechanism, which is inhibition of serotonin transporters. Ketamine has virtually no affinity for serotonin transports (Ki = 161,700 nM) as compared to NMDA receptors (Ki = 1,190 nM). The goal of this experiment was to determine if the antidepressant-like effects of ketamine in the DRL 72 s procedure are mediated by inhibition of serotonin transporters. The SSRI fluoxetine was used in combination with ketamine to evaluate the role of inhibiting serotonin transporters in the antidepressant-like effects of ketamine.

Atypical antipsychotic drugs, which are serotonin 5-HT_2 receptor antagonists, have been shown to be efficacious as adjunctive treatment and display rapid effects for the remission of depressive symptoms as compared to antidepressant drug treatment alone (Ostroff & Nelson, 1999; Tohen et al., 2010; Wright et al., 2013). The goal of this experiment was to determine if the antidepressant-like effects ketamine in the DRL 72 s procedure are mediated by 5-HT_{2A} receptor antagonism. Ketamine has appreciable binding at serotonin 5-HT_2 receptors (Ki = 15,000 nM) as compared to NMDA receptors (Ki = 1,190 nM); however, the functionality of ketamine at 5-HT_2 receptors is unknown (Kapur & Seeman, 2002). The serotonin 5-HT_2 receptor

antagonist ritanserin and serotonin 5-HT_2 receptor agonist quipazine were used to determine the role of serotonin 5-HT_2 receptors in the antidepressant-like effects of ketamine.

Experiment 3 Methods

Subjects

Thirty-six adult male Sprague-Dawley rats (Harlan Laboratories Inc, Frederick, MD) weighing between 300 and 350 grams at the start of the experiment were used in this study (see methods for experiment 1 for further details). All experimental procedures were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University (IACUC Protocol AM10215) and conducted in accordance with National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animals Resources, 2011).

Apparatus

Four operant conditioning chambers (29.2 x 30.5 x 24.1 cm) enclosed in sound attenuating cubicles were used (Model ENV-008-VP, Med-Associates). These operant conditioning chambers were the same operant chambers used in experiment 1 (see methods for experiment 1 for further details).

Drugs

The noncompetitive NMDA receptor antagonists (±) ketamine HCl (Sigma Aldrich), (+) MK-801 maleate (Sigma Aldrich), phencyclidine HCl (Sigma Aldrich), and memantine (Sigma Aldrich), and the 5-HT_2 agonist quipazine dimaleate (Research Biochemical International, Natick, MS) were dissolved in 0.9% physiological saline. The 5-HT_2 antagonist ritanserin (Sigma Aldrich) was dissolved in deionized water with two drops of 85% DL lactic acid. Sodium hydroxide (Sigma Aldrich) was used as a buffer to insure a pH level of approximately 7.0. The AMPA antagonist NBQX was suspended in deionized water with two drops of tween 80 (Sigma

Aldrich). All drugs were administered *intraperitoneally* at a volume of 1.0 ml/kg and doses represent the salt forms of the drugs. Testing of each drug was completed before initiating testing with another drug. Doses and pretreatment times were based on preliminary studies and previous studies in the literature (Carlezon & Wise, 1993; Hillhouse and Porter, 2014; Marek, Li, & Seiden, 1989a; Marek et al., 1989b; Sanger, 1992). Drugs, doses, and pretreatment times were as follows: ketamine (1.0-10.0 mg/kg; 5 min), MK-801 (0.032-0.18 mg/kg; 15 min), phencyclidine (0.32-10.0 mg/kg; 30 min), memantine (1.0-10.0 mg/kg; 30 min), quipazine (0.56-5.6 mg/kg; 30 min), ritanserin (0.32-10.0 mg/kg; 60 min), and NBQX (1.0-10.0 mg/kg; 30 min). Pretreatment time was the same for dose-effect and combination studies. Dose order was counterbalanced using a Latin-square design.

Training and Testing Procedures

All training and testing procedures were identical to experiment 1 (see experiment for further details). Test sessions occurred twice weekly (typically Tuesday and Friday) with a minimum of one training sessions prior to each test session. Testing of any one drug was completed before initiating testing with another drug, and a saline baseline was determined for each drug tested. Testing continued until each drug had been tested in groups of 6-8 rats.

Statistical Analysis

The dependent variables included 1) total number of earned reinforcers during each test session, 2) total number of responses during each test session, and 3) inter-response times (IRT) for all responses during each test session. All data were expressed as means (+/- standard error of the mean [S.E.M.]). Time bin data was not analyzed for this study. Response and reinforcer data were analyzed using a one-way repeated measures analysis of variance (ANOVA). The IRT distributions were obtained by recording responses in 25 6-s bins, with the first 6 s bin

representing “burst responding”. To determine if there was a shift in the IRT distribution, a peak location analysis was performed. Specifically, the median of the IRT distribution for each individual rat was determined after eliminating burst responses from the total number of responses. Medians (peak location) 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 s bin was found for each rat (total number of responses divided by number of responses for each time bin) and then averages were calculated for each bin. Planned multiple comparisons using Tukey post hoc tests were conducted after all significant ANOVAs, as appropriate. For combination studies, paired t-tests were conducted between control conditions and the reference dose to determine if the drug effect was maintained. One-way repeated measures ANOVA were conducted on reference dose and combinations (control condition was not included in the ANOVA). The criterion for significance was $p < 0.05$. Data were analyzed using GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, CA, USA).

Experiment 3 Results

DRL training and baseline performance. Twenty-eight rats met the DRL training criterion in a mean of 22.32 (± 1.12 SEM) training sessions. Eight rats failed to meet training criterion and, therefore, were not included in drug experiments.

Ketamine. The low-affinity NMDA receptor antagonist ketamine produced a significant dose-dependent increase in the number of reinforcers earned ($F[3,45] = 21.28$, $p < 0.001$; Figure 15a), decrease in the number of responses emitted ($F[3,45] = 21.09$, $p < 0.001$; Figure 15b), and rightward shift of the peak location on the IRT distribution ($F[3,45] = 22.38$, $p < 0.001$; Figure 15c). Specifically, compared to saline, treatment with 5.6 mg/kg ketamine increased reinforcers

($p < 0.05$), decreased responses ($p < 0.01$), and produced a rightward shift in the peak location of the IRT distributions ($p < 0.01$). Additionally, 10.0 mg/kg ketamine increased reinforcers, decreased responses, and produced a rightward shift in the peak location of the IRT distributions as compared to saline and all other doses ($p < 0.01$).

Phencyclidine. The high-affinity NMDA receptor antagonist phencyclidine produced a significant increase in the number of reinforcers earned ($F[3,24] = 7.77$, $p < 0.001$; Figure 15d) and significantly decreased the number of responses emitted ($F[3,24] = 4.64$, $p = 0.011$; Figure 15e), but did not produce a significant effect on the peak location of the IRT distributions ($F[3,24] = 2.44$, $p = 0.089$; Figure 15f). The 10.0 mg/kg dose of phencyclidine significantly increased reinforcers ($p < 0.001$) and decreased responses ($p < 0.05$) as compared to saline.

Memantine. The low-affinity NMDA receptor antagonist memantine produced a significant increase in the number of reinforcers earned ($F[3,18] = 16.00$, $p < 0.001$; Figure 15h), a significant decrease in the number of responses emitted ($F[3,18] = 11.99$, $p < 0.001$; Figure 15i), and produced a significant shift in the peak location of the IRT distributions ($F[3,18] = 13.51$, $p < 0.001$; Figure 15j). Specifically, treatment with the 10.0 mg/kg dose of memantine increased reinforcers ($p < 0.001$), decrease responses ($p < 0.05$), and produced a rightward shift in the peak location of the IRT disruptions ($p < 0.01$) as compared to saline and all other doses.

NBQX. The AMPA receptor antagonist NBQX significantly increased the number of reinforcers earned ($F[3,27] = 3.60$, $p = 0.026$) with the 3.2 mg/kg dose of NBQX increasing reinforcers as compared to saline ($p < 0.05$; Figure 16a). NBQX failed to significantly alter the number of responses emitted ($F[3,27] = 2.66$, $p = 0.068$; Figure 16b) or shift the peak location of the IRT distributions ($F[3,27] = 3.60$, $p = 0.026$; Figure 16c).

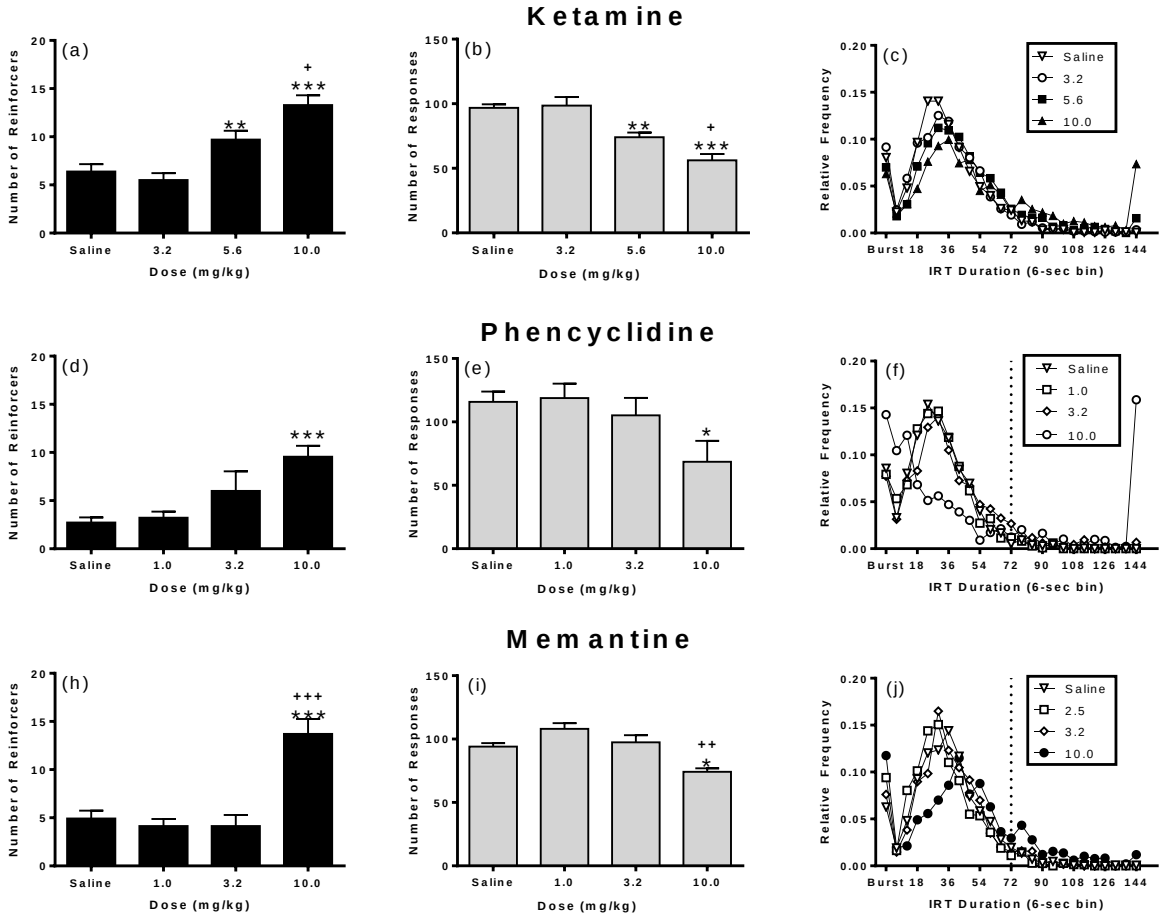

Figure 15. Effects of ketamine, phencyclidine, and memantine on DRL 72 s performance. Left panels (a, d, h) show drug effects on number of reinforcers earned. Center panels (b, e, i) show drug effects on number of responses emitted. Right panels (c, f, j) show drug effects on interresponse time (IRT) distributions. Filled points represent significant shifts in peak location after drug treatment as determined by peak location analysis followed by a Tukey post hoc test, $p < 0.05$. Other details as in Figures 4. Reinforcers and responses are expressed as means ± S.E.M for 7-16 rats (ketamine, n = 16; phencyclidine, n = 10; memantine, n = 7). IRT data are expressed as means. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus saline; ++$p < 0.01$, +++$p <$ 0.001 versus all doses.

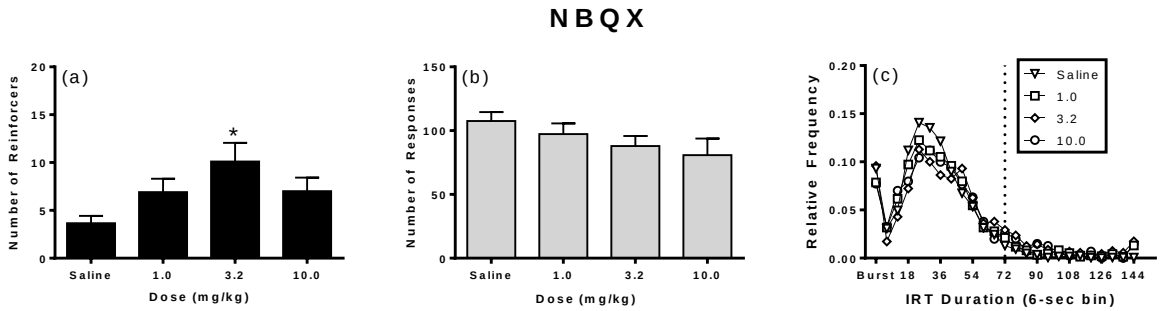

Figure 16. Effects of NBQX on DRL 72 s performance. Left panel (a) show drug effects on number of reinforcers earned. Center panel (b) show drug effects on number of responses emitted. Right panel (c) show drug effects on interresponse time (IRT) distributions. Other details as in Figures 4. Reinforcers and responses are expressed as means ± S.E.M for 10 rats. IRT data are expressed as means. *$p < 0.05$ versus saline.

Fluoxetine and ketamine combination. When administered alone, fluoxetine produced a significant increase in the number of reinforcers earned (F[3,18] = 9.16, $p < 0.001$; Figure 17a), a significant decrease in the number of responses made (F[3,18] = 7.09, $p = 0.002$; Figure 17b), and produced a significant rightward shift in the peak location of the IRT distributions (F[3,18] = 4.53, $p < 0.016$; Figure 17c). Consistent with experiment 1, the 10.0 mg/kg dose of fluoxetine increased reinforcers ($p < 0.001$), decreased responses ($p < 0.01$), and produced a rightward shift in the peak location of the IRT distributions ($p < 0.05$). To determine if the antidepressant-like effects produced by ketamine in the DRL 72 s procedure are mediated by blocking serotonin reuptake, an ineffective dose of ketamine (3.2 mg/kg) was administered in combination with fluoxetine (2.5-10.0 mg/kg). Treatment with saline + 3.2 mg/kg ketamine combination was not significantly different from the control treatment on reinforcers (t[6] = 0.83, $p = 0.436$), responses (t[6] = 0.12, $p = 0.907$), or the peak location of the IRT distributions (t[6] = 0.79, $p = 0.457$) (data not shown). The combination of fluoxetine + 3.2 mg/kg ketamine produced a significant increase in the number of reinforcers earned (F[3,18] = 3.86, $p = 0.027$; Figure 17d), but did not produce a significant effect on responses (F[3,18] = 2.93, $p = 0.062$; Figure 17e) or IRT distributions (F[3,18] = 1.52, $p = 0.242$; Figure 17f). Specifically, only the combination of 10.0 mg/kg fluoxetine + 3.2 mg/kg ketamine significantly increased reinforcers as compared to the saline + 3.2 mg/kg ketamine combination ($p < 0.05$).

Ritanserin and quipazine combination. When administered alone, ritanserin (1.0-10.0 mg/kg) did not produce a significant effect on reinforcers (F[4,28] = 0.34, $p = 0.846$; Figure 18a), responses (F[4,28] = 0.79, $p = 0.54$; Figure 18b), or IRT distributions (F[4,28] = 0.48, $p = 0.751$; Figure 18c). Ritanserin testing did not continue beyond the 10.0 mg/kg dose because this dose far exceeded doses of ritanserin (0.16-0.64 mg/kg) previously tested in DRL 72 (Marek et

al., 1989b) and drug solubility became an issue at doses exceeding 10.0 mg/kg. When administered alone, quipazine did not significantly alter the number of reinforcers earned (F[4,28] = 2.33, p = 0.08; Figure 18d), but quipazine did produce a significant decrease in the number of responses emitted (F[4,28] = 15.34, $p < 0.001$; Figure 18e) and produced a significant shift in the IRT distributions (F[4,24] = 2.76, p = 0.048; Figure 18f). Treatment with 5.6 and 10.0 mg/kg quipazine significantly decreased responses as compared to saline ($p < 0.05$). A Tukey post hoc test failed to reveal a significant difference between doses for the significant effect on IRT distributions; however, as shown in figure 18f, the 5.6 and 10.0 mg/kg dose of quipazine appear to be producing a rightward/downward shift in the IRT distribution.

Combination tests were conducted to determine that the doses of ritanserin tested were behaviorally active doses, and to further determine that both ritanserin and quipazine were exerting their behavioral effects at 5-HT_2 receptors. In order to determine if ritanserin could antagonize the decrease in responses produced by 10.0 mg/kg quipazine, ritanserin (0.1-1.0 mg/kg) was co-administered with the 10.0 mg/kg dose of quipazine. Treatment with ritanserin vehicle + 10.0 mg/kg quipazine combination did not significantly alter the number of reinforcers earned (t[6] = 1.72, p = 0.137), but did produce a significant decrease in the number of responses emitted compared to the control condition (t[6] = 7.23, $p < 0.001$). The ritanserin vehicle + 10.0 mg/kg quipazine combination failed to produce a significant shift in the peak location of the IRT distributions (t[6] = 2.26, p = 0.065) (data not shown). The combination of ritanserin + 10.0 mg/kg quipazine did not produce a significant effect in the number of reinforcers earned (F[3,18] = 1.60, p = 0.22; Figure 18h), but did produce a significant effect in the number of responses emitted (F[3,18] = 19.37, $p < 0.001$; Figure 18i) and produced a significant shift in the IRT distributions (F[3,18] = 4.68, p = 0.014; Figure 18j). Specifically, all three doses of ritanserin

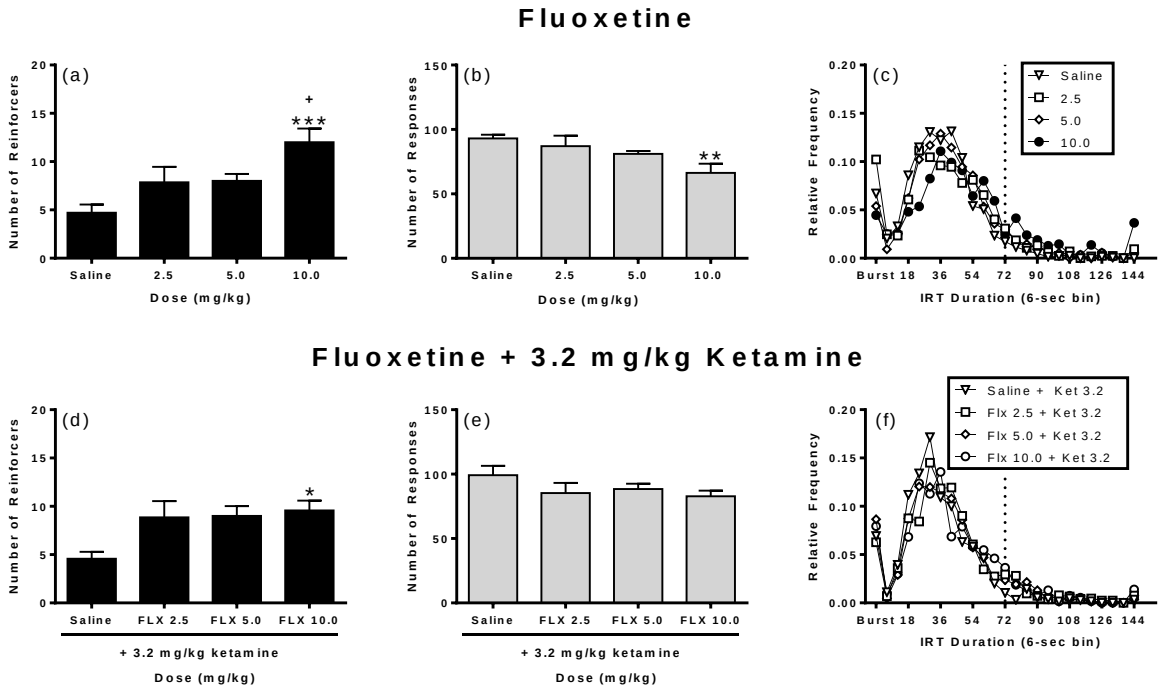

Figure 17. Effects of fluoxetine and the fluoxetine + 3.2 mg/kg ketamine combination on DRL 72 s performance. Left panels (a, d) show drug effects on number of reinforcers earned. Center panel (b, e) show drug effects on number of responses emitted. Right panels (c, f) show drug effects on interresponse time (IRT) distributions. Filled points represent significant shifts in peak location after drug treatment as determined by peak location analysis followed by a Tukey post hoc test, $p < 0.05$. Other details as in Figures 4. Reinforcers and responses are expressed as means ± S.E.M for seven rats. IRT data are expressed as means. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus saline (or saline + 3.2 mg/kg ketamine [d]); +$p < 0.05$ versus all doses.

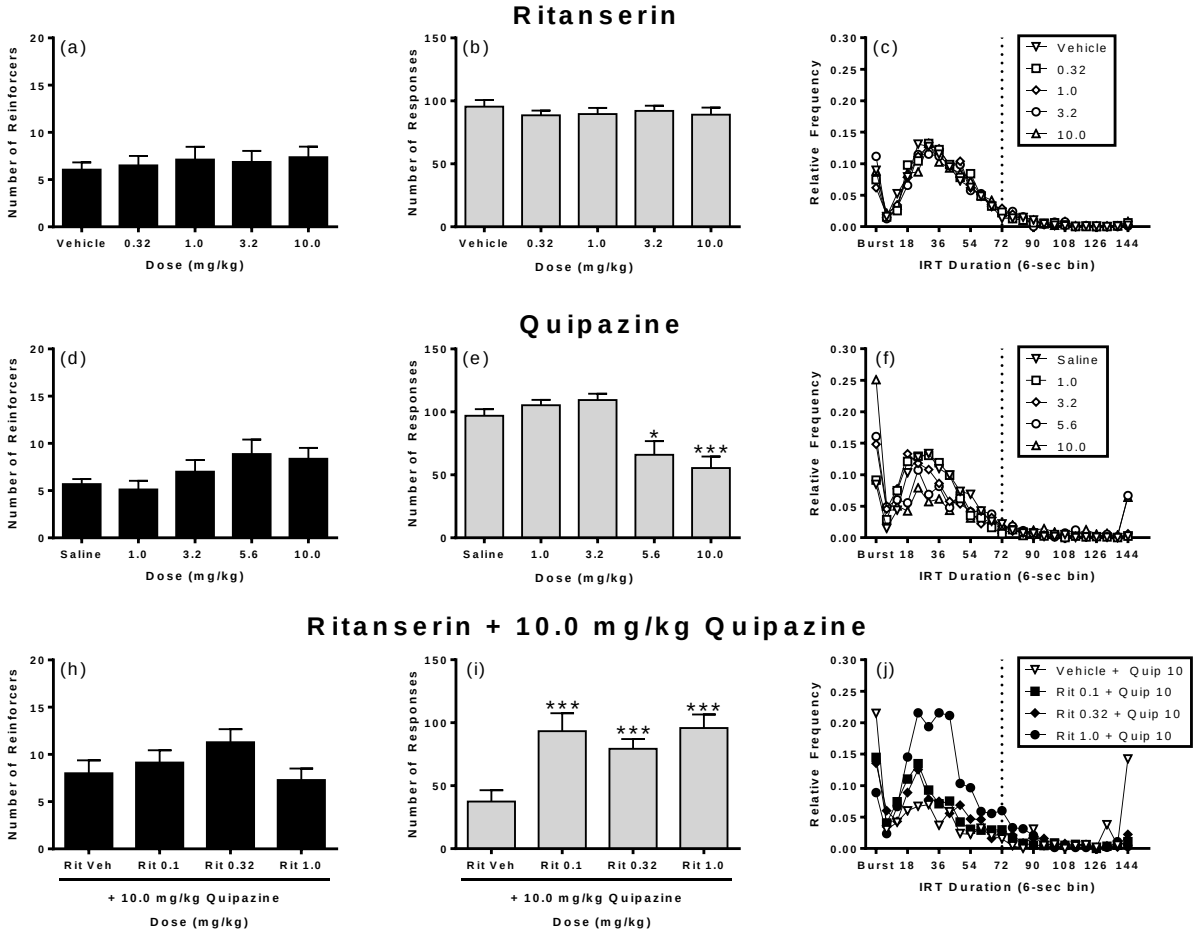

Figure 18. Effects of ritanserin, quipazine and the ritanserin + 10.0 mg/kg quipazine combination on DRL 72 s performance. Left panels (a, d, h) show drug effects on number of reinforcers earned. Center panel (b, e, i) show drug effects on number of responses emitted. Right panels (c, f, j) show drug effects on interresponse time (IRT) distributions. Filled points represent significant shifts in peak location after drug treatment as determined by peak location analysis followed by a Tukey post hoc test, $p < 0.05$. Other details as in Figures 4. Reinforcers and responses are expressed as means ± S.E.M for seven to eight rats (ritanserin, n = 8; quipazine, n = 8; ritanserin + 10.0 mg/kg quipazine, n = 7). IRT data are expressed as means. *$p < 0.05$, ***$p < 0.001$ versus vehicle (or vehicle + 10.0 mg/kg quipazine [i]); +$p < 0.05$ versus all doses.

(0.1-1.0 mg/kg) significantly increased responses ($p < 0.001$) and produced a leftward/upward shift in the peak location of the IRT distributions ($p < 0.05$) as compared to ritanserin vehicle + 10.0 mg/kg quipazine.

Ritanserin and ketamine combination. To determine if the antidepressant-like effects produced by ketamine in the DRL 72 s procedure were mediated by antagonism of 5-HT_2 receptors, an ineffective dose of ketamine (3.2 mg/kg) was administered in combination with the 5-HT_2 antagonist ritanserin (0.32-3.2 mg/kg). Treatment with ritanserin vehicle + 3.2 mg/kg ketamine combination was not significantly different from the control conditions on reinforcers ($t[7] = 0.43$, $p = 0.68$), responses ($t[7] = 0.06$, $p = 0.96$), or the peak location of the IRT distributions ($t[7] = 0.68$, $p = 0.52$) (data not shown). The combination of ritanserin + 3.2 mg/kg ketamine did not produce a significant effect (although there was a trend toward significance) in the number of reinforcer earned ($F[3,21] = 2.80$, $p = 0.065$; Figure 19a), but did produce a significant decrease on the number of responses emitted ($F[3,21] = 3.63$, $p = 0.029$; Figure 19b). The combination of ritanserin + 3.2 mg/kg ketamine did not produce a significant shift in the peak location of the IRT distributions ($F[3,21] = 1.78$, $p = 0.181$; Figure 19c). Specifically, the combination of 1.0 mg/kg ritanserin + 3.2 mg/kg ketamine significantly decreased responses as compared to the ritanserin vehicle + 3.2 mg/kg ketamine combination ($p < 0.05$).

Quipazine and ketamine combination. To determine if the antidepressant-like effects produced by ketamine in the DRL 72 s procedure were mediated by antagonism of 5-HT_2 receptors, an effective dose of ketamine (10.0 mg/kg) was administered in combination with the 5-HT_2 agonist quipazine (0.56-5.6 mg/kg). This combination was used to determine if quipazine could block the antidepressant-like effects of ketamine. As compared to control conditions, treatment with saline + 10.0 mg/kg ketamine combination significantly increased reinforcers

(t[7] = 3.87, p = 0.006), decreased responses (t[7] = 2.99, p = 0.02), and produced a rightward shift in the peak location of the IRT distributions (t[7] = 3.07, p = 0.018) (data not shown). The combination of quipazine + 3.2 mg/kg ketamine did not produce a significant effect in the reinforcer earned (F[3,21] = 0.58, p = 0.636; Figure 19d), but did produced a significant decrease on the number of responses emitted (F[3,21] = 10.47, p < 0.001; Figure 19e). The combination of quipazine + 3.2 mg/kg ketamine failed to significantly shift the peak location of the IRT distributions (F[3,21] = 1.88, p = 0.164; Figure 19f). Specifically, the combination of 5.6 mg/kg quipazine + 10.0 mg/kg ketamine significantly decreased responses as compared to all quipazine + 10.0 mg/kg ketamine combination (p < 0.01).

Ritanserin and MK-801 combination. The 5-HT_{2A} receptor antagonist M100907 has been shown to attenuate the psychostimulant-like effects (i.e. increase in responses, decrease in reinforcers and leftward shift in the IRT distributions) produced by MK-801 in the DRL 72 s procedure (Ardayfio et al 2008). In the present study, the combination of the 5-HT_2 receptor antagonist ritanserin and 0.18 mg/kg MK-801 was used to determine if ritanserin could block the behavioral effects of NMDA antagonists. Additionally, this combination was used to determine if 5-HT_2 receptors were mediating the dissociable effects produced by ketamine and MK-801 in the DRL 72 s procedure. Treatment with the ritanserin vehicle + 0.18 mg/kg MK-801 combination significantly increased responses (t[6] = 5.07, p = 0.002), but did not significantly alter reinforcers (t[6] = 1.68, p = 0.144) as compared to control conditions. Additionally, the ritanserin vehicle + 0.18 mg/kg MK-801 combination produced a leftward shift in the peak location of the IRT distributions (t[6] = 6.13, p < 0.001) as compared to control conditions (data not shown). Treatment with 1.0 mg/kg ritanserin + 0.18 mg/kg MK-801 combination significantly decreased responses (t[6] = 4.19, p = 0.006; Figure 20b), but did not significantly

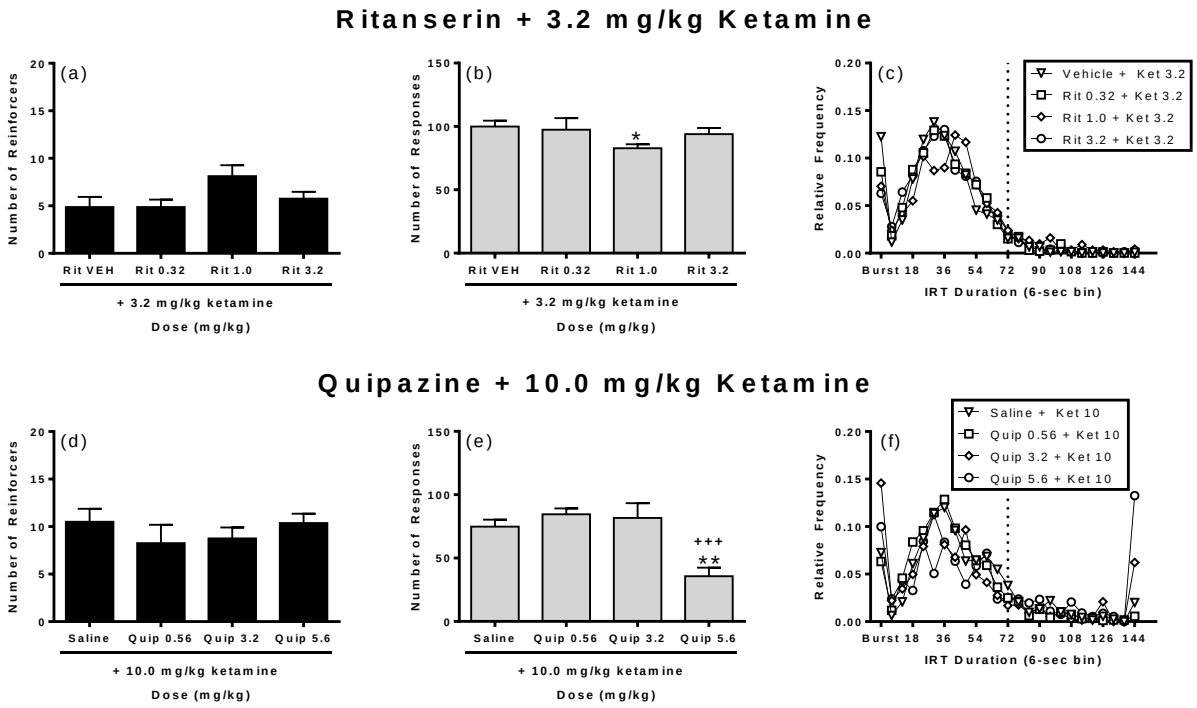

Figure 19. Effects of the ritanserin + 3.2 mg/kg ketamine and quipazine + 10.0 mg/kg ketamine combination on DRL 72 s performance. Left panels (a, d) show drug effects on number of reinforcers earned. Center panel (b, e) show drug effects on number of responses emitted. Right panels (c, f) show drug effects on interresponse time (IRT) distributions. Other details as in Figures 4. Reinforcers and responses are expressed as means ± S.E.M for eight rats. IRT data are expressed as means. *$p < 0.05$, **$p < 0.01$ versus vehicle + 3.2 mg/kg ketamine [b] (or vehicle + 10.0 mg/kg ketamine [e]); +++$p < 0.001$ versus all doses.

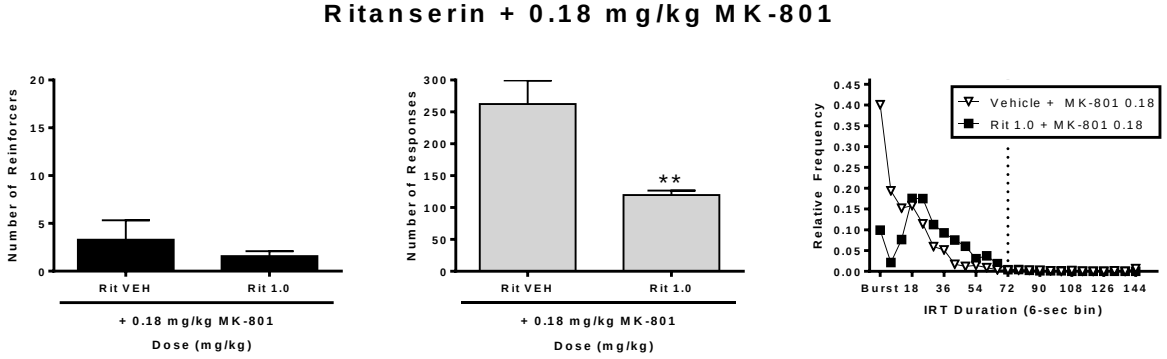

Figure 20. Effects of the ritanserin + 0.18 mg/kg MK-801 combination on DRL 72 s performance. Left panels (a) show drug effects on number of reinforcers earned. Center panel (b) show drug effects on number of responses emitted. Right panels (c) show drug effects on interresponse time (IRT) distributions. Other details as in Figures 4. Reinforcers and responses are expressed as means ± S.E.M for seven rats. IRT data are expressed as means. $^{**}p < 0.01$ versus vehicle + 0.18 mg/kg MK-801.

alter reinforcers (t[6] = 0.82, p = 0.441; Figure 20a). The 1.0 mg/kg ritanserin + 0.18 mg/kg MK-801 combination produced a significant rightward shift in the peak location of the IRT distributions (t[6] = 5.29, p = 0.002; Figure 20c).

Experiment 3 Discussion

Experiment 3 used a DRL 72 s procedure to compare the antidepressant-like effects of the noncompetitive NMDA receptor antagonists ketamine, phencyclidine, and memantine, as well as the AMPA receptor antagonist NBQX. Additionally, this study evaluated the involvement of serotoninergic mechanisms in the antidepressant-like effects of ketamine in the DRL 72 s procedure. There were three main findings. First, the three noncompetitive NMDA receptor antagonists, but not the AMPA receptor antagonist, produced antidepressant-like effects in the DRL 72 s procedure. Specifically, ketamine and memantine increased reinforcers, decreased responses, and produced a rightward shift in IRT distributions; whereas, phencyclidine increased reinforcers and decreased responses, but failed to shift the IRT distributions. The AMPA antagonist NBQX did increase reinforcers, but failed to alter responses or shift the IRT distributions. Second, the antidepressant-like effects of ketamine in the DRL 72 s procedure do not appear to be mediated by inhibiting the reuptake of serotonin via serotonin transporters or antagonism of 5-HT_2 receptors. Specifically, the SSRI fluoxetine and the 5-HT_2 receptor antagonist ritanserin failed to potentiate an ineffective dose of ketamine and the 5-HT_2 agonist quipazine failed to attenuate the antidepressant-like effects produced by 10.0 mg/kg ketamine in the DRL 72 s procedure. Third, the psychostimulant-like effects of MK-801 in the DLR 72 s procedure are, in part, mediated by 5-HT_2 receptors. Specifically, the 5-HT_2 receptor antagonist ritanserin attenuated the increase in responses and leftward shift in the IRT distribution for MK-801. Taken together, these findings suggest NMDA receptor antagonism, not AMPA receptor

antagonism, produces antidepressant-like effects in the DRL 72 s procedure. Additionally, the dissociable effects of ketamine and MK-801 in the DRL 72 s procedure may be mediated by 5-HT_2 receptors.

The present study is the first to report on the effects of phencyclidine in the DRL 72 s procedure. While the 10.0 mg/kg dose of phencyclidine increased reinforcers and decreased responses, it did not shift the IRT distribution. These phencyclidine effects are similar to effects of selective serotonin reuptake inhibitors (SSRIs), which increase reinforcement rate and decrease response rate, but often do not significantly shift the IRT distributions (O'Donnell et al., 2005; Sokolowski & Seiden, 1999). Other antidepressants, such as tricyclic antidepressants and MAO inhibitors, consistently increase reinforcement rate, decrease response rate, and produce a rightward shift in the IRT distribution (O'Donnell et al., 2005). In contrast to the antidepressant-like effects of phencyclidine in the preset study, a higher phencyclidine dose (15.0 mg/kg) did not alter time spent immobile in the forced swim test in rats, suggesting that phencyclidine did not produce an antidepressant-like effect in that procedure (Turgeon, Lin, & Subramanian, 2007).

The DRL 72 s behavioral profile produced by phencyclidine also differs from the effects of the other noncompetitive NMDA antagonists MK-801 and ketamine. Ketamine produces an antidepressant-like effect in rats maintained under a DRL 72 s schedule by increasing reinforcers, decreasing responses, and producing a rightward shift in the IRT distribution (in experiments 1 and 3), which is consistent with other preclinical studies evaluating the antidepressant-like effects of ketamine (Autry et al., 2011; Engin et al., 2009; Gigliucci et al., 2013; Li et al., 2011; Maeng et al., 2007; Tizabi et al., 2012). Conversely, MK-801 produces a stimulant-like behavioral profile in DRL 72 s, which is characterized by a decrease in

reinforcers, increase in responses, and a leftward shift in the IRT distribution. Thus, in the DRL 72 s procedure, phencyclidine effects were more similar to those of ketamine than to MK-801, although phencyclidine failed to produce the antidepressant-like rightward shift in IRT distributions.

The present study is the first to report on the effects of memantine in the DRL 72 s procedure. The results of memantine in the present study on DRL 72 s responding are similar to the effects produced by ketamine and other antidepressants (e.g. tricyclic antidepressants and MAO inhibitors) that increase reinforcement rate, decrease response rate, and produce a rightward shift in the IRT distributions. Furthermore, the antidepressant-like effects of memantine in the present study are consistent with the antidepressant-like effects produced by memantine in other preclinical procedures, such as forced swim test, chronic mild stress, and adrenocorticotropic hormone-induced behavioral deficits (Moryl, Danysz, & Quack, 1993; Quan, Zhang, Wang, Zhang, & Yang, 2011; Reus et al., 2010; Reus et al., 2012; Tokita, Fujita, Yamaji, & Hashimoto, 2012). For example, acute and chronic treatment with memantine in rats reduced time spent immobile in the forced swim procedure at doses that did not increase locomotor activity (Reus et al., 2012). However, the clinical efficacy of memantine remains unclear. Several clinical studies have reported that memantine was not superior to placebo (Lenze et al., 2012; Zarate et al., 2006); however, one study found memantine to be as effective as the SSRI escitalopram (Muhonen, Lonngvist, Juva, & Alho, 2008). Currently, memantine is being pursued as a possible adjunctive treatment for MDD.

In the present study, the AMPA receptor antagonist NBQX increased reinforcers, but failed to alter responses or IRT distributions. These results are different from the effects produced by the noncompetitive NMDA receptor antagonists ketamine, MK-801, phencyclidine,

and memantine, but are consistent with the behavioral effects of NBQX in other preclinical procedures used to assess the antidepressant-like effects of drugs such as the forced swim test, learned helplessness procedure and tail suspension test (Autry et al., 2011; Koike et al., 2011; Maeng et al., 2008). For example, a high dose of NBQX (10.0 mg/kg) did not alter time spent immobile in the forced swim (Autry et al., 2011; Maeng et al., 2008) or tail suspension test (Koike et al., 2011) tests in mice, and NBQX did not reduce the number of escape failures in the learned helplessness inescapable shock procedure in rats (Koike et al., 2011); however, ketamine did produce an antidepressant-like effect in these studies (Autry et al., 2011; Koike et al., 2011; Maeng et al., 2008). Taken together with the present findings, these results further support the hypothesis that NMDA receptor antagonism, not AMPA receptor antagonism, is responsible for the antidepressant-like effects of ketamine and other NMDA receptor antagonists.

In experiment 3, fluoxetine produced effects consistent with experiment 1 in which fluoxetine produced an antidepressant-like effect by increasing the number of reinforcers, decreasing the number of responses, and producing a rightward shift in the IRT distribution. The combination of fluoxetine + 3.2 mg/kg ketamine was used to determine the role of the inhibiting the serotonin transporter in the antidepressant-like effects of ketamine on DRL 72 s responding. This combination of 10.0 fluoxetine + 3.2 mg/kg ketamine produced an increase in the number of reinforcers, but did not alter responses or shift the IRT distribution. The lower doses of fluoxetine (2.5-5.0) did not potentiate the effects of the sub-effective ketamine on any of the dependent measures. These findings are in contrast to a previous report that the combination of the SSRI fluvoxamine and ketamine produced an additive effect by decreasing immobility time on the forced swim behavior in mice (Chaturvedi, Bapna, & Chandra, 2001). While the differences may be due to different drugs and procedures being used, another major difference

between these studies is that Chaturvedi et al. (2001) administered low, but effective doses of both fluvoxamine and ketamine when administered alone. The present study used an ineffective dose of ketamine and thus, it is possible that combining fluoxetine and 10.0 mg/kg ketamine may have yielded different results. Furthermore, ketamine is approximately 150-fold more selective for NMDA receptors as compared to serotonin transports (see table 5), and Martin et al. (1982) found that a high dose of ketamine (>80.0 mg/kg) is required to inhibit the serotonin transporter in rats. Thus, the results from the present study would suggest that inhibition of serotonin transporters does not appear to play a role in the antidepressant-like effects of ketamine in the DRL 72 s procedure.

In the experiment 3, the 5-HT_2 receptor antagonist ritanserin failed to significantly alter reinforcers, responses, and the peak location of the IRT distributions; whereas, the 5-HT_2 agonist quipazine decreased the number of responses, but did not significantly increase the number of reinforcers or produce a rightward shift in the IRT distributions. These results contrast previous reports that ritanserin increased reinforcers and decreased responses in rats in the DRL 72 s procedure (Marek et al., 1989b). Although ritanserin did not produce a behavioral effect when administered alone in the present study, low doses of ritanserin (0.1-1.0 mg/kg) blocked the decrease in responses produced by 10.0 mg/kg quipazine. The combination of ritanserin or quipazine with ketamine was used to determine if the antidepressant-like effects of ketamine in the DRL 72 s procedure was a result of antagonism of the 5-HT_2 receptors. Specifically, 1.0 mg/kg ritanserin + 3.2 mg/kg ketamine decreased the number of responses emitted, but did not significantly increase reinforcers (although there was a trend toward significance, $p = 0.06$) or shift the IRT distributions. Additionally, quipazine failed to block the antidepressant-like effects of ketamine in the DRL 72 s procedure, and was found to further decrease responding at a dose

that decreased responding when administered alone. The results from the present study are similar to the effects produced by the combination of the 5-HT_2 antagonist ketanserin and ketamine in the forced swim test in which this combination produced a modest, although not significantly different from when each drug was administered alone, additive decrease in time spent immobile on the forced swim behavior in mice (Chaturvedi et al., 2001). Several studies have shown that ketamine binds to serotonin 5-HT_2 receptors (Martin, Bouchal, & Smith, 1982; Kapur & Seeman, 2002; Sakai et al., 2013); however, the functionality of ketamine at 5-HT_2 receptors is unknown. The results from the present study suggest that 5-HT_2 receptors do not play a major role in the antidepressant-like effects of ketamine.

The results from the ritanserin + 0.18 mg/kg MK-801 combination data are consistent with a previous report that found the 5-HT_{2A} receptor antagonist M100907 attenuated the psychostimulant-like effects of MK-801 (Ardayfio et al 2008). In the present study, 1.0 mg/kg ritanserin attenuated the psychostimulant-like effects of MK-801 by decreasing responses to control levels and shifted the IRT distributions to the right. Thus, the dissociable effects of ketamine and MK-801 in the DRL 72 s procedure may be mediated by 5-HT_2 receptors. The results from the present study suggest that MK-801 activates the 5-HT_2 receptor, although it is unclear if MK-801 directly or indirectly activates the 5-HT_2 receptors; whereas, the effects of ketamine on 5-HT_2 receptors have yet to be fully elucidated.

General Discussion

The results from the present series of experiments add to the body of literature examining the behavioral effects of NMDA receptor antagonists, and found dissociable differences in terms of antidepressant-like and abuse-related effects of these drugs. The most notable difference found in the present series of experiments was between ketamine and MK-801. Specifically,

ketamine produced an antidepressant-like profile in rats responding under a DRL 72 s schedule of reinforcement, whereas MK-801 did not (experiments 1 and 3). Conversely, ketamine failed to produce an abuse-related facilitation of ICSS, whereas MK-801 did (experiment 2). Experiment 3 provided further evidence for dissociable behavioral effects in the DRL 72 s procedure in that the 5-HT_2 receptor antagonist ritanserin attenuated the psychostimulant-like effect produced by MK-801; whereas, ritanserin produced minimal changes in the behavioral effects of ketamine. Future studies need to evaluate other receptor mechanisms that may mediate these behavioral differences. For example, ketamine has moderate binding affinity for the sigma receptors as compared to NMDA receptors (see table 5) (Robson et al., 2012; Smith et al., 1987); whereas, MK-801 has no significant binding at sigma receptors (Okuyama et al., 1995). Additionally, the effects of ketamine at the sigma receptor appear to play a role in the discriminative stimulus properties of ketamine in rats and neurite outgrowth *in vitro* (Narita et al., 2001; Robson et al., 2012).

Second, future studies need to compare ketamine and MK-801 in several other preclinical procedures used to assess the antidepressant-like effects of drugs. For example, MK-801 has been shown to produce antidepressant-like effects in the forced swim test (Autry et al., 2011; Engin et al., 2009; Maeng et al., 2008); however, the present studies found that MK-801 did not produce an antidepressant-like effect in the DRL 72 s procedure. MK-801 has only served as a comparator for the antidepressant-like effects of ketamine in the forced swim test and DRL 72 s procedure. It will be important to compare ketamine and MK-801, as well as other NMDA antagonists, in the chronic mild stress, chronic unpredictable stress, and learned helplessness assays, which require repeated dosing of the clinically available antidepressant drug before deficits in anhedonia-like behaviors are reversed. Interestingly, a single administration of

ketamine can produce rapid and sustained antidepressant-like effects in these preclinical assays (i.e. chronic mild stress, chronic unpredictable stress, and learned helplessness) (Li et al., 2011; Maeng et al., 2007; Koike et al., 2011). The effects of MK-801 have not been assessed in any of these preclinical procedures (e.g. chronic mild stress, chronic unpredictable stress, and learned helplessness). To truly understand the antidepressant potential of these drugs, it is important to use a variety of preclinical procedures and to assess the data as a whole, rather than rely on only one or two procedures.

The results from the present series of experiments provided evidence that noncompetitive NMDA receptor antagonists, but not AMPA receptor antagonists, produce antidepressant-like effects in the DRL 72 s procedure. Specially, the two low-affinity NMDA receptor antagonists ketamine and memantine produced full antidepressant-like effects; whereas the higher-affinity NMDA receptor antagonist phencyclidine produced a partial antidepressant-like effect as it did not shift the IRT distributions. The results from the combinations studies suggest that the acute antidepressant-like effects of ketamine are not produced by inhibiting serotonin transporters or antagonism of 5-HT_2 receptors, which is consistent with a previous report that found serotonin was not required for the acute antidepressant-like effects of ketamine on forced swim behavior in rats (Gigliucci et al., 2013), and further suggest that mechanisms responsible for the antidepressant-like effects of ketamine may be novel and are not shared by fluoxetine.

To my knowledge, ketamine and memantine are the only two drugs from the present studies that have been evaluated in clinical research settings for antidepressant efficacy and ketamine has produced superior clinical efficacy over memantine. For example, several clinical studies have shown that ketamine produces rapid and sustained antidepressant effects following subanesthetic intravenous infusion in patients suffering from MDD (ann het Rot et al., 2010;

Berman et al., 2000; Murrough et al., 2013; Murrough et al., 2013; Zarate et al., 2006). Memantine, which is a selective low-affinity NMDA receptor antagonist, did not produce rapid or sustained antidepressant effects in clinical research. In fact, two clinical studies found that memantine was not superior to placebo (Lenze et al., 2012; Zarate et al., 2006). A single clinical study found that memantine produced antidepressant effects in patients suffering from MDD, but it was not superior to the SSRI escitalopram (Muhonen, Lonngvist, Juva, & Alho, 2008). Furthermore, memantine failed to produce rapid or sustained antidepressant effects in these studies. Although ketamine and memantine possess similar binding affinity for the NMDA receptors and produced similar effects in the DRL 72 s procedure, ketamine and memantine produce dissociable effects in clinical research.

While the present results demonstrated that phencyclidine produced partial antidepressant-like effects in the DRL 72 s procedure (experiment 3), there is little preclinical research assessing the antidepressant-like effects of phencyclidine, and it is unlikely that phencyclidine will be tested in human studies because of abuse liability concerns (Olthuis, Darredeau, & Barrett, 2013; Substance Abuse and Mental Health Services Administration, 2013). Several other NMDA receptor antagonists have been evaluated in clinical research settings for their antidepressant efficacy, but the results have been not been promising with most drugs failing to produce rapid and/or sustained antidepressant effects (Ibrahim et al., 2012; Preskorn et al., 2008; Zarate et al., 2013). For example, a study evaluating the antidepressant efficacy of the selective NMDA NR2B antagonist MK-0657 was terminated after only 5 patients completed the both phases of the study because of recruitment challenges and because the manufacturer discontinue the development of the compound (Ibrahim et al., 2012). In addition, the low-trapping NMDA channel blocker AZD6765 produced rapid (at 80 min) antidepressant

effects following a single infusion, but these antidepressant effects dissipated by 230 mins (Zarate et al., 2013). There is, however, one glutamatergic compound that has shown antidepressant efficacy similar to that of ketamine in patients suffering from MDD. The glycine-site partial agonist, GLYX-13, produced rapid and sustained antidepressant effects following a single infusion, and most importantly, did not produce psychotomimetic effects. The antidepressant effects of GLYX-13 are apparent at the end of day one and persist until day seven (Moskal et al., 2014). Currently, a 12-week repeated-dosing Phase IIb clinical trial is underway. Taken together, these results indicate that the underlying mechanisms responsible for the clinical antidepressant effects of ketamine may not be driven solely through NMDA receptor antagonism and may be mediated by other receptor targets.

In conclusion, results from the present series of experiments found that the noncompetitive NMDA receptor antagonists ketamine, MK-801, phencyclidine and memantine produced dissociable behavioral effects in the DRL 72 s operant procedure. Our findings provide further evidence that expression of these effects may be related to NMDA receptor affinity. For example, the low-affinity antagonists ketamine (Ki = 1,190 nM) and memantine (Ki = 690 nM) produced antidepressant-like effects in the DRL 72 s procedure with little evidence from ICSS procedures for abuse potential (Hillhouse et al., 2014; Tzschentke & Schmidt, 1999); whereas, the high-affinity antagonist MK-801 (Ki = 2.5 nM) failed to produce antidepressant-effects in the DRL 72 s procedure, but did produce abuse-related ICSS facilitation of ICSS (Hillhouse et al., 2014; Bresink et al., 1995). In comparison, phencyclidine has intermediate affinity at NMDA receptors (Ki = 42 nM) (Bresink et al., 1995), and it produced a mixed-profile of behavioral effects that included both partial antidepressant-like effects in the DRL 72 s procedure and abuse-related facilitation of ICSS. Furthermore, the dissociable effects of ketamine and MK-801

appear to be mediated by different pharmacological effects at 5-HT_2 receptors. Specifically, the psychostimulant-like effects of MK-801 in the DRL 72 s produce appear to be by 5-HT_2 receptor agonism; whereas, 5-HT_2 receptors do not appear to play a role in the antidepressant-like effects of ketamine in the DRL 72 s task. Taken together, these results indicate that the underlying mechanisms responsible for the clinical antidepressant effects of ketamine may not be driven solely through NMDA receptor antagonism and may be mediated by other receptor targets.

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Vita

Todd Michael Hillhouse was born on December 24, 1984, in Glendora, California and is an American citizen. Todd graduated from South Hills High School in West Covina California in 2003. He later moved to the upper peninsula of Michigan to attend Northern Michigan University in Marquette, Michigan. Todd received his Bachelor of Science and Master of Science in Psychology from Northern Michigan University in 2009 and 2011, respectively. After graduation with his Masters of Science, Todd took a research intern position in the Drug Development Department of the pharmaceutical company, Lundbeck in Paramus, New Jersey. In 2011, he began the biopsychology doctoral program at Virginia Commonwealth University in Richmond, Virginia. During his tenure at Virginia Commonwealth University, he published six peer-review articles and one book chapter.

Publications

Peer-Review Journal Publications

Hillhouse TM, Porter JH, Negus SS (2014) Dissociable effects of the noncompetitive NMDA receptor antagonists ketamine and MK-801 on intracranial self-stimulation in rats. *Psychopharmacology (Berl).* [Epub ahead of print]. doi: 10.1007/s00213-014-3451-3

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Book Chapters

Holly EN, LaCrosse AL, Hillhouse TM (In Press) Review of Group I and Group II metabotropic glutamate receptors, and their role in the pathophysiology of major depressive disorder. *Metabotropic Glutamate Receptors: Molecular Mechanisms, Role in Neurological Disorders, and Pharmacological Effects.* Ed. Foster M Olive, Nova Science Publishers (Hauppauge, NY, USA)