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**Modulating Ketamine's Locomotor Activating and Reinforcing Effects Through Drug  
Combinations**

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

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## **Abstract**

Major Depressive Disorder (MDD) affects approximately 280 million people worldwide. The standard of care for pharmacological treatment of MDD has been monoaminergic antidepressants (MAA). However, MAAs have serious clinical limitations including a relatively slow onset of action and up to 40% of patients failing to respond and being diagnosed as “treatment resistant”. Ketamine, an N-methyl-D-aspartate antagonist, was originally approved for use as an injectable anesthetic. In addition, ketamine has been shown to rapidly improve depressive symptoms in patients with treatment resistant depression. In 2019, (S)-ketamine was approved as a nasal spray (Spravato®) for patients with MDD and treatment-resistant depression in conjunction with their previously prescribed MAAs. However, ketamine has limitations of its own, such as producing sedation and dissociative effects as well as having known abuse liability. The goal of the current study was to identify potential drug combinations that optimize ketamine’s therapeutic use by decreasing its abuse-related behavioral effects. To address this goal, we tested the effects of ketamine alone and in combination with desipramine (a tricyclic antidepressant), D-cycloserine (a partial agonist at the NMDA receptor glycine-site) or naltrexone (opioid receptor competitive antagonist) in two behavioral assays in adult Sprague Dawley rats. The effects of our drug treatments on open field behavior was used to assess locomotor activation which has been linked to dopamine release in the brain. The ability of our test compounds to alter ketamine’s reinforcing effects was evaluated using intravenous (IV) ketamine self-administration. Effects on activity in an open field following ketamine administered alone were consistent with both published literature and previous work in this laboratory. This included locomotor activation at intermediate doses of ketamine (10 and 30 mg/kg) with a sex-dependent difference in sensitivity to these activating effects at the 10 mg/kg dose. Ketamine readily maintained IV self-administration with 0.3 and 0.56 mg/kg/infusion serving as positive reinforcers of behavior. Intermediate and high doses of desipramine (1 and 3 mg/kg) were found to decrease locomotor activity alone but did not alter ketamine’s locomotor activating effects. The high desipramine dose also reduced total ketamine intake in the self-administration procedure. D-cycloserine produced no effects on locomotor activity when administered alone nor any significant effect on ketamine

self-administration. Naltrexone alone decreased locomotor activity at 10 and 30 mg/kg. This non-selective suppression of behavior likely accounted for moderate decreases in ketamine-induced locomotor stimulation and self-administration that was observed. While data are too preliminary to rule out any of our three test compounds at this time, given its lack of disruption of behavior in the open field, potential to enhance antidepressant effects, and suggestion of modest decreases in ketamine self-administration, D-cycloserine remains the test drug of greatest interest.

**Key Words:** Ketamine, Desipramine, D-cycloserine, Naltrexone, Rats, Locomotor Activity, Self-administration

## Introduction

### Major Depressive Disorder & current MDD medications

Major depressive disorder (MDD), also known as clinical depression, is a serious mood disorder characterized by at least two weeks of persistent sadness or anxiety, feelings of hopelessness, irritability, loss of interest or pleasure in hobbies and activities, decreased energy, difficulty sleeping, appetite or weight changes, and potentially, thoughts of death, suicide attempts or suicide (Pitsillou et al. 2019). Not everyone who has MDD experiences every symptom or the same symptoms, some may experience a few symptoms while others may experience many (NIH, 2018). MDD affects approximately 280 million people worldwide spanning across all ages with ages 20+ making up 256 million of the affected population (WHO, 2021). Currently, those with MDD are usually treated with psychotherapy, cognitive behavioral therapies, and antidepressant medication. Antidepressant medications have typically focused on enhancing monoaminergic neurotransmission including; selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (Prozac), selective serotonin & norepinephrine inhibitors (SNRIs) like duloxetine (Cymbalta), tetracyclic antidepressants such as mirtazapine (Remeron), tricyclic antidepressants that act as norepinephrine/serotonin reuptake inhibitors like desipramine (Norpramin) and monoamine oxidase inhibitors (MAOIs) such as phenelzine (Nardil) (Ritter et al. 2018). The prescribed type of antidepressant differs from patient to patient, consistent with the many factors contributing to MDD, such as social, psychological, and biological factors resulting in different symptoms and different neurochemical imbalances (WHO, 2021). Though monoaminergic antidepressants (MAA) are among the best treatment approaches for moderate to severe MDD they also have serious clinical limitations. One of the limitations of most MAAs is their relatively slow onset of action, which on average takes 2-3 weeks for full effects but can take up to two months (Machado-Vieira et al., 2008). This can be a serious limitation as people with MDD may not be able to tolerate the delay in effects, especially if they are experiencing more serious symptoms including suicidal ideations. Additionally, for many years, the monoaminergic hypothesis has been relied upon to explain the pathophysiology of depression as being due to potential



deficiencies of serotonin (5HT) and norepinephrine (NE) in the Central Nervous System (CNS). However, more recent findings with drugs with non-monoaminergic mechanisms of action have demonstrated that we have a limited understanding of how we can treat MDD through the use of antidepressants (Matveychuk et al. 2020). For example, despite the many existing monoaminergic therapies, approximately 30-40% of patients fail to respond to therapy and ultimately are diagnosed with Treatment Resistant Depression (TRD), which is defined as not having achieved an adequate response following two or more different treatment attempts despite sufficient dosing and duration (Rush et al. 2006; Fava, 2003).

### Clinical Use of Ketamine

Ketamine is a nonbarbiturate dissociative anesthetic that was originally developed in 1962 as a replacement for phencyclidine (PCP). Ketamine is an arylcycloalkylamine which has a chiral carbon and exists in two stereoisomeric forms - (*S*) and (*R*) enantiomers. (*S*)-ketamine has about a three times higher affinity for the channel binding site than that of (*R*)-ketamine (Hollmann et al., 2001). The (*S*)-isomer of ketamine has also been reported to produce fewer psychotomimetic effects while having greater analgesic and anesthetic effects than the (*R*)-isomer in clinical studies (Peltoniemi et al., 2016; Andrade 2017). In preclinical studies consistent with its greater NMDA receptor binding affinity, the (*S*)-isomer also produces significantly more locomotor activation, ataxia, and head weaving in mice than (*R*)-ketamine does at the same dose (Masaki et al., 2019; Nishizawa et al., 2000). In the US, the currently marketed injectable formulation of ketamine is the racemate, in Europe both a racemic and an (*S*)-isomer formulation is available for injection. Relative to PCP, ketamine has faster pharmacokinetics including a shorter duration of action, as well as a decreased severity of emergence delirium resulting in an improved clinical profile (Li & Vlisides, 2016). Ketamine is currently FDA approved and traditionally used as an induction agent for anesthetic procedures, both for short-term and emergent medical procedures. Ketamine is sometimes preferred over other anesthetics due to its ability to preserve breathing and airway reflexes while actually stimulating the cardiovascular system, which avoids dangerous hypotension and bradycardia (Green et al., 2011). Additionally, ketamine also

has many other therapeutic uses such as pain management and most recently, for treating depression (Rosenbaum S., Gupta V., & Palacios J., 2021). Ketamine is being viewed as a breakthrough therapy for patients with treatment resistant depression. In a small clinical study by (Berman et al., 2000), ketamine was shown to significantly improve depressive symptoms in patients with MDD within 72 hours post ketamine infusion. Berman et al.'s (2000) discovery showed that a single, low dose infusion of ketamine (0.5 mg/kg via IV) was able to produce both rapid and prolonged antidepressant effects. Since that time, ketamine's rapid antidepressant effects have been demonstrated repeatedly in humans (Muller et al., 2016; Zanos & Gould, 2018; Matveychuk et al. 2020) culminating in an intranasal formulation of the (S)-isomer (esketamine, Spravato ®) being approved by the FDA for use in treatment resistant depression and suicidal ideation (Singh et al., 2020; Coyle & Laws, 2015; Aleksandrova et al., 2017). Preclinically, many studies revealed that ketamine can reverse depressive-like behaviors in rodents, as well as providing evidence of reversing dendritic atrophy and promoting synaptogenesis (Li et al., 2011; Burgdorf et al., 2015). In addition to being an important advancement in the treatment of MDD, ketamine, with its very different mechanism of action has made us rethink and reinvestigate the neurochemical changes which cause MDD and other mood disorders.

### Ketamine mechanism of action

Ketamine primarily acts as a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor, binding within the ion channel and preventing ion flow through the channel. NMDA receptors are one of the family of ionotropic, glutamate-activated receptors which also requires glycine binding as a co-agonist. NMDA receptors contain two glycine-binding (GluN1) subunits and two glutamate-binding (GluN2) subunits, which form a tetrameric GluN1/GluN2 receptor (Vyklícky et al., 2014). Uniquely in NMDA receptors, intracellular  $Mg^{2+}$  blocks the NMDA receptor at the resting membrane potential. This blockade must be removed from the receptor's pore in a voltage-dependent manner prior to opening of the channel. Upon ligand binding, depolarization of the neuron and relief of the  $Mg^{2+}$  block results in the opening of  $Ca^{2+}$  ion channels, resulting in an influx of  $Ca^{2+}$  (Kampa et al. 2004). Calcium

plays an important role in the intracellular signaling of the mTOR pathway and synaptogenesis (Nicoll & Malenka, 1999), but also plays a key role in the excitotoxicity of neurons if present in excess (Mattson M. 2003). Although ketamine is expected to block excitatory glutamate neurotransmission via NMDA receptor inhibition, it has been shown to acutely increase glutamate transmission within the synapse of the prefrontal cortex and enhance BDNF activity and downstream mTOR signaling (Abdallah et al., 2018; Rosenbaum S., Gupta V., & Palacios J., 2021) resulting in reversal of MDD-associated neurological changes. A plausible explanation to the increase in glutamate could be the disinhibition hypothesis. The disinhibition hypothesis postulates that there is a preferential inhibition of NMDA receptors on GABAergic interneurons. Blockade of these inhibitory neurons would result in an overall decrease in inhibition of glutamate releasing neurons leading to an overall enhancement of glutamate (Zanos P., & Gould T., 2018).

#### Limitations of ketamine

Despite ketamine's widespread use as a general anesthetic and its relatively new adoption as a rapid-acting antidepressant, ketamine still faces major challenges for clinical use, especially for long-term repeated use which may be necessary for both treatment of pain and depression.

Unfortunately, ketamine brings with it many use-limiting side effects such as: sedation, dissociative effects, memory impairment, motor impairment, and abuse liability. Wang et al. (2014) demonstrated that ketamine in rodent models not only causes learning and memory impairment in doses higher than 80 mg/kg, it increases the number of apoptotic cells in the hippocampal CA1 region, which led to slower performance in a spatial test of learning and memory (Vorhees & Williams, 2006). Additionally, ketamine is used recreationally, both in its powder and liquid forms, often referred to as "Special K", for its hallucinogenic and dissociative effects. It is a schedule III drug as classified by the FDA (Sassano-Higgins et al., 2016; DEA, 2022). Consistent with its abuse liability in humans, ketamine produces positive motivational effects in preclinical models of abuse-related effects including conditioned place preference and intravenous self-administration (Guo et al., 2016; Liu et al., 2016; Venniro et al., 2015). It is hypothesized that ketamine blocks NMDA receptors on GABA neurons

which leads to disinhibition of dopaminergic neurons, resulting in an overall increase in dopamine in reward-associated brain regions such as the medial prefrontal cortex, ventral striatum, nucleus accumbens, and hippocampus (Liu et al., 2016; Caffino et al., 2018). (Hancock and Stamford, 1999) also reported that ketamine increases dopamine efflux and inhibits dopamine uptake, contributing to the overall increase of dopamine in the brain. Dopamine is the major neurotransmitter responsible for controlling information processing in neurons that affect movement, attention and motivation (Mishra A., Singh S., & Shukla S., 2018) and specifically, it motivates and reinforces certain behaviors through activation of dopaminergic receptors in the mesolimbic/reward pathway (Baik, 2013). Elevations of dopamine levels in the nucleus accumbens have been associated with reinforcement of self-administration behavior (Willuhn et al., 2010), as well as increasing locomotor activity due to activation of  $\alpha 1$  adrenergic receptors and D2 dopamine receptors within the ventral-midbrain (Goertz et al., 2015; Baik 2013).

### Ketamine in Drug Combinations

There are multiple mechanisms to improve the therapeutic profile of medications and try to overcome or at least minimize their clinical limitations. One common approach is combining medications in order to enhance their therapeutic effects and/or minimize their adverse effects. Given ketamine's clinical relevance, we are interested in examining different drug combinations in an effort to optimize ketamine's therapeutic use by **decreasing its adverse effects**. In addition to identifying potentially useful drug combinations, the outcome of these studies may also suggest the contribution of different cellular mechanisms to the acute abuse-related behavioral effects of ketamine.

### Monoaminergic Antidepressant Desipramine

As previously mentioned, Spravato®, an intranasal form of the S-isomer of ketamine is used for treatment resistant depression and major depressive disorder, but it is specifically approved for use in combination with MAAs (Janssens, 2020). The majority of approved antidepressant medications for MDD act through monoaminergic mechanisms (Iadarola N., et al. 2015). Though many antidepressant

combinations are safe, there is still uncertainty about any enhanced or synergistic effects both good and bad that results from any combination (Dunner D. 2014). Desipramine (DSP) is a tricyclic antidepressant that is used to treat MDD and acts as a selective norepinephrine and, to a lesser extent, serotonin reuptake inhibitor in the presynaptic neuronal membrane (Maan J., Rosani A., & Saadabadi A., 2021). According to Thangathurai D et al. (2010), the combination of low-dose ketamine and desipramine has been shown to have a high success rate in alleviating noticeable symptoms in treatment-resistant depressed patients. Aligning with the clinical literature (Scheuing et al., 2015), tricyclic agents have also been shown to enhance ketamine's antidepressant effects in various preclinical studies. Further evidence of DSP enhancing ketamine effects was shown in a study done by (Ashleigh et al., 1990) which reported that DSP pre-treated rats required less ketamine to reach an anesthetic dose.

#### D-Cycloserine

D-cycloserine (DCS) is a partial agonist at the glycine site of the NMDA receptor. DCS binds to the glycine binding sites on GluN2A, GluN2B, and GluN2D subunits of the NMDA receptor, as well as being a full agonist at glycine binding sites on the GluN2C subunits (Newport et al., 2015). At low doses, DCS may function as an agonist but at high doses, has antagonist features (Guan et al., 2016). According to Heresco-Levy et al. (2013), a high dose of DCS (1000 mg via PO) combined with MAAs was able to produce significant antidepressant effects without producing the psychotomimetic or dissociative effects commonly associated with ketamine. In addition, DCS alone has been shown to produce modest antidepressant effects in a subset of patients with depression (Chen et al., 2019). DCS has also been shown to reverse some of the acute behavioral effects of ketamine and phencyclidine in preclinical models of schizophrenia, as well as being able to reverse the anesthetic effects of ketamine (Goff, 2017; Irifune et al., 1992). Therefore, theoretically DCS could potentiate the antidepressant effects of ketamine by producing similar therapeutic effects while possibly decreasing the adverse effects of ketamine.

## Naltrexone

Naltrexone (NTX) is a competitive opioid receptor antagonist that has been shown to block the antidepressant effects of ketamine in humans, as well as ketamine's effects in preclinical models of antidepressant-like activity (Williams et al., 2018; Zhang F. et al., 2021). Ketamine shows low binding affinity and efficacy at both mu and kappa opioid receptors. Combining this information with reports of naltrexone blocking ketamine's antidepressant effects sparked multiple hypotheses that ketamine's antidepressant effects were at least in part due to opioid receptor activation (Lavender et al., 2020). Interestingly, the same doses of naltrexone that blocked the antidepressant-like effects of ketamine in mice, failed to reverse ketamine's locomotor activating effects, an effect believed to be due to enhanced dopamine release in the brain and often used as a behavioral indicator of enhanced CNS dopamine (Zhang F. et al., 2021). No studies have investigated the effect of naltrexone on ketamine in self-administration in order to determine if opioid receptor activity might also contribute to ketamine's abuse-related effects.

## Study Aims and Hypotheses

The primary goal of the current study is to identify potential drug combinations that optimize ketamine's therapeutic use by decreasing its abuse-related and use-limiting effects. In order to address this goal, we tested the effects of ketamine alone and in combination with our compounds of interest on activity in an open field and on levels of IV self-administration of ketamine in rats across the following three Aims:

Aim1: Test the hypothesis that acute pretreatment with the traditional tricyclic antidepressant desipramine will decrease ketamine's locomotor activating effects and decrease total ketamine intake.

Aim 2: Test the hypothesis that acute pretreatment with D-cycloserine will not completely antagonize ketamine's abuse-related effects, but may attenuate ketamine's locomotor activating effects and IV self-administration levels, serving as a potential clinically useful combination therapy.

Aim 3: Test the hypothesis that acute pretreatment with the opioid antagonist naltrexone will not impact ketamine's locomotor activating effects or alter total IV ketamine intake except at high doses, where all behavior is non-selectively suppressed.

## **Methods and Materials**

### **Locomotor Activity**

Open field activity (OFA) is used to measure overall locomotor activity both under baseline conditions, following experimental manipulations as well as following drug administration. Activity levels may be measured either through measurement of the number of breaks in strategically placed photobeams (e.g., Med-Associates Infrared Photobeam systems, St. Albans, VT ) or through the use of video cameras and software designed to map and measure the actual movement of the subject (e.g. AnyMaze software Stoelting, Co. Wood Dale, IL). OFA behavior has been used to detect strain and/or genetic differences in basal levels of activity, the effects of experimental manipulations such as following brain injury or induction of a pathological model. Of particular interest for this project is the ability of OFA behavior to capture changes in activity levels following drug administration, in particular following administration of drugs which are abused. Psychomotor stimulants such as cocaine and amphetamines will typically cause locomotor stimulation and result in increased distances traveled at low to intermediate doses (Ciccarone, 2011; Wise & Bozarth, 1987). At high doses these drugs can induce stereotypical behavior and cause general behavioral disruption resulting in decreased distance traveled despite continued CNS stimulation (Wise & Bozarth, 1987). CNS depressant drugs with known abuse liability, such as opioids and ethanol, often show a biphasic dose response curve with low to intermediate doses actually causing increases in locomotor activity in rodents and higher doses suppressing locomotor activity (Correa et al., 2003; Nilges et al., 2019). The former is believed to be due to disinhibition of motor pathways. OFA testing can also be used to assess anxiety-like behavior

and willingness to explore a novel environment by measuring thigmotaxis, a rodent's innate behavior to stay close to the walls and avoid the middle of an open field (Zhang X. et al., 2021). These assessments are most typically performed in the presence of a stressor, such as very bright overhead lighting. Under these conditions, certain drugs such as the classical benzodiazepines and 5-HT<sub>1A</sub> receptor agonists which provide anxiolytic-like effects, reduce the stress-induced inhibition of exploration behavior and reduce thigmotaxis in an open field (Prut & Belzung, 2003).

According to previous studies, ketamine produces significant effects on locomotor activity, with some doses producing hyperactivity while higher doses cause inhibition of locomotor activity. (McDougall et al. 2019) demonstrated that ketamine increased locomotor activity which they attributed to an increase in dopamine levels in the prefrontal cortex and midbrain of rodents (Lindfors et al., 1997). (Irifune M., Shimizu T., & Nomoto M., 2019) demonstrated that an intraperitoneal (IP) injection of 30 mg/kg ketamine dose produced peak locomotor activity, while a high dose of 150 mg/kg ketamine significantly inhibited locomotor activity due to ketamine's anesthetic properties. The same study also showed that the activity of both low and high doses of ketamine are inhibited by a low dose of haloperidol, a dopamine receptor antagonist, at 0.10mg/kg. In a similar study by (Hetzler & Wautlet, 1985), ketamine stimulated locomotor activity in rats, with hyper locomotor activity occurring at 50mg/kg on average. (Beninger R., 1983) reported that changes to the overall activity of dopaminergic neurons in the CNS appear to correlate to changes in locomotor activity. This suggests that ketamine's dopaminergic effects are directly correlated with locomotor activity. These studies support that ketamine has dopaminergic properties which would explain the role it has in increasing motor activity and also in producing abuse-related effects.

### Subjects

For our open field testing (OFT), a total of 27 adult Sprague Dawley rats (Male = 14; Female = 13) were used to conduct the locomotor activity experiment. All rats completed ketamine dose response curve testing between 3 and 5 months of age and a subset of 8 rats (Male = 4; Female = 4) continued



testing of dose combinations completing all subsequent testing conditions by the age of 14 months. The rats were pair housed by sex in standard micro-isolator cages. The rats were maintained under conditions of a reverse 12-hour light/dark cycle (lights off at 0600 h, lights on 1800h) in a temperature (70-74°F) controlled vivarium. The experiments were performed during the dark portion of the cycle. The rats had free access to food and water at all times except during their experimental sessions. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University (IACUC Protocol AM10293) prior to the start of the study and were conducted in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 2011).

### Apparatus

All locomotor testing was conducted in a 43cm x 43cm x 30cm open field chamber with activity monitored and recorded through overhead digital cameras. The cameras were connected to a computer equipped with AnyMaze software (Version 4.99) that recorded and analyzed total distance traveled (in meters) and time spent in the center (central square 20 cm X 20 cm) of the open field. All subjects were acclimated to the laboratory setting and handling for approximately 2 weeks prior to starting any behavioral testing.

### Procedure

During each testing day, the animals were brought up and allowed to acclimate in the laboratory for a minimum of 30 minutes prior to any behavioral testing. The animals were tested at most twice a week, allowing a minimum of 72 hours between sessions in order to avoid any drug carryover effects, as well minimizing any sensitization to ketamine's effects and contact time with the open field. Prior to any drug administration, animals had undergone three habituation sessions in their assigned open field chambers. During the first two habituation sessions, the animals were placed in their assigned chamber for 30 min while their activity was recorded. On the third habituation session, the animals received an injection of saline intraperitoneally (IP) immediately prior to placement in the

chamber, which was consistent with the procedure followed during test sessions. The rationale behind choosing 30 minute testing sessions over other time frames was due to activity levels being the highest within the first 30 minutes in OFT (Swain et al, 2018), and subsequently fades towards baseline activity, which is consistent with previous literature (Piazza et al., 1989). Each animal was tested in the same chamber across all test sessions at approximately the same time of day. Separate chambers were designated as male or female. After each session, chambers were cleaned in an effort to reduce any odors that might impact behavior. The chamber floors were removed, washed with detergent, rinsed and dried while the interior walls of the chamber were cleaned with a 30% ethanol in water spray and wiped dry between testing of each subject.

During testing days, drug doses or dose combinations were administered using a counterbalanced approach within each dose response curve determination in order to minimize any order effect. On combination test days, all animals were administered the saline vehicle or a pretreatment drug (DSP, DCS, or NTX) and then returned to their home cage following the pretreatment administration (see Table 1). Once the appropriate pretreatment time was completed, the animals received either an injection of saline or ketamine and then immediately placed into the open field chamber following the second injection. Ketamine has various routes of administration with the rate of absorption being the fastest in intravenous (IV) > intraperitoneal (IP) > intramuscular (IM) > subcutaneous (SC) > oral (Levin-Arama et al., 2016). Intravenous administration of ketamine has the highest bioavailability in comparison to all other routes of administration, displaying ~100% bioavailability compared to intraperitoneal and subcutaneous bioavailability, which is ~30% and ~80-85%, respectively (Satyavert et al., 2021; Ganguly et al., 2016; Levin-Arama et al., 2016; Loo et al., 2016; Clements et al., 1982). The IP method of administration is typically used when IV administration is not feasible or is too challenging. With the IP administration, in small mammalian species, such as rats and mice, it is possible to administer large volumes of fluid safely without the need to anesthetize the animals beforehand (Turner et al., 2011). However, while IP administration has rapid absorption but is subject to hepatic first-pass metabolism and quickly gets degraded by the liver before being distributed throughout the body (Lukas et al., 1971). Similarly, with the SC method

of administration, the injections are absorbed at a slower rate compared to intravenous and intraperitoneal administration, providing a sustained effect and also avoiding the first pass effect in the liver (Turner et al., 2011). The subcutaneous space is also a large space that is optimal for large volumes of fluid in small animals. Both routes of administrations avoid technical difficulties that are sometimes seen with direct intravenous administration, such as requiring extensive skilled technique and training to ensure minimal blood loss and prevention of painful hematoma formation.

The specific drugs and their testing order are listed below. Following habituation, subjects were tested in the following order: =

1. Ketamine dose response curve. During these ketamine only testing days, the animals were given IP injections immediately prior to being placed in their open field chambers .
2. Desipramine dose response curve. Subjects were administered a dose of desipramine 30 min prior to a saline injection immediately before being placed into the open field.
3. Ketamine + Desipramine tests. On days where combination treatments were used, pretreatments of desipramine were administered 30 minutes prior to injection of saline or ketamine and immediately placed in the chamber.
4. Naltrexone dose response curve. Subjects were administered a dose of naltrexone 20 min prior to an IP injection of saline immediately prior to placement in the chamber.
5. Ketamine + Naltrexone tests. Pretreatments of naltrexone were administered 20 minutes prior to an injection of saline or ketamine and immediately placed in the chamber.
6. D-cycloserine (DCS) dose response curve. Subjects were administered a dose of DCS 20 min prior to a saline injection and then placed into the open field.
7. Ketamine + D-cycloserine tests. Subjects were administered a dose of DCS 20 min prior to an injection of ketamine or saline immediately prior to placement in the chamber.

**Table 1:** Overview of pretreatment drug and ketamine dosing in locomotor activity study

Test Drug	Ketamine (Ket)	Desipramine (DSP)	DSP + Ket	D-cycloserine (DCS)	DCS + Ket	Naltrexone (NTX)	NTX +Ket
Pretreatment Dose	N/A	Saline, 0.3 mg/kg, 1 mg/kg, 3 mg/kg	Saline, 0.3 mg/kg, 1 mg/kg, 3 mg/kg	Saline, 30mg/kg, 100mg/kg, 300mg/kg	Saline, 30mg/kg, 100mg/kg, 300mg/kg	Saline, 1mg/kg, 10mg/kg, 30mg/kg	Saline, 1 mg/kg, 10 mg/kg, 30mg/kg
Route of Administration	N/A	IP	IP	SC	SC	SC	SC
Dose of Ketamine	Saline, 3 mg/kg, 10mg/kg, 30mg/kg, 56mg/kg	Saline	Saline, 3 mg/kg, 10 mg/kg, or 30 mg/kg	Saline	Saline, 3 mg/kg, 10 mg/kg	Saline	Saline, 10 mg/kg
Route of Administration	IP	IP	IP	IP	IP	IP	IP
Pretreatment Time	N/A	30 minutes	30 minutes	20 minutes	20 minutes	20 minutes	20 minutes

## Data Analysis

Using OFT, we determined total distance traveled (in meters) as a measure of activity. Additionally, the time spent in the center (central square 20 cm X 20 cm) of the field was determined and used as a measure of thigmotaxis. Dependent measures were expressed as the mean of the test group plus or minus the standard error of the mean ( $\pm$ SEM). For expressing data as a percent of the control, each subject's test measure (distance traveled and time in the center zone) were divided by their corresponding values under saline conditions. To evaluate whether or not different treatment combinations were significantly different from controls, the data were first evaluated using a two-way mixed or repeated measure analysis of variance (ANOVA) evaluating factors of sex X dose condition. Significant main effects were explored for individual differences using Holm-Sidak (for  $n \geq 8$ ) or Fisher LSD (for  $n \leq 6$ ) post hoc analysis. For conditions where a significant main effect of sex was identified, an additional one-way ANOVA was determined within each sex to investigate significant effects of the treatment condition followed by multiple comparisons using Fisher's least significant difference (LSD) post-hoc test for all significant ANOVAs, as appropriate. For this study, differences were considered significant if the p value was less than 0.05.

## **Intravenous (IV) Self-Administration**

IV self-administration is a well-established operant assay used to evaluate the reinforcing effects of a drug. In this operant, a drug is delivered intravenously after a certain behavior is elicited, and in our experiment, this behavior is pressing a lever. There is a strong correlation between those drugs that are self-administered in animals and those that are abused by humans (O'Connor et al., 2011; Becker and Koob, 2016). For most drugs which produce rewarding effects in humans, the drug stimulus in animals will typically support an increase in the behavior which results in the drug's presentation and therefore serves as a positive reinforcer of behavior. A reinforcer is defined as the consequence that follows an operant response which increases the likelihood of that response reoccurring in the future. When behavior is modifiable by its consequences, it is described as an operant response (Barker et al., 2017). Rats specifically have been shown to self-administer a variety

of drugs with known abuse liability (Cicero et al., 2003; Garcia-Lecumberri et al., 2011; Sharp et al., 2021; Vollmer et al., 2021). Most relevant to the current study are those reports showing ketamine will maintain self-administration behavior in rats (Venniro et al., 2015; De Luca & Badiani, 2011). (O'Connor et al., 2011) completed a comprehensive review of rat self-administration using a multitude of different drugs in regards to abuse liability. Their study demonstrated that ketamine and other glutamatergic drugs, such as PCP and dextromethorphan, support the broad agreement that glutamatergic drugs share clinical indicators of abuse liability.

In self-administration procedures, as with many operant assays, drug presentation depends upon completion of responding under different schedules of reinforcements. One of the most commonly used approaches is use of a fixed ratio (FR) schedule in which the FR represents the number of responses required to elicit a given reward. This response requirement remains the same ('fixed') throughout the session. FR1 requires only one response to provide a single drug infusion while FR5 requires five responses to elicit that same drug infusion. This assay is useful in determining if a drug has positive reinforcing properties or not. In accordance with previous studies, there has been data supporting that NMDA receptor antagonists, such as phencyclidine (PCP), memantine, and ketamine, have positive reinforcing effects due to phasic increases in DA on dopaminergic D2 receptors (Jocham et al., 2014, Venniro et al., 2015; De Luca & Badiani, 2011).

## Subjects

A subset of rats that did not continue with locomotor testing (Male = 5; Female = 1), were transferred for testing in the self-administration study. These subjects were surgically implanted in a jugular or femoral vein with a chronic intravenous catheter (constructed from polyurethane tubing, 3.5 french, Access Technologies, Skokie, IL). For catheter implantation, the animals were anesthetized using isoflurane gas following morphine pretreatment. An incision was made over a jugular or femoral vein and a small section of the vein isolated and ligated. A small incision was made in the vein and the catheter inserted and secured in place with braided nylon ligatures. The distal end of the catheter was routed subcutaneously to connect with a cannula connector pedestal implanted in the animal's

mid-scapular region (Instech Laboratories, Plymouth Meeting, PA). After surgery, the animals were allowed a minimum of five days to recover prior to any behavioral sessions. During this post-operative recovery period, the rats were inspected and weighed each day to ensure their incisions were healing correctly and that they were not in distress. Additionally, the animals were given a tablet of carprofen (5mg/kg) daily for 72 hours postoperatively. To maintain the patency of the newly implanted IV catheter, the line was flushed with 0.2 mL of a heparin (250 U/ml)/cefazolin (50 mg/ml) solution daily throughout the study. Following recovery from surgery, the animals were trained to self-administer 0.3 mg/kg/infusion ketamine during daily 1-hour sessions in standard two-lever operant chambers as described below.

### Apparatus

The self-administration training and testing was conducted in eight standard computer-interfaced operant conditioning chambers (15 cm L x 11.5 cm D x 17.5 cm H; Model ENV-307A, Med Associates Inc., St. Albans, VT) which contained two retractable levers in the left and right positions (8 cm apart) on the front panel of the operant chamber. The levers extended 0.8 cm into the chamber and were positioned 2.5 cm above a grid floor constructed with parallel, stainless steel rods. In the center of the two levers, there was an inactive recessed food trough. During each session, the infusion tubing was connected to the back-mounted pedestal implanted in each rat. Infusions were delivered by a peristaltic pump located outside each chamber. Schedule parameters were controlled and responses recorded by MED-PC IV (Version 4.2, Med Associates Inc.) software running on MS Windows computers.

### General Procedure

Each day (5-6 days/week), the animals were brought to the laboratory and allowed to acclimate for a minimum of 30 minutes. During this time, all equipment was checked and calibrated. After acclimation and pretreatment drug administration (when appropriate), all animals were flushed with 0.2 mL of saline just prior to being placed in their assigned chambers. When placed in the

chambers, the drug line was connected to their chronic IV catheter through the Instech port system and the session initiated. When the self-administration session started, a central house light in the chamber was turned on and two levers extended into the chamber. The right lever was designated as the active lever, on which completion of the correct FR resulted in delivery of a 0.1 mL infusion of ketamine solution over 7-seconds. The left lever was designated the inactive lever and responding on the inactive lever reset the FR count on the active lever. Upon correct completion of the FR, a stimulus light over the right lever turned on and the peristaltic pump activated to deliver the infusion. When the infusion ended, the stimulus and house lights were turned off for a 3 second timeout period. During the drug delivery and the timeout period, the rats could still press the levers, but it did not count towards completing their FR. After the timeout ended, the house light came back on and the rats were able to respond for another infusion of drug. All training and test sessions lasted 60 min. At the end of the session, to maintain patency, the catheters were locked with 0.2 ml infusion of a heparin (250 U/ml)/cefazolin (50 mg/ml) solution and the animal returned to the home cage. Once the animals were trained to self-administer 0.3 mg/kg/infusion ketamine, a dose response curve was generated using a substitution procedure where saline or different concentrations of ketamine solution (0.1 mg/kg/infusion - 0.56 mg/kg/infusion) were substituted for the training solution of ketamine (0.3 mg/kg/infusion) for four consecutive sessions. Rats were returned to baseline training conditions for minimally three sessions between each substitution test.

### Training

During the initial training sessions (acquisition period), the animals were placed in the operant chamber with 0.3 mg/kg/infusion ketamine solution available under a FR1 schedule. Subjects were permitted up to 21 days to achieve the acquisition criteria (three consecutive sessions with >15 infusions earned and active lever responding minimally 70% higher than inactive lever responding). Any subject not achieving criteria within 21 days underwent active training including IV priming by research staff and baiting of levers for up to an additional 2 weeks. Once the subjects reached acquisition criteria under FR1 conditions and were exhibiting stable performance over 3 days (no



downward trends), the FR was increased. Over time as the animals learned, their FR was gradually increased to a terminal value of 5. Once at FR5 and responding reliably as indicated by 3 consecutive sessions with  $\geq 15$  infusions, no trends in infusion numbers, and individual session infusion numbers within 25% of the mean for the three sessions, subjects began testing as outlined below.

### Testing

(Ator and Griffiths, 2003) discussed the need for positive and negative controls, complete dose response curves, and other dependent variables such as locomotor activity in order to understand the reinforcing effects and whether a drug might maintain drug seeking and drug taking. The positive and negative controls are needed to reduce variability in the experiment and validate that our assay can capture a specific drug effect. The positive control results in an expected outcome and indicates that the test works. In our study, ketamine is designated to be our positive control, as it should maintain self-administration behavior and serve as a positive reinforcer. In contrast, a negative control fails to produce the expected positive outcome and serves to tell us what should happen if the drug lacks a particular action. In our study, saline was the negative control, as it lacks positive reinforcing effects and typically fails to maintain self-administration behavior. Saline as negative control is supported by numerous papers that tested various drugs of abuse in both animals and clinical trials (Venniro et al., 2015; Rezvani et al., 2018; Broadbear et al., 2004; Young & Woods, 1981). There were no differences between training sessions and testing sessions other than substitution of a different infusion solution or administration of a test compound as a pretreatment drug. A dose response curve for ketamine was determined using a substitution procedure. Once a subject was reliably responding for our training drug under FR 5 (described above), either saline (negative control) or different concentrations of ketamine were substituted for the training solution (0.3 mg/kg/infusion) over four consecutive daily sessions. After the four days, subjects were returned to baseline conditions.

For drug combination testing days, pretreatment doses were administered using a counter-balanced approach in an effort to minimize any order effect. The animals were administered the pretreatment drug either IP or SC followed by being returned to their home cage until the end of

the pretreatment time. At the end of the pretreatment time, the animal's IV line was flushed with saline, they were placed in the chamber, connected to the infusion system and the self-administration session started. Following each substitution and pretreatment testing session, the animals were returned to baseline training dose for a minimum of three days before being placed on another substitution or pretreatment test. The testing order was as follows:

1. Ketamine dose response curve. All subjects initially complete a dose response substitution curve for ketamine, where different concentrations of ketamine (0.1, 0.3 or 0.56 mg/kg/infusion) or saline, our negative control, were substituted for the training dose (0.3 mg/kg) of ketamine.
2. Ketamine + Desipramine (DSP). Subjects were administered a dose of DSP (saline, 0.3 mg/kg, 1 mg/kg, or 3 mg/kg) via IP injection, followed by a 30 minute pretreatment time prior to being placed into the self-administration chambers and the session started.
3. Ketamine + D-cycloserine (DCS). Subjects were administered a dose of D-cycloserine (saline, 30 mg/kg, 56 mg/kg, 100 mg/kg, or 300 mg/kg) via SC injection, followed by a 20 minute pretreatment time prior to being placed into the self-administration chambers and the session started.
4. Ketamine + Naltrexone. Subjects were administered a dose of naltrexone (saline, 0.1 mg/kg, 1 mg/kg, 10 mg/kg, or 30 mg/kg) via SC injection, followed by a 20 minute pretreatment time prior to being placed into the self-administration chambers and the session started.

#### Data analysis

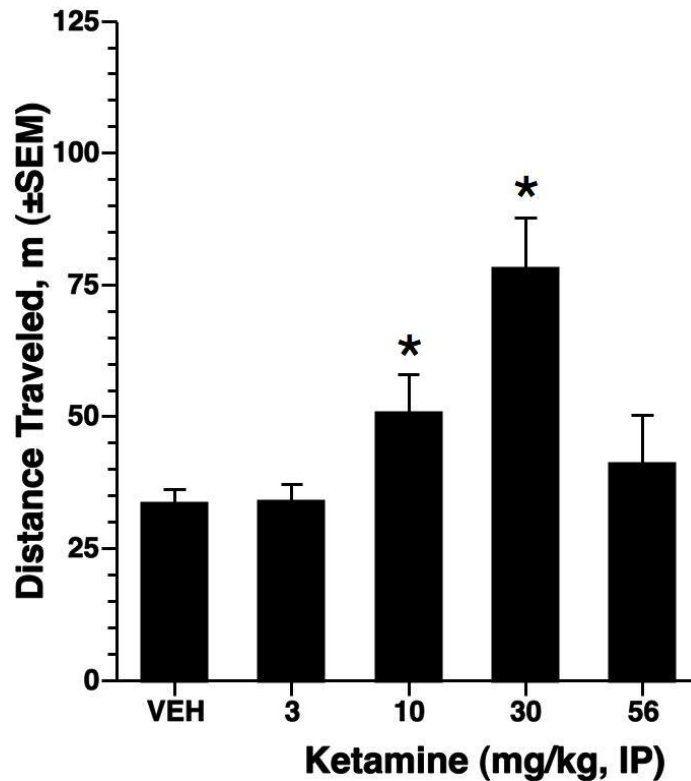
For the self-administration studies, our primary outcome measure of interest was the total number of ketamine infusions and the total amount of ketamine that the animals received during their one hour sessions. When analyzing the substitution data for the ketamine dose response curve, we only used the data generated on the last three of the four substitution days, as the first substitution day's data generally reflects a transition day. Number of infusions was analyzed over the entire 60 minute session. Dependent measures were expressed as the mean of the test group plus or minus the

standard error of the mean ( $\pm$ SEM). To evaluate whether or not different treatment combinations were significantly different from controls, the data were evaluated using a one-way analysis of variance (ANOVA) repeated or mixed measures approach. Dunnett post-hoc analysis was conducted after all significant ANOVAs. For this study, differences were considered significant if the p value was less than 0.05.

## **Results**

### **Locomotor Activity**

All subjects (n = 27, Male = 14; Female = 13) completed testing of the effects of ketamine on activity in an open field. Figure 1 and Table 2A present the mean distance traveled over a 30-minute session following administration of vehicle (saline) or different doses of ketamine (3 mg/kg to 56 mg/kg). A two-way (sex X dose) ANOVA comparing distance traveled showed a significant main effect of dose [ $F(2.204, 57.29) = 9.785, P=0.0001$ ] and a significant main effect of sex [ $F(1, 26) = 9.844, P=0.0042$ ], but no significant interaction ( $P=0.0773$ ). Ketamine produced a dose-dependent change in distance traveled with intermediate doses, specifically the 10 mg/kg ( $p=.0240$ ) and 30 mg/kg ( $p=.0002$ ) doses, producing a significant increase in locomotor activity relative to saline vehicle as determined using Holm-Sidak post hoc analysis. The 56 mg/kg dose did not result in a significant change from vehicle in terms of distance traveled ( $P=0.6614$ ).



**Figure 1:** Effect of different doses of ketamine on activity in an open field for 27 adult Sprague Dawley rats (Male = 14; Female = 13). Each bar represents the mean distance traveled (in meters)  $\pm$ SEM. \* denotes distance traveled was significantly different from saline (VEH) at  $p < 0.05$ .

**Table 2A:** Mean distance traveled, mean time spent in the center of the open field and corresponding SEM following saline (VEH) or varying doses of ketamine in Sprague Dawley rats ( $n=27$ ).

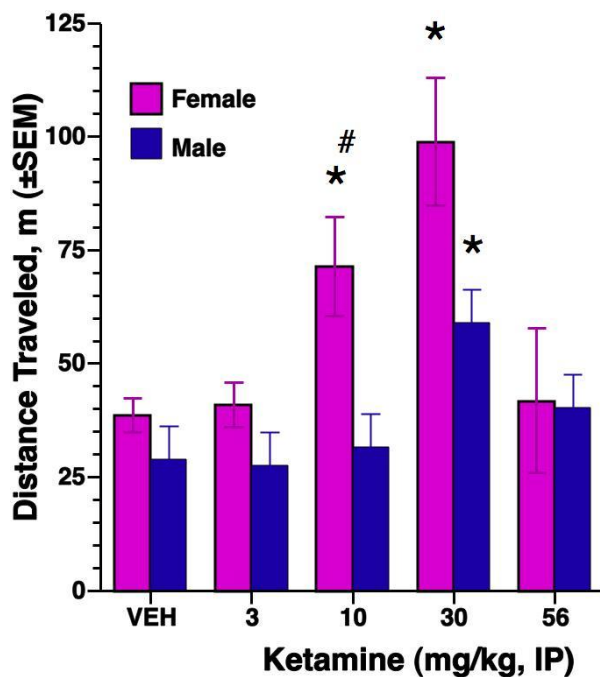
Ketamine Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
VEH	33.70	2.41	117.37	18.39
3	34.10	3.05	90.09	9.59
10	50.89	7.01	67.77	10.94
30	78.30	9.39	240.34	35.58
56	41.12	9.22	570.58	114.72

**Table 2B:** Mean distance traveled, mean time spent in the center of the open field and corresponding SEM following saline (VEH) or varying doses of ketamine in Sprague Dawley rats ( $n=27$ ) expressed as a percent of the VEH control distance.

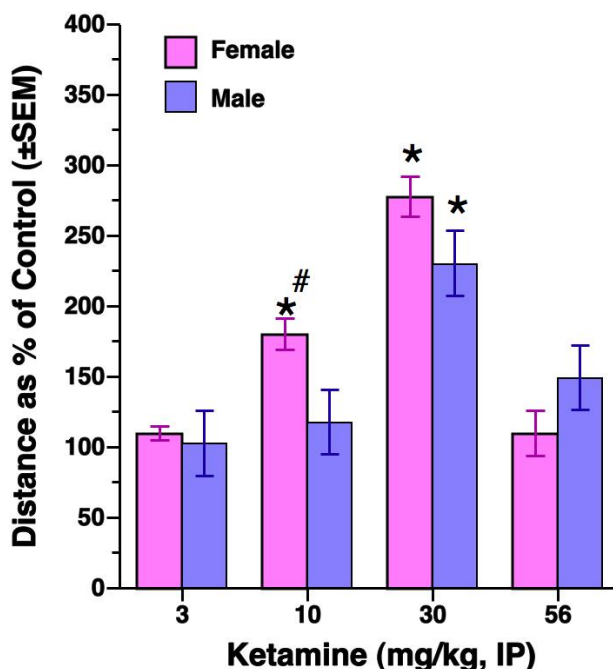
	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
3	106.06	7.54	149.99	48.82
10	147.01	14.30	89.18	19.23
30	276.63	40.57	600.55	327.25
56	130.17	26.45	640.98	160.70

Figure 2 and Tables 3A (female) and 3B (male) display the mean distance traveled with data presented separately for males and females. These data illustrate that there was a trend for female rats to travel longer distances than males under most treatment conditions, however this difference was only significant at the 10 mg/kg dose ( $P=0.0162$ ) where distance traveled by females was over twice the mean distance for males. Due to the sex differences observed, the distance traveled data were further analyzed using a one-way repeated measures ANOVA within each sex group. As shown in Figure 2 and Table 3B, the males had a main effect of dose [ $F(2.161, 28.09) = 4.333, P=0.0206$ ], with Dunnett's post hoc analysis determining the 30 mg/kg dose ( $P=0.0413$ ) caused a significant increase in distance traveled compared to saline pretreatment. Similarly the females showed a significant main effect of dose [ $F(1.850, 24.06) = 6.591, P=0.0061$ ]. Using Dunnett's post hoc analysis, it was determined that both the 10 mg/kg ( $P=0.0046$ ) and the 30 mg/kg doses ( $P=0.0023$ ) produced a significant increase in distance traveled compared to saline pretreatment in female rats.

Figure 3 presents the male and female data as a percent of the vehicle control distance. This permutation was done to account for any differences in baseline activity. Consistent with the actual values, when the data were normalized, we found a significant increase in distance traveled for females following doses of 10 ( $P=0.0275$ ) and 30 ( $P=0.0001$ ) mg/kg ketamine whereas only the 30 mg/kg dose ( $P=0.0005$ ) produced significant locomotor activation in the male rats.



**Figure 2:** Effects of different doses of ketamine on activity in an open field with data separated based on sex. Each bar represents the mean distance traveled (in meters)  $\pm$ SEM for male (N=14) and female (N=13) Sprague Dawley rats. \* denotes doses significantly different from saline (VEH) at  $p < 0.05$ . # denotes doses where distance traveled was significantly different between males and females at  $p < 0.05$ .



**Figure 3:** Effects of different doses of ketamine on activity in an open field with data separated based on sex expressed as a percent of the control data. Each bar represents the mean distance traveled as a percent of vehicle distance  $\pm$ SEM for male (N=14) and female (N=13) Sprague Dawley rats. \* denotes doses of ketamine that are significantly different from 100% at  $p < 0.05$ . # denotes the doses where distance traveled was significantly different between males and females at  $p < 0.05$ .

**Table 3A:** Mean distance traveled, mean time spent in the center of the open field and corresponding SEMs following saline (VEH) or varying doses of ketamine in female Sprague Dawley rats (n=13).

	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
VEH	38.61	3.81	117.58	32.39
3	40.97	4.93	84.39	12.62
10	71.46	10.87	84.36	19.07
30	98.91	14.09	281.25	52.44
56	41.85	15.97	774.90	199.35

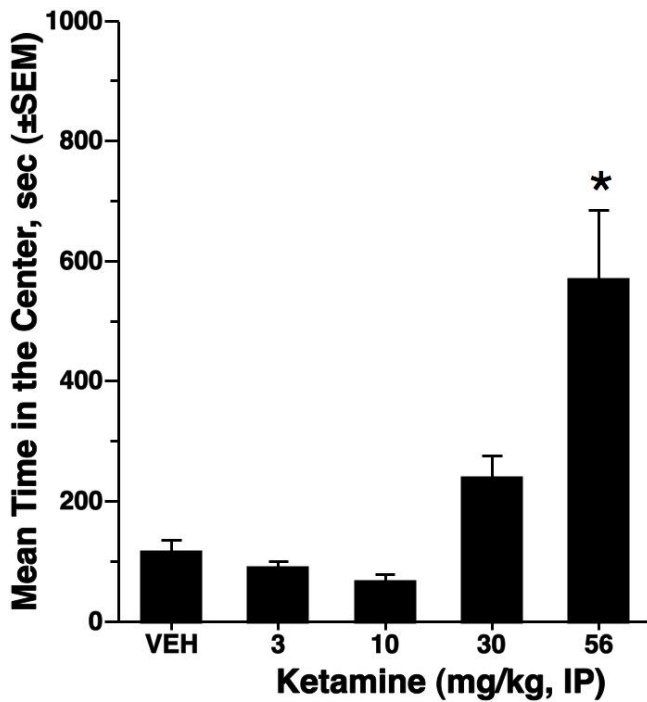
**Table 3B:** Mean distance traveled, mean time spent in the center of the open field and corresponding SEMs following saline (VEH) or varying doses of ketamine in male Sprague Dawley rats (n=14).

	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
VEH	29.13	2.59	117.16	20.13
3	27.72	2.61	95.38	14.25
10	31.80	4.73	52.36	9.41
30	59.16	9.91	202.35	45.97
56	40.44	9.60	380.86	91.28

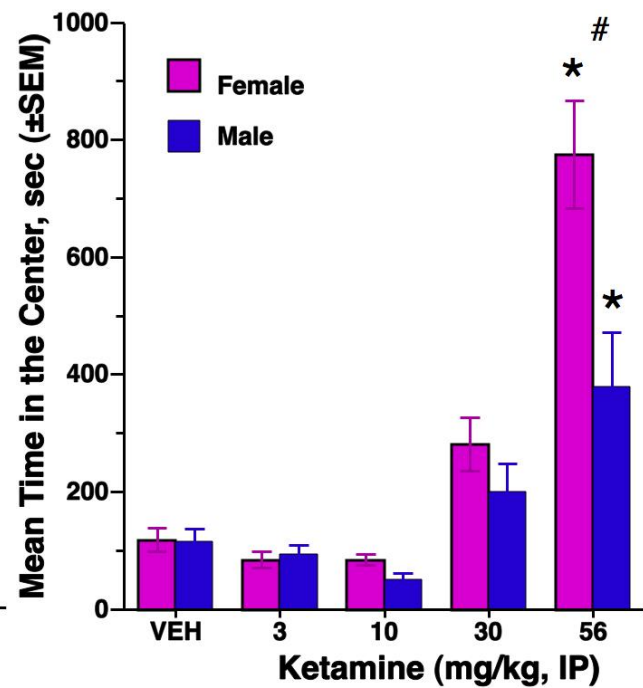
Figures 4A and 4B as well as Tables 2A, 3A and 3B present the mean time in the center over the 30-minute session following administration of VEH (saline) or different doses of ketamine (3 mg/kg to 56 mg/kg). In Figure 4A, the mean center time is shown for all 27 subjects across the different dosing conditions. Two-way mixed method ANOVA of the time spent in the center of the open field revealed a main effect of dose [ $F(1.257, 31.42) = 16.75, P=0.0001$ ] as well as a significant interaction between sex and dose [ $F(4, 100) = 2.661, P=0.0369$ ]. As shown in Figure 4A, the 56 mg/kg ( $P<0.0001$ ) dose produced a significant increase in time spent in the center of the open field reflecting over a 600% increase in time spent (see Table 2B) when compared to each subject's baseline time in the center. In figure 4B, the same data are presented except they are separated out based on sex. For both sexes, the 56 mg/kg dose of ketamine increased time in the center (females -  $P<0.0001$ , males -  $P=0.0093$ ) however, this effect was also significantly more pronounced in female rats ( $P=0.0002$ ) relative to the male rats.



4A)



4B)

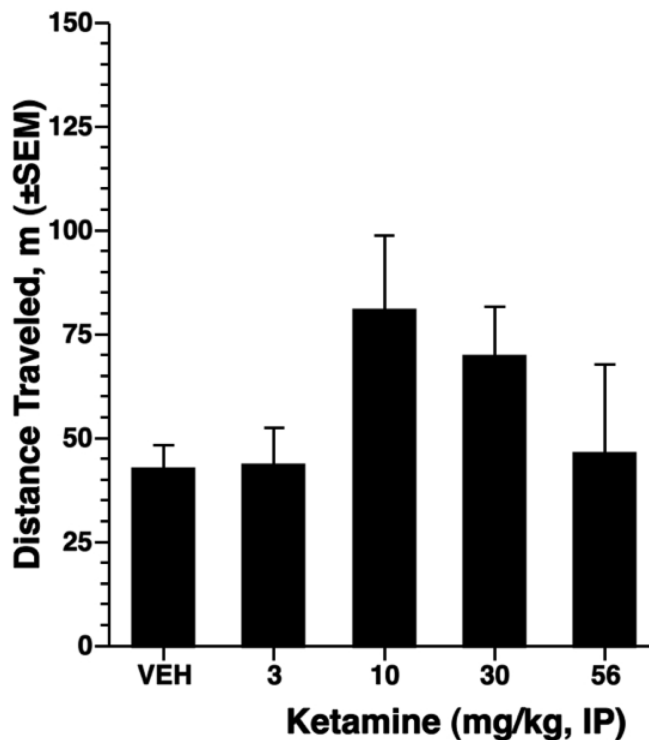


**Figure 4A:** Effects of different doses of ketamine on time spent in the center (20 x 20 cm) open field in adult Sprague Dawley rats (n=27). Each bar represents the mean time in the center  $\pm$ SEM. \* denotes doses of ketamine that resulted in times significantly different from VEH (saline) at  $p < 0.05$ .

**Figure 4B:** Effects of different doses of ketamine on time spent in the center (20 x 20 cm) open field in male (n=14) and female (n=16) Sprague Dawley rats. Each bar represents the mean time in the center  $\pm$ SEM. \* denotes doses of ketamine that resulted in times significantly different from VEH (saline) at  $p < 0.05$ .

A similar analysis of ketamine's effects on locomotor activity was performed using data from the subset of eight subjects (4 male, 4 female) that continued testing dose combinations in our OFT. Their data are presented in Figures 5 and 6 and Tables 4A through 5B. Given the much smaller sample size as well as the fact that some dose combination assessments are incomplete, we have utilized Fisher LSD post hoc analysis in order to identify potentially important drug interactions in this preliminary data set.

Figure 5 and Tables 4A and 4B present data from these 8 subjects collapsed across sex. Two-way repeated measures ANOVA of total distance traveled demonstrated that in this cohort there was a significant main effect of sex [ $F(1, 6) = 19.43, P=0.0045$ ] but there was no significant effect of dose when data were combined across sexes. Figure 6 presents the data separated based on sex. Significant differences in distance traveled were detected between males and females following administration of 10 mg/kg ketamine ( $P=0.0089$ ) using Fisher LSD post hoc analysis. The male and female data were broken out and analyzed within each sex using one-way repeated measures ANOVA. Analysis of the male subjects data failed to show a significant effect of ketamine on locomotor activity ( $p=0.3115$ ). Conversely the female data showed a significant effect of dose [ $F(1.200, 3.600)=11.64, P=0.0304$ ] with the 10 mg/kg dose producing an increase in distance traveled ( $P=0.0155$ ).



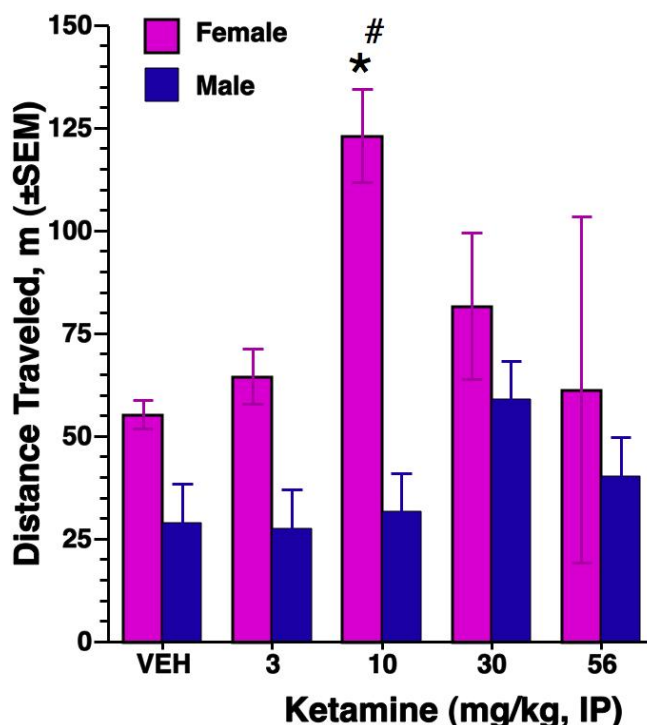
**Figure 5:** Effect of different doses of ketamine on activity in an open field for 8 adult Sprague Dawley rats (Male = 4; Female = 4). Each bar represents the mean distance traveled (in meters)  $\pm$ SEM. \* denotes distance traveled was significantly different from saline (VEH) at  $p < 0.05$ .

**Table 4A:** Mean distance traveled, mean time spent in the center of the open field and corresponding SEMs following saline (VEH) or varying doses of ketamine in Sprague Dawley rats (n=8).

Ketamine Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
VEH	42.80	5.56	119.89	22.35
3	43.57	8.91	68.30	15.26
10	81.04	17.78	97.43	29.48
30	69.85	11.87	291.76	59.26
56	46.52	21.31	541.71	218.06

**Table 4B:** Mean distance traveled, mean time spent in the center of the open field and corresponding SEMs following saline (VEH) or varying doses of ketamine in Sprague Dawley rats (n=8) when expressed as a percent of the subject's VEH control distances.

Ketamine Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
3	94.90	10.33	77.25	23.32
10	176.99	30.13	105.08	45.07
30	178.77	33.60	314.85	83.98
56	116.65	43.17	393.06	124.62



**Figure 6:** Effects of different doses of ketamine on activity in an open field with data separated based on sex. Each bar represents the mean distance traveled (in meters)  $\pm$ SEM for male (n=4) and female (n=4) Sprague Dawley rats. \* denotes doses significantly different from saline (VEH) at  $p < 0.05$ . # denotes doses where distance traveled was significantly different between males and females at  $p < 0.05$ .

**Table 5A:** Mean distance traveled, mean time spent in the center of the open field and corresponding SEMs following saline (VEH) or varying doses of ketamine in female Sprague Dawley rats (n=4).

	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
VEH	55.36	3.52	128.68	38.71
3	64.61	6.76	90.90	22.33
10	123.10	11.38	162.83	32.70
30	81.79	17.83	356.63	93.66
56	61.35	42.10	926.55	339.38

**Table 5B:** Mean distance traveled, mean time spent in the center of the open field and corresponding SEMs following saline (VEH) or varying doses of ketamine in male Sprague Dawley rats (n=4).

	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
VEH	30.23	5.18	111.10	27.97
3	22.54	5.48	45.70	15.73
10	38.97	12.91	32.03	11.64
30	57.91	15.63	226.90	69.35
56	31.69	14.15	156.88	89.43

## DESIPRAMINE

Figure 7 and Tables 6 through 9 show how different doses of desipramine alone and in combination with ketamine alter locomotor activity. In the figure specifically, across the X-axis are the varying doses of ketamine (3, 10 and 30 mg/kg) and saline which were administered immediately prior to placement in the open field. The four individual columns for each of these treatments represent the distance traveled following the different desipramine pretreatments administered 30 min prior to the test session. The black bars represent saline pretreatment, the red bar = 0.3 mg/kg of desipramine, turquoise = 1 mg/kg of desipramine, and dark blue = 3 mg/kg desipramine. Similarly, the four tables provide mean values for distance and time in the center for each ketamine dosing condition across the four desipramine pretreatment doses.

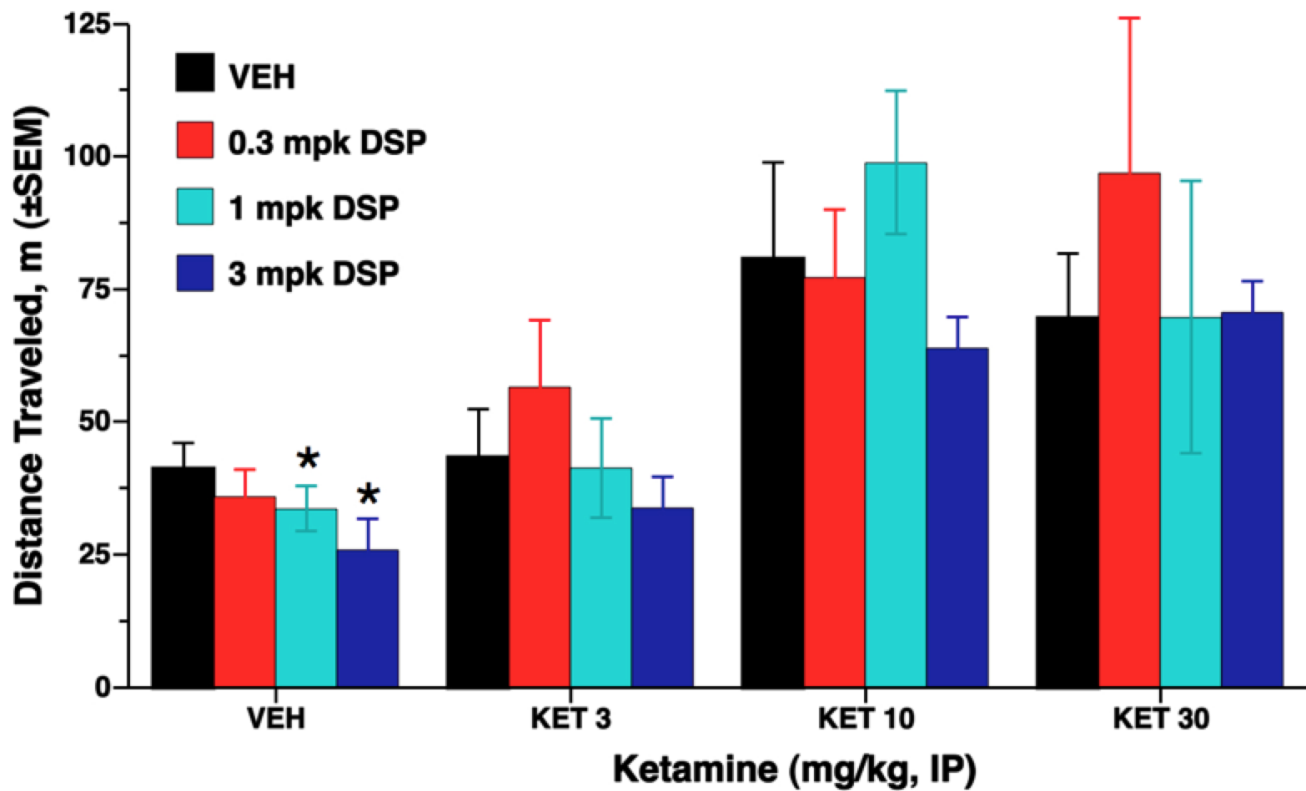
When administered alone, as shown by the bars above the VEH treatment in Figure 7 and in Table 6, desipramine produced a dose-dependent decrease in distance traveled. Analysis of these data using a two-way repeated measures ANOVA revealed a significant main effects of dose [ $F(2.163, 12.98) = 8.934, P=0.0032$ ] and sex [ $F(1, 6) = 7.936, P=0.0305$ ] but no significant interaction between the two. Post hoc analysis confirmed that both the 1 and 3 mg/kg dose of desipramine significantly decreased activity relative to the vehicle control. Based on the sex differences, the data was broken out and analyzed within each sex using 1-way repeated measures analysis. With the female group, a one-way repeated measures ANOVA showed a significant effect of dose [ $F(1.776, 5.327) = 6.017, P=0.0449$ ] however post hoc analysis was unable to identify any individual difference. Additionally, the male group was analyzed using a one-way repeated measures ANOVA, which failed to show a significant effect of desipramine on locomotor activity ( $p=0.0751$ ).

For data in Table 7 and shown above KET 3 in Figure 7, all desipramine dose combinations included 6 to 8 subjects (Males = 2 to 4; Females = 4). A two-way mixed method ANOVA of distance traveled was performed which identified a significant main effect of sex [ $F(1, 6) = 9.573, P=.0213$ ] but no effect of dose condition. The data was further separated based on sex and underwent one-way repeated (females) or mixed methods (males) ANOVA to detect significant effects within sex. One-way repeated measures ANOVA showed no effect of desipramine on distance traveled following

administration of 3 mg/kg ketamine in female ( $P=0.3325$ ) or male ( $P=0.4014$ ) rats.

For data in Table 8 and shown above KET 10 in Figure 7, all dose combinations included 6 to 8 subjects (4 females and 2 to 4 males). A two-way mixed method ANOVA analysis of activity was performed which identified a significant main effect of sex [ $F(1, 6) = 9.129, P=0.0234$ ], a significant main effect of dose [ $F(2.630, 13.81) = 5.466, P=0.0128$ ], and a significant interaction [ $F(4, 21) = 4.653, P=0.0076$ ]. Fisher LSD post hoc analysis identified that females were significantly more active at the VEH + KET10 condition than the males ( $P=0.0086$ ). The data was then separated based on sex and underwent one-way repeated (female) or mixed methods (male) ANOVA to detect significant effects within each sex. In the female group, the one-way repeated measures ANOVA showed no effect of desipramine on distance traveled when combined with 10 mg/kg ketamine [ $F(1.744, 5.233) = 2.573, P=0.1676$ ]. In the male group, the one-way repeated measures ANOVA showed no effect of desipramine on distance traveled when combined with 10 mg/kg ketamine, ( $P=0.5040$ ).

In Table 9 and shown above KET 30 in Figure 7, the test dose combinations with 30 mg/kg ketamine was the most limited in terms of subject numbers, with only 4 to 8 subjects (2- 4 female and 2-4 male) completing different test combinations of the DSP + KET 30. Two-way mixed method ANOVA failed to detect a significant main effect of dose or sex.



**Figure 7:** Effects of saline or different doses of ketamine in combination with desipramine pretreatment on activity in an open field for 8 Sprague Dawley rats (Male = 4; Female = 4). Each bar represents the mean distance traveled (in meters) ± SEM. \* denotes the mean distance traveled was significantly different from VEH+VEH at  $p < 0.05$ .

**Table 6:** Effect of desipramine alone on distance traveled and time spent in the center zone in 8 (Male = 4; Female = 4) Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
VEH	41.45	4.54	148.28	25.09
DSP 0.3	35.94	5.04	195.16	37.99
DSP 1	33.70	4.31	184.66	52.96
DSP 3	26.00	2.78	130.98	34.90

**Table 7:** Effect of desipramine combined with 3 mg/kg ketamine on distance traveled and time spent in the center zone in 6 to 8 (Male = 2-4; Female = 4) Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
VEH + VEH	41.45	4.54	148.28	25.09
VEH + KET 3	43.57	8.91	68.30	15.26
DSP 0.3 + KET 3	56.69	12.57	129.40	37.10
DSP 1 + KET 3	41.35	9.31	132.62	36.77
DSP 3 + KET 3	33.89	8.27	87.96	49.32

**Table 8:** Effect of desipramine combined with 10 mg/kg ketamine on distance traveled and time spent in the center zone in 6 to 8 (Male = 2-4; Female = 4) Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
VEH + VEH	41.45	4.54	148.28	25.09
VEH + KET 10	81.04	17.78	97.43	29.48
DSP 0.3 + KET 10	110.34	15.74	141.55	41.03
DSP 1 + KET 10	87.39	9.60	199.33	29.30
DSP 3 + KET 10	63.95	13.58	147.46	33.53

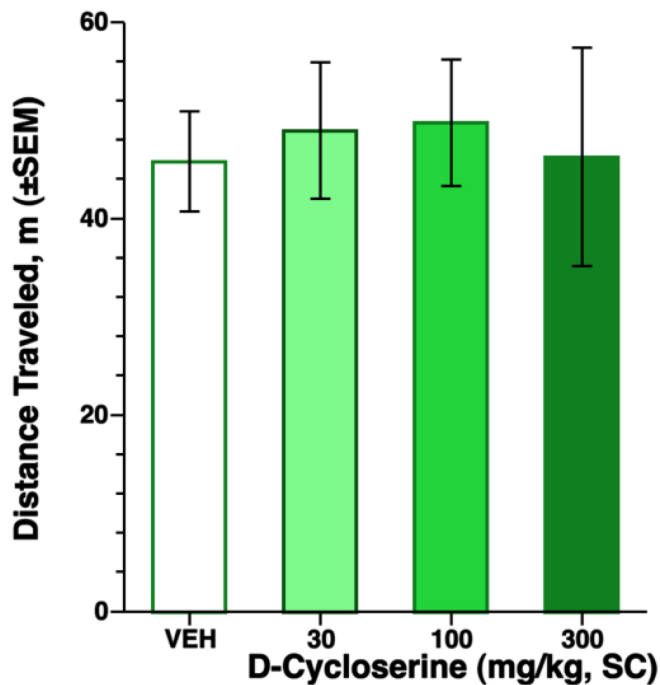
**Table 9:** Effect of desipramine combined with 10 mg/kg ketamine on distance traveled and time spent in the center zone in 6 to 8 (Male = 2-4; Female = 4) Sprague Dawley rats.-8 (Male = 2-4; Female = 2-4) Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
VEH + VEH	41.45	4.54	148.28	25.09
VEH + KET 30	69.85	11.87	291.76	59.26
DSP 0.3 + KET 30	96.90	29.22	342.87	189.18
DSP 1 + KET 30	69.76	25.61	421.20	152.09
DSP 3 + KET 30	70.74	37.78	364.25	159.84



## **D-CYCLOSERINE**

The effects of different doses of DCS on locomotor activity are shown in Figure 8 and Table 10. All test data are the results in 4 female adult Sprague Dawley rats. One-way repeated measures ANOVA failed to show a significant effect of DCS alone on locomotor activity ( $p=0.6910$ ), as well as failing to show a significant effect on time in the center ( $P=0.1589$ ), which is shown in Table 10. At this time there is insufficient DCS + ketamine combination data to warrant statistical analysis and inclusion in this document, however the DCS data alone was included to permit comparison to the effects of DCS pretreatment on ketamine self-administration.



**Figure 8:** Effects of different doses of DCS alone on activity in an open field for a subset of 4 female adult Sprague Dawley rats. Each bar represents the mean distance traveled (in meters)  $\pm$ SEM. \* denotes the mean distance traveled is significantly different from VEH at  $p < 0.05$ .

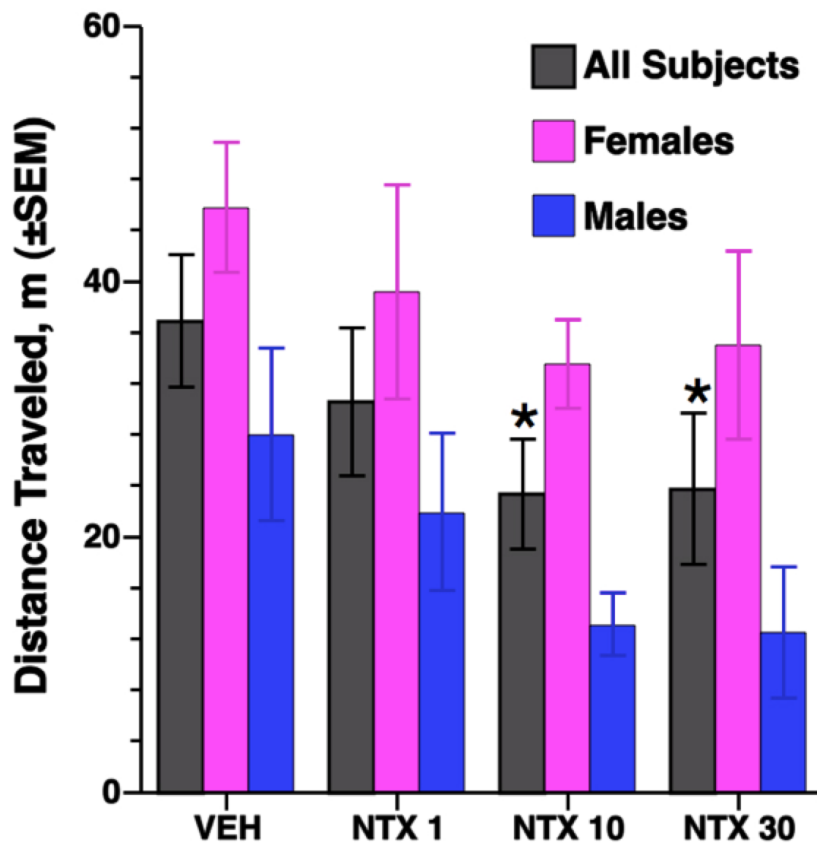
**Table 10:** Effect of DCS alone on distance traveled and time spent in the center zone the subset of 4 female Sprague Dawley rats.

D-Cycloserine Dose (mg/kg, SC)	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
0 (Saline)	45.78	5.07	258.93	91.13
30	48.97	6.94	426.88	115.04
100	49.75	6.41	434.55	142.05
300	46.24	11.10	316.28	148.42

## NALTREXONE

Figure 9 and Table 11 present the effects of different doses of naltrexone alone on locomotor activity. The data in Figure 9 are shown both for the group as a whole, collapsed across sex, as well as for males and females separately. The grey bars represent all subjects, while the pink and blue bars represent female only and male only subjects, respectively. A two-way ANOVA revealed a significant main effect of dose [ $F(2, 130, 12.78) = 7.317, P=0.007$ ] and sex [ $F(1, 6) = 7.221, P=0.0362$ ] but no significant interaction between the two. Post hoc analysis confirmed that both the 10 mg/kg ( $p=0.0113$ ) and 30 mg/kg ( $p=0.0279$ ) doses of naltrexone significantly decreased activity relative to the vehicle control. Based on the sex differences, the data was broken out and analyzed within each sex using one-way repeated measures analysis. Within the female group, a one-way repeated measures ANOVA was performed and failed to show a significant effect of naltrexone on locomotor activity ( $p=0.1580$ ). Within the male group, a one-way repeated measures ANOVA was also performed, which failed to show a significant effect of naltrexone on locomotor activity ( $p=0.0957$ ) despite a trend for a decrease in distance traveled.

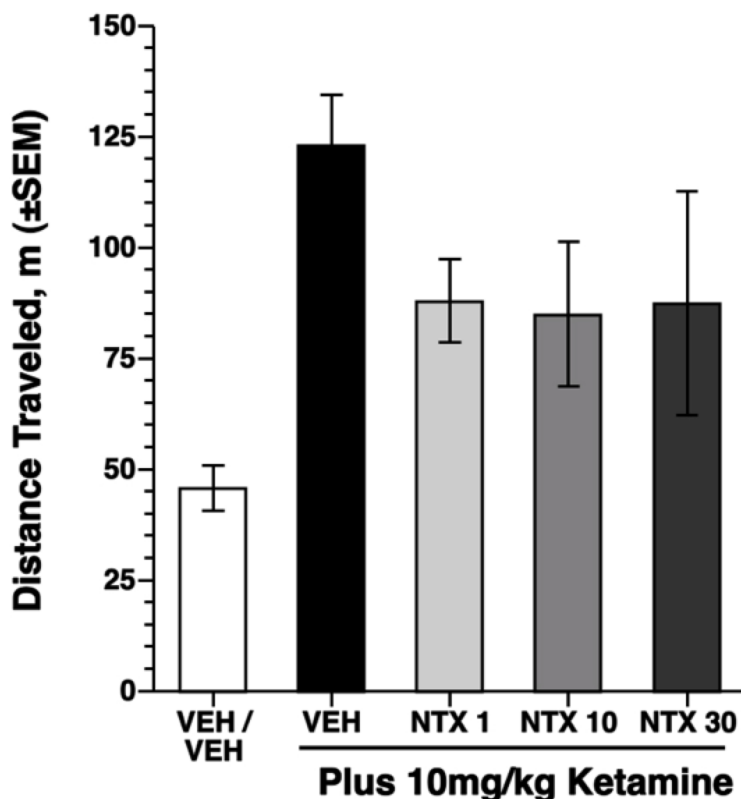
Figure 10 and Table 12 show the effect of pretreatment with different doses of naltrexone on ketamine's locomotor effects when compared to the baseline conditions of VEH + VEH. Across the X-axis are the varying doses of naltrexone (1 mg/kg - 30 mg/kg) in addition to 10 mg/kg ketamine, as well as the negative control, saline (VEH/VEH). One-way repeated measures ANOVA comparing combinations of vehicle or naltrexone with 10 mg/kg ketamine failed to detect any significant change from the distance traveled following the VEH + VEH (saline + saline) combination ( $p=0.4208$ ).



**Figure 9:** Effects of different doses of naltrexone (1 mg/kg - 30 mg/kg) alone on activity in an open field for 8 (Male = 4; Female = 4) adult Sprague Dawley rats. Each bar represents the mean distance traveled (in meters)  $\pm$ SEM. \* denotes mean distance traveled significantly different from VEH (saline) at  $p < 0.05$ .

**Table 11:** Effect of naltrexone alone on distance traveled and time spent in the center zone in 8 (Male = 4; Female = 4) adult Sprague Dawley rats.

Naltrexone Dose (mg/kg, SC)	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
0 (Saline)	28.04	6.78	105.65	21.13
1	21.95	6.14	41.78	33.46
10	13.15	2.42	65.63	31.34
30	12.52	5.13	42.15	20.63



**Figure 10:** Effects of 10 mg/kg ketamine following pretreatment with varying doses of naltrexone (1 mg/kg - 30 mg/kg) on distance traveled in an open field for 8 adult Sprague Dawley rats (Male = 4; Female = 4). Each bar represents the mean distance traveled (in meters)  $\pm$ SEM. \* denotes the mean distance traveled is significantly different from VEH+VEH at  $p < 0.05$ .

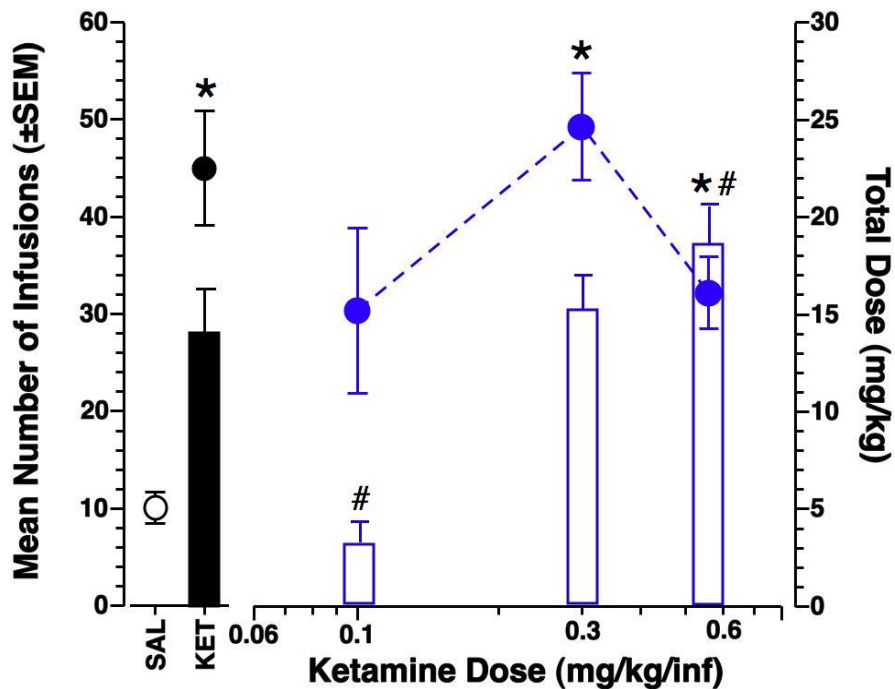
**Table 12:** Effect of different NTX + 10 mg/kg ketamine dosing conditions on distance traveled and time spent in the center zone of an open field in 8 (Male = 4; Female = 4) adult Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM	Time in Center (s)	Time SEM
VEH+VEH	45.78	5.07	258.93	91.13
VEH + KET10	123.10	11.38	162.83	32.70
NTX 1 + KET10	87.95	9.43	147.85	31.74
NTX 10 + KET10	84.98	16.21	150.08	44.89
NTX 30 + KET10	87.32	25.25	109.30	29.66

## **Self-Administration**

All self-administration studies were performed in 6 adult Sprague Dawley rats (5 male, 1 female). Because of the limited number of female subjects, all data were analyzed collapsed across sex. Figure 11 presents the mean number of infusions received during 60-min self-administration sessions with saline (SAL) or varying doses of ketamine (0.1, 0.3 and 0.56 mg/kg/infusion) as shown with symbols (circles) and measured by the left-hand Y-axis. Performance during training sessions between substitution tests served as the positive control. The mean number of infusions received during these sessions is shown above KET in the graph. The data point above SAL shows the mean number of infusions received when saline, the negative control, was available. The remainder of the graph shows the mean number of infusions received during ketamine substitution tests. Mean number of infusions and standard errors shown in the graph were calculated based on the last three sessions of each four-day substitution. When analyzing the mean number of infusions, a repeated measures one-way ANOVA was performed with post hoc analysis using Dunnett's, which revealed that there was a significant effect of dose [ $F(1.688, 8.442) = 9.394$ ,  $P=0.0085$ ]. Using Dunnett's post hoc, all comparisons were made against the negative control, saline. The training dose of ketamine maintained infusion levels that were significantly greater than the levels maintained by saline ( $P=0.0107$ ). Similarly, when tested under the 4-day substitution test conditions, the 0.3 ( $P=0.0043$ ) and 0.56 ( $P=0.0183$ ) mg/kg/infusion test doses also maintained infusion numbers significantly above saline. While the number of infusions during testing of the 0.1 mg/kg/infusion dose was greater than saline levels, it was also associated with highly variable levels of intake between as well as within an individual subject across test days. The total drug of ketamine is represented by the bars in Figure 11 and measured by the righthand Y-axis. When analyzing the total ketamine intake, a repeated measures one-way ANOVA was performed with post hoc analysis using Dunnett's, revealing a significant effect of dose [ $F(1.406, 7.028) = 52.36$ ,  $P<0.0001$ ]. Using Dunnett post hoc comparison, total dose received when saline and different doses of ketamine were available was compared to intake when the baseline dose of 0.3 mg/kg/infusion ketamine was available. Saline ( $P=0.0018$ ) and 0.1 mg/kg/infusion ketamine ( $P=0.0073$ ) solutions resulted in significantly lower total dose than under

the ketamine baseline conditions. The 0.3 mg/kg/infusion ketamine test dose was not different from the total doses self-administered during the baseline training sessions. The 0.56 mg/kg/infusion ketamine dose maintained a total dose intake significantly higher ( $P=0.0021$ ) than during the baseline training sessions.



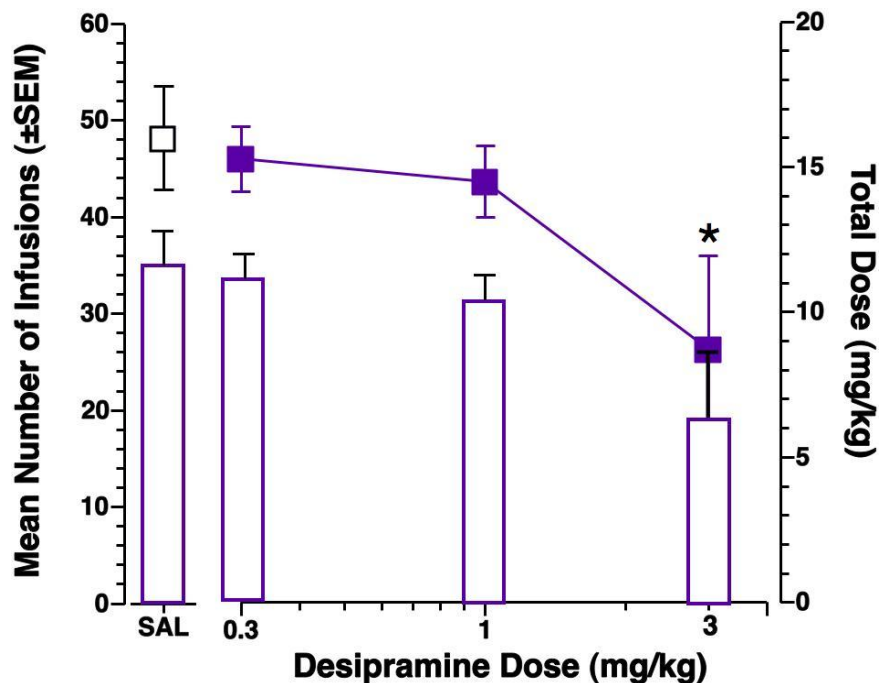
**Figure 11:** The symbols (circles) show mean number of infusions under baseline training conditions (KET) and when different ketamine concentrations and saline (SAL) were available in 6 adult Sprague-Dawley rats (Male = 5; Female = 1) trained to self-administer 0.3 mg/kg/infusion ketamine during daily 1-hour sessions. The bars show the mean total dose of ketamine self-administered calculated based on infusion numbers. \* denotes infusion numbers significantly different from saline at  $p < 0.05$ . # denotes total intake significantly different from mean total dose during training sessions.

**Table 13:** Mean number of lever responses, total number of infusions, and total dose of ketamine administered during self-administration of different ketamine concentrations and saline in 6 adult Sprague-Dawley rats (Male = 5; Female = 1) trained to self-administer 0.3 mg/kg/infusion ketamine.

	Inactive Lever	Inactive SEM	Active Lever	Active SEM	Infusions	Infusions SEM	Total Dose	Total Dose SEM
Saline	4.8	1.1	55.6	7.3	10.1	1.6	0.0	0.0
Ketamine Baseline	11.3	1.8	248.2	40.1	45.0	5.9	13.5	1.8
Ket 0.1	6.7	2.2	179.4	45.7	30.4	8.5	3.0	0.9
Ket 0.3	21.8	6.7	323.4	81.0	49.3	5.5	14.8	1.7
Ket 0.56	11.1	3.1	170.8	16.7	32.2	3.7	18.0	2.1



Figure 12 presents the mean number of ketamine infusions received during 60-min self-administration sessions following pretreatment with saline (SAL) or varying doses of desipramine (0.3, 1, and 3 mg/kg). The symbols (squares) and left-hand Y-axis show the mean number of infusions the animals received under the FR5 work requirement, while the right-hand Y-axis and the bars convey the total amount of ketamine that was self-administered in mg/kg. The X-axis shows the dose of desipramine that was administered IP 30 minutes prior to the start of the session. The data point above SAL shows the mean number of infusions the animals received when similarly pretreated with saline. Mean number of infusions and standard errors shown in the graph were calculated based on that one session. The mean number of infusions self-administered following different doses of desipramine pretreatment was compared to the mean number of infusions following saline pretreatment using a one-way repeated measures ANOVA followed by Dunnett post hoc analysis. This comparison revealed a significant main effect of dose [ $F(1.186, 5.930) = 6.488, P=0.0410$ ] and identified that the number of infusions was significantly lower following the 3 mg/kg dose of desipramine ( $P=0.0462$ ) than following saline pretreatment. Because the total dose self-administered is directly related to the number of infusions, a similar effect was outcome was found for Total Dose; there was a significant main effect of dose [ $F(1.186, 5.930) = 6.488, P=0.0410$ ] with pretreatment with the 3 mg/kg dose of desipramine resulting in a significantly lower total dose ( $P=0.0462$ ).

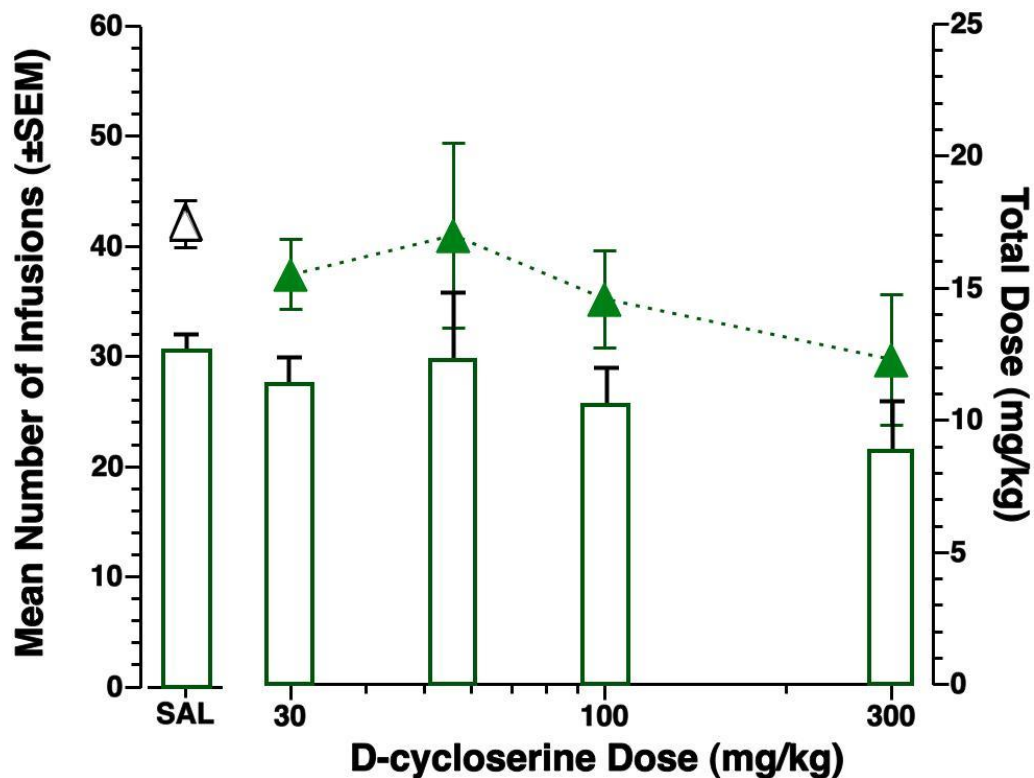


**Figure 12:** Ketamine self-administration levels (purple squares) and total dose self-administered following pretreatment with saline (SAL) or different doses of desipramine in 6 adult Sprague-Dawley rats (Male = 5; Female = 1) trained to self-administer 0.3 mg/kg/infusion ketamine. \* denotes infusion numbers significantly different from saline pretreatment at  $p < 0.05$ . # denotes total intake significantly different from mean total dose following saline.

**Table 14:** Mean number of lever responses, total number of infusions, and total dose of ketamine self-administered following pretreatment with saline or different doses of desipramine in 6 adult Sprague-Dawley rats (Male = 5; Female = 1) trained to self-administer 0.3 mg/kg/infusion ketamine.

Desipramine Dose (mg/kg)	Inactive Lever	Inactive SEM	Active Lever	Active SEM	Infusions	Infusions SEM	Total Dose (mg/kg)	Total Dose SEM
0 (Saline)	32.17	7.62	262.17	24.35	48.17	5.39	14.45	1.62
0.3	12.50	2.60	238.50	15.91	45.83	3.35	13.75	1.01
1	13.00	4.20	227.33	17.49	43.50	3.70	13.05	1.11
3	11.17	3.72	141.17	50.67	26.17	9.67	7.85	2.90

Figure 13 presents the mean number of ketamine infusions received during 60-min self-administration sessions following pretreatment with saline (SAL) or varying doses of DCS (30, 56, 100, and 300 mg/kg) in five male Sprague Dawley rats. The symbols (green triangles) and left-hand Y-axis show the mean number of infusions the animals received under the FR5 work requirement, while the right-hand Y-axis and the bars convey the total amount of ketamine that was self-administered in mg/kg. The X-axis shows the dose of DCS that was administered SC 20 minutes prior to the start of the session. The data point above SAL shows the mean number of infusions the animals received when similarly pretreated with saline. Mean number of infusions and standard errors shown in the graph were calculated based on that one session. When comparing the mean number of infusions following different doses of DCS to the mean number of infusions following saline, a one-way mixed measures ANOVA revealed that there was no effect of DCS ( $P=0.1214$ ). When analyzing the total ketamine intake, again, because it is directly proportional to the mean number of infusions taken, there was no effect of DCS pretreatment ( $P=0.1214$ ).

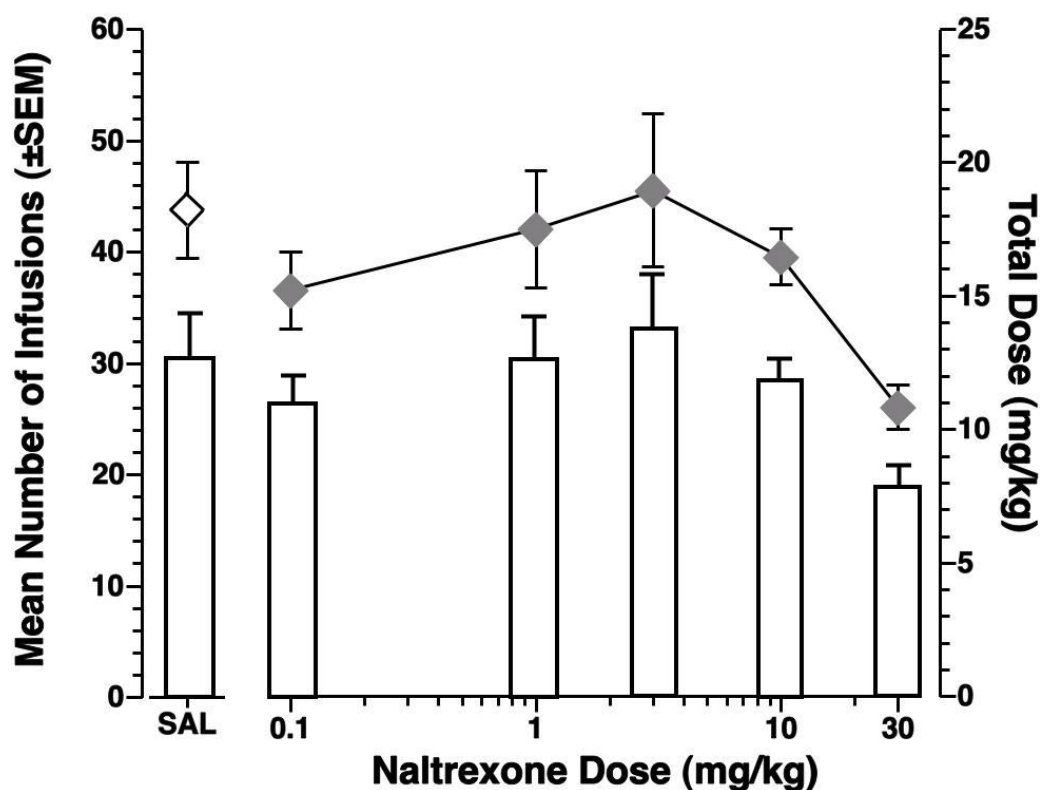


**Figure 13:** Ketamine self-administration levels (green triangles) and total dose self-administered following pretreatment with saline (SAL) or different doses of DCS in 5 male Sprague-Dawley rats trained to self-administer 0.3 mg/kg/infusion ketamine during daily 1-hour sessions. Each data point is based on n= 4-5 rats. \* denotes infusion numbers significantly different from saline pretreatment at  $p < 0.05$ . # denotes total intake significantly different from mean total dose following saline.

**Table 15:** Mean number of lever responses, total number of infusions, and total dose of ketamine administered during self-administration in combination with saline or different doses of D-cycloserine in 4-5 adult male Sprague-Dawley rats (Male = 5) trained to self-administer 0.3 mg/kg/infusion ketamine.

D-cycloserine Dose (mg/kg)	Inactive	Inactive SEM	Active	Active SEM	Infusions	Infusions SEM	Total Dose	TD SEM
0 (Saline)	13.20	5.76	222.80	13.06	42.00	2.14	12.60	0.64
30	11.50	3.23	203.25	17.02	37.25	3.20	11.18	0.96
56	9.40	5.70	220.00	44.60	40.80	8.40	12.24	2.52
100	10.00	3.36	182.80	23.16	35.00	4.40	10.50	1.32
300	9.75	5.81	158.25	33.59	29.50	5.92	8.85	1.78

Figure 14 presents the mean number of ketamine infusions received during 60-min self-administration sessions following pretreatment with saline (SAL) or varying doses of naltrexone (0.1, 1, 3, 10, and 30 mg/kg). The symbols (gray diamonds) and left hand Y-axis show the mean number of infusions the animals received under the FR5 work requirement, while the right hand Y-axis and the bars convey the total amount of ketamine that was self-administered in mg/kg. The X-axis shows the dose of NTX that was administered SC 20 minutes prior to the start of the session. The data point above SAL shows the mean number of infusions the animals received when similarly pretreated with saline. Mean number of infusions and standard errors shown in the graph were calculated based on that one session. Testing is still ongoing and the data points on the graph reflect data from only 2 to 6 subjects. Analysis to compare the mean number of infusions following different doses of naltrexone to the mean number of infusions following saline, a one-way mixed measures ANOVA revealed that there was no effect of naltrexone [ $F(1.578, 3.551) = 5.020, P=0.0942$ ]. Similarly, analysis of the total ketamine intake across pretreatments found no effect of naltrexone pretreatment [ $P=0.0942$ ].



**Figure 14:** Self-administration levels for saline and different doses of Naltrexone pretreatment in 2-6 adult Sprague-Dawley rats (Male = 5; Female = 1) trained to self-administer 0.3 mg/kg/infusion ketamine during daily 1-hour sessions. \* denotes doses significantly different from saline at  $p < 0.05$ .

**Table 16:** Mean number of lever responses, total number of infusions, and total dose of ketamine administered during self-administration in combination with saline or different doses of Naltrexone in 1-6 adult Sprague-Dawley rats (Male = 5; Female = 1) trained to self-administer 0.3 mg/kg/infusion ketamine.

Naltrexone Dose (mg/kg)	Inactive	Inactive SEM	Active	Active SEM	Infusions	Infusions SEM	Total Dose	TD SEM
0 (Saline)	13.20	5.31	219.40	16.95	41.60	2.98	12.48	0.89
0.1	16.00	5.00	198.00	19.00	36.50	3.50	10.95	1.05
1	17.60	5.46	230.40	26.24	42.00	5.22	12.60	1.57
3	20.00	10.12	246.75	31.53	45.50	6.84	13.65	2.05
10	15.50	4.50	219.50	14.50	39.50	2.50	11.85	0.75
30	7.50	3.50	142.00	5.00	26.00	2.00	7.80	0.60

## Discussion

**Ketamine baseline locomotor behavior.** Based on multiple previous studies evaluating ketamine's effects on locomotor activity (Irifune et al., 1991; Usun et al., 2013; Yamamoto et al., 2016; McDougall et al., 2017) we expected to see minimal or no change in overall locomotor activity at low, saline-like doses of ketamine. At intermediate doses of ketamine, we expected to see an increase in locomotor activity potentially due to ketamine-induced increases in dopaminergic activity as has been previously reported (Beninger, 1983; Hetzler & Wautlet, 1985; Lindefors et al., 1997). Finally, at high doses we expected to see a decrease in distance traveled due to ketamine's anesthetic/CNS depressant effects (Marland et al., 2013; Kurdi et al., 2014). Our results are consistent with these predicted outcomes (Figures 1, 2, 5 and 6). When evaluating all subjects (n=27), locomotor activity following the intermediate ketamine doses of 10 mg/kg and 30 mg/kg was significantly increased compared to activity following saline administration. The effect was most pronounced following 30 mg/kg ketamine where distance traveled increased almost 3-fold (Tables 2A and 2B). This could be explained by either a direct or indirect ketamine-induced increase in dopaminergic activity (Can et al., 2016; Hancock and Stamford, 1999; Irifune et al., 1991; Uchihashi et al., 1992; Usun et al., 2013). The data also suggest that activity levels have returned to baseline levels at the 56 mg/kg dose of ketamine. However, results at this high dose actually represent the mean of the subjects in which this dose produced an activating effect which led to an increase in distance traveled, while conversely, in other subjects it produced a strong sedative effect and limited total distance traveled. Previous studies by Hetzler and Wautlet (1985) and Irifune et al. (1991) demonstrated that adult rats and mice that were injected with moderate to high doses of ketamine exhibited hypoactivity, which is presumed to be due to the drug's anesthetic effects through blockade of NMDA receptors and decreased neuronal excitation. The variability in response across subjects, some being stimulated and some being sedated, explains why the mean activity level at 56 mg/kg dose was not significantly different compared to saline levels of activity. This variability was especially pronounced in the female rats and is consistent with the relatively large SEMs shown in Figures 2 and 6.

In the current study, female rats showed a trend for increased levels of activity across multiple doses

of ketamine and even under vehicle control conditions. Overall, only the difference at the 10 mg/kg dose achieved statistical significance (Figure 2 and Tables 3A and 3B) for the full group of 27 subjects. Male rats showed a trend for lower levels of activity across all conditions with a significant increase in locomotion only at the 30 mg/kg dose whereas females displayed significantly increased activity at 10 mg/kg as well as 30 mg/kg. When the data were expressed as a percent of the saline control values (Figure 3), thereby taking into account any differences in baseline behavior, the difference in response across sex was minimized but the sex-based difference at 10 mg/kg ketamine remained. The trend for sex-associated differences in locomotor activity were even more apparent in the subset of animals completing the combination testing (Figure 6; compare Tables 5A and 5B). However, the small sample sizes and between subject variability prevented detection of significant differences. The data in Figures 2 and 6 suggest a difference not only in level of response to ketamine but also show a leftward shift in the curve for females relative to the males suggesting an enhanced potency or sensitivity for ketamine. Our results are consistent with previous behavioral studies in rodents which have shown differences in the locomotor response to ketamine based on sex with ketamine being more potent in females than in males (Wilson et al., 2007; Wiley et al., 2011; McDougall et al., 2017; 2019; Crawford et al., 2020 and Páleníček et al., 2011). The study by Wiley et al. (2011) demonstrated that ketamine and other NMDA receptor antagonists have sex-dependent effects and that female rodents have higher activity levels when compared to male rodents. Wilson et al. (2007) also demonstrated that female rats tend to have higher activity levels when treated with 10 mg/kg ketamine compared to males under the same conditions. The reason behind this sex difference may be due to pharmacodynamic differences in NMDA receptor numbers and/or subunit composition. It may also simply reflect inherent differences in behavior between males and females that are not specific to the glutamate system. Alternatively, the increase in locomotor activity could be due to pharmacokinetic factors resulting in higher blood levels in females, as has been seen in acute ketamine treatment studies by Saland & Kabbaj (2018) and Páleníček et al. (2011).

In addition to impacting distance traveled, as the ketamine dose increased, there was also more total time spent in the center of the open field with a significant effect noted at the 56 mg/kg dose (Figure



4A and Tables 2A and 4A). Consistent with other responses to ketamine, this effect was significantly more robust in females (Figure 4B and compare Tables 5A and 5B). Interestingly, there was a trend for the 10 mg/kg dose to decrease time in the center however this effect was not significant. Interpretation of these ketamine-associated effects is complicated because of several confounding characteristics of this study. Increased time in the center zone (and therefore decreased thigmotaxis) has often been linked with anti anxiety-like behavior and is increased by drugs with anxiolytic effects. This interpretation is more appropriate when stress (e.g., bright overhead lights) has been applied, creating anxiety-like behavior which can be counteracted by anxiolytic drugs. We did not use a stressor in this study so cannot necessarily explain the increased center time as an anti anxiety-like effect. Additionally, while time in the center was significantly increased at the highest dose tested, this was likely an artifact of placing the subjects in the center of the open field at the session start, and those that were sedated then spent most of the 30 min in the center of the field. The increased time in the center following 30 mg/kg ketamine is less likely to be due to sedative effects. However, it is also not simply due to more activity as the increase reflects an approximately 4 to 6-fold increase in *time* spent, not simply increased distance traveled in the center. It is true that ketamine has been shown to produce anti anxiety-like effects in preclinical models (Engin et al., 2009; Papp et al., 2017). To determine if this increase in center time did reflect a change in anxiety-like behavior due to decreased concern about being in the open part of the field, it would require additional studies that specifically measure anxiety-like behavior and anti anxiety-like effects of drugs (e.g., light/dark box, elevated plus maze, novelty suppressed feeding).

**Ketamine baseline self-administration behavior.** A complete dose response curve is essential in understanding the safety and potency of a drug, as it explains the relationship between the effect of a drug and the amount of drug given and allows accurate comparisons of potency and efficacy between drugs as well as changes in potency and efficacy for the same drug under different pretreatment conditions (Currie 2018; Ralston et al., 2018). All these factors were taken into consideration when designing and performing our self-administration studies. Ketamine has been repeatedly shown to serve as a positive reinforcer in preclinical studies (Collins et al., 1984; Winger et al., 1989; Broadbear

et al., 2004; Van der Kam et al., 2009; De Luca & Badiani, 2011; De Luca et al., 2012; Guo et al., 2016; Venniro et al., 2015; Rezvani et al., 2018), therefore in our self-administration studies, we expected to see ketamine serve as a positive reinforcer and maintain IV self-administration behavior. Consistent with prior studies, all six subjects acquired IV ketamine self-administration behavior when 0.3 mg/kg/infusion was made available under FR1 conditions (data not shown). Maintenance behavior under FR5 conditions demonstrated a significant decrease in mean number of infusions when saline (VEH), the negative control, was substituted for the 0.3 mg/kg/infusion ketamine training solution (Figure 11 and Table 13), decreasing from a mean of ~45 infusions/session under baseline conditions to ~10 infusions for saline. The testing of different doses of ketamine produced a classic inverted U-shaped self-administration curve. Responding for infusions of the 0.1 mg/kg/infusion dose of ketamine was highly variable, likely because this lower dose of ketamine failed to produce sufficient CNS effects in some subjects and therefore failed to maintain self-administration behaviors. As the dose was increased, ketamine maintained self-administration behavior increased to levels significantly above saline levels with both 0.3 and 0.56 mg/kg/infusion serving as positive reinforcers. While infusion numbers decreased at the 0.56 mg/kg/infusion dose, the total dose self-administered continued to increase supporting that the infusion decrease was due to a potency increase and not due to a decrease in reinforcing efficacy. The maintenance of ketamine self-administration has been attributed to ketamine increasing the release of glutamate in the frontal cortex and nucleus accumbens, which in turn stimulates dopamine release (Masuzawa et al., 2003) as well as potential blockade of dopamine uptake (Hancock and Stamford, 1999) in the nucleus accumbens overall increasing dopamine levels in reward circuitry.

## **DESIPRAMINE (DSP)**

**DSP effects on locomotor behavior.** Interestingly, relatively few studies have evaluated the effects of desipramine or its parent compound, imipramine, on locomotor activity following acute dosing in “normal” rats. The majority of the literature focuses on the effects of repeated, subchronic dosing and/or the effects of desipramine in rodent models of depression or attention deficit hyperactivity

disorder where baseline activity is altered because of the model. Those few studies with comparable testing conditions to our study suggest that desipramine and other tricyclic antidepressants will decrease activity levels (Estrada-Camarena et al., 2004; Umehara et al., 2013). Based on this, we expected to see desipramine produce a dose-dependent decrease in distance traveled. Furthermore, we also hypothesized that when desipramine was administered as a pretreatment for ketamine, we would see a decrease in the locomotor activity induced by ketamine. There have been no studies looking at the effects of desipramine or any monoaminergic antidepressant on ketamine-induced locomotor activity. A recent study by Lamanna et al., (2021) suggested that desipramine disrupts dopaminergic neurotransmission in rodents and an older study with imipramine in cats proposed that imipramine might be interfering with the reward system in felines (Zagrodzka et al., 1987). Based on these studies in combination with the assumption that elevated dopamine contributes to ketamine-induced increases in locomotor activity, we believed it likely that desipramine would decrease ketamine's locomotor activating effects in an open field.

Under conditions where desipramine was administered alone (desipramine pretreatment followed by saline immediately before placement in the open field) we saw a significant dose dependent decrease in activity (Figure 7 and Table 6). Unfortunately, all dose combinations with ketamine were associated with a much greater degree of variability than when testing desipramine alone. This prevented identification of any dose combination significantly different from VEH + VEH conditions although there was a trend for doses of 10 and 30 mg/kg ketamine alone and in combination with desipramine to increase locomotor activity. The 3 mg/kg dose of ketamine failed to increase locomotor activity and when desipramine preceded 3 mg/kg ketamine, there were modest increases and decreases in distance traveled, but none of these test conditions were significantly different from VEH + VEH conditions. The most clinically relevant information from these data is that desipramine, which may be administered in combination with ketamine in the treatment of depression, does not appear to enhance ketamine-induced locomotor activation and therefore likely does not increase ketamine-induced increases in dopamine in the brain.

**DSP effects on ketamine self-administration behavior.** Desipramine has been shown to decrease the rewarding/reinforcing effects of drugs of abuse in humans and in preclinical models (Lima et al. Wee et al., 2006; Fuchs et al., 1998; Paterson et al., 2008) while not producing any reinforcing effects itself (Wee et al., 2006; Tzschenke et al., 2006). However, these results have not been consistent across all studies and are more likely to be noted when desipramine is administered repeatedly (Lima et al., 2003; Paterson et al., 2008; Tzschenke et al., 2006). There have been no studies looking at the effects of desipramine or any monoaminergic antidepressant on ketamine self-administration.

In this study, desipramine did cause a decrease in mean number of infusions and total ketamine intake (Figure 12), at the 3 mg/kg pretreatment dose. Table 14 reinforces the findings shown in Figure 12, as we see the mean number of infusions decreases almost 50% from ~48 following VEH pretreatment to ~26 following 3 mg/kg desipramine, as well as total dose self-administered decreasing from 14.45 mg/kg with VEH pretreatment to 7.85 mg/kg when pretreated with 3 mg/kg desipramine. There was no significant effect of the 0.3 mg/kg and 1 mg/kg doses of desipramine detected at this time. Given the ability of desipramine to alter the reinforcing properties of other drugs of abuse, it may be that similarly, through alteration in dopamine neurotransmission, the 3 mg/kg dose of desipramine altered ketamine's reinforcing effects making the drug less effective as a reinforcer. However, the activity suppressant effects of desipramine identified in the locomotor activity study also raise the possibility that desipramine is simply causing a nonselective suppression of behavior so that *any* behavior would be suppressed, not just IV drug self-administration. This lack of selectivity could decrease the potential therapeutic benefits of ketamine + desipramine. To better address the relative selectivity of desipramine's effects, testing the effect of desipramine pretreatment on food maintained responding could provide useful information regarding the reason for decreased ketamine self-administration and desipramine's potential therapeutic use combined with ketamine.

### **D-cycloserine (DCS)**

**DCS effects on locomotor behavior.** DCS functions as a glycine-site partial agonist. It has relatively high efficacy as a partial agonist so at low to moderate doses functions similar to glycine-site agonists.

At very high doses where its concentrations are much greater than glycine, a full agonist at the glycine-site, DCS can decrease NMDA receptor activation, basically functioning like an antagonist. This dual nature of DCS has made it of great interest in treating disorders where too much or too little NMDA receptor activation is occurring. For example, a study performed by Heresco-Levy et al. (2013), found that a high dose of DCS (1000 mg/d) was able to produce significant antidepressant effects when combined with MAAs and it did so without producing ketamine-like psychotomimetic and dissociative effects. Goff et al. (1995) also found DCS in combination with conventional antipsychotic agents was able to improve negative symptoms of schizophrenia, as well alleviate ketamine-exacerbated schizophrenia (Heresco-Levy & Javitt, 2004). Most pertinent to the current study, Irifune et al., (1992) demonstrated that DCS could reverse ketamine's anesthetic effects. Therefore we anticipated that DCS, while not completely antagonizing ketamine's effects, might be able to improve the therapeutic effects of ketamine by moderately reducing ketamine's locomotor activating and reinforcing effects.

At this time, we have only completed determination of a DCS dose response curve alone in our 8 OFT subjects. There are insufficient data points to include the few dose combination tests which have been completed. Across a wide range of low to moderate doses DCS administered alone failed to produce any effect on locomotor activity (Figure 10 and Table 12). These findings are in agreement with a study performed by Gaiardi et al. (2010) which demonstrated that chronic and acute pretreatment with DCS had no influence on the acute locomotor behavior after amphetamine administration. However, a conflicting study by Carlsson et al. (1994) found that DCS induced hyperlocomotion in mice at doses of 80 and 160 mg/kg as well as potentiated the locomotor stimulation produced by NMDA antagonists (uncompetitive = MK-801 and competitive = D-CPPene) combined with clonidine. Further testing of DCS in combination with ketamine is warranted to clarify the relationship between DCS and locomotor activity. These studies are ongoing.

**DCS effects on ketamine self-administration.** DCS showed a trend to dose-dependently decrease ketamine intake but effects so far are modest and do not reach significance (Figure 13). This analysis was preliminary given that we had a limited number of subjects (n=5) and not all animals had

completed all dose conditions (increasing our variability and decreasing our statistical power). With an increase in our subject numbers (projected to complete testing with minimally 12 subjects), we may be able to identify doses which significantly decrease ketamine intake. Even moderate attenuation of the undesirable effects of ketamine in combination with previous studies showing that DCS can produce and enhance antidepressant effects in patients with depression (Heresco-Levy et al. 2013, Chen et al., 2019), thereby improving the therapeutic index of the drug overall, makes this drug combination very interesting.

### **Naltrexone (NTX)**

**NTX effects on locomotor behavior.** Ketamine demonstrates low binding affinity and moderate efficacy at mu and kappa opioid receptors (Zanos et al., 2018). A study performed by Williams et al. (2018), demonstrated that a dose (50 mg/kg, IV) of NTX attenuated a low dose (0.5 mg/kg) of ketamine's antidepressant effect in depressed patients and proposed that opioid receptor activation played a key role in ketamine's antidepressant effects. Similarly Zhang et al., 2021 showed that NTX could block the antidepressant-like effects of ketamine in mice in differential reinforcement of low rates of responding (DRL). However, Zhang & Hashimoto (2019) demonstrated that a 10 mg/kg dose of NTX failed to block the antidepressant-like effects of 10 mg/kg ketamine in mice in the forced swim test. The conflicting findings could be due to the different routes of administration as that affects bioavailability, as well as different clinical and preclinical models. No studies have been performed to determine whether or not opioid receptors play any role in either the locomotor activating or the reinforcing effects of ketamine. When tested alone, NTX produced a significant decrease in distance traveled in all subjects at the 10 and 30 mg/kg dose of NTX (Figure 8). Table 10 allows numerical visualization of that significant decrease in distance traveled at those doses. In Figure 9, we show that no dose of NTX was able to significantly counteract the locomotor activation produced by a 10 mg/kg dose of ketamine.

**NTX effects on self-administration behavior.** Naltrexone has been tested in multiple preclinical self-administration studies for its ability to reverse the reinforcing effects of common drugs of abuse

(Collins et al., 1984). No studies have been performed studying the effects of NTX on ketamine self-administration. We did see a trend for a dose-dependent decrease in the mean number of ketamine infusions and a 30% decrease in total dose self-administered following pretreatment with 30 mg/kg NTX (Figure 14 and Table 16) but this effect was not significant. Studies are ongoing to increase our total subject number as well as explore more dose combinations. Regardless, 30 mg/kg NTX was associated with significant behavioral suppression in the open field test, therefore it is likely that the effects in the self-administration assay are the result of nonselective behavioral suppression. Similar to results with desipramine, the lack of selectivity could decrease the potential therapeutic benefits of ketamine + NTX combination. Testing of the effect of NTX pretreatment on food-maintained responding could provide useful information regarding the selectivity of NTX to decrease operant behavior maintained by different reinforcers.

### **Conclusion**

Overall, we saw effects with ketamine alone which were consistent with both published literature and previous work in this laboratory. This included locomotor activation at intermediate doses of ketamine with a sex-dependent difference in sensitivity to these activating effects and IV self-administration, with ketamine serving as a positive reinforcer of behavior. Our overall goal was to explore the potential for our three test compounds, DSP, DCS and NTX to decrease the locomotor activating effects of ketamine and/or ketamine self-administration. Desipramine produced a dose-dependent decrease in ketamine self-administration but the effects of desipramine on ketamine-induced locomotion were modest and inconsistent. Additionally desipramine suppressed activity when administered alone. DCS produced no effects on locomotor activity when administered alone but as yet, no significant effect on ketamine self-administration. NTX administered alone suppressed activity at moderate to high doses. This non-selective suppression of behavior likely accounted for the moderate decreases in ketamine-induced locomotor stimulation and self-administration that was observed. While data are too preliminary to rule out any of our three test compounds at this time, given its lack of disruption of behavior in the open field, potential to enhance antidepressant effects, and suggestion of modest

decreases in ketamine self-administration, DCS remains the test drug of greatest interest.



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