CALIFORNIA STATE UNIVERSITY SAN MARCOS

THESIS SIGNATURE PAGE

THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF ARTS

IN

PSYCHOLOGY

THESIS TITLE: Antidepressant Effects of Ketamine in Wistar and Sprague Dawley Rats

AUTHOR: Michelle Calderwood

DATE OF SUCCESSFUL DEFENSE: 1/31/2015

THE THESIS HAS BEEN ACCEPTED BY THE THESIS COMMITTEE IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ARTS IN
PSYCHOLOGY.

Dr. Keith Tujillo
THESES COMMITTEE CHAIR

SIGNATURE

DATE

4/22/16

Dr. Angelica Rocha
THESES COMMITTEE MEMBER

SIGNATURE

DATE

5/25/16

Dr. Sergio Iniguez
THESES COMMITTEE MEMBER

SIGNATURE

DATE
CALIFORNIA STATE UNIVERSITY SAN MARCOS

THESIS SIGNATURE PAGE

THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF ARTS

IN

PSYCHOLOGY

THESIS TITLE: Antidepressant Effects of Ketamine in Wistar and Sprague Dawley Rats

AUTHOR: Michelle Calderwood

DATE OF SUCCESSFUL DEFENSE: 1/31/2015

THE THESIS HAS BEEN ACCEPTED BY THE THESIS COMMITTEE IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ARTS IN
PSYCHOLOGY.

Dr. Keith Trujillo
THESIS COMMITTEE CHAIR
SIGNATURE
DATE

Dr. Angelica Rocha
THESIS COMMITTEE MEMBER
SIGNATURE
DATE

Dr. Sergio Iñiguez
THESIS COMMITTEE MEMBER
SIGNATURE
DATE 4/25/16
The Antidepressant Effects of Ketamine in Wistar and Sprague Dawley Rats

Michelle Calderwood

California State University San Marcos
# Table of Contents

**Abstract**  
**Introduction**  
**Ketamine**  
**The antidepressant effects of ketamine in humans**  
**The antidepressant effects of ketamine in rodents**  
**Preliminary Studies**  
**Preliminary Study 1: Effect of Ketamine in the Forced Swim Test in Sprague Dawley Rats**  
**Methods**  
**Results**  
**Summary and Discussion**  
**Preliminary Study 2: Comparison of the Effect of Ketamine in the Forced Swim Test in Sprague Dawley and Wistar Rats**  
**Methods**  
**Results**  
**Summary and Discussion**  
**Preliminary Study 3: Locomotor Effects of Ketamine Following Intraperitoneal versus Subcutaneous Injection**  
**Methods**  
**Results**  
**Summary and Discussion**  
**Overall Summary and Discussion**  
**Thesis Studies**  
**Methods**  
**Animals**  
**Apparatus**  
**Results**  
**Experiment 1: Results**  
**Experiment 1: Summary and Discussion**  
**Experiment 2: Results**  
**Experiment 2: Summary and Discussion**  
**Experiment 3: Results**  
**Experiment 3: Summary and Discussion**  
**Discussion**  
**Tables & Figures**  
**Figure PS1.1**  
**Figure PS1.2**  
**Figure PS2.1**  
**Figure PS2.2**  
**Figure PS2.3**  
**Figure PS2.4**  
**Figure PS3.1
Abstract

Over the last decade, a number of studies have demonstrated that ketamine has immediate and long-lasting antidepressant effects in humans and rodent models of depression. However, our lab and others have been unable to replicate these effects using the Forced Swim Test (FST). Therefore, we investigated the antidepressant effects of ketamine, with attention to methodological factors that may influence these effects including strain of animal and route of administration. We expected that we would see antidepressant like effects in three separate experiments using a single low dose, a repeated low dose and a single high dose of ketamine, all administered subcutaneously. We also expected Wistar rats to show greater antidepressant like effects across doses. In addition, we expected Wistar rats to have increased locomotor response to ketamine when compared to Sprague Dawley rats across doses. Surprisingly we found no reliable antidepressant effects of ketamine at a low dose administered once or repeatedly. We did see antidepressant effects in response to a large dose of ketamine for both strains. We observed increases activity in response to ketamine at all doses and notable difference between strains. Wistar animals were significantly more active for a longer period of time in response to ketamine.
Introduction

Clinical depression is a recurrent and lifelong illness characterized by depressed mood, diminished interest in activities, anhedonia (or lack of pleasure), fatigue and inappropriate feelings of guilt. This disorder can also cause psychomotor agitation and interfere with an individual’s ability to solve problems or make decisions. According to the National Institute of Mental Health, depression is a leading cause of disability in the United States. Approximately 16.5% of Americans will be diagnosed with clinical depression at some point in their lives (Kessler, Chiu, Demler, & Walters, 2005).

The antidepressant drugs that are most commonly used to treat depression, known as “typical antidepressants,” work by increasing the neurotransmitters serotonin and norepinephrine in the brain. Doctors have used these typical antidepressants to treat patients with depression for over 50 years. Typical antidepressants are effective in approximately 70% of depressed patients; this leaves 30% of depressed patients who are treatment resistant (Kessler, Chiu, Demler, & Walters, 2005). Individuals who are prescribed typical antidepressants need to take them continuously for 4 to 6 weeks before they experience any therapeutic effects even though they start increasing serotonin and norepinephrine immediately. The delayed therapeutic effect suggests that downstream mechanisms are more likely responsible for therapeutic effects than are the initial increases of serotonin and norepinephrine.

More recently, the glutamatergic system has received attention in research regarding the neurobiology of depression. Glutamate is the primary excitatory neurotransmitter in the brain, thought to play a major role in learning and plasticity. The glutamate system has three main ionotropic receptors: N-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainate receptors (Machado-Vieira, Manji, & Zarate, 2009; White & Ryan, 1996). Research with ketamine, which acts by blocking NMDA
receptors demonstrates that this drug has potent and uniquely long-lasting antidepressant effects (Domino, 2010; Machado-Vieira, Salvadore, Diazgranados & Zarate, 2009; Murrough & Charney, 2010).

**Ketamine**

Ketamine is a dissociative anesthetic and a derivative of phencyclidine (PCP or “angel dust”). It was developed in the 1960s as an anesthetic to replace PCP, which had problematic side effects (Domino, 2010; Sun et al., 2011; Wolff & Winstock, 2000). It is referred to as a dissociative drug because it creates a state of disconnect between the user and his or her surroundings (Annetta, Iemma, Garisto, Tafani & Proietti, 2005). Ketamine is considered to be very safe because, unlike other anesthetics, it does not cause respiratory or cardiovascular depression (Trujillo et al., 2011; Wolff & Winstock, 2000). Ketamine continues to be used widely for anesthesia and analgesia.

Ketamine’s effects are largely due to its action on NMDA receptors. NMDA receptors are ionotropic receptor cation channels that are activated by the neurotransmitter glutamate. Ketamine acts to block the open ion channel in the receptor and prevent calcium influx (Trujillo et al., 2010; White & Ryan, 1996).

Aside from its clinical use, ketamine is a popular drug of abuse due to its psychedelic and euphoric effects. The drug was popularized for its psychedelic effects in the 1970s. Later ketamine’s popularity grew as a “club drug” used at raves and dance clubs (Trujillo et al., 2010; Wolff & Winstock, 2006). The Drug Enforcement Administration classified ketamine as a Schedule III drug in 1999 because of its increasing recreational use (Drug Enforcement Administration, 1999). According to the DEA, Schedule III drugs have the potential for abuse and moderate or low physical and psychological dependence.
In addition to ketamine’s medical and recreational uses, it is used to investigate schizophrenia. Ketamine’s effects mimic positive and negative symptoms of schizophrenia better than other drug models of the disorder (Moghaddam, Adams, Verma & Daly, 1997). A dose of ketamine can enhance symptoms in schizophrenic volunteers and mimic them in non-schizophrenic individuals (Lahti, Weiler, Michaelidis, Parwani & Tamminng, 2001). Because of this, it is used as an animal model for of schizophrenia in order to investigate possible treatments and neurobiology of schizophrenia (Becker et al., 2003).

**The antidepressant effects of ketamine in humans**

Ketamine has been found to have rapid, robust and long-lasting antidepressant effects. Its effects are superior to typical antidepressants because one dose decreases depression symptoms immediately and the antidepressant effects can last up to two weeks. In contrast, typical antidepressants need to be administered continuously for approximately 4 to 6 weeks to exert therapeutic effects. Researchers have replicated the antidepressant effects of ketamine in humans and in rodents.

Sappington, Corssen, Becker, and Tavakoli (1979) discovered ketamine’s potential for antidepressant effects when they observed that the drug reduced negative affect in non-depressed individuals. Since then, published case reports and single participant studies have repeatedly demonstrated that ketamine can relieve depression. Moreover, the antidepressant effects of ketamine have been demonstrated using case studies with one or two patients as well as larger clinical populations (Caddy et al., 2014; Corell & Futter, 2006; Covvey et al., 2012; Irwin & Iglewicz, 2010; Liebrenz et al., 2007; Messer et al., 2010).

More recent attention given to ketamine as an antidepressant was initially driven by Berman et al. (2000). Berman found large and long-lasting antidepressant effects in a controlled
clinical study. Berman used seven depressed patients who had been unresponsive to treatment with typical antidepressant medications. The patients had a rapid and robust antidepressant response to a low dose of intravenous ketamine that lasted for up to two weeks post treatment. Studies have replicated the Berman study several times with larger sample sizes, different groups and various measures for depression (Abdallah, et al., 2013; Aan het Rot et al., 2010; Berman et al., 2000; Dakwar et al., 2014; Diazgranados et al., 2010; Lai et al., 2014; Loo et al., 2012; Machado-Vieira et al., 2010; Okamoto et al., 2010; Phelps et al., 2009; Price et al., 2009; Rassmusin et al., 2013; Salvador et al., 2009; Salvador et al., 2012; Scheidegger et al., 2012; Shah Carreno & Frazer, 2014; Thakurta et al., 2012; Trial et al., 2013; Valentine et al., 2011; Wang et al., 2012; Zarate et al., 2006; Zarate et al., 2012).

The antidepressant effect of ketamine in humans is a robust and replicable effect that supports a role for glutamate in depression. Studies have demonstrated ketamine's antidepressant effects using various doses, administered acutely or chronically in untreated or treatment-resistant patients. However, ketamine is not an ideal medication to be made widely available for the treatment of depression. Ketamine has the potential for abuse and it has a number of psychotropic effects such as dissociation. In addition, there is evidence that ketamine can cause cognitive deficits and long-term health complications. Mice display memory deficits after chronic treatment and frequent ketamine users display deficits in both short-term and long-term memory (Morgan & Curran, 2006; Morgan & Curran, 2011). Long-term effects of ketamine include deficits in pain sensitivity, neuromuscular strength and lower urinary tract damage (Chu, Ma, & Wong, et al., 2008; Sun, Lam, Wong, Lam, Tang, et al., 2011). The exact mechanism by which ketamine exerts its antidepressant effects is unknown. It is important to continue to investigate these antidepressant effects in order to elucidate the role of glutamate in the
neurobiology of depression and to discover new treatments for this disabling psychiatric disorder.

The antidepressant effects of ketamine in rodents

Researchers use animal models of depression to better investigate the neurobiology of depression and ketamine’s antidepressant effects. Rodents used in these studies are genetically similar and have very similar life experiences, eliminating many of the variations that compliment and confound research with humans. There are several animal models of depression often used in research including the learned helplessness paradigm, the forced swim test, the tail suspension test and the chronic mild stress/sucrose preference test. Scientists have demonstrated the antidepressant effects of ketamine using a variety of rodent models of depression (Autry et al., 2011; Bechtolt et al., 2011; Benoit, Chenu, & Bourin, 2005; Charliane et al., 2010; Garcia et al., 2009; Lindholm et al., 2011; Maeng et al., 2008; Wang et al., 2011). However, the forced swim test is the most frequently used paradigm used to screen for antidepressant efficacy (Browne & Lucki, 2013; Yan et al., 2010).

For the forced swim test procedure, researchers introduce animals to an inescapable cylinder full of water and record their behavior. Animals treated with antidepressant drugs are less immobile, and they climb more and swim more than untreated animals. Immobility in the forced swim test is most often reported in studies on the antidepressant effects of ketamine. Secondary behaviors on the FST include climbing (attempts to escape the cylinder) and swimming. Climbing and swimming behaviors are also important because differences between those two behaviors have been shown to reflect different drugs and their mechanism of action. Serotonergic drugs typically increase swimming behavior but noradrenergic drugs tend to increase climbing behavior (Cryan, Valentino, & Lucki, 2005).
Ketamine’s antidepressant-like effects have been demonstrated on the rat forced swim at various doses, administered acutely or chronically (Table 1). The antidepressant-like effects in animals are comparable to the antidepressant effects of ketamine found in humans. Similar to human studies, ketamine’s antidepressant effect is evident within thirty minutes to one hour after treatment, whereas typical antidepressants, such as imipramine and desipramine, require multiple days of repeated treatment (Meang et al. 2008). The long-term effect of ketamine seen in human studies has also been demonstrated in rats. For example, one study showed that rats had antidepressant-like effects up to ten days post treatment with an anesthetic dose of ketamine (Yilmaz, Schulz, Aksoy & Canbeyli, 2002).

Ketamine has immediate and long-lasting antidepressant effects in humans and in animals. Furthermore, ketamine shows uniquely long lasting effects compared to other NMDA antagonists. Drugs similar to ketamine such as MK-801, a more selective NMDA antagonist, demonstrate immediate antidepressant effects. However, ketamine’s antidepressant-like effects have been found to last longer (Maeng et al., 2008). Taken together, theses studies support a strong role for glutamate in the neurobiology of depression and a unique ability of ketamine to exert immediate, robust and long-lasting antidepressant effects.

The leading hypothesis for ketamine's antidepressant-like effects has focused on its blockade of NMDA receptors. However, recent evidence suggests that ketamine as well as other NMDA antagonists have a range of effects in the brain, including causing increased glutamate release and subsequent AMPA receptor activation, suggesting that ketamine may act via glutamatergic hyperactivity. According to the glutamate hyperactivity hypothesis, blockade of NMDA receptors leads to increased levels of glutamate in the synapse, which can activate AMPA receptors; activation of AMPA receptors is ultimately responsible for ketamine’s
antidepressant effects (Homayoun & Moghadam, 2007; Koike, Iijima & Chaki, 2011; Lindholm et al., 2011; Machado-Vieira, Manji & Zarate 2009; Maeng, 2008; Schmidt, 2008; Skolnick, 2008). Furthermore, brain derived neurotrophic factor (BDNF), a protein that regulates synaptic plasticity is also increased in the hippocampus as well as prefrontal cortex in mice and rats following ketamine injections (Garcia et al., 2008; Rues et al., 2010; Yang et al., 2012; Yang et al., 2013; Zhou et al., 2013). The mammalian target of rapamycin (mTOR) as well as BDNF are thought to underlie the antidepressant response of ketamine. The mTOR, when activated, regulates cell growth and neural plasticity, suggesting that neurogenesis and long-term potentiation may be occurring in response to ketamine. The evidence suggests that NMDA antagonists like ketamine cause increased AMPA throughput and neurogenesis that is mediated via BDNF and mTOR (Du, et al. 2006; Dwyer & Duman, 2012; Dwyer, Lepack & Duman, 2011; Hashimoto, 2011, Jernigan et al., 2011; Li et al., 2010, Zhou et al., 2013).

Despite the excitement and many positive findings regarding the antidepressant effects of ketamine there are studies that have been unable to fully replicate ketamine’s antidepressant effects in some rodent models. In several experiments, Popik, Kos, Sowa-Kucma and Nowak (2008) tried to replicate antidepressant effects of ketamine in rodent models of depression, in both rats and mice. Mice were tested in the FST immediately after, and two weeks after ketamine treatment. The mice that received the highest dose (50 mg/kg) had a significant reduction in immobility 30 minutes after treatment but no effects of ketamine were seen two-weeks later. Furthermore, Popik et al. (2008) tried to replicate the long-term effects of an anesthetic dose of ketamine but found no long-term antidepressant effects 6 and 7 days after treatment.

In addition, Bechtolt et al. (2011) tested the long lasting, antidepressant effects of ketamine with two different strains of mice at a range of doses in the forced swim test and the
tail suspension test. No dose of ketamine reduced immobility in CD/1 mice seven days after treatment. In Balb/Cj mice only the highest anesthetic dose of ketamine produced antidepressant effects an hour after treatment and no significant effects were seen seven days after treatment. The authors concluded that the antidepressant effects of ketamine might be specific to certain strains of mice. It is not uncommon to find differential effects of drugs between animal strains. Furthermore, in speaking with scientists at conferences, we’ve learned that some have had difficulties detecting antidepressant effects of ketamine in the forced swim test, and that more specific methods may be necessary to achieve positive results. Taken together, the evidence suggests that antidepressant effects of ketamine can be demonstrated in animal models, but these may be particularly sensitive to methodological factors (Table 1).

**Preliminary Studies**

Three preliminary studies were performed to investigate the behavioral effects of ketamine: 1) the antidepressant-like effects of ketamine were examined in Sprague Dawley rats using the rat forced swim test; 2) the antidepressant-like effects of a low dose ketamine were compared in Sprague Dawley and Wistar rats using the rat forced swim test, and the open field locomotor monitoring system 3) differences in behavior induced by ketamine were examined following intraperitoneal and subcutaneous administration. Preliminary Study 1 found no effect of ketamine in the forced swim test. This study used a low intraperitoneal (IP) dose of ketamine in Sprague Dawley rats. Given the null results, the subsequent study was designed to assess differences in strain on the FST.

Methodological factors can greatly affect the outcome of behavioral paradigms with rodents, including the strain of the animals. While some studies, including Engin et al. (2011), have found antidepressant effects of ketamine on the forced swim test using Sprague Dawley
rats, it has been more commonly reported using Wistar rats (Table 1). Other studies have found differences between mouse strains in response to ketamine on tail suspension test and the forced swim test (Bechtolt et al. 2011). Preliminary Study 2 found some antidepressant-like effects of ketamine in Wistar, but not in Sprague Dawley rats. This study again used a low IP 10 mg/kg dose of ketamine.

In addition to animal strain, another methodological factor that can impact behavioral responses to drug is, the route of injection. Therefore, Preliminary Study 3 investigated the effect of route of administration on behavioral response to ketamine and revealed greater effects following subcutaneous administration, when compared to intraperitoneal injection.

**Preliminary Study 1: Effect of Ketamine in the Forced Swim Test in Sprague Dawley Rats**

The purpose of this study was to attempt to replicate the antidepressant-like effects of ketamine using the FST and Sprague Dawley rats. We expected that animals that received ketamine treatment would be less immobile on the forced swim test than saline treated animals, reflecting antidepressant effects of the ketamine. In addition, we used this preliminary study to measure baseline depressive behavior (increase in immobility) from day one to day two. Climbing and swimming behaviors were also collected. Increased climbing and swimming behaviors reflect antidepressant-like behavior but are typically secondary measures to immobility.

**Methods.**

*Animals.* Thirty-four Adult Sprague Dawley rats were used in this experiment. Animals were habituated to the vivarium for one week prior to testing. The vivarium is run on a twelve-hour light/dark cycle and animals have free access to food and water in their home cages.
Forced Swim Test. The rat forced swim test procedure was modified from Porsolt, Anton, Blavet and Jalfre (1977). The forced swimming apparatus is a Plexiglas cylinder (19cm diameter, 40.3cm height) filled with 30 cm of water (Kinder Scientific, Poway, CA). Water is kept at a temperature of 25 degrees Celsius.

Procedure. Animals were handled for five minutes each for the three days prior to the start of experiments. On Day 1, animals were brought to testing room one at a time in transport cages and were placed in swimming apparatus for 15 minutes. They were then towel dried and placed in a heated incubator, kept at approximately 32 degrees Celsius for thirty minutes before being returned to the vivarium. On Day 2, animals were brought to the testing room in the same order and manner as day one, except the animals were placed in the apparatus for only 5 minutes. On Day 3, animals were again brought to the testing room in the same order and manner as Day 2. On Day 3 animals received either intraperitoneal ketamine treatment (10 mg/kg) or saline (control) one hour before testing. All swimming sessions were video recorded (with the camera placed to the side of the swim apparatus) and behavior was scored in seconds as time spent swimming, climbing or immobile. Climbing behavior was scored when the animal’s forelimbs had contact with the apparatus walls and the rat appeared to be trying to climb out. Swimming behavior was scored when the animal was rapidly paddling front and back limbs. Immobility was scored when the animal was moving just enough to keep its head above the water.

Drugs. Ketamine hydrochloride (Sigma-Aldrich) was used in this study. It was dissolved in physiological saline (0.9% NaCl) and injected in a volume of 10 ml/kg.

Statistics. One matched samples t-test was used to assess immobility from Day 1 to Day 2. Three independent samples t-tests compared saline and ketamine groups on each behavior on Day 3 (swimming, climbing and immobility).
Results.

As expected, increased depressive like behaviors (greater immobility) was observed between Day 1 and Day 2 (Figure PS1.1). Contrary to the hypothesis we did not see decreased immobility in rats that received ketamine compared to control rats that received saline. Also, contrary to the hypothesis we did not see increased climbing or swimming behaviors in animals that received ketamine compared to animals that received saline. Statistical analyses confirmed these observations (PS1.2). There were significant depressive effects of the FST as measured by immobility. Animals were significantly more immobile on Day 2 than they were on Day 1, \( t(16)=3.59, p<.01 \) (Figure PS1.1). On Day 3, saline and ketamine groups were not significantly different on the forced swim test with regard to immobility, \( t(15)=.80, p=.43 \), climbing behavior, \( t(15)=1.20, p=.25 \), or swimming behavior \( t(15)=1.38, p=.19 \) (Figure PS1.2).

Summary and Discussion

Despite good reason to believe we would detect antidepressant effects of a low dose of ketamine, there were no significant differences between the control and ketamine groups for any behavior measured in the FST. Given the number of studies that have found effects of ketamine, there are a number of concerns and possible explanations that should be considered.

First, the forced swim test is a well-validated and reliable animal model for assessing antidepressant effects of drugs (Porsolt, 1977; Yan, Cao, Das, Zhu & Gao, 2010). In addition, as expected, in this preliminary study there was significant depression of behavior in the FST observed before any drug treatment. Regarding the power of the current study, the number of animals per group was selected based on previous studies. In addition, extraneous variables are well controlled in studies done with rats due to the similarity of the animal’s genes and environment so a larger numbers of animals are not essential to detecting drugs effect. Moreover,
the trend in the results was opposite to what was predicted, with a slight increase in immobility and a decrease in swimming induced by ketamine. The rat forced swim test should be a valid and powerful test for investigating the antidepressant effects of ketamine, but such effects were not seen in the current study.

A potential explanation for these results is that methodological factors may have interfered with the ability to detect effects of ketamine in this experiment. For example, the strain of rat selected may be insensitive to the antidepressant effects of ketamine. It is well known that there are behavioral differences seen between strains of rats on various paradigms. Additionally, there are strain differences seen on the forced swim test in response to typical antidepressants in mice (Borsini & Meli, 1988) and rats (Cryan, Valentino & Lucki, 2005; Vetulani, Nalepa, Popik, 1991). Furthermore, many of the studies that reported significant effects of ketamine on the FST at low doses, used Wistar rats rather than Sprague Dawley rats – in fact, 14 out of the 18 rat FST studies cited here used Wistar rats (Table 1). Subsequently, Preliminary Study 2 was designed to investigate the effects of rat strain on ketamine response in the FST.

Preliminary Study 2: Comparison of the Effect of Ketamine in the Forced Swim Test in Sprague Dawley and Wistar Rats

As discussed above, previous research has revealed antidepressant effects of ketamine on the forced swim test. Engin et al. (2011) used Sprague Dawley rats and found antidepressant effects of a low and moderate dose of ketamine. However, in investigations of the antidepressant effects of ketamine, the use of Wistar rats on the forced swim is noticeably more common; 14 out the 18 rat FST studies identified in literature used Wistar rats (Table 1). The purpose of this preliminary study was to investigate the difference in sensitivity between Sprague Dawley and Wistar rats on the forced swim test in response to ketamine, and furthermore to investigate
differences in the locomotor response to ketamine between the two rat strains. We hypothesized that Wistar rats would show an antidepressant effect in response to a low dose of ketamine that is not effective in Sprague Dawley rats. Specifically, we expected that Wistar rats but not Sprague Dawley rats treated with ketamine would show significantly less immobility compared to their corresponding control rats that received saline. We also expected Wistar rats treated with ketamine to climb and swim more than Wistar control rats. Finally, to further explore behavioral differences between the two strains of rats, we examined the locomotor effects of ketamine and hypothesized that Wistar rats would show greater locomotor stimulant effects of ketamine than Sprague Dawley rats.

**Methods.**

**Animals.** Sixteen adult male Sprague Dawley rats and sixteen adult male Wistar rats were used for this study (n=8/group). Animals were habituated to the vivarium for one-week prior to experiments. The vivarium is on a 12-hour light/dark cycle and food and water is freely available.

**Forced Swim Test.** The forced swim test was used to measure depressive like behavior in rats and as described above. All swimming sessions were video recorded. Behavior was scored in seconds immobile, climbing and swimming as described above.

On Day 1, animals were brought to the testing room and placed in the swimming apparatus for 15 minutes. Then they were towel dried and placed in an incubator for 30 minutes. The following day (Day 2), animals received (10 mg/kg) of ketamine or saline one hour before testing. They were brought to the testing room in the same manner as pretest day and placed in the forced swim test apparatus for five minutes.
**Locomotor Monitor.** A Kinder Scientific Smart Frame Cage Rack system was used to assess locomotor activity. This system consists of photo beam arrays (7 x 16) into which standard plastic rat cages (10.5” x 19” x 8”) are placed. The photo beam arrays are interfaced with a personal computer for data collection. The computer collects photocell beam breaks as an assessment of activity. Successive beam breaks indicate forward movement referred to here as ambulations.

The animal’s locomotor response to ketamine was measured on two separate occasions, one and two weeks after the forced swim test, in response to 25 mg/kg (week 1) and 50 mg/kg (week 2). On locomotor testing days, the animals were weighed, transported to the testing room, and allowed 30 minutes of habituation to the room. Then animals were placed in the locomotor apparatus and given 30 minutes to habituate before they were injected with either ketamine (25 mg/kg or 50 mg/kg) or saline, placed back in the apparatus, and their locomotor behavior was assessed for 90 minutes.

**Drugs.** Ketamine hydrochloride (Sigma-Aldrich) was used in this study. It was dissolved in physiological saline (0.9% NaCl) and injected intraperitoneal (IP) in a volume of 1 ml/kg.

**Statistics.** A matched sample t-test was used to compare behaviors on the forced swim test from on day 1 and day 2 in control animals. Two by two (rat strain by treatment) ANOVAs were used to analyze behaviors measured in the forced swim test in response to ketamine including time spent immobile, climbing and swimming. Planned comparisons were used to compare Wistar ketamine and saline groups, and Sprague Dawley ketamine and saline groups for each analysis.
Two by two (strain by treatment) ANOVA’s were used to analyze total locomotor activity (ambulations) in response to ketamine. Two t-tests were used to assess differences between strains in response saline and to ketamine for each ANOVA.

**Results.**

First, as in Preliminary Study 1, there was evidence that the forced swim test was effective in producing depressive behaviors in control animals from day 1 to day 2 (Figure PS2.1). Second, there appeared to be some differences in antidepressant response to ketamine based on strain. Wistar rats that received ketamine appeared to spend less time immobile and more time climbing than Wistar rats that did not receive ketamine (Figure PS2.2). In contrast, Sprague Dawley rats showed no effects of ketamine in the FST. Also, Wistar rats showed evidence of a greater locomotor stimulant response to ketamine than Sprague Dawley rats.

Statistical analyses confirmed the depressant effect in the FST from day 1 to day 2. There were significant differences in immobility from day one to day two for saline control animals, Wistar rats \( t(14)=2.51, p=.02 \), and Sprague Dawley rats \( t(14)=2.42, p=.04 \) (Figure PS2.1).

On day 2, there were no significant effects of treatment on immobility, \( F(1,28)=.02, p=.89 \), or by strain on immobility, \( F(1,28)=.07, p=.79 \) (Figure PS2.2A). There was also no interaction between treatment and strain, \( F(1,28)=2.73, p=.11 \) for immobility. Although there appeared to be a decrease in immobility compared to Wistar rats, planned comparisons revealed that Wistar rats that received ketamine did not spend significantly more time immobile than Wistar rats that received saline \( t(14)=1.12, p=.28 \). Sprague Dawley rats that received ketamine did not spend more time immobile than Sprague Dawley rats that received saline \( t(14)=1.21, p=.25 \).
There were no significant effects of treatment, $F(1,28)=1.88$, $p=.18$, or strain, $F(1,28)=.05$, $p=.83$ for time spent climbing (Figure PS2.2B). There was also no interaction between treatment and strain, $F(1,28)=2.29$, $p=.18$ for time spent climbing. Planned comparisons revealed that Wistar rats that received ketamine spent more time climbing than Wistar rats that received saline $t(14)=2.85$, $p=.01$. However, there was no significant difference between Sprague Dawley ketamine and saline groups $t(14)=.08$, $p=.93$.

Finally, there were no significant effects of treatment, $F(1,28)=2.19$, $p=.15$, or strain, $F(1,28)=.55$, $p=.46$ for time spent swimming. There was also no interaction between treatment and strain, $F(1,28)=.48$, $p=.49$ for time spent swimming (Figure PS2.2C). Planned comparisons revealed no differences for Wistar ketamine and saline groups, $t(14)=.51$, $p=.61$, or Sprague Dawley ketamine and saline groups $t(14)=1.68$, $p=.11$.

The locomotor data, in this case the number of ambulations during the test session were analyzed using a two (Wistar vs. Sprague Dawley) by two (saline vs. ketamine 25 mg/kg) ANOVA (Figure PS2.3A & PS2.4A). The ANOVA showed strong main effects of treatment for number of ambulations, $F(1, 28)=21.99$, $p<.01$. There was no significant effect of strain $F(2,28)=2.23$, $p=.15$, and no significant interaction of strain and treatment, $F(1,28)=1.64$, $p=.21$. Planned comparisons showed that Wistar rats that received ketamine were not significantly more active than Sprague Dawley rats that received ketamine $t(14)=1.40$, $p=.18$, there were no differences between saline groups, $t(14)=.74$, $p=.47$. Although the overall effect was not statistically significant, ketamine-induced locomotion in Wistar animals appeared more prolonged than that in Sprague Dawley animals (Figure PS2.2).

Similar effects were seen with the higher dose of ketamine in locomotor behavior (Figure 2.3B & 2.4B). There was a significant effect of treatment, $F(1, 28)=119.75$, $p<.01$. There was no
significant effect of strain, $F(1,28)=46, p=.50$ and there was no interaction effect of strain and treatment, $F(1,28)=.35, p=.56$. Planned comparisons revealed no differences between Wistar and Sprague Dawley ketamine groups, $t(14)=.64, p=.53$.

**Summary and Discussion**

As hypothesized, the results showed no effect of ketamine in the FST for Sprague Dawley rats but did reveal an effect in Wistar rats. Wistar rats treated with ketamine showed increased climbing behavior relative to Wistar rats treated with saline. Surprisingly, no significant differences were seen for immobility or swimming. These results tentatively suggest that Wistar rats are more sensitive to the antidepressant effects of ketamine than Sprague Dawley rats.

We further explored strain differences in the locomotor response to ketamine. As expected, there was a significant, dose-dependent stimulant effect of ketamine in both strains. There was no significant effect of strain or a significant interaction effect of treatment and strain. Nevertheless, Wistar rats that received ketamine showed a more prolonged stimulate effect than Sprague Dawley rats that received ketamine at the lower dose (25 mg/kg: Figure PS2.3B).

The results tentatively suggest that the antidepressant effects of ketamine in the FST are dependent on the strain of the animal used, with Wistar rats more sensitive than Sprague Dawley. Also, Wistar rats showed a trend toward greater ketamine-induced activity; however, the modest difference does not appear to be sufficient to explain the differences in the FST. Further research on strain differences in the FST and in locomotor activity in response to ketamine are therefore warranted.
Preliminary Study 3: Locomotor Effects of Ketamine Following Intraperitoneal versus Subcutaneous Injection

Preliminary Study 3 was designed to investigate the differences in behavioral response to ketamine based on route of administration. The most common routes of administration used in animal studies with rodents are subcutaneous (SC) and intraperitoneal (IP) injections. Ketamine is most often injected using the intraperitoneal route in behavioral studies on rats. Drugs injected this way are subject to first pass metabolism, also known as the first pass effect. The first pass effect refers to the reduction in availability of a drug to the brain due to metabolism by the liver. Subcutaneous injection avoids the first pass, resulting in more drug available to the brain following injection (Turner, Brabb, Pekow & Vasbinder, 2011).

There is evidence that suggests that the effects of dissociative drugs differ based on route of injection. For instance, Kalinichev et al. (2008) found that Sprague Dawley rats given subcutaneous injections of PCP were significantly more active than rats given the same dose using the IP route of administration and that total blood concentrations and total brain concentrations of PCP were higher for SC than for IP. Based on previous research and the demonstrated greater availability of the drug to the brain using SC administration, we hypothesized that animals that receive SC injections of ketamine would be significantly more active than animals that received IP injections of the same dose of ketamine.

Methods.

**Animals.** Twenty-four adult male Sprague Dawley rats were used for this study. There were eight animals per treatment group. Animals arrived approximately two weeks prior to the study in order to habituate to the vivarium. The vivarium is on a twelve-hour light/dark cycle. Animals have free access to food and water in their home cages.
**Kinder Scientific Open Field Motor Monitor.** Each enclosure (16” x 16” x 15”) contains two rows of photocells to measure activity; the bottom row measures horizontal activity and the top row measures vertical activity (rearing). Each enclosure is attached to a personal computer to collect data. Animals were measured for locomotor activity (ambulations) for a 120-minute time course. Ambulations are interruptions of successive photocell beams associated with forward motion.

**Procedure.** Animals were handled for five minutes a day for three days prior to testing and were counterbalanced each week into treatment groups. Animals were tested over a study period of three weeks. We had a total of eight treatment groups overall and six animals per group each week of the study. The same 24 animals were counterbalanced into treatments groups for each week, with six animals in each group. Treatment groups included ketamine (10 mg/kg) IP and SC on week one, ketamine (25 mg/kg) IP and SC or 50 ketamine (50 mg/kg) IP and SC on week 2 and finally ketamine (100 mg/kg) IP and SC on week 3.

On testing day, the animals were transported to the testing room in their home cages and then habituated to the testing room for thirty minutes. Subsequently, the animals were habituated to locomotor chambers for thirty-minutes. Animals were administered drug immediately following the habituation period and observed in chambers for 120 minutes post-injection. White noise is played during testing to mask potential background noise.

**Drugs.** Ketamine hydrochloride (10, 25, 50 and 100 mg/kg) was used in this study. It was dissolved in physiological saline (0.9% NaCl) and injected in a volume of 1 ml/kg.

**Statistics.** Four unpaired t-tests were used to compare route of administration for each treatment group. The comparisons included animals that received SC and IP injections of 10 mg/kg of ketamine, animals that received SC and IP injections of 25 mg/kg of ketamine, animals
that received SC and IP injections of 50 mg/kg of ketamine and animals that received either SC or IP of 100 mg/kg of ketamine.

Results.

As predicted, different locomotor responses were obtained from the same dose of ketamine using different routes of administration. Overall, there was a dose dependent increase in locomotor activity in response to ketamine. Animals that received subcutaneously administered doses showed a greater increase locomotor activity compared to intraperitoneally administered doses of ketamine (Figure PS3.2). There was also a dose dependent increase in duration of locomotor effect as well as a large increase in duration of effect for animals that received SC ketamine compared to IP ketamine at the same dose (Figure PS3.1) For the 10-mg/kg dose of ketamine there was a significant difference for locomotor activity based on route of administration SC and IP, $t(118)=2.53$, $p=.0127$ (Figure PS3.1). Similarly, at the 25-mg/kg dose, there was a significant difference for locomotor activity between route of administration SC and IP, $t(118)=4.717$, $p<.0001$, and for the 50 mg/kg dose $t(118)=5.27$, $p<.01$ (Figure PS3.1). At the 100 mg/kg dose there were no significant differences between route of administration when comparing the total activity of SC and IP, $t(118)=1.363$, $p = .18$ (Figure PS3.1). However, in examining the time course of the locomotor response, it was clear that there were differences between the two routes of administration, with the effects of SC administration delayed relative to IP. Thus, the first half and the second half of the time course were analyzed separately. Using this approach, there were significant differences between the 100 mg groups. An independent t-test demonstrated a significant difference for the first 60 minutes post injection SC and IP, $t(58)=5.51$, $p<.0001$ and for the second 60 minutes post injection $t(58)=9.93$, $p<.0001$ (Figure PS3.1).
Summary and Discussion.

As seen previously by others (See table 1), ketamine produced a robust, dose-dependent stimulation of locomotor activity. As hypothesized, there was a significant difference in locomotor behavior depending on route of administration. Specifically, for all doses administered using the subcutaneous route locomotor activity peaked later and lasted longer than doses administered using the intraperitoneal route. The SC dose at 25 mg/kg also peaked higher and lasted longer than intraperitoneal dose that was twice as large. The 100 mg/kg dose did not reveal a significant effect when comparing the full time course. However, the groups show a very different time course (Figure PS3.2). Significant differences were observed after analyzing the first and last parts of the time course separately.

Ketamine is widely used in preclinical research investigating schizophrenia, major depressive disorder, and addiction. Subcutaneous and intraperitoneal are the most common routes of administration in animal research using rodents. The evidence in this study suggests some important differences in locomotor effects of ketamine depending on route of injection. Subcutaneous doses produce later peaks in effect and longer effects than the corresponding intraperitoneal dose. These differences are probably due to greater bioavailability of ketamine to the brain in a manner similar to that previously reported for phencyclidine.

These results have implications for animal research using ketamine. The antidepressant effects of ketamine have been found in humans using intravenous administration of ketamine. Intravenous injections are more similar to subcutaneous injections because they both avoid the first pass effect (Turner, et al. 2011). The first pass effect changes the amount of active drug that is available to the brain. Therefore, using subcutaneous injections instead of intraperitoneal may allow us to better detect effects of ketamine in the FST.
**Overall Summary and Discussion**

Studies have shown ketamine to have robust and long-lasting antidepressant effects in depressed patients at low intravenous doses. Berman et al. (2000) found that a group of depressed and treatment resistant patients had significantly lower depression scores only two hours after injection and this effect lasted up to two weeks. Many have replicated the Berman study using different populations and measures. Similarly, several studies have replicated the antidepressant effects of ketamine in animal models, most notably the rat FST. Despite positive results, however, there are contradicting studies that show no significant antidepressant effects of ketamine, or only short-lived effects, in the FST (Becholt et al., 2011; Popik, et al., 2008).

Several factors could affect the ability of the forced swim test to detect ketamine’s antidepressant effects, including strain of animal and route of injection.

The preliminary studies lay the foundation for a more complete assessment of factors that may influence ketamine’s antidepressant effects in the rat forced swim test. Preliminary study 1 failed to replicate positive results for the antidepressant effects of ketamine using the FST. In this study, we used Sprague Dawley rats and intraperitoneal administration. Other studies have demonstrated strain differences with regard to behavior on the FST (Bechtolt et al., 2011). Furthermore, many studies that found antidepressant effects of ketamine used Wistar rats.

Preliminary study 2 was designed to test the differences between Wistar and Sprague Dawley rats in response to ketamine on the forced swim test; we also assessed locomotor behavior to determine if any strain differences could be found on other ketamine-induced behaviors. We found that Wistar rats treated with ketamine spent more time climbing and a trend toward less time immobile than the Wistar saline control group. No differences were seen between saline and ketamine treated Sprague Dawley rats.
Preliminary study 3 was designed to explore the differences in behavior due to route of injection of ketamine. It was found that the total activity, as well as the pattern of activity, differs based on route of injection, with greater effect following subcutaneous injection compared to intraperitoneal injection. With these findings as a starting point we will determine the differences in the antidepressant effects of ketamine in the FST between Sprague Dawley and Wistar rats using subcutaneous administration. Given the past literature and the preliminary studies, there is reason to believe that changing key methodological components of our experiment will allow us to detect the antidepressant effects of ketamine.

**Thesis Studies**

Given the preliminary results and past research, there is reason to believe that antidepressant effects of ketamine can be obtained in animal models, but that these effects are sensitive to methodology in the FST. The purpose of this thesis was to explore methodological factors that might contribute to antidepressant effects of ketamine in rats. Thus, we compared two commonly used rat strains and a range of ketamine doses for possible antidepressant effects in the forced swim test. Given the results of the route of injection study (Preliminary Study 3), as well as discussions with knowledgeable scientists at conferences, we replaced intraperitoneal injections with subcutaneous injections. We believed that the pharmacokinetic change induced by the switching the route of injection would increase our opportunity to detect antidepressant effects of ketamine. In addition, we tested the effects of a repeated low dose of ketamine administration on the FST. Furthermore, the locomotor effects in response to ketamine were measured in order to assess other potential differences in ketamine-induced behaviors in the two rat strains and to determine if locomotor effects might confound any antidepressant effects. Three experiments were done to investigate the antidepressant response to different dose
regimens of ketamine in Wistar and Sprague Dawley rats. Each experiment compared Wistar and Sprague Dawley rats in the FST and in locomotor behavior.

Experiment 1 tested the effects of one low dose of ketamine (10 mg/kg). In the preliminary studies with 10 mg/kg of ketamine, we saw antidepressant-like effects of ketamine for Wistar rats but not for Sprague Dawley rats. Wistar rats showed antidepressant effects in a secondary measure on the forced swim, not on the primary measure immobility. We believed that changing the route of administration from intraperitoneal injections to subcutaneous injections would allow us to detect antidepressant effects of this dose of ketamine. So, we hypothesized that Wistar rats but not Sprague Dawley rats treated with ketamine would show antidepressant effects on the forced swim test. We also expected to see short-lived locomotor stimulant effects of ketamine, with slightly greater effects in Wistar than Sprague Dawley rats.

Experiment 2 tested the effects the same dose of ketamine (10 mg/kg) administered twice, 24 hours before in addition to one hour before the FST in Wistar and Sprague Dawley rats. Parise et al., (2013) found that a single low dose of ketamine (5 or 10 mg/kg) showed no reliable antidepressant effects in adolescent rats but that two injections of these low doses (one injection 24 hours before the second in the forced swim test) revealed significant antidepressant effects at these doses. We, therefore, expected that animals that received ketamine would show significant antidepressant effects of ketamine in both strains of animal but that Wistar rats would show greater antidepressant effects when compared to Sprague Dawley rats. Also, we expected to see short-lived locomotor stimulant effects and greater stimulant effects in Wistar rats compared to Sprague Dawley rats.

Experiment 3 tested the antidepressant effects of a higher dose of ketamine (30 mg/kg). At this higher dose we expected to detect antidepressant effects of ketamine in Wistar rats and
Sprague Dawley rats, with Wistar rats showing greater antidepressant-like effects on forced swim test compared to Sprague Dawley rats. Similarly, we expected an increased locomotor response to ketamine in both rat strains.

**Methods**

**Animals.**

Twenty-four adult male rats were used in each experiment, 12 Wistar rats and 12 Sprague Dawley rats. Animals habituated to the vivarium for one-week prior to experiments. The vivarium is on a 12-hour light/dark cycle and food and water is freely available. There were eight animals in each ketamine group and four animals in each control/saline group. We used a smaller number of saline control animals in order to reduce the number of animals needed for the study. The number of animals per group was chosen based on previous research done with rats on the forced swim test (Porsolt, 1978; Garcia et al. 2008; Reus et al., 2011). In addition, six to ten animals per group is traditionally thought to be large enough to detect differences in psychopharmacology studies while minimizing the number of animals needed. Rats are all very similar genetically and have had nearly identical environments since birth so fewer subjects are needed to find effects compared to human studies.

**Apparatus**

*Forced Swim Test.* Animals were tested on the forced swim test (Porsolt, 1978). The forced swim apparatus is an inescapable Plexiglas cylinder that is 19cm in diameter and 40.3cm in height and filled with 30 cm of water (Kinder Scientific, Poway, CA).

*Locomotor Test.* A Kinder Scientific Open Field Motor Monitor was used for testing locomotor behavior. Each enclosure (16” x 16” x 15”) contains two arrays of photocells to measure activity; the bottom array measures horizontal activity and the top array measures
vertical activity (rearing). Each enclosure is attached to a computer to collect data. The
computer collects photocell beam breaks as an assessment of activity. Successive beam breaks
indicate forward movement referred to here as ambulations. When the animals breaks beams
from the top array of photocells it indicate that they are on their hind legs and this behavior is
recorded as rearing. The apparatus also detects repeated beam breaks that are recorded as fine
movements. Time active is also recorded and collected by the apparatus.

Procedure. The procedure was the same for all three experiments. Animals were handled for five
minutes each for three days before testing in order to habituate to the experimenter. The
experimenter handled each animal in exactly the same manner on each day. The forced swim test
consisted of two swimming sessions over two days (Figure 1.1). On Day 1, Animals were
brought to a room adjacent to the testing room in transport cages and received subcutaneous
injections of saline or ketamine. The animals were placed in the swimming apparatus exactly one
hour after injections for 15 minutes. Upon completion of the swim test, they were towel dried
and placed in an incubator kept at 32 degrees Celsius for 30 minutes. Finally, animals were
returned to the vivarium using a transport cage. No drug was administered on day one for
Experiments 1 or 3. Animals in Experiment 2 did receive an SC injection of saline or 10 mg/kg
of ketamine on day one and day two.

On Day 2, Animals were transported to a room adjacent to the test room in the same
order and manner as day one. The animals were treated with SC injections of ketamine or saline
one hour before being placed in the forced swimming apparatus. Animals in Experiment 1 and 2
received saline or 10 mg/kg of ketamine and animals in Experiment 3 received saline or 30
mg/kg of ketamine. Thus, in Experiment 1, animals received a single injection of ketamine (10
mg/kg) one hour prior to the FST on Day 2; in Experiment 2, animals received an injection of
ketamine (10 mg/kg) 24 hours and 1 hour prior to the FST on Day 2; and in Experiment 3, animals received a single injection of ketamine (30 mg/kg) one hour prior to the FST on Day 2. One hour following injection on Day 2 rats were placed in the apparatus for five minutes. Then, they were removed and placed in an incubator kept at 32 degrees Celsius for 30 minutes before being returned to the vivarium. All swimming sessions were video recorded. Behaviors on test days were scored in seconds immobile, climbing and swimming. Treatment groups were counter balanced to apparatus across experiments and scored one week later to minimize experimenter bias. The forced swim test and procedure are taken from Porsolt (1978) and Slattery & Cryan (2012).

On day 8, the locomotor response to the same ketamine treatment was measured. On test day the animals were transported to the testing room in their home cage and given a thirty-minute habituation period to the testing room. Subsequently, the animals were habituated to locomotor chambers for thirty-minutes, injected with ketamine, and locomotor activity monitored for 90 minutes post-injection.

**Drugs.** Ketamine hydrochloride (Sigma Aldrich) were used in this study. It was dissolved in physiological saline (0.9% NaCl) and injected in a volume of 1 ml/kg.

**Analysis.** Forced Swim Test: A trained researcher scored the data at least one week after the end of the final experiment. To minimize experimenter bias, the animals were identified by number. Behaviors were scored in a manner similar to those described by Cryan, Valentino & Lucki, (2005). The animal was considered immobile when it was not using its front paws and only making minimal movements necessary to keep its head above the water. Climbing behavior was identified as upward movements of forepaws against the side of the swim apparatus, as if trying to escape. Finally, the animal was swimming if it was paddling with its forepaws, and
moving across the swim chamber. The data was analyzed using three 2 by 2 factorial ANOVAs for the total number of seconds of each behavior during the five minute session. The factorial analysis included strain (Sprague Dawley versus Wistar) by treatment group (saline versus ketamine). Using factorial analysis allows us to look at effects of treatment across strain as well as any interaction between the two independent variables. Three planned comparisons were then preformed using Student’s t-test for each behavior.

**Locomotor activity.** The locomotor data was similarly analyzed using three 2 by 2 factorial ANOVAs for total ambulations, fine movements, rears and time active. The total activity counts used were for 30-minutes post-injection for Experiments 1 and 2 and 75-minutes post-injection sums for Experiment 3 (to account for the differing duration of drug effects at the different doses). These timeframes were selected based on the time course data. Three planned comparisons (Students t-tests) were run between Wistar saline and Wistar ketamine groups, Sprague Dawley saline and Sprague Dawley ketamine groups, and between Wistar ketamine and Sprague Dawley ketamine groups for each type of locomotor behavior including ambulations, fine movements, rears and time active. Finally, locomotor activity was examined from 60-65 minutes post injection, the time corresponding to placement in the forced swim test. A five-minute total of ambulations from 60-65 minutes post-injection was analyzed using a two-way factorial ANOVA and three planned comparisons.

**Results**

Saline control groups from all three studies were compared across experiments for immobility, climbing, and swimming. This analysis was done to assess the consistency of the forced swim test on the animals across experiments, to determine if control animals could be collapsed into a single group across experiments and increase the power of the statistical tests. A
two-way ANOVA was done on two strains of control animals from three experiments. There was no effect of experiment day on time immobile $F(2,18)=1.82, p=.18$, there was an effect of strain $F(1,18)=4.43, p=.05$ but no interaction effect $F(2,18)=.45, p=.64$. Although there was a significant effect of strain bonferoni post hoc test revealed no significant differences between any of the groups. As for climbing there was no significant effect of experiment day $F(2,18)=1.71, p=.21$, strain $F(1,18)=.38, p=.54$, or interaction effect $F(2,18)=.43, p=.66$. There was also no effect of experiment day $F(2,18)=.02, p=.98$, strain $F(1,18)=2.82, p=.10$, or interaction effect for swimming $F(2,18)=1.71, p=.21$. Therefore, we combined the saline groups within strain, which made control groups of 12 animals to compare to treatment groups of 8 animals in each of the three experiments described here. Second, we compared saline animals from day 1 on the FST to day 2 (test day) on the FST to determine if a depressive effect of the forced swim test for saline animals (Figure 1.2). Both Wistar and Sprague Dawley animals appeared to show increased immobility on day two compared to day one (Figure 1.2). Wistar animals on day two showed increased immobility but it was not significant according to a matched t-test, $t(11)=1.48, p=.17$. However, Sprague Dawley animals showed a significant increase in immobile behavior from day 1 to day 2, $t(11)=2.74, p=.01$.

To determine reliability of experimenter scoring of the data, test-re-test reliability for immobility was assessed in a small group of animals ($N=5$). The first and second tests were significantly correlated, $r=.98, p<.0001$. 
Experiment 1: Results.

Forced Swim Test: There were no apparent antidepressant effects of a single, low, SC dose of ketamine measured by immobility or climbing (Figure 1.3 & 1.4). There was a trend toward an antidepressant effect observed in swimming behavior but only for Sprague Dawley animals.

The results for immobility showed no significant effect of treatment, $F(1,36)=.06$, $p=.80$, a significant effect of strain $F(1,36)=5.34$, $p=.03$ but no interaction of treatment and strain, $F(1,36)=.18$, $p=.67$ (Figure 1.3A & 1.4A). Further, planned comparisons revealed that there was no significant difference between the Wistar ketamine and Wistar saline controls with respect to immobile behavior on the FST, $t(18)=.51$, $p=.61$, and there was no difference in immobility between the Sprague Dawley ketamine and Sprague Dawley saline control group, $t(18)=.12$, $p=.91$ There was no significant difference between Wistar ketamine and Sprague Dawley ketamine groups, $t(14)=1.30$, $p=.21$.

For climbing behavior on the FST, there were no effects of treatment, $F(1,36)=.25$, $p=.62$, or strain $F(1,36)=.27$, $p=.60$, and interaction effects $F(1,36)=.07$, $p=.79$, (Figure 1.3B & 1.4B). Planned comparisons confirmed that there was no significant difference between Wistar ketamine and Wistar saline control groups, $t(18)=.15$, $p=.88$. There were no significant differences between Sprague Dawley ketamine and Sprague Dawley saline groups $t(18)=.58$, $p=.57$ and no differences were detected between ketamine groups, $t(14)=.17$, $p=.87$ between the two strains.

There was a trend toward a significant effects of treatment for swimming in the FST following a 10 mg/kg dose of ketamine $F(1,36)=4.04$, $p=.05$. There was no effect of strain $F(1,36)=.05$, $p=.82$ but there was a trend toward an interaction effect $F(1,36)=3.29$, $p=.08$.
According to the planned comparisons Sprague Dawley ketamine animals did swim significantly more than the Sprague Dawley saline control animals, $t(18)=2.64, p=.02$. However, the Wistar ketamine group did not swim significantly more than the Wistar saline control group $t(18)=.14, p=.89$, and there were no significant differences between the ketamine groups $t(14)=.94, p=.36$ for the two strains.

Locomotor Behavior: Locomotor effects of the same dose of ketamine (single SC 10 mg/kg) were tested one week after the forced swim test. We observed and analyzed four separate measures of locomotor effects in the open field monitoring system: ambulations, fine movements, rearing and time active. Ambulations revealed significant locomotor effects of ketamine compared to saline for both groups and an increased effect of Wistar animals compared to Sprague Dawley animals (Figure 1.5A & 1.6A). Fine movements revealed a significant effect of ketamine for Wistar rats but not for Sprague Dawley rats and in this case there was no significant difference found between Wistar and Sprague Dawley ketamine animals (Figure 1.5B & 1.6B). There were significant decreases in rearing behavior for both strains of rat that received ketamine and no difference between the Sprague Dawley and Wistar ketamine groups (Figure 1.5C & 1.6C). Because the stimulant effect of ketamine was short-lived, the total activity during the first 30 minutes of the session was analyzed with a two-way ANOVA for each type of activity. Stimulant effects of ketamine were seen for all four types of activity, as hypothesized. Also in support of our hypotheses, there were significant differences in response to ketamine depending on strain of rat.

Ambulations are successive beam breaks in the open field monitoring system and are reflective of forward motion. For ambulations, there were significant effects of treatment, $F(1,36)=97.83, p<.0001$ and of strain $F(1,36)=12.73, p=.001$, but no interaction of treatment and
strain $F(1,36)=1.98, p=.17$ (Figure 1.5A & 1.6A). We again performed planned comparisons using t-tests. There was a significant increase in ambulatory behavior for Wistar animals that received ketamine compared to Wistar saline control animals, $t(18)=8.09, p<.0001$. There was also a significant increase in ambulations for Sprague Dawley animals that received ketamine compared to Sprague Dawley saline control animals $t(18)=5.93, p<.0001$. Wistar animals that received ketamine were significantly more active than Sprague Dawley animals that received ketamine $t(14)=2.18, p=.04$.

Fine movements are reflective of repetitive movements, such as grooming or sniffing. There was a significant effect of treatment for fine movements $F(1,36)=13.81, p=.0007$, there was no significant effect for strain $F(1,36)=1.91, p=.17$, and there was no significant interaction for treatment and strain $F(1,36)=1.64, p=.21$ (Figure 1.5B & 1.6B). According to planned comparisons, there was a significant increase of fine movements for Wistar animals that received ketamine compared to Wistar saline controls, $t(18)=2.92, p=.001$. However, there was no significant difference for Sprague Dawley animals that received ketamine compared to Sprague Dawley controls, $t(18)=1.96, p=.07$, although there was a trend showing that Sprague Dawley animals that received ketamine displayed more fine movements than Sprague Dawley saline controls. There was also no significant difference but a strong trend showing that Wistar ketamine animals showed more fine movements than Sprague Dawley ketamine animals, $t(14)=.06, p=.95$.

Rears are photo beam breaks detected by the upper photocell array in the open field locomotor monitor. Rears are typically measured when the animal stands on its hind legs. Ketamine has been demonstrated to reduce rearing behavior in rats. There do not seem to be any differences with respect to strain for this behavioral effect of ketamine (Figure 1.5C & Figure
1.6C). There was a significant effect of treatment for rears $F(1,36)=62.13, p <.0001$, there was no effect for strain $F(1,36)=.08, p=.77$, and no significant interaction for treatment and strain $F(1,36)= .30, p=.58$. Wistar ketamine animals showed significantly fewer rears than Wistar control animals, $t(18)=4.62, p=.0002$, as did Sprague Dawley animals that received ketamine compared to Sprague Dawley controls, $t(18)=6.92, p<.0001$. No other comparisons were significant. There were no significant differences ketamine groups of both strains, $t(14)=1.44, p=.17$.

Time active measures the amount of time the animals were moving during each session. For time active, there was a significant effect of treatment $F(1,36)=28.32, p<.0001$, a strong trend but insignificant effect of strain $F(1,36)=3.94, p=.06$, and there was a significant interaction effect of treatment and strain $F(1,36)=25.95, p<.0001$ (Figure 1.5D & 1.6D). Planned comparisons showed that there was a significant increase in time active for Wistar ketamine compared to Wistar saline control rats, $t(18)=6.91, p<.0001$. However this effect was not significant for Sprague Dawley ketamine animals compared to Sprague Dawley saline controls, $t(36)=.17, p=.86$. There was a significant difference between Wistar animals that received ketamine and Sprague Dawley animals that received ketamine $t(14)=4.83, p=.0003$.

Finally, an additional analysis was done to assess locomotor activity from 60-65 minutes post injection, the timeframe during which the FST was performed. This analysis allows us to determine if ketamine had a locomotor stimulation effect that might confound interpretation of the FST. The total activity during this five-minute interval was analyzed using a two-way ANOVA (Figure 1.7A). This data demonstrated that ketamine did not significantly increase locomotor activity at 60 minutes after injection but interestingly Sprague Dawley ketamine animals showed a significant decrease in ambulations and time active compared to there relative
controls. There were no significant effect detected for fine movements, there was still a significant decrease in rearing behavior for Sprague Dawley animals but not Wistar animals compared to their corresponding control groups.

For ambulations, there was no significant but a trending effect of treatment $F(1,36)=3.19$, $p=.08$, strain $F(1,36)=.036$, $p=.85$, or an interaction between treatment and strain $F(1,36)=.43$, $p=.52$ (Figure 1.7A). Planned comparisons revealed that there were no significant differences between Wistar ketamine and Wistar saline control animals $t(18)=.69$, $p=.50$, but Sprague Dawley ketamine animals showed a significant decrease in ambulations compared to Sprague Dawley saline control animals $t(18)=2.14$, $p=.04$. There were also no significant differences between ketamine groups $t(14)=.37$, $p=.71$.

There were no significant effects found for fine movements analyzed 60-65 minutes post injection (Figure 1.7B). There was no effect of treatment $F(1,36)=3.35$, $p=.07$, strain $F(1,36)=.06$, $p=.80$, or interaction effect $F(1,36)=08$, $p=.78$. Wistar ketamine animals were not different from Wistar saline controls $t(18)=1.80$, $p=.09$. and Sprague Dawley ketamine animals were not different from Sprague Dawley saline controls $t(18)=96$, $p=.35$. In addition, there were no significant differences between ketamine animals $t(14)=.01$, $p=.99$.

Rears were suppressed for both strains an hour after ketamine administration. There was a significant effect of treatment, $F(1,36)=7.01$, $p=.02$ but not for strain, $F(1,36)=.23$, $p=.63$, or interaction of treatment and strain, $F(1,36)=.27$, $p=.60$ (Figure 1.7C). Planned comparisons shoed that ketamine Wistar animals did not show significantly less rears than Wistar saline controls, $t(18)=1.31$, $p=.21$ but Sprague Dawley ketamine animals did show significantly fewer rears than Sprague Dawley saline controls, $t(18)=2.71$, $p=.01$. There were no differences between ketamine groups, $t(14)=.05$, $p=.96$. 
For time active there was an interesting and significant effect of treatment 60-65 minutes post injection for Sprague Dawley animals but not for Wistar animals (Figure 1.7D). There was a significant effect of treatment $F(1,36)=5.56, p=.02$, but no significant effect for strain $F(1,36)=.54, p=.47$, or interaction between treatment and strain, $F(1,36)=1.34, p=.26$. Wistar ketamine animals showed no significant differences from Wistar saline controls $t(18)=.78, p=.44$. However, Sprague Dawley ketamine animals spent significantly less time active than Sprague Dawley saline controls $t(18)=2.78, p=.01$. There were no significant differences between Wistar ketamine or Sprague Dawley ketamine animals, $t(14)=.31, p=.76$.

**Experiment 1: Summary and Discussion**

For locomotor behavior, we expected locomotor effects of ketamine in both strains and we expected Wistar animals to be more sensitive than Sprague Dawley animals in response to ketamine. For ambulations, we saw a significant effect of ketamine in both strains. Although there were no significant interaction effects for treatment or strain for ambulatory behavior, Wistar animals that received ketamine were significantly more active than Sprague Dawley animals that received ketamine. In addition, Wistar animals peaked in ambulatory behavior later than Sprague Dawley animals and the increased ambulations lasted longer in response to ketamine (Figure 1.5A). The data for fine movements, however, was more complex. There was a significant effect of treatment but not of strain or interaction effect. Wistar ketamine animals showed a significant increase in fine movements compared to Wistar controls while Sprague Dawley animals did not. Sprague Dawley fine movements peaked higher than Wistar animals but dropped back to baseline sooner (Figure 1.5B). Strain differences were also evident in time active. The time the animals spent active in response to ketamine depended on whether they were Wistar or Sprague Dawley animals. Wistar animals spent more time active in response to
ketamine than Sprague Dawley animals. Rears showed a reliable suppression that did not depend on strain but Time active showed significant effects of treatment, strain and a significant interaction effect of treatment and strain. These results demonstrate a robust locomotor response to ketamine that is complex and differs according to strain.

Further analysis on a five-minute time interval, which occurred one hour after treatment, showed that there were no significant increases locomotor effects for Wistar or Sprague Dawley animals. Interestingly, there was a decrease in locomotor activity an hour after injection of ketamine that was significant for Sprague Dawley ketamine but not Wistar ketamine rats. In fact, there was a significant decrease in activity for Sprague Dawley animals according to ambulations and time active and while there was no longer a significant decrease in suppression of rears for Wistar rats there was a suppression or rearing behavior for Sprague Dawley rats. This effect of ketamine an hour after administration on activity could potentially explain the inconsistent results found in the FST. The suppression of locomotor activity found an hour after a low dose of ketamine could make it more difficult to overcome immobility in the FST. This data demonstrates the complex locomotor response to ketamine, some of which continues one hour after ketamine administration and differs between strains.

**Experiment 2: Results.**

Forced Swim Test: In Experiment 2, the antidepressant-like effects of two SC doses of ketamine (10 mg/kg) were tested. Drug was administered 24 hours and one hour before the second forced swim test. The results were similar to those seen in Experiment 1, with little evidence of effects of ketamine in the FST.

For immobility, there was no significant effect of treatment $F(1,36)=1.71, p=.20$. However, there was a significant effect of strain $F(1,36)=6.97, p=.01$. There was no interaction
ANTIDEPRESSANT EFFECTS OF KETAMINE

Effect of treatment and strain $F(1,36)=.03, p=.85$ (Figure 2.1A & 2.2A). The planned comparisons revealed that there were no significant differences between the Wistar ketamine and Wistar saline control groups, $t(18)=1.06, p=.30$ or the Sprague Dawley ketamine and Sprague Dawley saline control groups $t(18)=.80, p=.44$. There was also no significant difference but a trend that showed that Wistar ketamine animals were less immobile than Sprague Dawley ketamine animals, $t(18)=1.82, p=.09$.

For climbing behavior on the FST, there were no significant effects of treatment, $F(1,36)=.0005, p=.98$, strain $F(1,36)=1.22, p=.28$, or interaction $F(1,36)=.06, p=.80$ (Figure 2.1B & 2.2B). Planned comparisons confirmed there was no significant difference between Wistar ketamine and Wistar saline controls, $t(18)=.15, p=.88$. There was no significant difference between Sprague Dawley ketamine and Sprague Dawley saline controls, $t(18)=.21, p=.84$. No differences were detected between ketamine groups, $t(18)=1.07, p=.30$.

Similarly, there was no significant effect of treatment for swimming in the FST $F(1,36)=.31, p=.58$. There was no effect of strain $F(1,36)=2.76, p=.10$ and there was no interaction between treatment and strain, $F(1,36)=.24, p=.62$ (Figure 2.1C & 2.2C). According to the planned comparisons Sprague Dawley ketamine group did not swim significantly more than the Sprague Dawley saline control group, $t(18)=.05 p=.96$. The Wistar ketamine group did not swim significantly more than the Wistar saline control group, $t(18)=.69, p=.49$. There were no significant differences between the Wistar or Sprague Dawley ketamine groups $t(14)=.74, p=.47$.

Locomotor effects: The locomotor assessment revealed short-lived stimulant effects of ketamine, similar to those in Experiment 1. Increases in activity were seen as measured by ambulations, fine movements, and time active while decreases in activity were seen for rears. Wistar animals that received ketamine showed greater locomotor effects than Sprague Dawley
animals that received ketamine as measured by ambulations, rears and time active. For ambulations, there was a significant effect of treatment $F(1,36)=45.24, p<.0001$ and of strain $F(1,36)=15.20, p=.0004$ and no significant but some indication for an interaction of treatment and strain $F(1,36)=3.16, p=.08$ (Figure 2.3A & 2.4A). We again performed planned comparisons using t-tests. There was a significant increase in ambulatory behavior for Wistar animals that received ketamine compared to Wistar saline control animals, $t(18)=5.08, p<.0001$. There was also a significant increase in ambulations for Sprague Dawley animals that received ketamine compared to Sprague Dawley saline control groups, $t(18)=4.53, p=.0003$. Wistar animals that received ketamine were significantly more active than Sprague Dawley animals that received ketamine $t(36)=2.48, p=.03$.

For fine movements, there were significant effects of treatment, $F(1,36)=17.74, p = .0002$, and strain $F(1,36)=4.57, p=.04$, but there was no interaction for treatment and strain $F(1,36)=.73, p=.39$ (Figure 2.3B & 2.4B). Planned comparisons showed that Wistar animals that received ketamine showed significantly more fine movements than Wistar controls, $t(18)=3.24, p=.004$. Sprague Dawley animals that received ketamine showed significantly more fine movements compared to Sprague Dawley controls, $t(18)=2.39, p=.03$. There was no significant difference between Wistar ketamine and Sprague Dawley ketamine animals, $t(14)=.88, p=.39$.

As expected, ketamine reduced rearing behavior. There was a significant effect of treatment for rears $F(1,36)=51.22, p<.0001$, no effect for strain $F(1,36)=1.61, p=.21$, and there was no significant interaction for treatment and strain $F(1,36)= 2.36, p=.13$ (Figure 2.3C & 2.4C). Planned comparisons revealed that there was a significant effect of ketamine for Wistar animals when compared to Wistar saline control animals, $t(18)=3.50, p=.002$, and for Sprague Dawley animals that received ketamine compared to Sprague Dawley saline controls, $t(18)=7.28,$
p<.0001. Additionally, there were significant differences between ketamine groups, $t(14)=4.31$, $p=.00007$, Wistar animals showed significantly more rears than Sprague Dawley animals.

For time active, there was a significant effect of treatment $F(1,36)=30.88, p<.0001$ but no effects of strain $F(1,36)=.61, p=.44$. There was a significant interaction of treatment and strain $F(1,36)=13.79, p=.0007$ (Figure 2.3D & 2.4D). Planned comparisons revealed that there was a significant increase in time active for Wistar ketamine compared to Wistar control rats, $t(18)=5.98, p<.0001$; however this effect was not significant for Sprague Dawley ketamine animals compared to Sprague Dawley controls, $t(18)=1.46, p=.16$. There was a significant difference between Wistar animals that received ketamine and Sprague Dawley animals that received ketamine $t(14)=2.79, p=.01$. Specifically Wistar animals that received ketamine were significantly more active than Sprague Dawley animals that received ketamine.

Lastly, the locomotor activity from 60-65 minutes was analyzed using a two-way ANOVA for ambulations, fine movements, rears and time active. Locomotor activity of Wistar rats was mildly stimulated by ketamine during this timeframe while the activity of Sprague Dawley rats was mildly suppressed. For ambulations, there were no significant effects of treatment $F(1,36)=.15, p=.70$, or strain $F(1,36)=1.93, p=.17$. However, there was a significant interaction between treatment and strain $F(1,36)=4.28, p=.04$ (Figure 2.5A). T-tests revealed that there were no significant differences between Wistar ketamine and Wistar saline control animals $t(18)=1.40, p=.18$, or Sprague Dawley ketamine and Sprague Dawley saline control animals $t(18)=1.76, p=.10$. There were no significant differences between Wistar animals that received ketamine and Sprague Dawley animals that received ketamine but Wistar ketamine animals showed a trend for increased ambulations compared to Sprague Dawley animals $t(14)=1.95, p=.07$. 
Fine movements revealed a locomotor stimulant effect of ketamine one hour after injection. There was a significant effect of treatment, $F(1,36)=22.15$, $p<.0001$, no effect of strain, $F(1,36)=.28$, $p=.59$, and no interaction effect, $F(1,36)=1.09$, $p=.30$ (Figure 2.5B). Planned comparisons showed that Wistar animals that received ketamine showed significantly more fine movements than Wistar animals that did not receive ketamine $t(18)=4.42$, $p=.0003$. Sprague Dawley animals that received ketamine showed significantly more fine movements than their corresponding control group $t(18)=2.41$, $p=.03$. There were no significant differences between ketamine groups $t(14)=.95$, $p=.36$.

Rears continued to show some suppression but it was no longer significant one hour after treatment. There was some indication of an effect of treatment for rears there was no but it was not significant, $F(1,36)=3.16$, $p=.08$, no significant effect of strain, $F(1,36)=.003$, $p=.96$ or interaction effect, $F(1,36)=.84$, $p=.37$ (Figure 2.5C). Rearing behavior for Wistar ketamine animals was not significantly less than Wistar control animals $t(18)=.51$, $p=.61$, Sprague Dawley ketamine animals did show significantly fewer rears than Sprague Dawley controls, $t(18)=2.44$, $p=.02$. There were no significant differences between ketamine groups $t(14)=.83$, $p=.42$.

Time active showed no significant effects of ketamine. No effects of treatment, $F(1,36)=.14$, $p=.71$, strain $F(1,36)=.0002$, $p=.98$, or interaction $F(1,36)=3.34$, $p=.07$ (Figure 2.5D). The planned comparisons showed that there were no significant effects for Wistar ketamine compared to saline controls $t(18)=1.37$, $p=.19$ or Sprague Dawley ketamine compared to saline controls $t(18)=1.23$, $p=.23$. There were also no differences between ketamine groups $t(14)=1.24$, $p=.24$. 

Experiment 2: Summary and Discussion.

Overall, Experiment 2 did not support the hypothesis that ketamine at low repeated doses produces an antidepressant effect. In addition, this experiment did not replicate the partial antidepressant effects that were found in swimming behavior for Sprague Dawley rats in Experiment 1. The data here suggests that there are not reliable antidepressant effects of ketamine at 10 mg/kg even when an additional injection is added.

Locomotor results for Experiment 2 were very similar to those found in Experiment 1. Ketamine increased ambulations for both Wistar and Sprague Dawley rats, and the response in Wistar animals was greater than in Sprague Dawley. The pattern of response was also similar to Experiment 1, Sprague Dawley animals peaked in ambulatory behavior sooner and dropped back down to baseline faster than Wistar animals (Figure 2.3A & 2.4A).

A similar effect was seen for fine movements as well. Wistar animals had more fine movements overall but in Sprague Dawley animals the peak in fine movements was higher than in Wistar rats and dropped back to baseline more rapidly (Figure 2.3B).

As in Experiment 1, ketamine increased time active for both Wistar and Sprague Dawley rats. Wistar animals that received ketamine spent significantly more time active than Sprague Dawley animals that received ketamine. Thus, across different measures of activity, Wistar animals showed an increased sensitivity to locomotor effects of ketamine.

Finally, activity from a five-minute interval 60-65 minutes post-injection was analyzed. This five-minute interval corresponds to the time that the animals would otherwise be placed in the FST. The results differed somewhat from those seen in Experiment 1. Wistar animals showed a mild increase in behavior, while Sprague Dawley showed a mild decrease in behavior, resulting in a significant difference between the two strains. However, fine movements were increased for
both Sprague Dawley and Wistar rats that received ketamine compared to their respective control groups. Like experiment one, these results demonstrate the complex locomotor response to ketamine in which some but not all behavioral responses depend on strain.

**Experiment 3: Results.**

Forced Swim Test: There were significant antidepressant effects measured here by immobility and swimming. There were significant effects of ketamine in the FST in response to 30 mg/kg of ketamine as demonstrated by immobility and swimming. For immobility, there were significant effects of treatment, $F(1,36)=18.52, p=.0001$ and strain $F(1,36)=5.79, p=.02$, but no interaction effect of treatment and strain $F(1,36)=.39, p=.53$ (Figure 3.1A and 3.2A). There was a significant decrease in immobility for Wistar animals that received ketamine compared to Wistar saline controls, $t(18)= 2.66, p=0.02$, and for Sprague Dawley animals compared to saline controls $t(18)=3.41, p=0.003$. However, there was no significant differences between Wistar ketamine and Sprague Dawley ketamine groups, $t(14)=1.66, p=0.12$.

For climbing behavior on the FST, there were no effects of treatment, $F(1,36)=1.70, p=.20$, strain $F(1,36)=.03, p=.85$, or interaction $F(1,36)=1.00, p=.32$ (Figure 3.1B & 3.2B). Planned comparisons confirmed there was no significant difference between the Wistar ketamine and Wistar saline group, $t(18)=1.58, p=.13$. There was no significant difference between the Sprague Dawley ketamine and Sprague Dawley saline group, $t(18)=.22, p=.83$. No differences were detected between ketamine groups, $t(18)=.84, p=.42$.

There were significant effects of treatment for swimming in the FST following a 30 mg/kg dose of ketamine $F(1,36)=67.03, p<0.0001$, and for strain $F(1,36)=7.43, p=.009$ but there was no interaction of treatment and strain $F(1,36)=1.61, p=.21$ (Figure 3.1C & 3.2C). The Wistar ketamine animals spent significantly more time swimming than the Wistar saline controls,
ANTIDEPRESSANT EFFECTS OF KETAMINE

$t(18)=5.79, p<.0001$. Sprague Dawley ketamine animals swam significantly more than the Sprague Dawley saline controls, $t(18)=6.00, p<.0001$. There was no significant difference between the ketamine groups $t(14)=1.89, p=.08$, but Wistar ketamine animals showed a trend and spent more time swimming than Sprague Dawley ketamine animals.

Locomotor Behavior: Locomotor activity increased in response to ketamine for both strains as measured by ambulations, fine movements and time active. Time active showed that Wistar animals were especially sensitive to the effect of ketamine. Rears were suppressed for both Wistar and Sprague Dawley rats that received ketamine. Since the duration of the stimulant effect was longer lasting at this higher dose than at the lower dose, the locomotor effects were assessed using the total activity for the 75 minutes post injection instead of 30 minutes. For ambulatory behavior, there was a significant effect of treatment $F(1,36)=249.1, p<.0001$ and of strain $F(1,36)=6.07, p=.02$, but no interaction of treatment and strain $F(1,36)=1.92, p=.17$ (Figure 3.3A & 3.4A). Planned comparisons showed that there was a significant increase in ambulatory behavior for Wistar animals that received ketamine compared to Wistar saline control animals, $t(18)=11.93, p<.0001$. There was also a significant increase in ambulations for Sprague Dawley animals that received ketamine compared to Sprague Dawley saline control animals, $t(18)=10.36, p<.0001$. Wistar animals that received ketamine were not more active than Sprague Dawley animals that received ketamine $t(14)=1.69, p=.12$.

For fine movements there was a significant effect of treatment $F(1,36)=35.05, p<.0001$, there was no significant effect of strain $F(1,36)=.03, p=.85$, and there was a significant interaction for treatment and strain $F(1,36)=5.86, p=.02$ (Figure 3.3B and 3.4B). Wistar animals that received ketamine preformed more fine movements compared to Wistar saline controls, $t(18)=5.92, p<.0001$. Sprague Dawley animals that received ketamine also preformed more fine movements.
movements compared to Sprague Dawley saline controls, \( t(36)=2.46, p=.02 \). Although there was a significant interaction planned comparisons showed no indication of it and there were no significant differences between Wistar ketamine and Sprague Dawley ketamine animals, \( t(14)=1.43, p=.18 \).

As with the lower dose of ketamine, the higher dose suppressed rearing behavior. Not surprisingly, there were significant effects of treatment for rears \( F(1,36)=65.14, p<.0001 \), there were no effects for strain \( F(1,36)=.70, p=.40 \), and there was no significant interaction for treatment and strain \( F(1,36)=.006, p=.94 \) (Figure 3.3C and 3.4C). Planned comparisons showed there was a significant effect of ketamine for Wistar animals when compared to Wistar control animals, \( t(18)=4.96, p<.0001 \), and for Sprague Dawley animals that received ketamine compared to Sprague Dawley controls, \( t(18)=6.87, p<.0001 \). No other comparisons were significant. There were no significant differences between ketamine animals \( t(14)=2.02, p=.06 \).

There were robust effects of ketamine for time active. There were significant effects of treatment \( F(1,36)=83.71, p<.0001 \), and strain \( F(1,36)=4.25, p=.04 \). There was also significant interaction of treatment and strain, \( F(1,36)=18.91, p<.0001 \) (Figure 3.3D and 3.4D). Planned comparisons showed that there was a significant increase in time active for Wistar ketamine compared to Wistar control rats, \( t(18)=10.88, p<.0001 \). Sprague Dawley ketamine animals also showed a significant increase in time active compared to Sprague Dawley controls, \( t(18)=3.06, p=.006 \). There was a significant difference between Wistar animals that received ketamine spent significantly more time active than Sprague Dawley animals that received ketamine \( t(14)=4.14, p=.001 \).

Given the robust locomotor stimulation induced by ketamine at this dose, lasting up to 75 minutes following injection, it is not surprising that we found significant stimulation of activity
during the 60-65 minute timeframe. There was a significant effect of treatment when comparing ambulations 60-65 minute sums post injection, $F(1,36)=32.87, p=.0001$ but no effects of strain, $F(1,36)=.66, p=.55$ or interaction, $F(1,36)=2.42, p=.25$ (Figure 3.5A). Planned comparisons showed that Wistar ketamine animals were significantly more active than Wistar saline control animals $t(18)=3.70, p=.002$. Sprague Dawley ketamine animals also show increased ambulatory behavior compared to Sprague Dawley saline control animals, $t(18)=2.30, p=.03$. There were no differences between ketamine groups, $t(14)=.86, p=.40$.

In addition, fine movements revealed significant effects of treatment $F(1,36)=18.31, p=.0001$, no significant effects of strain $F(1,36)=.47, p=.49$ or significant interaction $F(1,36)=1.17, p=.29$ (Figure 3.5B). Planned comparisons revealed that Wistar animals that received ketamine showed significantly more fine movements than Wistar saline controls $t(18)=3.99, p=0.009$. Sprague Dawley animals that received ketamine also showed significantly more fine movements than Sprague Dawley controls $t(18)=2.16, p=.04$. There were no differences detected between ketamine groups $t(14)=.86, p=.40$.

Rears were significantly suppressed by ketamine in both strains. There was a significant effect of treatment, $F(1,36)=12.18, p=.001$, but not for strain $F(1,36)=.22, p=.64$ and no interaction $F(1,36)=.36, p=.55$ (Figure 3.5C). Wistar ketamine animals showed less rearing than Wistar saline controls but it was not significant, $t(18)=1.76, p=.09$. However, Sprague Dawley ketamine animals did show significantly fewer rears than Sprague Dawley saline controls, $t(18)=3.59, p=.002$. There were no significant differences between ketamine groups $t(14)=1.00, p=.33$.

Time active at 60-65 minutes post injection revealed a significant effect of treatment $F(1,36)=8.68, p=.006$, no significant effect of strain $F(1, 36)=1.15, p=.29$ but a significant
interaction effect of treatment and strain $F(1, 36)=9.20, p=.005$ (Figure 3.5D). Wistar animals that received ketamine spent more time active than Wistar saline controls, $t(18)=3.74, p=.001$, however, Sprague Dawley animals that received ketamine did not spend more time active than Sprague Dawley saline controls $t(18)=0.07, p=.95$. Wistar animals that received ketamine spent significantly more time active than Sprague Dawley animals that received ketamine $t(14)=3.29, p=.005$.

**Experiment 3: Summary and Discussion.**

Results of Experiment 3 revealed significant antidepressant-like effects of ketamine on the forced swim test as measured by immobility and swimming behavior but not for climbing behavior. Also, there was an effect of strain for Swimming behavior, Wistar ketamine animals swam more than Sprague Dawley animals. This higher dose of ketamine demonstrated decrease immobility and increased swimming, which is consistent with antidepressant-like effects. Furthermore, Wistar rats showed some evidence that they may more sensitive to those effects. These results suggest that Wistar rats may be more sensitive to antidepressant effects of ketamine, especially at higher doses.

Ketamine stimulated locomotor activity in both Wistar and Sprague Dawley animals and there was a greater locomotor response in Wistar animals. Wistar animals showed a greater peak in locomotor activity and the increased activity lasted longer in response to ketamine, and ambulations as well as fine movements reflect this pattern. Furthermore, time active showed significant effects of treatment, strain as well as an interaction of treatment and strain, which demonstrates that the locomotor effects of ketamine differ depend upon strain. The results here support our hypothesis that ketamine would have increased locomotor effects on Wistar animals compared to Sprague Dawley animals.
Locomotor activity for the 60-65 minute interval clearly demonstrate that lingering stimulant effects that may confound the effects seen on the forced swim test as measured by ambulations, fine movements and time active. Wistar rats showed increased effects of ketamine compared with Sprague Dawley rats. However, rears were still equally and significantly suppressed for both strains demonstrating a long lasting and complex locomotor response to ketamine in which only activated behaviors are dependent on strain while ketamine suppressed rearing is robust in both.

**Discussion**

Overall, the results of these experiments show mixed findings regarding the antidepressant effects of ketamine in Wistar and Sprague Dawley rats. Experiment 1 showed no significant antidepressant effects of a low dose of ketamine for immobility or climbing. However, Sprague Dawley ketamine animals swam significantly more than Sprague Dawley controls. This effect could potentially be due to blunted responses in Wistar rats compared to Sprague Dawley rats even though the effect was lost in Experiment 2. Experiment 2 showed no antidepressant effects of ketamine using two repeated doses of ketamine, for immobility, climbing or swimming behavior. Experiment 3, using a higher dose of ketamine, showed significant and robust antidepressant-like effects of ketamine for both Wistar and Sprague Dawley animals for immobility and swimming but not for climbing. However, as discussed below, strong stimulant effects of ketamine at the higher dose may have confounded the antidepressant effects.

Antidepressant effects are seen here only at the highest dose of ketamine for both Wistar and Sprague Dawley strains. These results are unexpected due to the ability of past research to find strong antidepressant effects across a range of doses of ketamine (Table 1). In fact, the 10
mg/kg dose has repeatedly been reported to produce significant antidepressant effects in the FST (Table 1). On the other hand, it is important to note that others have reported difficulties in obtaining antidepressant effects at low doses of ketamine. For example, Popik, Kos, Sowa-Kucma, and Nowak (2008) found antidepressant effects of ketamine in mice only a very high 50 mg/kg dose of ketamine (and they found no enduring antidepressant effects of ketamine in mice or rats). Similarly, Bechtholt et al., (2011) found no enduring antidepressant effects of ketamine in Balb-C mice or CD-1 mice and only acute effects one hour after a 160 mg/kg dose of ketamine. Gigliucci et al., (2013) found antidepressant effects of a 25 mg/kg dose of ketamine in Sprague Dawley rats but no effect for a 10 mg/kg dose given one hour before FST. Finally, Tizabi et al., (2012) found no antidepressant effect of ketamine in female Wistar rats thirty minutes after low doses ranging from 0.5 to 5.0 mg/kg; they did, however, find significant effects of ketamine in female Wistar-Kyoto rats. While the forced swim test has been widely used to research the antidepressant effects of ketamine, the present results, together with others, demonstrate some inconsistent results with this approach.

Given the reliability, validity and power of the FST, we expected to find antidepressant effects of ketamine. It is important to note that the direction and the magnitude of effects in Experiments 1 and 2 make it clear that a larger number of animals would not have produced a significant response in support of antidepressant effects. For example, in Experiment 2 the direction was a small increase in immobility, the opposite of what would be expected with antidepressant effects. Moreover, we did indeed find significant effects at the high dose of ketamine in Experiment 3 using the same number of animals. Thus, the studies were well controlled and used reliable methodology and a sufficient number of animals to achieve
statistical significance. The lack of response at the lower dose of ketamine suggests that hidden variables may have impacted on the studies.

An example of a ‘hidden variable’ may be related to anxiety or stress in the animals. In discussions with other scientists working on the FST (personal communication, Society for Neuroscience Conference, 2013), it was suggested mild stress in rats may increase the ability to detect effects of ketamine on the FST. Indeed, increased stress using food deprivation, restraint, social defeat or chronic mild stress enhances depressive effects in the rat FST (Bogdonov, Kanecar, D’Anci, & Renshaw, 2013). Although previous studies using ketamine have not reported exposing animals to stress before the FST, it is possible that differences in animal handling procedures could contribute to subtle differences in stress that impact on the results. It is unclear how animals in each of these studies were handled leading up to the study. Animals were either not handled at all leading up to the study or for an unknown period of time in all studies reported here. It is typical in our laboratory to handle animals for 3 to 4 days leading up to a study. This approach could potentially have minimized stress, making it more difficult to detect the effects of ketamine. Further research should elaborate on the effects of mild stress on the FST with ketamine administration.

Another “hidden variable” could involve increased social exposure for the animals and or “enriched” environments. Exposure to enriched environments such as a running wheel can decrease immobility and enhance antidepressant effects of a typical antidepressant. Although the animals in the current studies were not exposed to “enriched” environments, they are socially housed in large cages and they show little to no signs of anxious behavior. Potentially, our Sprague Dawley and Wistar rats are exposed to a form of social enrichment in our lab environment, decreasing salience of ketamine’s antidepressant effects. In the current thesis
studies, rats were housed on a 12-hour light/dark cycle and food available ad libitum across studies. On the other hand, the forced swim studies cited here showed antidepressant effects of ketamine housed singly and socially. Previous studies have shown that greater social stimulation has no influence over rats on immobility (Bogdonova, Kanecar, D’Anci, & Renshaw, 2013). Future research should elaborate on the effects of environment on the response to ketamine in the FST and other paradigms for depression. It is a possibility that either decreased stress following handling procedures or some other variable such as social housing could have influenced behavior on the FST such that antidepressant effects would be undetectable.

Given the sensitivity of the FST to be influenced by hidden or extraneous variables, further research should pay close attention and include detailed procedural accounts of all environmental variables involved, especially those that relate to stress. Additional paradigms such as sucrose preference or foot shock should be included or added when testing potential antidepressant effects of ketamine or similar glutamatergic drugs.

Next, it is important to consider possible confounding effects of ketamine on the forced swim test. Ketamine causes increased locomotor activity across a range of doses. Although ketamine has a short half-life and low to moderate doses of ketamine do not typically have locomotor effects that last longer than an hour, our results demonstrate that locomotor effects of these low doses can be detected during a five minute interval, an hour after administration. Experiment 1 and 2 revealed some interesting evidence that there were lingering locomotor effects of ketamine one hour after administration in response to a low dose that is commonly used to test the antidepressant effects of ketamine. Experiment 1, unexpectedly revealed a suppression of ambulatory behavior for Sprague Dawley animals but not for Wistar animals, while both strains showed an increase in fine movements one-hour post injection in response to a
single low dose of ketamine. Experiment 2, revealed that Wistar animals showed an increase in ambulatory behavior one hour after the second injection while Sprague Dawley animals showed a mild suppression of ambulatory behavior. Both strains showed increases in fine movements and suppression of rears one hour after a low dose of ketamine. An increase in locomotor effects can be observed most clearly at the higher dose of 30 mg/kg, which confounds the decreased immobility or increased swimming usually representative of antidepressant-like effects on the FST. These results demonstrate the complex locomotor response to ketamine across a range of doses. This is particularly important when assessing a test such as the FST in which locomotor suppression or activation can be mistaken for a depressive like or antidepressant-like response. Given these results, further investigation is needed with respect to locomotor response to ketamine and its relationship to the FST. Finally, when comparing different strains in the FST it’s important to pay attention to potential differences in the locomotor response to ketamine.

FST results and confounding locomotor effects aside, the strain differences in locomotor activity and antidepressant effects detected are interesting by themselves. The locomotor response to ketamine differed between strains of rat in a manner predicted by our hypotheses. As expected we found significant locomotor effects of ketamine as indicated by ambulations, fine movements, rears and time active for both Wistar and Sprague Dawley rats. In general, Wistar animals showed a greater peak in activity and/or a longer duration of activity in response to ketamine than Sprague Dawley animals, while rearing behavior was equally suppressed in both strains of rat. Furthermore, Experiment 1 showed a response to ketamine in swimming behavior for Sprague Dawley rats but not for Wistar rats. It is known that there are pharmacological and behavioral differences between strains of rats in response to a variety of drugs (Andrews, 1995). These strain differences should be taken into consideration when designing studies with rats.
It would be of interest to explore the neurobiological differences between Wistar and Sprague Dawley rats that underlie the behavioral differences. Wistar-Kyoto rats, a strain developed from Wistar rats specifically for their increased stress response, anxious behavior and increased depressive like behavior (Walker et al., 2009), have been compared to Sprague Dawley rats in response to stressors. Wistar-Kyoto rats and Sprague Dawley rats show differences in behavioral response to desipramine and fluoxetine on the forced swim test (Lopez-Rubalcava & Lucki, 2000). Wistar-Kyoto rats showed greater immobility overall but significant decreases at lower doses for both SNRIs and SSRIs while SD rats showed significant increases in climbing in response to an SNRI and significant increases in swimming in response to an SSRI. Wistar-Kyoto and Sprague Dawley rats also show differences in serotonin transporters, norepinephrine transporters and dopamine transporters in several brain regions (Pare & Tenjani-Butt, 1996; Jiao, Pare, and Tenjani-Butt, 2003). To my knowledge there are no studies comparing NMDA receptors between Wistar, Wistar-Kyoto or Sprague Dawley rats. Given the differences in response to ketamine demonstrated in the present experiments, future studies should investigate differences in NMDA receptors and downstream pathways across the strains. Additionally, it is important to note that there are several animal models of depression and while the forced swim test is the most common it is possible that this model is not the best model to assess non-traditional antidepressant drugs. Other models include chronic unpredictable stress and or maternal deprivation and subsequent testing on immobility measures such as the tail suspension or forced swim, as well as sucrose preference, which is thought to measure anhedonia in rats (a symptom of depression).

Results of these thesis studies demonstrate differences in locomotor response to ketamine between Wistar and Sprague Dawley rats. Results of all three experiments demonstrated a
greater locomotor activation in Wistar rats compared to Sprague Dawley rats and a different pattern of response between strains. There were strain differences in locomotor behavior seen an hour after injection in response to the low, low repeated and a higher dose of ketamine. Particularly interesting was that Sprague Dawley rats showed some indication of locomotor depression an hour after a low dose of ketamine while Wistar animals did not and strains showed increased fine movements and significantly decreased rears in response to all doses of ketamine one hour later. These results supported our predictions for the strain differences in response to ketamine and these differences should be considered when designing studies, which test the short-term behavioral effects of ketamine.

It is important to consider that the forced swim test is one of many animal models used to screen antidepressants. It is reliable and it is the test used most commonly, but it has poor construct validity and the expression and etiology of depression in the animals is not easily comparable to human behavior. Other paradigms model depression more efficiently by using chronic mild stress, which more accurately reflects thoughts regarding etiology. The chronic unpredictable stress procedure includes 21 days of mild stressors, such as tilted or overcrowded cages, food deprivation, light/dark cycle changes, and others. Testing on sucrose preference or swim test are normally performed after chronic stress. Animals that are depressed tend to prefer sucrose less than non-depressed rats. Ketamine can rapidly reverse the effects of chronic unpredictable stress (Li, Liu, Dwyer, Banasr, & Lee, et al., 2010). Another model, which may be appropriate, is the learned helplessness paradigm. The learned helplessness procedure involved exposing a rodent to inescapable foot shocks on day 1 and then exposing them again to escapable foot shocks on the following day (Koike, Iijima, Chake, 2011). Rodents that are depressed will be slower to escape than animals that have not been previously exposed or are resilient due to
ketamine injections. Early life stress/maternal deprivation is yet another animal model that produced depressive like behavior in animals. Animals are deprived of their mother for postnatal days 1 through 10 and are tested as adults using either the forced swim or sucrose preference. Ketamine has been shown to reverse behavioral effects of maternal deprivation as well as oxidative stress markers, which happen in response to maternal deprivation (Reus, Nacif, Abelaira, Tomaz, dos Santos, & Carlessi et al., 2015; Reus, Carlessi, Titus, Abelaira, & Ignacio, et al., 2015). Finally social defeat model is another stress-based model that produces depressive symptoms that are then measured by immobility in the forced swim test. Pretreatment with ketamine makes mice resilient to the development of depressive like symptoms in response to social defeat (Brachman, McGowan, Perusini, Lim, & Pham, et al., 2015). Antidepressant effects of ketamine have been shown in all of these models separately. In this case our lab could not replicate the effects of ketamine in the forced swim test. However, adding a stressful component before forced swim and using sucrose preference as well would be a good way to proceed with this research.

The present study was aimed at determining if, under sensitive conditions, the antidepressant effects of ketamine could be replicated, and if strain of the rat would affect the response. Much of the literature demonstrates that low doses of ketamine have rapid, robust and long-lasting antidepressant effects in depressed patients. In addition, ketamine has antidepressant effects in a range of rodent models of depression across a range of doses. However, the current studies were unable to replicate these findings except at the highest dose, which was confounded by locomotor stimulant effects. Based on these FST results alone it seems the forced swim test, under the current conditions, is not reliable in detecting antidepressant effects of low doses ketamine that do not also stimulate locomotor activity. Nonetheless, strain differences in
behavioral responses to ketamine were obtained. These findings are important due to the
continued interest in the preclinical behavioral effects of ketamine for depression, analgesia,
anesthesia, drug abuse and addiction.
Figure PS1.1. Time immobile from day 1 to day 2 demonstrating depressive like behavior on day 2 of the FST.
Figure PS1.2.

*Figure PS1.2.* Effects of ketamine and saline on day three of testing on the FST. A.) Time spent immobile B.) Time spent climbing C.) Time spent swimming

Figure PS2.1.

*Figure PS2.1.* Immobility from day 1 and day 2 for Wistar and Sprague Dawley control animals demonstrating depressive like behavior on day 2 of the FST.
Figure PS2.2. Effects of ketamine and saline on day three of testing on the FST. A.) Time spent immobile  B.) Time spent climbing  C.) Time spent swimming

Figure PS2.3. A.) Total ambulations for Wistar and Sprague Dawley rats treated with ketamine 25 mg/kg or saline.  B.) Total ambulations for Wistar and Sprague Dawley rats treated with ketamine 50 mg/kg or saline.
Figure PS2.4. A.) Total ambulations for Wistar and Sprague Dawley rats treated with ketamine 25 mg/kg or saline. B.) Total ambulations for Wistar and Sprague Dawley rats treated with ketamine 50 mg/kg or saline.

Figure PS3.1.
Figure PS3.1. Ambulations for SC and IP saline, ketamine 10 mg/kg, 25 mg/kg, 50 mg/kg & 100 mg/kg.

Figure 1.1.

![Timeline depicting the general protocol for the thesis experiments.](image)

Figure 1.1. Timeline depicting the general protocol for the thesis experiments.

Figure 1.2.
Figure 1.2. Effect of saline treatment on day 1 and day 2 of the forced swim test for Wistar and Sprague Dawley rats.

Figure 1.3. A.) Time course of time immobile one hour after a single injection of ketamine (10 mg/kg) or saline for Wistar and Sprague Dawley animals. B.) Time course of climbing. C.) Time course of swimming.

Figure 1.4. A.) Time spent immobile B.) Time spent climbing C.) Time spent swimming for Wistar and Sprague Dawley animals in response to a single SC 10 mg/kg dose of ketamine or saline.
Figure 1.5. A.) Ambulations B.) Fine movements C.) Rears D.) Time active; Time course 30 minutes reinjection up to 90 minutes post injection in response to a single SC dose of ketamine 10 mg/kg or saline for Wistar and Sprague Dawley animals.
Figure 1.6. Sums for 0-30 minutes post injection selected based on time course data for A.) Ambulations B.) Fine movements C.) Rears and D.) Time active. Measurements were collected in response to 10 mg/kg of ketamine or saline for Wistar and Sprague Dawley animals.
Figure 1.7. Five-minute sums for 60-65 minutes post-injection of 10 mg/kg of ketamine. A.) Ambulations B.) fine movements C.) Rears and D.) Time active.
**Figure 2.1.**

*Figure 2.1. A.) Time course of time immobile one hour after injection of ketamine (Dble10 mg/kg) or saline for Wistar and Sprague Dawley animals. B.) Time course of climbing. C.) Time course of swimming.*

**Figure 2.2.**

*Figure 2.2. A.) Time spent immobile B.) Time spent climbing C.) Time spent swimming for Wistar and Sprague Dawley animals in response to SC 10 mg/kg dose of ketamine or saline administered 24 hours and one hour prior to the FST.*

**Figure 2.3.**
Figure 2.3. A.) Ambulations B.) Fine movements C.) Rears D.) Time active, Time course 30 minutes reinjection up to 90 minutes post injection in response to a repeated SC dose of ketamine 10 mg/kg or saline for Wistar and Sprague Dawley animals.
Figure 2.4. Sums for 0-30 minutes post injection selected based on time course data for A.) Ambulations B.) Fine movements C.) Rears and D.) Time active. Measurements were collected in response to a repeated 10 mg/kg of ketamine or saline for Wistar and Sprague Dawley animals.
Figure 2.5

Figure 2.5. Five-minute sums for 60-65 minutes post-injection of two 10 mg/kg of ketamine. A.) Ambulations B.) fine movements C.) Rears and D.) Time active.

Figure 3.1

Figure 3.1. A.) Timecourse of time immobile one hour after a single injection of ketamine (30
mg/kg) or saline for Wistar and Sprague Dawley animals. B.) Timecourse of climbing. C.) Timecourse of swimming.

Figure 3.2.

Figure 3.2. A.) Time spent immobile B.) Time spent climbing C.) Time spent swimming for Wistar and Sprague Dawley animals in response to a single SC 30 mg/kg dose of ketamine or saline.
Figure 3.3. A.) Ambulations B.) Fine movements C.) Rears D.) Time active, Time course 30 minutes reinjection up to 90 minutes post injection in response to a single SC dose of ketamine 10 mg/kg or saline for Wistar and Sprague Dawley animals.
Figure 3.4. Sums for 0-75 minutes post injection selected based on time course data for A.) Ambulations B.) Fine movements C.) Rears and D.) Time active. Measurements were collected in response to 30 mg/kg of ketamine or saline for Wistar and Sprague Dawley animals.
Figure 3.5. Five-minute sums for 60-65 minutes post-injection of 30 mg/kg of ketamine. A.) Ambulations B.) fine movements C.) Rears and D.) Time active.
Table 1.

<table>
<thead>
<tr>
<th>Author &amp; Date</th>
<th>Dose</th>
<th>Methods</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akinfiores &amp; Tizabi, (2013)</td>
<td>0.25, 0.50 mg/kg</td>
<td>Male Wistar-Kyoto rats received IP injections of ketamine for 10 days and were tested 20-22 hours after the last injection. Animals were housed in pairs through out experiment. Observer was blind to treatment.</td>
<td>The 0.50 dose of ketamine significantly decreased immobility and increased swimming behavior on the FST. The 0.25 mg/kg dose had no effect on behavior on the FST</td>
</tr>
<tr>
<td>Assis, Rezin, &amp; Comim, et al. (2009)</td>
<td>5, 10, 15 mg/kg</td>
<td>Male Wistar rats received IP injections one hour before testing. Animals were housed 4 per cage. Unknown if observer/s were blind to treatment.</td>
<td>A 5 mg/kg dose decreased immobility compared to saline animals. 10 and 15 mg/kg significantly and dose dependently decreased immobility</td>
</tr>
<tr>
<td>Engin, Treit, &amp; Dickson (2009)</td>
<td>10, 50 mg/kg</td>
<td>Male Sprague Dawley rats received single IP injections 30 minutes before testing. Animals were individually housed. Unknown if observer/s were blind to treatment.</td>
<td>Ketamine treated rats were significantly and dose dependently less immobile than saline treated animals. Ketamine decreased depressive behaviors on the FST</td>
</tr>
<tr>
<td>Garcia, Comim, &amp; Valvassori, et al., (2008)</td>
<td>Acute 5, 10, 15 mg/kg</td>
<td>Male Wistar rats received IP injections one hour before testing. Animals were housed 5 per cage. Unknown if observer/s were blind to treatment.</td>
<td>A 5 mg/kg dose decreased immobility compared to saline animals. 10 and 15 mg/kg significantly and dose dependently decreased immobility or depressive behavior</td>
</tr>
<tr>
<td>Garcia, Comim, &amp; Valvassori, et al., (2008)</td>
<td>Chronic 5, 10, 15 mg/kg</td>
<td>Male Wistar rats received IP injections. Animals received a final injection one hour before testing. Animals were housed 5 per cage. Unknown if observer/s were blind to treatment.</td>
<td>Doses 5, 10 &amp; 15 significantly reduced immobility time or depressive behavior compared to saline. Ketamine significantly increased climbing and swimming behaviors compared to saline</td>
</tr>
<tr>
<td>Gigliucci et al., (2013)</td>
<td>10, 25 mg/kg</td>
<td>Male Sprague Dawley rats were assigned to one of five groups and received ketamine 10 or 25 mg/kg one hour before FST. Ketamine 25 mg/kg 24 hours or 24, 5, and 1-hour prior to the FST. Animals were singly housed three days prior to experiment. Unknown if observer/s were blind to treatment.</td>
<td>Ketamine at 25 mg/kg given one hour before FST reduced immobility but 10 mg/kg did not. Ketamine at 25 mg/kg decreased immobility given either one hour or 24 hours prior to testing but not after three injections given 24, 5 and 1 hour prior to FST</td>
</tr>
<tr>
<td>Kojik &amp; Chaki, (2014)</td>
<td>1, 3 10 mg/kg</td>
<td>Male Sprague Dawley rats were tested 24 hours after IP injection. Unknown if observer/s were blind to treatment.</td>
<td>The 10 mg/kg dose of ketamine significantly decreased immobility but the 1 and 3 mg/kg doses did not</td>
</tr>
<tr>
<td>Parise et al., (2013)</td>
<td>5, 10, 20 mg/kg</td>
<td>Adolescent Male Sprague Dawley rats (PI 35-49) were tested 24 hours after 2 IP injections of ketamine given 4 hours apart. Observer blind to treatment.</td>
<td>Only the 20 mg/kg dose of ketamine showed a significant decrease in time spent immobile and an increased latency to immobility 24 hours after final injection. Behavioral counts also revealed a decrease in immobility as well as an increase in climbing and swimming behavior</td>
</tr>
<tr>
<td>Popik, Kos, Sowa-Kucma &amp; Nowak, (2008).</td>
<td>50, 160 mg/kg</td>
<td>Male Wistar rats were injected intraperitoneally with and tested 6 and 7 days after treatment. Male Wistar rats received chronic IP injections. Animals were tested 40 minutes post-injection Observer blind to treatment.</td>
<td>There were no significant differences between ketamine and control animals on day 6 or 7 for immobility, swimming or climbing. Acute and chronic treatment with ketamine reduced immobility/depression and increased climbing and swimming. However, the antidepressant like effects were reduced compared to the acute effects of ketamine suggesting that some tolerance to antidepressant effects occurred</td>
</tr>
</tbody>
</table>
## Antidepressant Effects of Ketamine: The Rat Forced Swim Test

<table>
<thead>
<tr>
<th>Author &amp; Date</th>
<th>Dose</th>
<th>Methods</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reus, Stringar, &amp; Ribeiro, et al. (2010)</td>
<td>5, 10 mg/kg</td>
<td>Male Wistar rats were treated with IP injections one hour before testing. Animals were housed 5 per cage. Unknown if observer/s were blind to treatment.</td>
<td>Ketamine 5 and 10 mg/kg reduced immobility/depressed behavior compared to saline animals. Reduction was only significant for ketamine 10mg/kg.</td>
</tr>
<tr>
<td>Tizabi, Bhatti, Manaye, Das, &amp; Akinfiresoy (2012)</td>
<td>.50, 2.5 or 5 mg/kg</td>
<td>Female Wistar-Kyoto rats and Wistar rats IP injected acutely or chronically. Animals received treatment 20 minutes before a 10-minute locomotor test and subsequent FST or they received treatment for 10 days and animals were tested 22-24 hours post injection. Animals were handled once daily for one week prior to experiments. Unknown if observer/s were blind to treatment.</td>
<td>In the acute study only the Wistar-Kyoto rats showed significant decreased immobility in response to ketamine 2.5, and 5 mg/kg. The chronic study revealed antidepressant effects for ketamine at .50 and 2.5 mg/kg but again only for Wistar-Kyoto rats, not Wistar rats.</td>
</tr>
<tr>
<td>Wang, X., Yang, Y. &amp; Zhou, X et al. (2011).</td>
<td>15 mg/kg</td>
<td>Male Wistar rats received IP injections one hour before testing. Behavior scored by expert observers. Unknown if observer/s were blind to treatment.</td>
<td>Ketamine significantly decreased immobility time on the FST.</td>
</tr>
<tr>
<td>Wang et al., (2014)</td>
<td>10 mg/kg</td>
<td>Male Wistar rats received IP injections 30 minutes before testing. Animals were house 4 per cage. Behavior scored by expert observers. Unknown if observer/s were blind to treatment.</td>
<td>Ketamine significantly decreased immobility time on the FST.</td>
</tr>
<tr>
<td>Yang et al., (2012)</td>
<td>5, 10 &amp; 15 mg/kg</td>
<td>Male Wistar rats received IP injections 30 minutes before testing. The rats then received a second injection placed in the FST again. Animals were housed 4 per cage. Behavior scored by one expert observer. Unknown if observer/s were blind to treatment.</td>
<td>The second forced swim test showed significantly decreased immobility for all doses of ketamine tested.</td>
</tr>
<tr>
<td>Yang, Li, &amp; Wang, et al. (2012)</td>
<td>10mg/kg</td>
<td>Male Wistar rats received IP injections 30 minutes before testing. The rats then received a second injection placed in the FST again. Animals were housed 4 per cage Behavior scored by one expert observer. Unknown if observer/s were blind to treatment.</td>
<td>Ketamine significantly decreased immobility time or depressive behavior compared to saline.</td>
</tr>
<tr>
<td>Yilmaz, Schulz, Aksoy &amp; Canbeyli (2002)</td>
<td>160 mg/kg</td>
<td>Male Wistar rats were injected intraperitonealy and tested 3, 7, 10 days post injection. Unknown if observer/s were blind to treatment.</td>
<td>Ketamine animals were more immobile or less depressed than saline on all three test days.</td>
</tr>
<tr>
<td>Zhang et al., (2013)</td>
<td>3, 10 mg/kg</td>
<td>Male Wistar rats received intraperitoneal injections 60 minutes before subsequent locomotor testing, immediately followed by the FST. Animals were housed 5 per cage. Blind expert observers scored the last five minutes of a six-minute test.</td>
<td>Ketamine significantly decreased immobility for both the 3 mg/kg and 10 mg/kg doses.</td>
</tr>
<tr>
<td>Zhou, et al., (2013)</td>
<td>10 mg/kg</td>
<td>Male Wistar rats received intraperitoneal injections 60 minutes before subsequent locomotor testing, immediately followed by the FST. Animals were housed 5 per cage Blind expert observers scored the last five minutes of a six-minute test.</td>
<td>Ketamine significantly decreased immobility</td>
</tr>
</tbody>
</table>

*Table 1. Summary of methods and results from studies using the Rat FST.*
References


Caddy, C., Giaroli, G., White, T. P., Shergill, S. S., & Tracy, D. K. (2014). Ketamine as the prototype glutamatergic antidepressant: pharmacodynamic actions, and a systematic
ANTIDEPRESSANT EFFECTS OF KETAMINE


kетамин на нормальных и шизофренических добровольцев. *Neuropsychopharmacology*, 25(4), 455-467. doi:10.1016/S0893-133X(01)00243-


Maeng, S., Zarate, C.A. (2007), The role of glutamate in mood disorders: results from the ketamine in major depression study and the presumed cellular mechanism underlying its antidepressant effects. *Current Psychiatry, 9*(6), 467-474.


Blockade to Dopaminergic and Cognitive Disruptions Associated with the Prefrontal Cortex. *Neuroscience Letters, 17*(8), 2921–2927.


Pałucha-Poniewiera, A., Szewczyk, B., & Pilc, A. (2014). Activation of the mTOR signaling pathway in the antidepressant-like activity of the mGlu5 antagonist MTEP and the mGlu7 agonist AMN082 in the FST in rats. *Neuropharmacology, 82*, 59–68. doi:10.1016/j.neuropharm.2014.03.001


