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Effects of (R,S)-ketamine and (2R,6R)-hydroxynorketamine: a preclinical study

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Effects of (*R*,*S*)-ketamine and (2*R*,6*R*)-hydroxynorketamine: a preclinical study

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Dedicated to the mice, may the findings herein be worthy of their sacrifice; with love to my mother, who taught me to be curious; with love to my father, who taught me to take pride in my work; with love to Aly, for her unbounded support and brilliance; to my friends Dr. Priyodarshan Goswamee and Fan Zhang, your friendship has helped me in many ways beyond this work; to my committee (Dr. Joseph H. Porter, Dr. A. Rory McQuiston, Dr. Caroline O. Cobb, Dr. Katherine L. Nicholson, and Dr. Mary E. Loos) and previous mentors, your insight and advice have made me a better scientist; to all of the laboratory technicians, graduate students, and undergraduate students, thank you for your hard work and trust in my ideas.

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Abstract

In 2017, 7.1% of US adults were diagnosed with depression, and 50% of patients received medication to treat their depression. Depression can cause severe interruptions in an individual's cognitive functioning and behaviors like sleeping, eating, working, and socializing. Unfortunately, approximately 40% of patients do not respond to treatment with monoaminergic medications (e.g. Prozac®) and therapeutic effects may be delayed 2-8 weeks. Due to these therapeutic shortcomings, faster acting and more efficacious treatments are needed. Recent preclinical findings indicate potential for glutamatergic drugs like (*R*,*S*)-ketamine and (2*R*,6*R*)-hydroxynorketamine to produce more rapid and longer-acting therapeutic effects. The antidepressant effectiveness of (*R*,*S*)-ketamine for many patients is undisputed; however, the antidepressant efficacy of the ketamine metabolite (2*R*,6*R*)-hydroxynorketamine is disputed. Despite an incomplete understanding of the mechanism of action, the FDA approved (*S*)-ketamine (esketamine; Ketanest®, Spravato®) for treatment-resistant depression in March 2019. Excitement for a better treatment, however, is lessened by reports indicating that some patients may experience severe side effects like reduced cognitive functioning a day or more after (*S*)-ketamine treatment. In sum, some glutamatergic drugs like (*R*,*S*)-ketamine are effective in treating depression, but potential side-effects and abuse liability may limit their clinical use. This provides a strong impetus to further examine and compare the antidepressant efficacy and side-effects of (*R*,*S*)-ketamine, the ketamine isomers (*R*)-ketamine, (*S*)-ketamine, and the ketamine metabolite (2*R*,6*R*)-hydroxynorketamine. The current study measured the effects of (*R*,*S*)-ketamine, (*R*)-ketamine, (*S*)-ketamine, and (2*R*,6*R*)-hydroxynorketamine on C57bl/6 mice in three preclinical behavioral assays; differential-reinforcement-of-low-rate responding (DRL), drug discrimination, and spontaneous alternation in a Y-maze. These assays provide measures of

antidepressant-like effects, discriminative stimulus effects, and cognitive effects of drugs, respectively. Results indicated that (*R*,*S*)-ketamine, (*S*)-ketamine, and (2*R*,6*R*)-hydroxynorketamine produced significant antidepressant-like effects in the DRL task. No significant effect was found with (*R*)-ketamine in the DRL task. The lack of effect in the DRL task may be due to the slower metabolism of (*R*)-ketamine compared to (*S*)-ketamine and the 10-minute pretreatment time used in the present study. In the drug discrimination experiment, both isomers substituted for (*R*,*S*)-ketamine; the effective dose of (*S*)-ketamine was lower than (*R*)-ketamine and this indicates greater potency in this experiment. (2*R*,6*R*)-Hydroxynorketamine did not substitute for (*R*,*S*)-ketamine at the tested doses. In the Y-maze experiment, (*R*,*S*)-ketamine and (*S*)-ketamine reduced spontaneous alternation 24-hours after injection. No effect on spontaneous alternation was observed with (*R*)-ketamine and (2*R*,6*R*)-hydroxynorketamine at the tested doses. The results from the Y-maze experiment indicate that (*R*)-ketamine and (2*R*,6*R*)-hydroxynorketamine may have less effect on cognition than (*R*,*S*)-ketamine and (*S*)-ketamine. The present study reports novel findings regarding the antidepressant-like effects, subjective effects, and cognitive effects of (*R*,*S*)-ketamine, its isomers, and the metabolite (2*R*,6*R*)-hydroxynorketamine. The results of the present study and many other preclinical studies suggest that clinical research is warranted for the (*R*)-ketamine (arketamine) isomer and the ketamine metabolite, (2*R*,6*R*)-hydroxynorketamine.

Effects of (*R*,*S*)-ketamine and (2*R*,6*R*)-hydroxynorketamine: a preclinical study

Major depressive disorder is the most common mental health disorder and a major cause of disability-adjusted life years lost worldwide (Murray & Lopez, 1996; Kessler, 2012; World Health Organization [WHO], 2019). In 2012, the U.S. lifetime prevalence rate for depression was over 16% (Kessler et al., 2012). During 2013-2016, 8.1% of persons in the U.S. aged 20 and over had depression in any given two-week period (Brody, 2018). In 2005, treatment and loss of productivity due to depression was estimated to have cost the US $173.2 billion; which increased to $210.5 billion in 2010 (Greenberg, Fournier, Sisitsky, Pike, & Kessler, 2015). About 80% of adults with depression in the U.S., report some difficulty with work and social activities because of their depression (Brody, 2018). Depression is associated with persistent symptoms that extend well beyond momentary sadness (National Institute of Mental Health [NIMH], 2016). Depression can cause severe interruptions in an individual's thoughts, by affecting emotions and cognitive functioning, and behaviors like sleeping, eating, working, and socializing (NIMH, 2013). Symptoms may range from mild to severe and to be diagnosable as defined by the Diagnostic and Statistical Manual (DSM-5) of the American Psychiatric Association, symptoms must persist for at least two weeks (see Table 1; NIMH, 2013; Uher, Payne, Pavlova, & Perlis, 2014; American Psychiatric Association, 2017). Fortunately, some patients are successful in reversing their depressive symptoms with treatment (Marken & Munro, 2000; Rush et al. 2004).

Table 1. DSM-5 criteria for major depressive disorder

Symptom	Frequency requirements
Depressed mood (subjective or observed)	Most of the day, nearly every day
Loss of interest or pleasure (anhedonia)	Most of the day, nearly every day
Change in weight or appetite	Appetite: nearly every day
Insomnia or hypersomnia	Nearly every day
Psychomotor retardation or agitation	Nearly every day
Loss of energy or fatigue	Nearly every day
Worthlessness or guilt	Nearly every day
Impaired concentration or indecisiveness	Nearly every day
Thoughts of death or suicide attempt	Thoughts: recurrent Suicide attempt: any

At least five symptoms must be present during the same two-week period and one must be either depressed mood, or loss of interest or pleasure (symptoms are summarized here; for use in a clinical setting, refer to the DSM-5 manual; American Psychiatric Association, 2013; Uher et al., 2014).

Treatment of depression

Clinicians may decide to treat depressed patients with pharmacological treatments, non-pharmacological treatments, or both; a commentary on the factors important for this decision are beyond the scope of the present study, however, the Sequenced Treatment Alternative to Relieve Depression (STARED) multicenter trial offers insight on supplementation strategies or the use of non-pharmacological treatments (see Rush et al., 2004). For a patient's initial episode of depression, a treatment regimen that includes both medication and therapy is the most successful regimen option for treating depressive symptoms (Bandelow et al., 2015). Medication alone is more successful than therapy alone, and physical exercise or taking a placebo is better than doing nothing (Bandelow et al., 2015). Treatment of depression may include administration of monoaminergic antidepressant drugs, generally classified as selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), or monoamine oxidase inhibitors (MAOIs) (Williams et al., 2000; Rush et al., 2004). SSRIs may be chosen as an initial treatment because of the higher tolerability and near absent risk for overdose lethality observed with SSRI treatment (MacGillivray et al., 2003). Unfortunately, between 34% and 46% of patients do not respond to treatment with these drugs (MacGillivray et al., 2003; Hillhouse & Porter, 2015). In addition, the effectiveness of monoaminergic drugs, newer and older, does not significantly differ (Williams et al., 2000). Moreover, therapeutic effects associated with monoaminergic drugs require daily administration, and therapeutic effects may be delayed by 2-8 weeks; this delay may further worsen disability-adjusted life year loss (Marken & Munro, 2000; MacGillivray et al., 2003). If a patient has no improvement after the first-line treatment, then clinicians may augment with a second component (e.g., cognitive behavioral therapy) or replace with another treatment (Rush et al., 2004). A patient with depression may be considered to have treatment-resistant depression

when six-weeks of continuous antidepressant treatment fails to produce alleviate symptoms (Fava & Davidson, 1996). Treatment options for patients beyond or in conjunction with medication may include psychotherapy and electroconvulsive therapy (Bandelow et al., 2015).

Patients diagnosed with major depressive disorder have deficits in general cognitive performance, attention, visual learning, and memory compared to the performance of healthy controls (Roca et al., 2015). Patients with cognitive symptoms have earlier onset and longer episodes of depression (Papakostas, 2014). Patients with cognitive symptoms are further negatively impacted by treatment with monoaminergic drugs, like SSRIs, which have been reported to worsen cognitive symptoms for over 50% of patients with depression (Papakostas, 2014; Popovic et al., 2015). The study by Papakostas (2014) reported that treatment with Paroxetine, Fluoxetine, Citalopram, Escitalopram, and Sertraline (all SSRIs) worsened cognitive symptoms for patients diagnosed with depression. Development of treatment options with fewer side-effects, like worsening memory, is imperative for health outcomes (Papakostas, 2014).

Non-pharmacological treatment options, like electroconvulsive therapy, are also effective in treating depression (Fink, 2001). Notably, electroconvulsive therapy is effective for about 50% of patients with treatment-resistant major depressive disorder (Dierckx et al., 2012); however, clinical use of electroconvulsive therapy is limited by adverse side-effects like impaired memory and confusion (Calev et al., 1991; Kujala et al., 2002). Another emerging non-pharmacological treatment, transcranial magnetic stimulation, has potential as a non-invasive and low-cost treatment (George & Post, 2011). A recent meta-analysis of randomized, double-blinded, and sham-controlled trials reported mixed results with active transcranial magnetic stimulation (Moffa et al., 2019). The effect of transcranial magnetic stimulation on memory or cognition was not measured and the researchers recommended further large scale randomized

clinical trials (Moffa et al., 2019). Like monoaminergic treatment, transcranial magnetic stimulation or therapy may not work for every patient and the reasons for this are unclear (MacGillivray et al., 2003; Bandelow et al., 2015; Moffa et al., 2019).

Pathogenesis of depressive symptoms

Depressive symptoms and depressive-like behavior, for which chronic exposure to stressful stimuli is a major risk factor, are associated with changes in the structure and function of the mammalian (human and rodent) nervous system (Citri & Malenka, 2008; Grandjean et al., 2016; Ochs-Ross et al., 2019). The neural plasticity mechanisms important for these changes play an important role in the ability of the nervous system to change in response to environmental stimuli, behavior, thought, and emotions (Citri & Malenka, 2008; Ganguly & Poo, 2013). These changes can be observed at multiple levels, from *in vivo* animal behavior to a single neuron, and the molecular mechanisms responsible are well conserved across all organisms (Hegde, Goldberg, & Schwartz, 1993; Kültz, 2005). Preclinical research, whether with sea slugs or rodents, allows researchers to make inferences from the effects of stressful stimuli and antidepressants on non-human animals for human health (Hegde, Goldberg, & Schwartz, 1993; Duman et al., 2016). Both human and animal studies expand understanding of the mechanisms underlying depression.

Depressive symptoms and depressive-like behaviors are associated with changes in the hippocampus, prefrontal cortex, amygdala, and other brain regions in both humans and mice, respectively (Liston et al., 2006; Drevets et al., 2008; Liu et al., 2017; Ochs-Ross et al., 2019). Post-mortem human studies have indicated that depressive symptoms are associated with reduced grey matter within the hippocampus, prefrontal cortex, amygdala, and other brain regions (Drevets et al., 2008; Kang et al., 2012). Preclinical studies also have demonstrated that

stressful stimuli cause protein degradation in these same brain regions (McEwen, 2001; Ulrich-Lai & Herman, 2009). Conversely, protein synthesis is positively correlated with successful treatment of depression and therapeutic effects with monoaminergic drug treatment (Drevets, Price, & Furey, 2008; Kang et al., 2012).

Brain-derived neurotrophic factor (BDNF) is one protein implicated in the cellular processes associated with depressive symptoms (Duman et al., 1999; Duman et al., 2016). This protein, BDNF, is important for maintaining existing neurons and the growth of new neurons and new synapses (Huang & Reichardt, 2001). Exposure to stressful stimuli and the stress hormone corticosterone have both been demonstrated to decrease expression of BDNF (Warner-Schmidt and Duman 2006). Decreased BDNF expression leads to decreases in dendritic spines, which are small protrusions on a neuron's dendrite that help transmit electrochemical signals between neurons (Duman and Duman 2015). The medication ketamine, historically used for anesthesia, has been demonstrated to rapidly increase dendritic spine numbers via increasing BDNF expression (Duman and Duman 2015). Taken together, these findings support the hypothesis that depression may result from inappropriate neuronal structuring.

Ketamine

In 2019, the U.S. Food and Drug Administration's (FDA) approval of SPRAVATO™, or (*S*)-ketamine (esketamine), marked the introduction of a new rapid-acting antidepressant drug (FDA, 2019). Unlike all other FDA approved antidepressant drugs, (*S*)-ketamine is an arylcyclohexylamine, a noncompetitive potent N-methyl-D-aspartate receptor (NMDAR) antagonist, and is effective within hours following a single dose (Marken & Munro, 2000; Roth et al., 2013). Whether or not NMDAR antagonism is important for the antidepressant effects of (*S*)-ketamine remains unclear, however (Zanos et al., 2016; Yamaguchi et al., 2018). One

hypothesis, the disinhibition hypothesis, proposes that reversal of depressive symptoms is produced by ketamine inhibiting NMDARs on gamma-Aminobutyric acid (GABA) producing interneurons. The inhibition of GABAergic interneurons is hypothesized to disinhibit glutamatergic pyramidal neurons and increase synaptic protein translation, which leads to newly synthesized proteins like BDNF (Aleksandrova, Wang, & Phillips, 2017). Future studies should further examine this hypothesis.

Despite an incomplete understanding of the mechanism of action, the potential benefits of (*S*)-ketamine led the FDA to designate (*S*)-ketamine as a breakthrough therapy, meaning the FDA grants priority to the development of (*S*)-ketamine as a therapeutic drug for treatment-resistant depression (Ionescu & Papakostas, 2017; FDA, 2019). One benefit over traditional monoaminergic antidepressants, which require daily drug administration, is that treatment with (*S*)-ketamine begins with drug administration twice a week for four weeks and then shifts to once a week or once every two weeks (Marken & Munro, 2000; Janssen Pharmaceutical Companies, 2019). The more infrequent dosing may be made possible by long-lasting changes in the structure of neurons and synapses (Duman and Duman 2015). In a recent phase 3, double-blind, randomized clinical study with 297 adults, treatment-resistant patients treated with intranasal (*S*)-ketamine plus oral antidepressant (an SSRI) were significantly less likely to experience relapse of depressive symptoms than patients treated with an intranasal placebo plus oral SSRI (Patel & Holle, 2019). Excitement for a better alternative treatment, however, is lessened by reports indicating that some patients may experience severe side effects like hallucinations, reduced cognitive functioning, and nausea for a few hours after (*S*)-ketamine treatment (Zanos et al., 2018; Patel & Holle, 2019). Other arylcyclohexylamines, like (*R*)-ketamine and (2*R*,6*R*)-

hydroxynorketamine may have reduced effect on cognition than (*S*)-ketamine (Yang et al., 2016; Zanos et al., 2018), but that remains to be determined.

Ketamine, or (*R*,*S*)-ketamine, is a racemic mixture with equal parts of two stereoisomers ((*R*)-ketamine and (*S*)-ketamine, see Figure 1, left panel). Stereoisomerism, or spatial isomerism, is a term used to describe molecules with the same molecular formula, but different spatial orientation (International Union of Pure and Applied Chemistry, McNaught, & Wilkinson, 1997). (*R*)-Ketamine and (*S*)-ketamine are optical isomers; a subtype of stereoisomerism where the two molecules are mirror images of one another but are non-superimposable (e.g. human hands are a common macro example). (*R*)-Ketamine and (*S*)-ketamine are identical in molecular formula and only differ in the direction they rotate in space; *R* or *S*, referring to Latin Rectus for right, clockwise, or Latin Sinister for left, counterclockwise, respectively (International Union of Pure and Applied Chemistry, McNaught, & Wilkinson, 1997). Furthermore, (*R*,*S*)-norketamine and (2*R*,6*R*;2*S*,6*S*)-hydroxynorketamine are two major metabolites of (*R*,*S*) ketamine, and each have distinct stereoisomers (see Figure 1, right two panels). These stereoisomers, like other stereoisomers, have differing effects on biological entities (PubChem, 2020). Like the isomers of ketamine, the isomers of the metabolites (i.e., (*R*)-norketamine, (*S*)-norketamine, (2*S*,6*S*)-hydroxynorketamine, and (2*R*,6*R*)-hydroxynorketamine, see Figure 1, right two panels) may differ in antidepressant efficacy. For example, one preclinical study reported greater antidepressant efficacy with (2*R*,6*R*)-hydroxynorketamine treatment over (2*S*,6*S*)-hydroxynorketamine in the forced swimming test (FST; Zanos et al., 2016). Other preclinical studies have reported mixed antidepressant effects with the metabolites in the FST (Sałat et al., 2015; Yamaguchi et al., 2018; Xiong et al., 2019). While no controlled clinical trials with ketamine's metabolites have been published, a recent open-label pilot study with seven

treatment-resistant depression patients was conducted with (*R*)-ketamine (arketamine) in Brazil (Leal et al., 2020). Following a single intravenous injection of 0.5 mg/kg arketamine, rapid and robust antidepressant effects were evident in all seven subjects, peaking at 240 minutes and persisting in six of the patients for seven days. A measure of the dissociative effects of arketamine were minimal or non-existent for six patients with one patient displaying a brief increase in effects at 40 minutes post-infusion. The authors concluded that "Arketamine might produce fast-onset and sustained effects humans with favorable safety profile, like previously reported with animals; further controlled trials are needed." Obviously, controlled double-blind clinical trials will the necessary to determine the validity of these preliminary findings. There also are plans to conduct clinical trials with (2*R*,6*R*)-hydroxynorketamine (Thomas, 2018).

Figure 1. Ketamine metabolic pathway - simplified

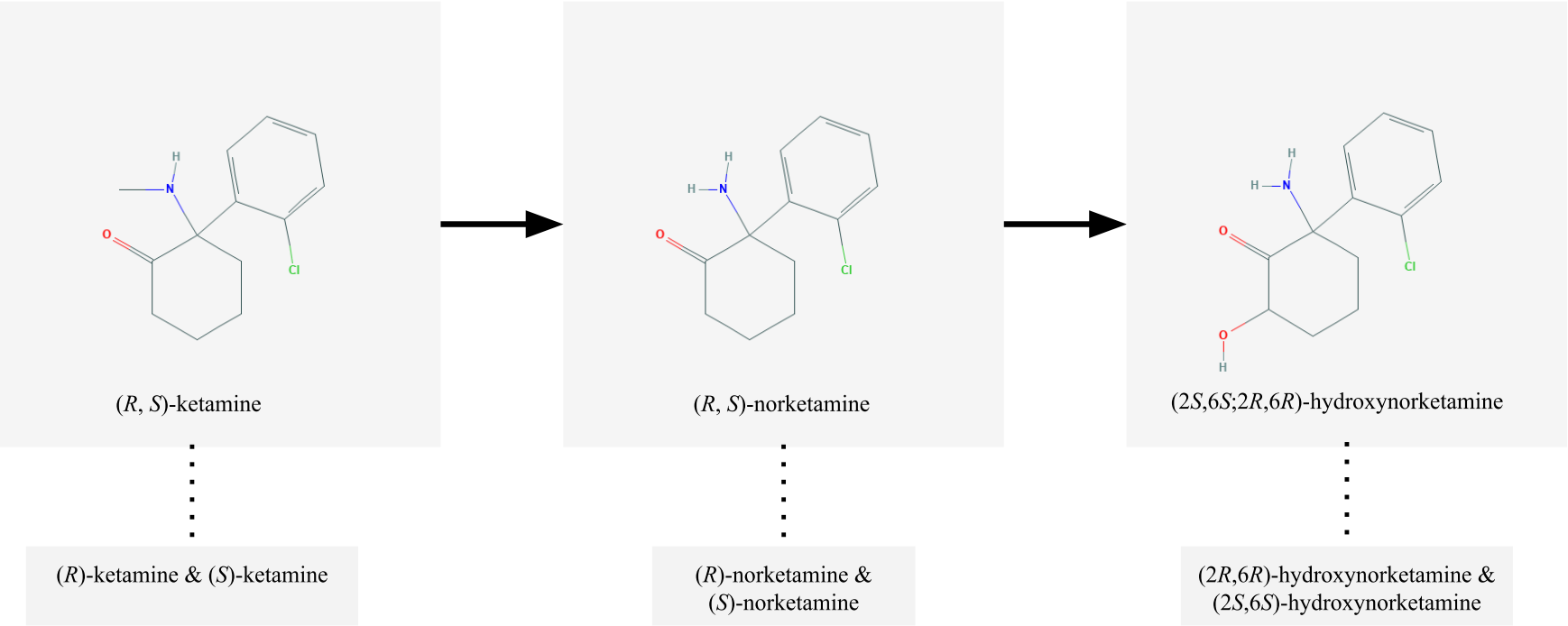

Figure 1 represents the metabolic pathway from (*R*, *S*)-ketamine to (*R*, *S*)-norketamine or (2*R*,6*R*;2*S*,6*S*)-hydroxynorketamine. Below each racemic mixture are the isomers. Chemical structure images provided by the PubChem open source database (Kim et al., 2019).

Determining which mechanism of action is responsible for its antidepressant effect may be difficult because (*R*,*S*)-ketamine is a "dirty" drug. A dirty drug is an informal term in pharmacology for drugs that bind to many different molecular targets. Beyond NMDAR antagonism, (*R*,*S*)-ketamine has been reported to be an antagonist at nicotinic acetylcholine receptors, an agonist at dopamine receptors, an antagonist at serotonin receptors, and an agonist at opioid receptors (see table 3 in Zanos et al., 2018). Further compounding the difficulty in ascertaining mechanism of action is that the ketamine isomers differ in their respective receptor binding profiles. For instance, (*S*)-ketamine has greater affinity for NMDARs than does (*R*)-ketamine (Zanos et al., 2016). Interestingly, the ketamine metabolite (2*R*,6*R*)-hydroxynorketamine does not bind to NMDARs (Zanos et al., 2016). Current evidence suggests that (2*R*,6*R*)-hydroxynorketamine appears to be a more selective drug than (*R*,*S*)-ketamine; one type of molecular target for HNK has been identified thus far: nicotinic acetylcholine receptors (Moaddel et al., 2013, Zanos et al., 2018). Further, all drugs discussed in the present study have actions on the nicotinic acetylcholine receptor; thus, this receptor may be important for the antidepressant effects seen in the present study.

One known clinical trial has tested a α7 nicotinic acetylcholine receptor agonist (Gandelman et al., 2018). Gandelman et al. (2018) examined the effect of the daily use of transdermal nicotine patches among patients with late-life depression in a recent open-label study. Participants were placed on a dose escalation regimen from 3.5mg/day to a maximum of 21mg/day over the course of 12 weeks. Study outcomes included self-reported mood and cognitive symptoms. Results indicated that 86.7% participants (13 of 15) exhibited less severe depressive symptoms and improved cognitive performance over the 12 weeks (Gandelman et al.,

2018). Future double-blind randomized clinical trials may support or refute the role of nicotinic acetylcholine receptors and/or arylcyclohexylamines on mood and cognition.

Preclinical behavioral assays

Preclinical research is the first step in assessing novel candidate molecules for efficacy and safety. Historically, preclinical behavioral assays have been foundational techniques to screen monoaminergic antidepressants, like SSRIs, TCAs, or MAOIs, for antidepressant-like effects (Williams et al., 2000; Krishnan & Nestler, 2011). The distinction between human and non-human animal, antidepressant effects and antidepressant-**like** effects, respectively, is made to caution researchers away from anthropomorphic language and toward objectivity (Shapiro, 1993). The differential-reinforcement-of-low-rate (DRL) task is one behavioral assay used to screen for antidepressant-like effects (O'Donnell et al., 2005).

The differential-reinforcement-of-low-rate procedure does not stress subjects like despair-based assays (i.e. the FST and the tail suspension test; TST). The DRL task uses an operant conditioning procedure to train subjects to withhold a response for a predetermined amount of time, or inter-response time requirement, and then pressing a lever for presentation of the reinforcer (i.e. food reward). Importantly, the DRL task is not a model of depression, rather, it is used to screen antidepressant drugs. That said, increased impulsivity is positively correlated with severity of depressive symptoms in humans and antidepressant drugs may decrease impulsivity in laboratory animals (O'Donnell et al., 2005). The primary outcomes (dependent variables) in the DRL task are total responses and total reinforcers. Compared to control conditions, an antidepressant drug would be hypothesized to increase the number of reinforcers and to decrease the number of responses. Premature lever responses, before the inter-response time requirement is met, will reset the inter-response time requirement will not result in

presentation of reinforcement. The inter-response time requirement is gradually increased throughout training in the differential-reinforcement-of-low-rate task to a terminal value.

A 72-second interval has been used successfully to screen SSRIs and other monoaminergic antidepressant drugs (O'Donnell et al., 2005). Sanger and Blackman (1989) reviewed the utility of the DRL 72s operant task for screening antidepressant drugs and drugs from other classifications. They cited studies that tested 18 different tricyclic and MAOI antidepressant drugs. All of these drugs produced an antidepressant-like profile by dose-dependently increasing reinforcers and decreasing responses. In contrast, drugs from other classes, including alcohol, pentobarbital (barbiturate), chlordiazepoxide (anxiolytic), morphine (opiate), chlorpromazine and haloperidol (typical antipsychotics), clozapine (atypical antipsychotic), and diphenhydramine (antihistamine) did not produce an antidepressant-like profile. The only antidepressant that Sanger & Blackman (1989) reported as a false negative was bupropion, which is an atypical antidepressant that acts as a norepinephrine–dopamine reuptake inhibitor. A more recent review by O'Donnell et al. (2005) also reported that selective serotonin reuptake inhibitors and norepinephrine reuptake inhibitors, as well as a number of atypical antidepressants increase reinforcers and decrease responses in the DRL 72s task. They also reported that drugs from other psychotherapeutic classes (e.g. anxiolytics, opiates, etc.) do not generally produce antidepressant-like effects in the DRL 72s task. They concluded that activation of noradrenergic and/or serotonergic mechanisms were responsible for producing antidepressant-like effects in this task (they also reported that bupropion is a false negative in the DRL procedure).

(*R*,*S*)-Ketamine also has been reported to produce significant antidepressant-like effects in the 72-second DRL task (Hillhouse et al., 2014). Hillhouse et al. (2014) demonstrated that

10.0 mg/kg (*R*,*S*)-ketamine significantly increased reinforcers and reduced responses in rats. Further, D-amphetamine produced a psychostimulant effect in the DRL task, decreasing the number of reinforcers and increasing the number of responses. This indicates that the DRL task may have advantages for screening antidepressant drugs because stimulant-like drugs do not produce false-positives (Hillhouse et al., 2014). No studies to date have reported on the effects of the ketamine isomers (*R*)-ketamine, (*S*)-ketamine, or the ketamine metabolite (2*R*,6*R*)-hydroxynorketamine in the DRL 72-second task. Other antidepressant screening assays include FST and TST, which are both behavioral despair-based assays.

FST and TST measure the effects of antidepressant drugs on animals faced with an inescapable stressor (Petit-Demouliere, Chenu, & Bourin, 2005; Cryan, Mombereau, & Vassout, 2005). FST, also known as Porsolt's test or behavioral despair test, is initiated by placing an animal in an inescapable water filled chamber (Porsolt, Le Pichon, & Jalfre, 1977; Porsolt, Bertin, & Jalfre, 1977). A drug-naïve animal is hypothesized to adopt an immobile posture more quickly and retain immobility for longer than an animal treated with an efficacious antidepressant drug. This behavior, immobility, is often referred to as a depressive-like behavior and is believed to reflect an involuntary defeat strategy phylogenetically consistent with human depression (Anisman & Zacharko, 1990; Sloman, 2008). TST is also based on the observation that animals will adopt an immobile posture when faced with an inescapable stressor (Thierry, Steru, Chermat, & Simon, 1984). During TST, rodents are hung by their tail for a short session (typically 6 minutes), and they will eventually develop an immobile posture (i.e. the animals stop struggling to escape). The length of immobility (typically measured in seconds) can then be compared between treatment groups (Cryan et al., 2005). In sum, an efficacious antidepressant

drug would be hypothesized to reduce immobility in both tests, and this is inferred to be an antidepressant-like effect.

FST and TST are high-throughput screening assays with high interlaboratory reliability. Both assays measure unconditioned behavior and require minimal equipment. The validity and translatability of FST and TST is not without criticism. For example, drugs with stimulant-like effects may produce false positives and drugs with sedative-like effects may produce false negatives (Steru et al. 1985; Slattery and Cryan 2012). Another major criticism is that monoaminergic drugs require repeated administration in humans to reverse depressive symptoms. In these preclinical screening assays, FST and TST, monoaminergic drugs may be given once before the session and an antidepressant-like effect is then observed (Carey et al., 2017). The disconnect between acute and repeated antidepressant drug administration also hinders translation to human depression.

Screening for antidepressant-like effects is important to understand potential efficacy of a candidate drug for treating human depression; however, understanding the subjective effects of drugs can also be informative. Drug discrimination is a preclinical behavioral assay used to assess the subjective effects of novel candidate molecules. The subjective effects of a drug results from the drug's actions within an organism. Therefore, understanding the subjective effects of a drug can be informative of the drug's actions, as subjective effects of drugs are usually (but not always) mediated by relatively specific actions at central neurotransmitter receptors (Young, 2009). Drug discrimination has allowed researchers to gain insight on the subjective effects and pharmacology of drugs for over 50 years (Young, 2009; Porter, Prus, and Overton, 2018). In this task, subjects are typically trained to distinguish the subjective effects of a drug from vehicle (two-lever drug discrimination) (Porter et al., 2005; Solinas et al., 2006). A

two-lever drug discrimination study, hereinafter referred to as drug discrimination, commonly relies on rodents (and other animals) trained to perform a specific operant response like lever pressing for a reinforcer. Pressing the drug-paired lever leads to reinforcement only during training sessions in which the subject was treated with the training drug. After receiving vehicle injections, the subjects must respond on the vehicle-paired lever in order to receive a reinforcer. After training is complete, and subjects accurately discriminate between the training drug or vehicle by choosing the condition-appropriate lever, other drugs can be administered for substitution testing. Reinforcement is presented after responses on either lever during test sessions. Other test drugs are considered to share subjective effects with the training drug if subjects respond on the drug-paired lever. Drugs that substitute for the training drug are then inferred to share subjective effects and discriminative stimulus properties with the training drug. A study by Zanos et al. (2016) trained mice to discriminate between 10 mg/kg (*R*, *S*)-ketamine and vehicle. During testing, mice were injected with 10.0 mg/kg and 50.0 mg/kg (2*R*,6*R*)-hydroxynorketamine and were presented with the drug-paired lever and vehicle-paired lever. Results indicated that mice responded primarily on the vehicle-paired lever (Zanos et al., 2016). Thus, it was inferred that (2*R*,6*R*)-hydroxynorketamine does not share subjective effects or discriminative stimulus properties with 10 mg/kg (*R*, *S*)-ketamine. Further, mice do not self-administer (2*R*,6*R*)-hydroxynorketamine, unlike (*R*, *S*)-ketamine (Zanos et al., 2016). The results from the drug discrimination experiment and self-administration experiment indicate (2*R*,6*R*)-hydroxynorketamine may not share the ketamine-like abuse liability.

Other preclinical behavioral assays are useful for assessing cognitive functions, such as spatial memory (Snigdha et al., 2013). Some of these assays have been demonstrated to be sensitive to disruption in medial prefrontal cortex and hippocampus activity (Kesner et al., 2008),

which interestingly, are brain regions implicated in human depressive symptoms and rodent depressive-like behavior (Liston et al. 2006; Drevets et al., 2008). A study by Kesner et al. (2008) demonstrated that lesions in the hippocampus of trained rats caused poorer performance in a spatial memory recall task. Studies also have indicated that altered hippocampal function is associated with age-related memory deficits in humans (Yassa et al., 2011; Holden et al., 2012). This parallel between rodents and humans contributes to the validity of preclinical studies measuring spatial memory and allow for interpretation of drug effects on rodent cognitive functioning that may translate to humans (Snigdha et al., 2013).

The preclinical behavioral assays used to measure the spatial memory of rodents may include measurements of unconditioned or conditioned behavior. Because training is not needed for measurements of unconditioned behavior (in contrast to conditioning tasks), these tasks allow for relatively quick studies and high through-put screening (Snigdha et al., 2013). One example is the spontaneous alternation assay, which uses a maze to measure unconditioned behavior (Miedel et al., 2017). The maze may be shaped as a Y, T, or + sign. The subject, commonly a mouse or rat, is placed in the maze and the subject can explore the entire maze. A calculated percentage of total arm entries and the sequence of arm entries is an outcome measured in this assay. Subjects with lower scores than control conditions are considered to have impairment in spatial memory.

Conceptually, the spontaneous alternation assay measures the efficiency of an organism's search for resources. Organisms like mice are driven by an innate curiosity to explore new environments (Kraeuter et al., 2019). Mice with normal spatial memory and normal activity in the medial prefrontal cortex and hippocampus will exhibit efficient exploration by remembering previously explored environments (Kesner et al., 2008; Kraeuter et al., 2019). An organism using

the optimal search pattern would never repeat arm entries during the same search sequence (e.g. arm A to arm B to arm C). Conversely, inefficient search patterns are indicative of spatial memory deficits. One example of an inefficient search pattern would proceed from arm A to arm A to arm A.

Preclinical studies have consistently demonstrated that certain doses of (*R*, *S*)-ketamine reduce rodent performance in spatial memory tasks (Garfield et al., 1985; Wang et al., 2006; Duan et al., 2013; Khanegheini et al., 2019). The study by Garfield et al. (1985) reported that administration of 15 mg/kg (*R*, *S*)-ketamine and 15 mg/kg (*S*)-ketamine impaired performance in a +-shaped maze. (*S*)-ketamine at a dose of 15 mg/kg had the greatest effect; whereas, no deficits were observed with 15 mg/kg (*R*)-ketamine. No significant effects were observed at a dose of 7.5 mg/kg for any of these drugs. Taken together, these findings indicate that (*R*)-ketamine does not alter normal spatial memory in mice at the same dosages of (*R*, *S*)-ketamine and (*S*)-ketamine. Nonetheless, no study has yet reported on the effects of (2*R*,6*R*)-hydroxynorketamine in the spontaneous alternation assay.

Rationale

The present study expanded understanding of (*R*,*S*)-ketamine and the metabolite (2*R*,6*R*)-hydroxynorketamine. One important issue is that the antidepressant-like effects of the metabolite (2*R*,6*R*)-hydroxynorketamine treatment is disputed. Zanos et al. (2016) was the first study to report on the antidepressant-like effects of (2*R*,6*R*)-hydroxynorketamine in FST. Some preclinical studies have supported the findings by Zanos et al. (2016) by indicating significant antidepressant-like effects with (2*R*,6*R*)-hydroxynorketamine (Pham et al., 2018; Zanos et al., 2019), while other studies have reported finding no antidepressant-like effects (Shirayama & Hashimoto, 2017; Yang et al., 2017; Yamaguchi et al., 2018; Xiong et al., 2019). None of these studies, nor any other study to date, have used the DRL task to assess the antidepressant-like effects (2*R*,6*R*)-hydroxynorketamine. In this task, (*R*,*S*)-ketamine has been reported to produce antidepressant-like effects in rats (Hillhouse et al., 2014), however, no studies have reported on the effects of the ketamine isomers (*R*)-ketamine, (*S*)-ketamine or the metabolite (2*R*,6*R*)-hydroxynorketamine in the DRL task. In the present study, the aim of experiment 1 was to measure the effects of (*R*,*S*)-ketamine, its isomers (*R*)-ketamine, (*S*)-ketamine, and the metabolite (2*R*,6*R*)-hydroxynorketamine in the DRL task. Experiments 2 and 3 were designed to measure other important behavioral effects of (*R*,*S*)-ketamine, its isomers, and the metabolite (2*R*,6*R*)-hydroxynorketamine.

(*R*,*S*)-Ketamine was approved as a dissociative anesthetic in 1970 and reports of abuse of ketamine began to appear as early as 1971 (see Siegel 1978). Reports of (*R*,*S*)-ketamine abuse continued throughout the 1980s and 1990s (e.g. Kamaya & Krishna 1987) and in 1999 the DEA designated (*R*,*S*)-ketamine as a Schedule III therapeutic drug recognizing its abuse liability. A number of reviews and commentaries have addressed the recreational use and abuse potential of

(*R*,*S*)-ketamine (Expert Committee of Drug Dependence, WHO 2014; Liu et al. 2016; Sassano-Higgins et al 2016; Hillhouse et al., 2019). Recent studies (both preclinical and clinical) on the use of ketamine as a rapid treatment for depression typically mention abuse liability as a possible concern (Zanos et al., 2018; Patel & Holle, 2019). No study to date has reported whether (2*R*,6*R*)-hydroxynorketamine is abused by humans.

Unlike (*R*,*S*)-ketamine, mice do not self-administer (2*R*,6*R*)-hydroxynorketamine (Zanos et al., 2016). This difference may be due to differences in the subjective effects associated with each drug. (2*R*,6*R*)-hydroxynorketamine may lack the ketamine-associated subjective effects that lead to abuse (Zanos et al., 2016). Drug discrimination is a useful assay for helping to identify the potential abuse liability of drugs. Drugs of abuse typically produce strong subjective effects in both humans and animals and to the extent that these subjective effects relate to abuse liability, studying the subjective effects (i.e., discriminative stimulus properties) of drugs in animals provides a good translational approach to this problem that has application to potential abuse issues in humans. Thus, testing novel candidate molecules for shared subjective effects with drugs known to be abused by humans is an important step in medication development.

To understand the subjective effects of (*R*,*S*)-ketamine, a drug discrimination study was conducted in Experiment 2. Previously, Zanos et al. (2016) trained mice to discriminate between 10 mg/kg (*R*,*S*)-ketamine and saline to test whether (2*R*,6*R*)-hydroxynorketamine shares discriminative stimulus properties with (*R*,*S*)-ketamine. After training, when mice were tested with (2*R*,6*R*)-hydroxynorketamine, results indicated that (2*R*,6*R*)-hydroxynorketamine (10.0 mg/kg and 50.0 mg/kg doses) did not share discriminative stimulus properties with a 10 mg/kg (*R*,*S*)-ketamine training dose. These results suggested that (2*R*,6*R*)-hydroxynorketamine lacks the abuse potential associated with (*R*,*S*)-ketamine. Zanos et al. (2016) did not test (*R*)-ketamine and

(*S*)-ketamine in that study. Training dose has been shown to be an important determinant of the properties of discriminative stimuli and different patterns of substitution for test drugs may be evident with different training doses of the training drug (see Stolerman et al. 2011). Therefore, the current study used a lower training dose, 5.0 mg/kg (*R*,*S*)-ketamine, in a two-lever drug discrimination procedure to determine if this altered the discriminative stimulus properties of (*R*,*S*)-ketamine and whether (2*R*,6*R*)-hydroxynorketamine shared discriminative stimulus properties with (*R*,*S*)-ketamine at this lower training dose. In addition, we tested the ketamine isomers, (*R*)-ketamine and (*S*)-ketamine to determine if they share discriminative stimulus properties with (*R*,*S*)-ketamine.

Finally, the present study measured the effects of (*R*,*S*)-ketamine, (*R*)-ketamine, (*S*)-ketamine, and (2*R*,6*R*)-hydroxynorketamine on cognition 24 hours after treatment. (*R*,*S*)-Ketamine treatment (20 mg/kg and 30 mg/kg) has been reported to produce significant cognitive impairment in rodent spatial memory 15 minutes after treatment (Verma & Moghaddam, 1996). (*S*)-Ketamine, but not (*R*)-ketamine, also has been demonstrated to produce deleterious effects on cognition 5 minutes after treatment (Garfield et al., 1985). Also, the recent approval of (*S*)-ketamine for treatment-resistant depression, has raised concerns by some that some patients may experience reduced cognitive functioning for several hours after each treatment (Zanos et al., 2018; Patel & Holle, 2019). No published study has reported on the cognitive effects of (2*R*,6*R*)-hydroxynorketamine in either animals or humans.

An ideal antidepressant drug improves depressive symptoms without hindering cognitive performance. Furthermore, a longer pretreatment time to measure effects on cognition may be warranted because multiple studies have demonstrated that the antidepressant-like effects associated with (*R*,*S*)-ketamine treatment have been observed 24-hours (and longer) after

treatment (Duman et al., 2016; Franceschelli et al., 2015; Fukumoto et al., 2017; Zanos et al., 2019). To this end, the present study treated subjects 24 hours before testing in the Y-maze.

Taken together, these gaps in understanding provide a strong impetus to test (*R*,*S*)-ketamine, its isomers (*R*)-ketamine, (*S*)-ketamine, and the metabolite (2*R*,6*R*)-hydroxynorketamine in the DRL task to test for antidepressant-like effects, a drug discrimination study with a lower 5.0 mg/kg (*R*,*S*)-ketamine training dose to test for shared subjective effects (and potential for abuse liability), and a spontaneous alternation Y-maze task to test for cognitive effects.

Aims & hypotheses

Aim 1 (Experiment 1): Differential-reinforcement-of-low-rate (DRL)

To determine if (*R*,*S*)-ketamine, the ketamine isomers, (*S*)-ketamine and (*R*)-ketamine, and the ketamine metabolite (2*R*,6*R*)-hydroxynorketamine produce an antidepressant-like profile similar to (*R*,*S*)-ketamine in the differential-reinforcement-of-low-rate 72-second task (DRL 72-sec) with C57BL/6 mice.

Hypothesis 1: (*R*,*S*)-ketamine will produce significantly fewer mean responses and significantly more mean reinforcers compared to vehicle. The current study tested doses of 5.0, 10.0, 17.8, and 32.0 mg/kg (*R*,*S*)-ketamine. This dose range was based on a previous study in rats by Hillhouse et al. (2014). In that study (*R*,*S*)-ketamine demonstrated an antidepressant-like profile at 10.0 mg/kg (*R*,*S*)-ketamine, but not at 1.0 and 3.2 mg/kg doses of (*R*,*S*)-ketamine.

Hypothesis 2: (*S*)-ketamine and (*R*)-ketamine will produce significantly fewer mean responses and significantly more mean reinforcers compared to vehicle. Changes in mean responses and mean reinforcers are hypothesized to be dose-dependent: thus, doses of 10.0, 17.8, and 32.0 mg/kg were tested for each isomer.

Hypothesis 3: (2*R*,6*R*)-hydroxynorketamine will produce significantly fewer mean responses and significantly more mean reinforcers compared to vehicle. Zanos et al. (2016) demonstrated (2*R*,6*R*)-hydroxynorketamine can be safely administered in doses above 56.0 mg/kg. Therefore, a dose of 56.0 mg/kg was tested.

Aim 2 (Experiment 2): Drug discrimination

To determine if 0.625, 1.25, 2.5, 5.0, 10.0 mg/kg (*R*)-ketamine, 0.3125, 0.625, 1.25, 2.5, 5.0 mg/kg (*S*)-ketamine, and 10.0 mg/kg, 56.0 mg/kg (2*R*,6*R*)-hydroxynorketamine share

discriminative stimulus properties with 5.0 mg/kg (*R*,*S*)-ketamine in a two-lever drug discrimination task.

Hypothesis 1: C57BL/6 mice will discriminate between 5.0 mg/kg (*R*,*S*)-ketamine and vehicle in a two-lever drug discrimination task. Successful discrimination is indicated when subjects respond ≥80% on the drug paired-lever when treated with 5.0 mg/kg (*R*,*S*)-ketamine and ≤ 20% on the drug paired-lever when treated with vehicle. A dose response generalization curve was determined by testing 0.625, 1.25, 2.5, 5.0, and 10.0 mg/kg (*R*,*S*)-ketamine.

Hypothesis 2: The ketamine isomers (*R*)-ketamine and (*S*)-ketamine, but not the ketamine metabolite (2*R*,6*R*)-hydroxynorketamine will share discriminative stimulus properties with (*R*,*S*)-ketamine. No studies have reported on whether (*R*)-ketamine, (*S*)-ketamine, or (2*R*,6*R*)-hydroxynorketamine will substitute for 5.0 mg/kg (*R*,*S*)-ketamine in a drug discrimination task.

Aim 3 (Experiment 3): Spontaneous alternation in a Y-maze

To determine if (*R*,*S*)-ketamine, the ketamine isomers, (*S*)-ketamine and (*R*)-ketamine, and the ketamine metabolite (2*R*,6*R*)-hydroxynorketamine produce cognitive deficits in spontaneous alternation in a Y-maze task in C57BL/6 mice. The following doses were tested: 10.0 and 32.0 mg/kg (*R*,*S*)-ketamine; 16.0 and 32.0 mg/kg (*S*)-ketamine; 16.0 and 32.0 mg/kg (*R*)-ketamine; 10.0, 32.0, and 56.0 mg/kg (2*R*,6*R*)-hydroxynorketamine. Garfield et al. (1985) previously reported significant spatial memory impairment (spontaneous alternation in Y-maze) in mice after treatment with 15.0 mg/kg (*R*,*S*)-ketamine and 15.0 mg/kg (*S*)-ketamine.

Hypothesis 1: (*R*,*S*)-Ketamine and (*S*)-ketamine are hypothesized to significantly reduce % spontaneous alternation in the Y-maze task 24 hours after treatment.

Hypothesis 2: (*R*)-Ketamine and (2*R*,6*R*)-hydroxynorketamine are hypothesized to not significantly reduce % spontaneous alternation in the Y-maze task 24 hours after treatment.

Methods

All procedures were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University (IACUC Protocol AM10284) and all studies were performed in agreement with the guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Subjects

All studies used adult male C57BL/6 mice (Envigo, Indianapolis, IN) weighing 21-32 grams. Mice were maintained on a 12/12-hour light-dark cycle (lights on 0600 h). Mice were allowed two weeks to adapt the new environment before experimentation. The mice were housed individually in a temperature-controlled vivarium at 22-24° Celsius. Nesting material, cardboard tubes, and ad libitum access to water were provided in their home cage. After two weeks, mice were handled for 5 minutes daily for one week.

Drug

All drugs were administered subcutaneously at a volume of 10 ml/kg. All drugs were dissolved in 0.9% saline. Doses and pretreatment times were based on previous studies in the literature (Hillhouse & Porter, 2014; Zanos et al., 2016) and studies in our laboratory with C57BL/6 mice (unpublished data). (*R, S*)-Ketamine was purchased from Sigma-Aldrich Chemical Company (St Louis, Missouri, USA). (*R*)-Ketamine, and (*S*)-ketamine were purchased from Cayman Chemical Company, 1180 East Ellsworth Road, Ann Arbor, MI 48108 USA. (2*R*,6*R*)-Hydroxynorketamine was graciously provided in collaboration with the National Center for Advancing Translational Sciences in Bethesda, Maryland, USA.

Experiment 1: Differential-reinforcement-of-low-rate (DRL)

Subjects

Free-feeding (100%) bodyweights were established for nine adult male C57BL/6 mice by using the highest free-feeding body weight before withdrawing free access to food. Their weights were maintained at 85-90% of their 100% body weights via food restriction. Because this study lasted for 18 months, subjects were placed on ad libitum food and not trained or tested for two weeks during month 6 and month 12. The new 100% body weights were established at the end of each free-feeding period.

Materials

Apparatus

The DRL training and testing were conducted in six standard computer-interfaced operant conditioning chambers (15 cm L X 11.5 cm D X 17.5 cm H; Model ENV-307A, Med Associates Inc., St. Albans, Vermont, USA), each containing two retractable levers in the left and right position (8 cm apart) on the front panel of the operant chamber. The levers extended 0.8 cm into the chamber and were positioned 2.5 cm above a grid floor constructed of parallel stainless-steel rods. Centered between them was a recessed food trough into which a liquid dipper delivered 0.02 mL of sweetened milk (by volume: 80 mL powdered milk, 80 mL sugar, and 200 mL tap water). Test chambers were housed in sound attenuating cubicles equipped with ventilation fans. MED-PC software (Version 4.2, Med Associates Inc.) was used to control the operant sessions and record data.

Procedure

Magazine training

All mice were first given two sessions of magazine training, during which the house light was on, but no lever was extended. Magazine training consisted of one reinforcer (i.e., sweet milk) being delivered non-contingently, according to a fixed-time 60s schedule for 30 min. On

the third day, a 20 min single lever training session was applied, during which the mice were trained to press either the left or right lever under a fixed ratio 1 (FR 1) food reinforcement schedule, in which each level press resulted in delivery of a reinforcer. The position of the lever associated with the FR 1 schedule was counterbalanced, with half of the subjects assigned the right lever and half assigned to the left lever. The criteria for passing the FR 1 training was 30 reinforcers delivered in 20 min. On day 7, after all mice met the criteria, the DRL training began.

Differential-reinforcement-of-low-rate training

Under the DRL schedule, a response produced a reinforcer only after a specified inter-response interval had elapsed. Responses emitted before the end of the inter-response interval reset the timer and did not produce a reinforcer. All DRL training and testing sessions were set to end at 60 min or when the animal received 50 reinforcers. The inter-response interval was gradually increased over 12-16 sessions; from an initial value of 4.5s, to a terminal value of 72s. To clarify, mice were initially trained on a DRL 4.5s schedule for the first session, and all mice reached the 50-reinforcer goal within the 60 min session time. The next day, the DRL schedule was increased to 9s. Then, the DRL schedule was increased to 18s for 2 sessions and then to 36s for 5 sessions. Finally, the DRL schedule was increased to the final schedule value of 72s until performance stabilized (the number of responses for each mouse did not vary by more than 10% of the mean for five or six consecutive sessions). Test sessions occurred twice weekly (typically Tuesdays and Fridays) with a minimum of one training session between each test session. A vehicle baseline, which consisted of two vehicle test days, was established before and after each drug test.

Experiment 1 used a within-subjects design; therefore, all subjects were treated with each test drug and dose. Testing began with (*R, S*)-ketamine with doses of 5.0 mg/kg, 10.0 mg/kg,

17.8 mg/kg, and 32 mg/kg. Then, (*S*)-ketamine and (*R*)-ketamine were tested at 10.0 mg/kg, 17.8 mg/kg, 32.0 mg/kg doses. Finally, (2*R*, 6*R*)-hydroxynorketamine was tested at a dose of 56 mg/kg. All drugs were administered subcutaneously 10 minutes prior to testing. The order of dose was randomly counterbalanced with between two groups of subjects, one group in ascending doses and the other group of mice tested in descending doses.

Data analysis

The dependent variables were reinforcers (total number of earned reinforcers during each test session) and responses (total number of responses during each test session). All data are expressed as means. One-way repeated measures analyses of variance (ANOVA) were conducted to test for statistically significant differences between each drug condition and saline. When the ANOVA was significant, Dunnett's post hoc tests were used to compare the drug doses to saline. The criterion for significance was set at $p < 0.05$ and all data were analyzed using GraphPad Prism version 8.3 (GraphPad Software, San Diego, California, USA) on a Windows 10 PC.

Experiment 2: Drug discrimination

Subjects

Twelve adult male C57BL/6 mice (Envigo, Indianapolis, IN) weighing 21-34 grams were used in this study. The same acclimation and housing procedures used in experiment 1 were followed. The 100% bodyweights were established using the highest free-feeding body weight before withdrawing free access to food, and their weights were maintained at 85-90% of their body weights via food restriction. Experiment 2 lasted for 12 months; therefore, mice were placed on free feed during month 6 for 2 weeks. The new 100% body weights were established at the end of the free-feeding period.

Materials

Apparatus

See apparatus in Experiment 1.

Procedure

Magazine training

See procedure in Experiment 1.

Single lever training

The mice were initially trained to lever press using a modified autoshaping procedure to respond on a single extended lever (the vehicle-condition appropriate lever) for 0.02 mL of sweetened milk (Barrett & Vanover, 2004; Vanover & Barrett, 1998). Following autoshaping of the lever press response, single-lever training began. A single lever (the vehicle-paired lever) was extended inside the chamber. Each subject was placed in the operant chamber for a 15-minute session and trained on a FR 1 reinforcement schedule. The value of the FR was gradually increased over the next 11-16 sessions until FR 10 was obtained. Animals were then trained using errorless training, in which a single condition-appropriate lever (i.e. vehicle or drug lever) was extended inside the chamber. First, the mice were trained with only the vehicle lever with vehicle injection (10 min pre-session). After response rates stabilized under vehicle condition (5 sessions), the mice were then trained with 5.0 mg/kg (*R, S*)-ketamine injection (10 min pre-session) with the opposite (i.e., drug) lever until response rates stabilized (6 sessions). All lever assignments were counterbalanced across mice to avoid possible confounding by olfactory cues. To this end, the response levers were cleaned with a 10% ethanol solution after each test session (Extance & Goudie, 1981). Following completion of single-lever training, two-lever drug discrimination training began.

Drug discrimination acquisition training

During the two-lever drug discrimination training, both levers were extended into the operant chamber. The subjects were administered a training dose of 5.0 mg/kg (*R*, *S*)-ketamine or vehicle according to a double alternation sequence of drug (D) and vehicle (V) (i.e., DDVVDDVV). On days when the drug was administered, only responding on the drug-associated lever was reinforced. Conversely, on days that vehicle was administered, only responding on the vehicle-associated lever was reinforced. A response on the incorrect lever before completing ten responses on the condition-appropriate lever reset the FR counter. Subjects received two-lever drug discrimination training until the discrimination training criteria were passed for five of six consecutive sessions. Successful discrimination training was evaluated and assessed according to three criteria: (1) the first completed fixed ratio of the FR 10 schedule was completed on the condition-appropriate lever, (2) ≥80% of total responses made during the session occurred on the condition-appropriate lever, and (3) response rate for the session was greater than or equal to 10 responses per minute.

Generalization and substitution testing

Once drug discrimination training was completed, generalization testing with the training drug began. During test sessions, switching levers before completion of the FR10 requirement reset the FR counter. However, completion of the FR 10 requirement on either lever resulted in delivery of a reinforcer. Mice were required to pass control tests for vehicle and (*R*, *S*)-ketamine (according to the training criteria above) before the start of generalization dose-effect curve for each test drug. Additionally, mice were required to meet the training criteria for vehicle and (*R*, *S*)-ketamine on two consecutive training days before becoming eligible for a test day.

The (*R*, *S*)-ketamine generalization dose-effect curve was established with doses of 0.625 mg/kg, 1.25 mg/kg, 2.5 mg/kg, 5.0 mg/kg, and 10 mg/kg, with subcutaneous injections 10 minutes prior to testing. The order of dose was randomly counterbalanced with between two groups of mice; half of the mice were tested in ascending dose order and the other half were tested in descending dose order. For the tests of (*R*)-ketamine and (*S*)-ketamine, the subjects were randomly counterbalanced into two groups for substitution testing of (*R*)-ketamine and (*S*)-ketamine. Subjects were treated with 0.625 mg/kg, 1.25 mg/kg, 2.5 mg/kg, 5.0 mg/kg, 10.0 mg/kg (*R*)-ketamine or 0.3125 mg/kg, 0.625 mg/kg, 1.25 mg/kg, 2.5 mg/kg, 5.0 mg/kg (*S*)-ketamine, 10 minutes prior to testing. For the substitution test with (2*R*,6*R)*-hydroxynorketamine, mice were treated with 10.0 mg/kg and 56.0 mg/kg. (2*R*,6*R)*-Hydroxynorketamine was tested first at 10-minute injection time and then at 60-minute injection time; the 60-minute pretreatment time for (2*R*,6*R)*-hydroxynorketamine was chosen for exploratory purposes.

Data analysis

The Med-PC software was programmed to calculate the percent drug-lever responding (% DLR) on the condition-appropriate lever by dividing the number of responses on that lever by the total number of responses on both levers and then multiplying the result by 100. Responses per minute were calculated by taking the total number of responses on both levers and dividing by 15 mins (i.e. the session length). Full substitution to the (*R, S*)-ketamine stimulus cue was defined as ≥ 80% DLR on the (*R*, *S*)-ketamine lever. Partial substitution to (*R*, *S*)-ketamine stimulus cue was defined as ≥ 60% DLR. Vehicle-appropriate responding was defined as ≤ 20% DRL. One-way repeated measures analyses of variance (ANOVA) were conducted on response rates to test for statistically significant differences between each drug condition and saline. When the ANOVA was significant, Dunnett's post hoc tests were used to compare the drug doses to saline. The

criterion for significance was set at $p < 0.05$ and all data were analyzed using GraphPad Prism version 8.3 (GraphPad Software, San Diego, California, USA) on a Windows 10 PC.

Experiment 3: Spontaneous alternation in a Y-maze

Subjects

One-hundred and twenty adult male C57BL/6 mice (Harlan, Indianapolis, IN) weighing between 19 and 26 g, were used in this experiment. The same acclimation and housing procedures used in experiments 1 and 2 were followed, the only exception in Experiment 3 was that the mice were never food deprived. Upon arrival, mice habituated to the vivarium for two weeks before experimentation. Mice were maintained on a 12/12-hour light-dark cycle (lights on 0600). Mice were group housed with four mice per cage. Nesting material, cardboard tubes, and ad libitum access to water were provided in their home cage. After two weeks, mice were handled for 5 minutes daily for one week. Mice were aged 8-14 weeks for testing.

Materials

Apparatus

The apparatus was a 3-arm maze and each arm spaced at an angle of 120 degrees (Figure 2). Each arm was 35 cm long, 7 cm wide, and the walls were 20 cm tall. The arms were clearly labelled ‘A’, ‘B’, and ‘C’. The walls and floor are clear acrylic and were painted white. To avoid shadows, the maze was well lit from above. Each session was digitally recorded with a GoPro Hero 7 camera. The maze was cleaned with a 10% ethanol solution between each session.

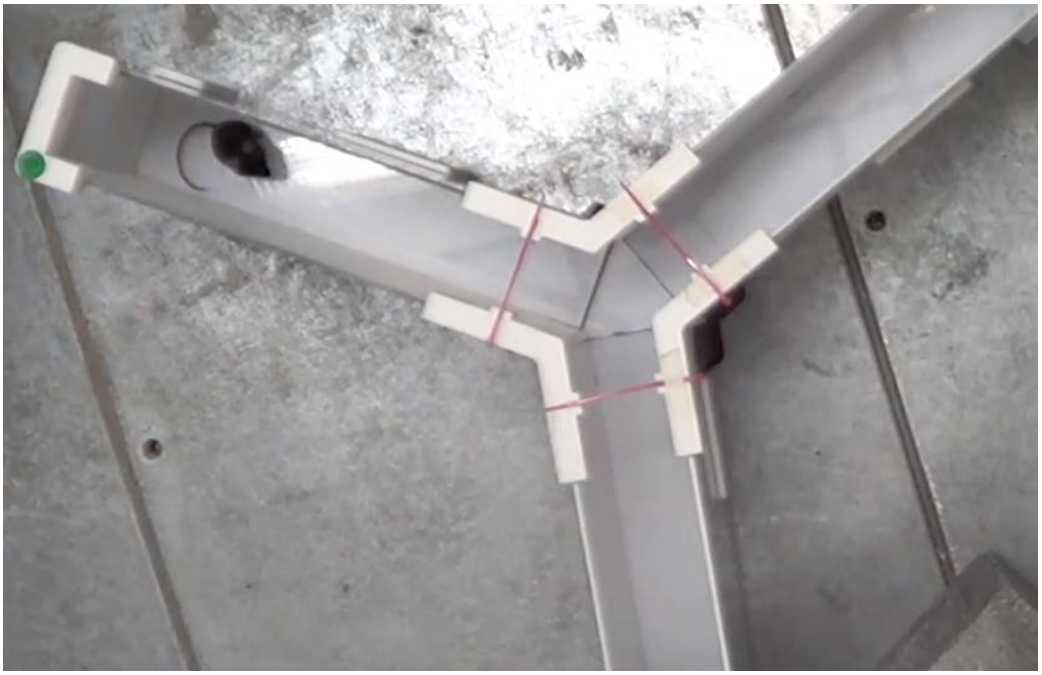

Figure 2. Y-maze was made from clear acrylic, cut with a computer numerical control router, and polylactic acid 3D printed components.

Procedure

Spontaneous alternation is an unconditioned behavior. Therefore, no training is required to test short-term memory in this procedure. Mice were habituated to the room with the maze for at least 30 minutes prior to testing. After habituation, video recording began, and each mouse was gently placed into the same maze arm to start each test session. The maze was cleaned with 10% ethanol between sessions to reduce the likelihood of olfactory cues carry-over between sessions. Test sessions lasted for 10 minutes, during which time the mice could freely explore the maze.

A between-subjects design was used with mice randomly assigned to ten groups (12 per group); saline, (*R*, *S*)-ketamine 10.0 mg/kg, (*R*, *S*)-ketamine 32.0 mg/kg, (2*R*,6*R)*-hydroxynorketamine 10.0 mg/kg, (2*R*,6*R)*-hydroxynorketamine 32.0 mg/kg, (2*R*,6*R)*-hydroxynorketamine 56.0 mg/kg, (*R*)-ketamine 16.0 mg/kg, (*R*)-ketamine 32.0 mg/kg, (*S*)-ketamine 16.0 mg/kg, and (*S*)-ketamine 32.0 mg/kg. All drugs were administered subcutaneously

24 hours prior to testing because we wanted to assess whether cognitive deficits persisted beyond elimination of the drug (Rajagopal et al., 2016). Persistent effects on cognition would indicate a neuronal structural change and dysregulation of network activity in the brain beyond acute effects while the drug is in the organism. This is important for treating human patients. Ideally, treatment would not cause long-term cognitive impairments to patients who may already be suffering from cognitive deficits associated with depression.

Data analysis

Sessions were digitally recorded and then arm entries were scored by one undergraduate student masked to the test conditions. Then, a second student (also blinded to test conditions) would watch the videos and ensure arm entries were scored correctly. A mouse was considered to have made a spontaneous alternation when all four paws entered a different arm (i.e. leaving the center of the maze and crossing the threshold to a maze arm) of the maze in three consecutive alternations, or arm entries. Percent spontaneous alternation was then calculated by dividing the spontaneous alternation count during the 10-minute session by total number of arm entries, minus two, during the 10-minute session. Subtracting two arm entries from the denominator (the total number of arm entries) accounts for the first two arm entries, which could not have counted as a spontaneous alternation (Miedel et al., 2017). One-way between-subject analyses of variance (ANOVA) were conducted for each drug to test for statistically significant differences between each drug condition and the saline, vehicle condition (which was used for each analysis). When the ANOVA was significant, Dunnett's post hoc tests were used to compare the drug doses to vehicle. The criterion for significance was set at $p < 0.05$ and all data were analyzed using GraphPad Prism version 8.3 (GraphPad Software, San Diego, California, USA) on a Windows 10 PC.

Results

Experiment 1: Differential-reinforcement-of-low-rate (DRL)

Figure 3 shows the effects of (*R*,*S*)-ketamine on mean number of reinforcers and number of responses. (*R*,*S*)-Ketamine treatment produced a significant effect on reinforcers ($F(4, 44) = 9.43$, $p = 0.0014$). A Dunnett's multiple comparisons test indicated that 32 mg/kg (*R*,*S*)-ketamine significantly increased the mean number of reinforcers as compared to vehicle ($p < 0.0041$). For number of responses, (*R*,*S*)-ketamine produced a significant effect ($F(4, 44) = 4.27$, $p = 0.015$). A Dunnett's multiple comparisons test indicated that 32 mg/kg (*R*,*S*)-ketamine significantly decreased the mean number of responses as compared to vehicle ($p < 0.033$).

(*R*)-Ketamine (Figure 4) 10.0 mg/kg, 17.8 mg/kg, and 32.0 mg/kg produced no significant effects on reinforcers or responses at any of the tested doses ($F(3, 35) = 1.69$, $p = 0.21$); ($F(3, 35) = 0.55$, $p = 0.58$), respectively). (*S*)-Ketamine (Figure 5) produced a significant effect on reinforcers ($F(3, 35) = 8.23$, $p = 0.014$). A Dunnett's multiple comparisons test indicated that both 17.8 mg/kg and 32.0 mg/kg (*S*)-ketamine significantly increased the mean number of reinforcers as compared to vehicle ($p = 0.022$, $p = 0.030$, respectively). For number of responses, (*S*)-ketamine produced a significant effect ($F(3, 35) = 17.45$, $p < 0.0001$). A Dunnett's multiple comparisons test indicated that both 17.8 mg/kg and 32.0 mg/kg (*S*)-ketamine significantly decreased the mean number of responses as compared to vehicle ($p = 0.0023$, $p = 0.0006$, respectively).

The ketamine metabolite (2*R*,6*R*)-hydroxynorketamine (Figure 6), at a dose of 56 mg/kg, produced a significant increase in the mean number of reinforcers ($t(8) = 2.8$, $p = 0.023$) and a significant decrease in the mean number of responses ($t(8) = 3.51$, $p = 0.0079$).

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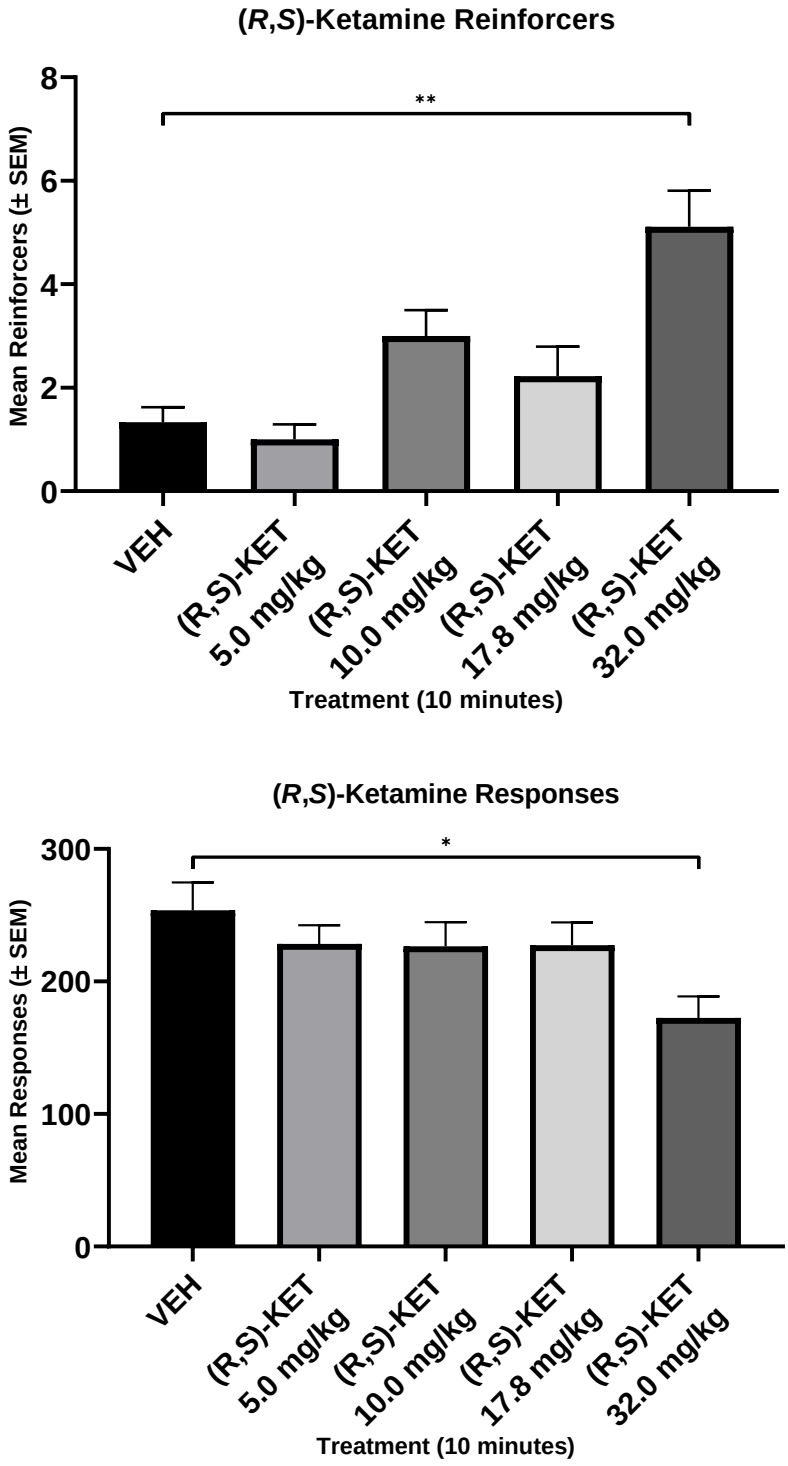

Figure 3. Effects of (*R*,*S*)-ketamine on mean number of reinforcers and number of responses (n = 9). Asterisks represent significant differences from vehicle, and all data are expressed as means ± standard error of the mean (±SEM). The alpha level was set to 0.05. For all graphs: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, and **** = $p < 0.0001$.

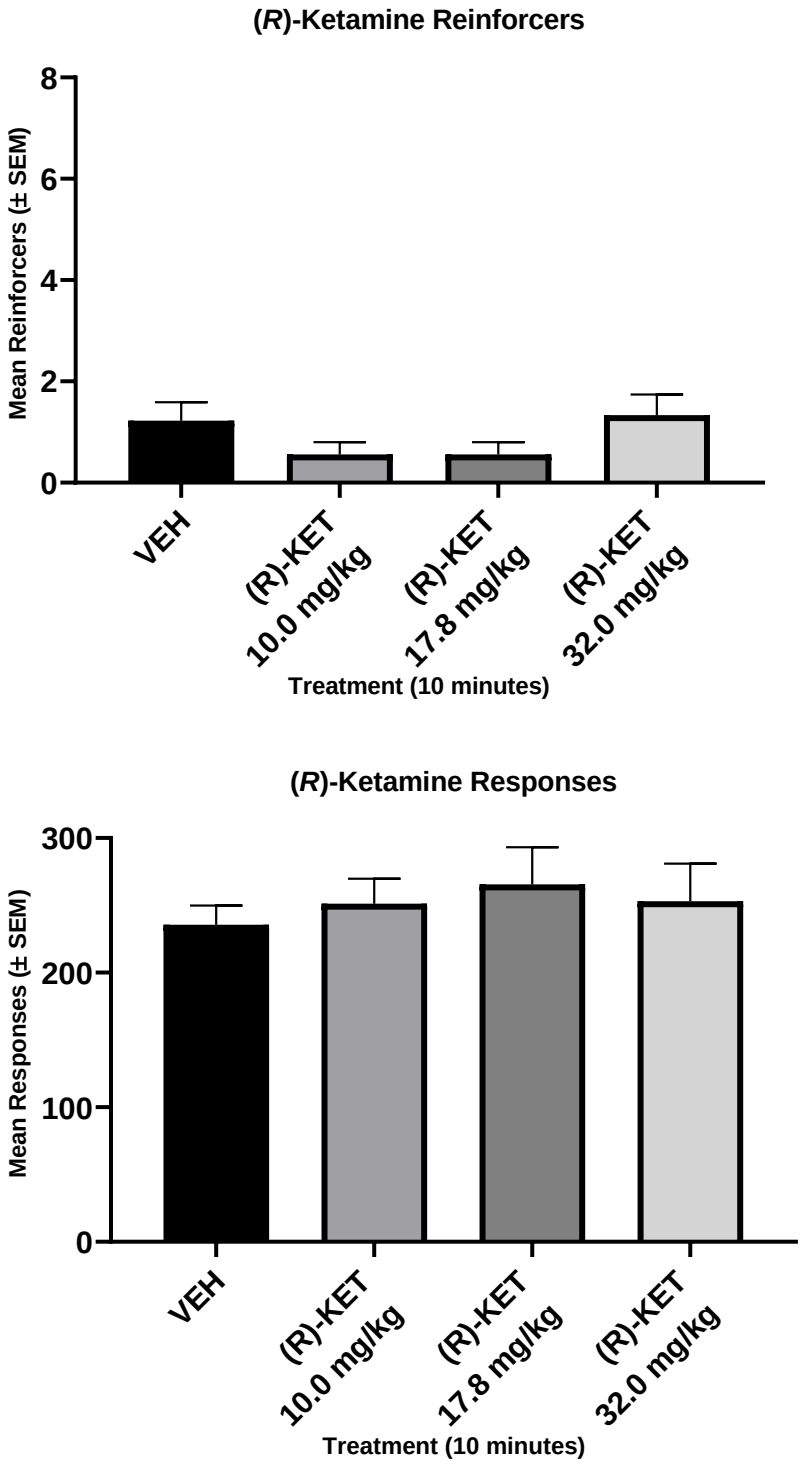

Figure 4. Effects of (*R*)-ketamine on mean number of reinforcers and number of responses (n = 9). See Figure 2 for other details.

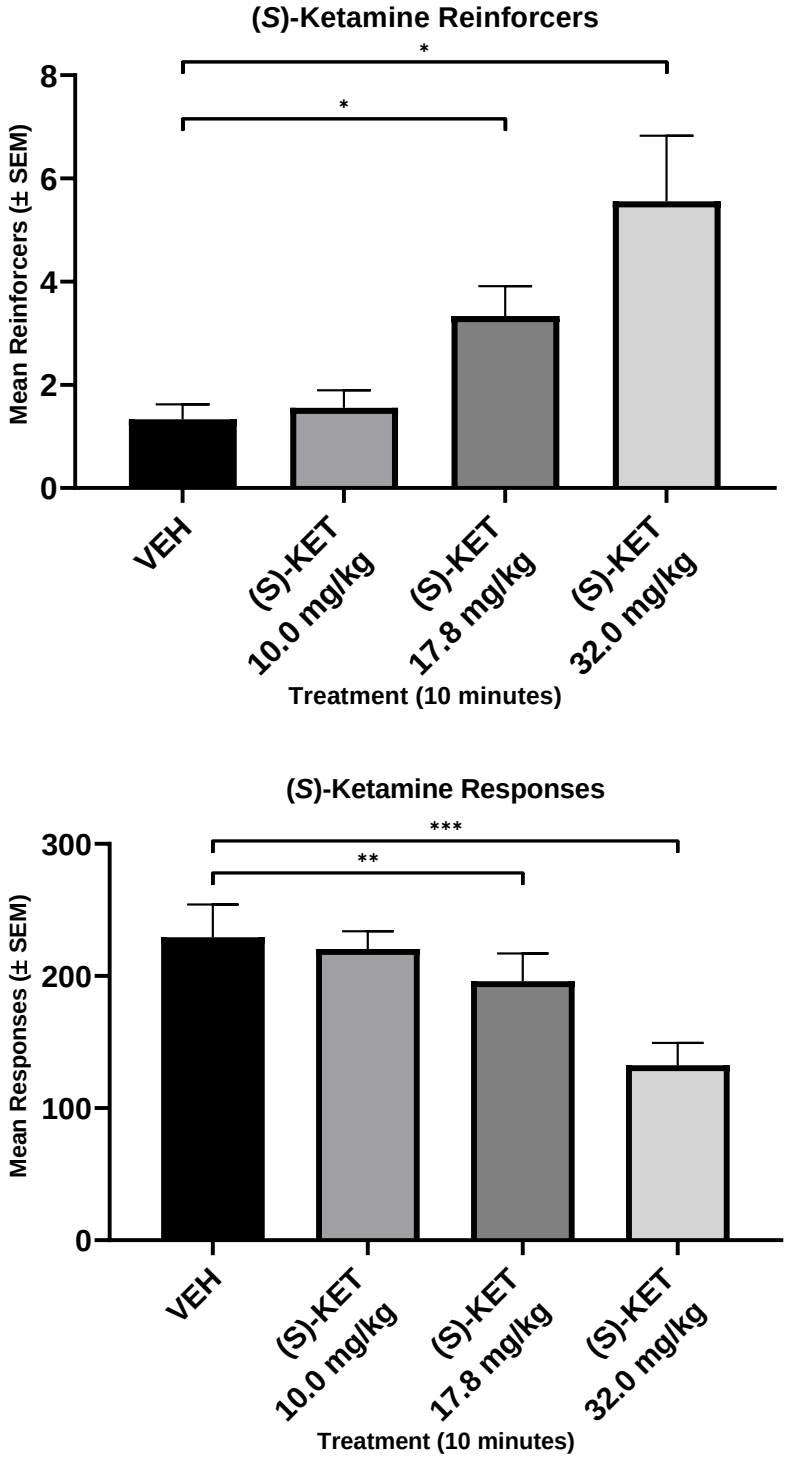

Figure 5. Effects of (*S*)-ketamine on mean number of reinforcers and number of responses (n = 9). See Figure 2 for other details.

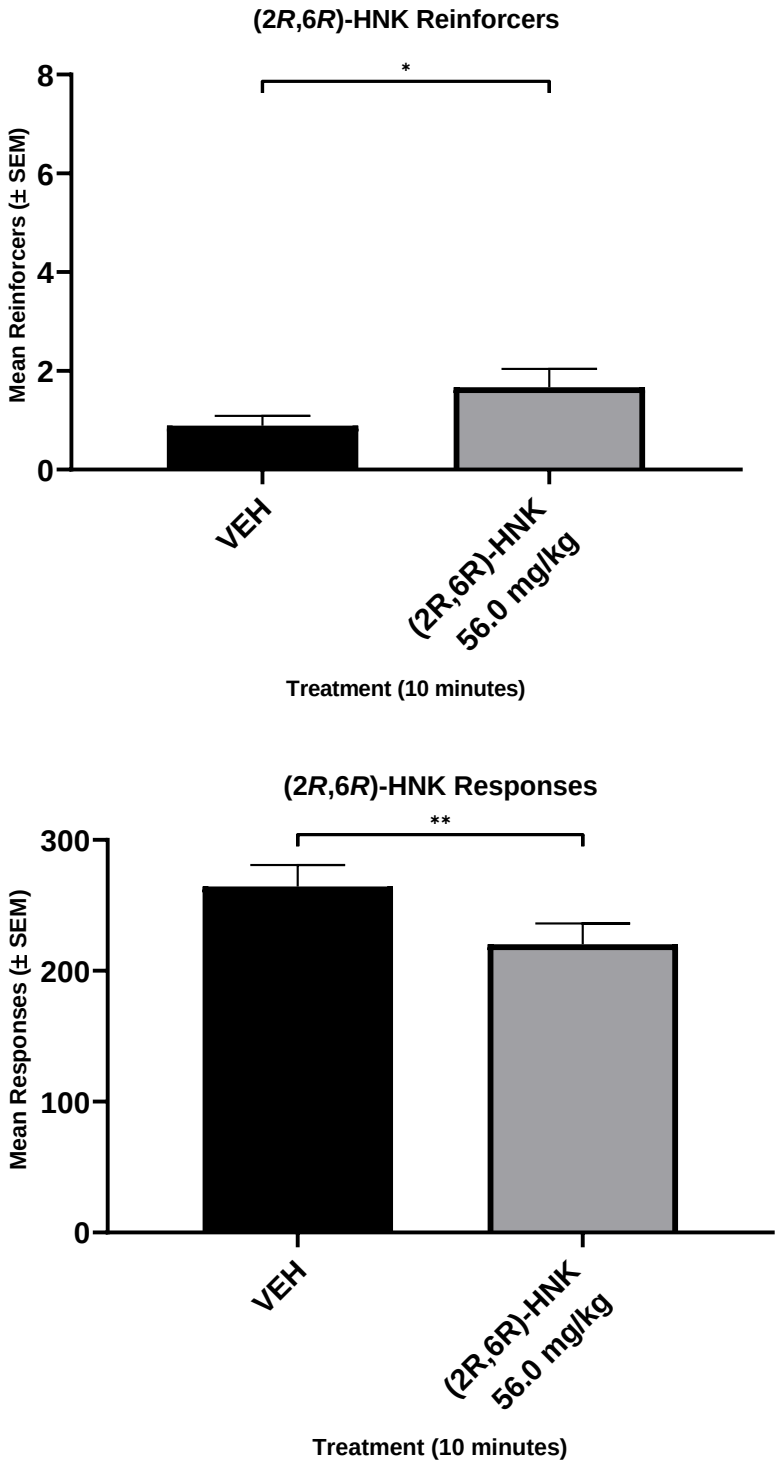

Figure 6. Effects of (2*R*,6*R*)-hydroxynorketamine on mean number of reinforcers and number of responses (n = 9). See Figure 2 for other details.

Experiment 2: Drug discrimination

Figure 7 shows the (*R*,*S*)-ketamine generalization dose effect curve for C57BL/6 mice trained to discriminate 5.0 mg/kg (*R*,*S*)-ketamine from vehicle. Subjects were successfully trained to discriminate 5.0 mg/kg (*R*,*S*)-ketamine and vehicle in a two-lever drug discrimination task (average 19.6 sessions; range = 8 – 29 sessions). Generalization testing with (*R*,*S*)-ketamine yielded an ED_{50} = 2.03 mg/kg (95% confidence interval (C.I.) = 1.71 – 2.42 mg/kg). (*R*,*S*)-Ketamine (fully generalized (80% or greater responding on the drug-paired lever) at 2.5 mg/kg, 5.0 mg/kg (the training dose), and 10.0 mg/kg (*R*,*S*)-ketamine. Responding above 80% on the drug-paired lever, is considered full generalization to the training dose. A one-way repeated measures ANOVA indicated a significant effect of treatment on the mean number of responses per minute ($F (5, 55) = 3.66$, $p = 0.0063$. A Dunnett's multiple comparisons test revealed that 0.625 mg/kg and 1.25 mg/kg (*R*,*S*)-ketamine produced small but significant increases in responses per minute when compared to vehicle. This increase in responses may be attributed to changes in locomotor behavior associated with (*R*,*S*)-ketamine treatment. (*R*,*S*)-Ketamine treatment has been observed to increase locomotor behavior in an open-field (Zanos et al., 2016).

Both the (*R*) and (*S*) ketamine isomers shared discriminative stimulus properties with (*R*,*S*)-ketamine 5.0 mg/kg. The (*R*) isomer (Figure 8) fully substituted at 10 mg/kg, but not at lower doses (ED_{50} = 2.69 mg/kg, 95% C.I. = 1.43 - 5.05 mg/kg). The (*S*) isomer (Figure 9) fully substituted at 2.5 mg/kg and 5.0 mg/kg (ED_{50} = 1.07 mg/kg, 95% C.I. = 0.69-1.63 mg/kg). Neither the (*R*) or (*S*)-ketamine isomers produced any significant changes in response rates ($F (5, 25) = 1.33$, $p = 0.30$; $F (6, 36) = 1.41$, $p = 0.28$, respectively).

Figures 10 and 11 present results for the (2*R*,6*R*)-hydroxynorketamine substitution testing at 10-minute and 60-minute injection times, respectively. Both 10.0 mg/kg and 56.0 mg/kg

(2*R*,6*R*)-hydroxynorketamine, at 10-minute ($F(2, 10) = 1.63$, $p = 0.24$) and 60-minute pretreatment ($F(2, 10) = 1.72$, $p = 0.23$) failed to substitute for 5.0 mg/kg (*R*,*S*)-ketamine, producing only vehicle-appropriate responding.

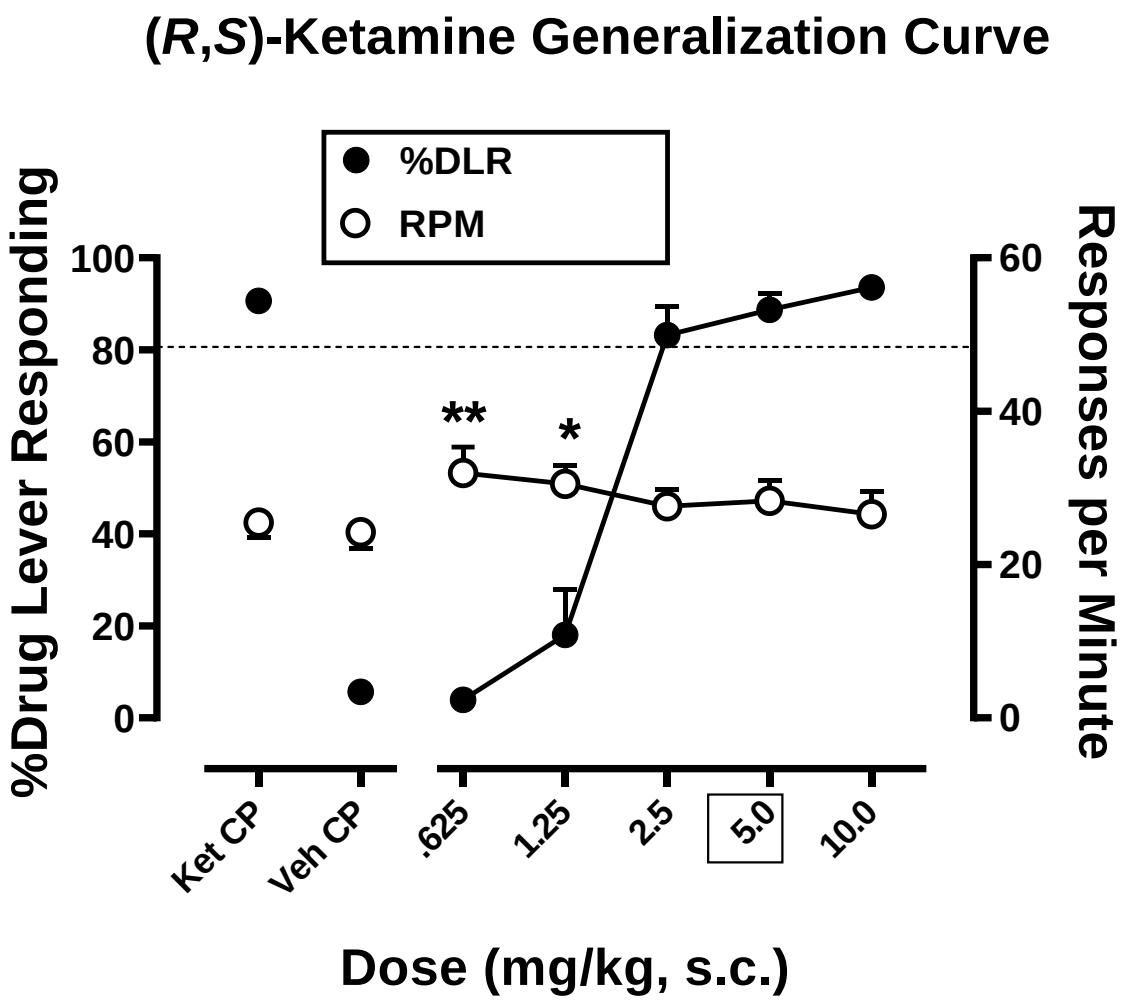

Figure 7. The (*R*,*S*)-ketamine generalization dose effect curve for C57BL/6 mice trained to discriminate 5.0 mg/kg (*R*,*S*)-ketamine from vehicle (n = 12). The dashed line at 80% indicates the threshold to meet full generalization criteria, mean responses above this line are considered full generalization. Control test sessions (Ket CP and Veh CP) were conducted prior to testing of other doses and indicate that the discrimination training was successful. The left vertical axis and filled circles represent the percentage of drug lever responses (%DLR) on the (*R*,*S*)-ketamine-paired lever. The right vertical axis and open circles represent response rate, or responses per minute (RPM). The figure shows mean percent drug lever responding (± SEM) and mean responses per minute (± SEM) for (*R*,*S*)-ketamine. Significantly different from vehicle, * = $p < 0.05$, ** = $p < 0.01$.

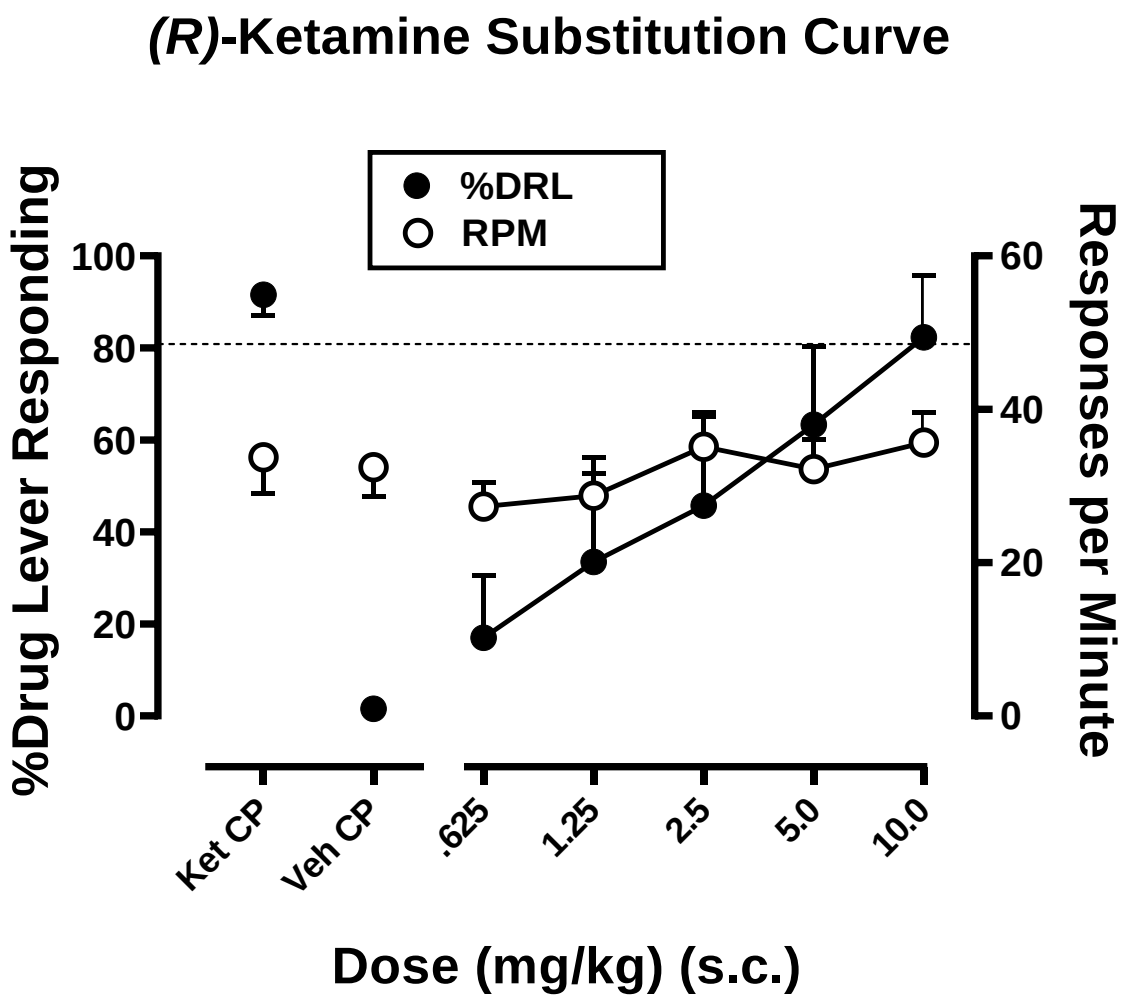

Figure 8. (*R*)-ketamine generalization curve. The figure shows mean percent drug lever responding (± SEM) and mean responses per minute (± SEM) for (*R*)-ketamine (n = 6). See Figure 7 for more details.

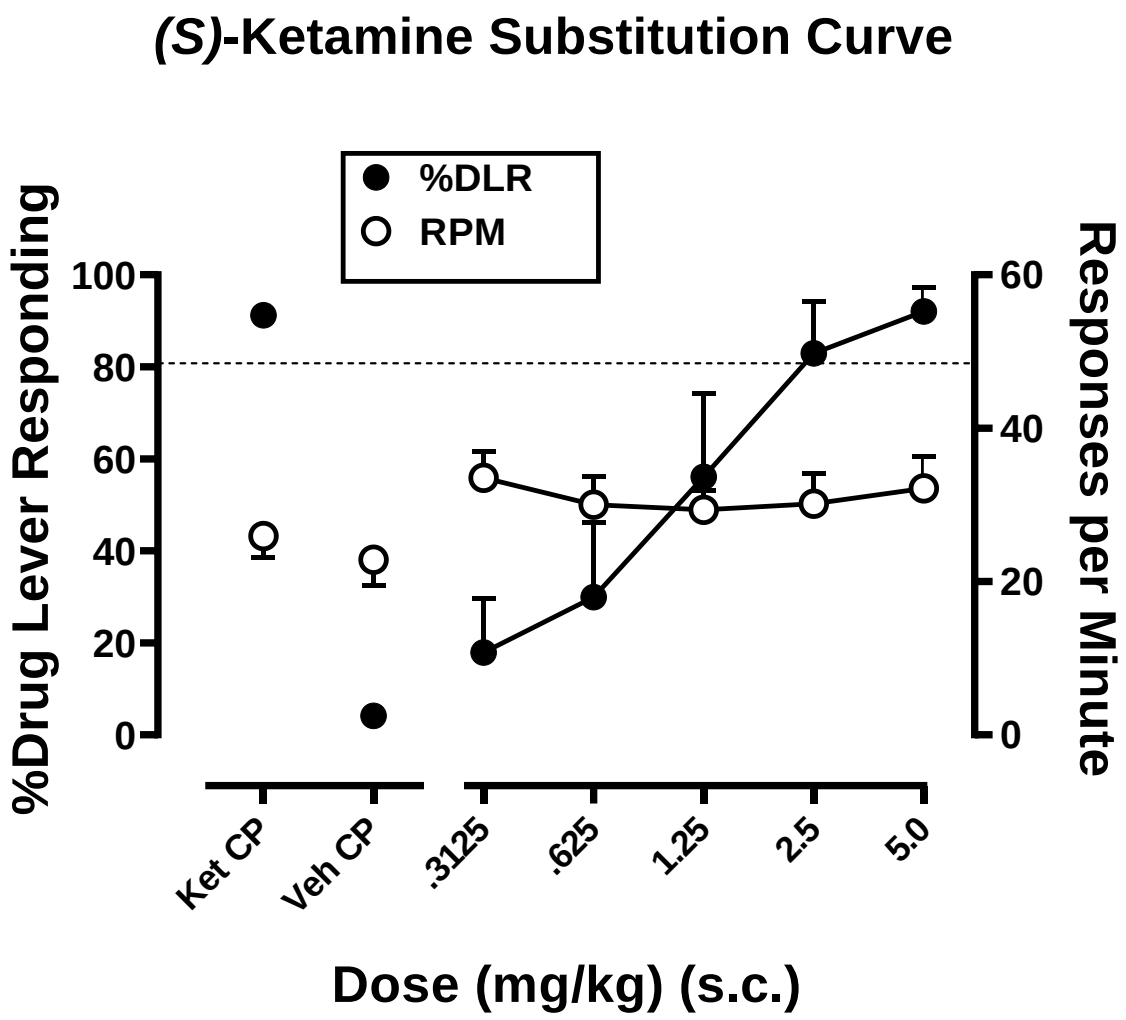

Figure 9. (*S*)-ketamine generalization curve. The figure shows mean percent drug lever responding (± SEM) and mean responses per minute (± SEM) for (*S*)-ketamine (n = 7). See Figure 7 for more details.

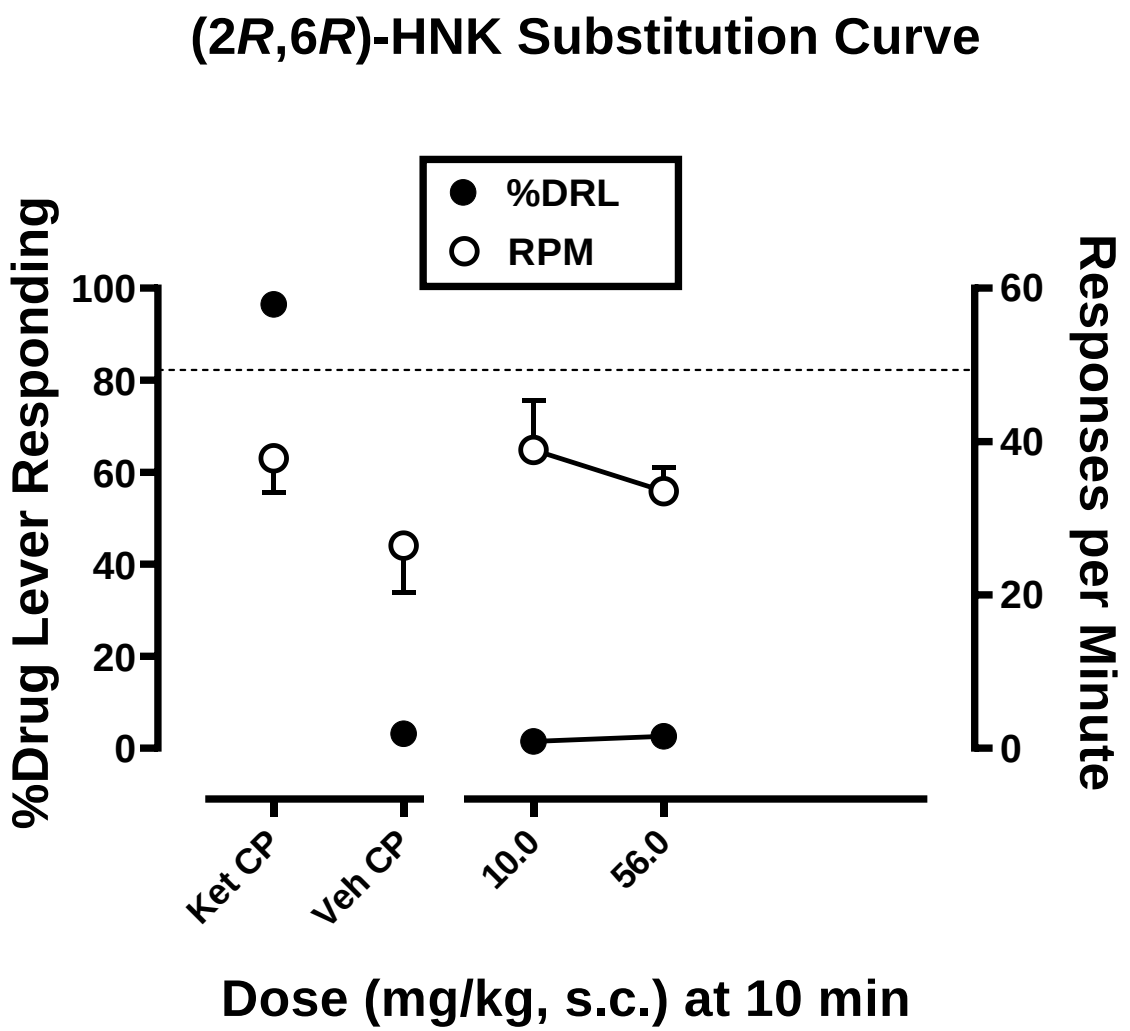

Figure 10. (2*R*,6*R*)-hydroxynorketamine generalization curve. The figure shows mean percent drug lever responding (± SEM) and mean responses per minute (± SEM) for (2*R*,6*R*)-hydroxynorketamine (n = 6). See Figure 7 for more details.

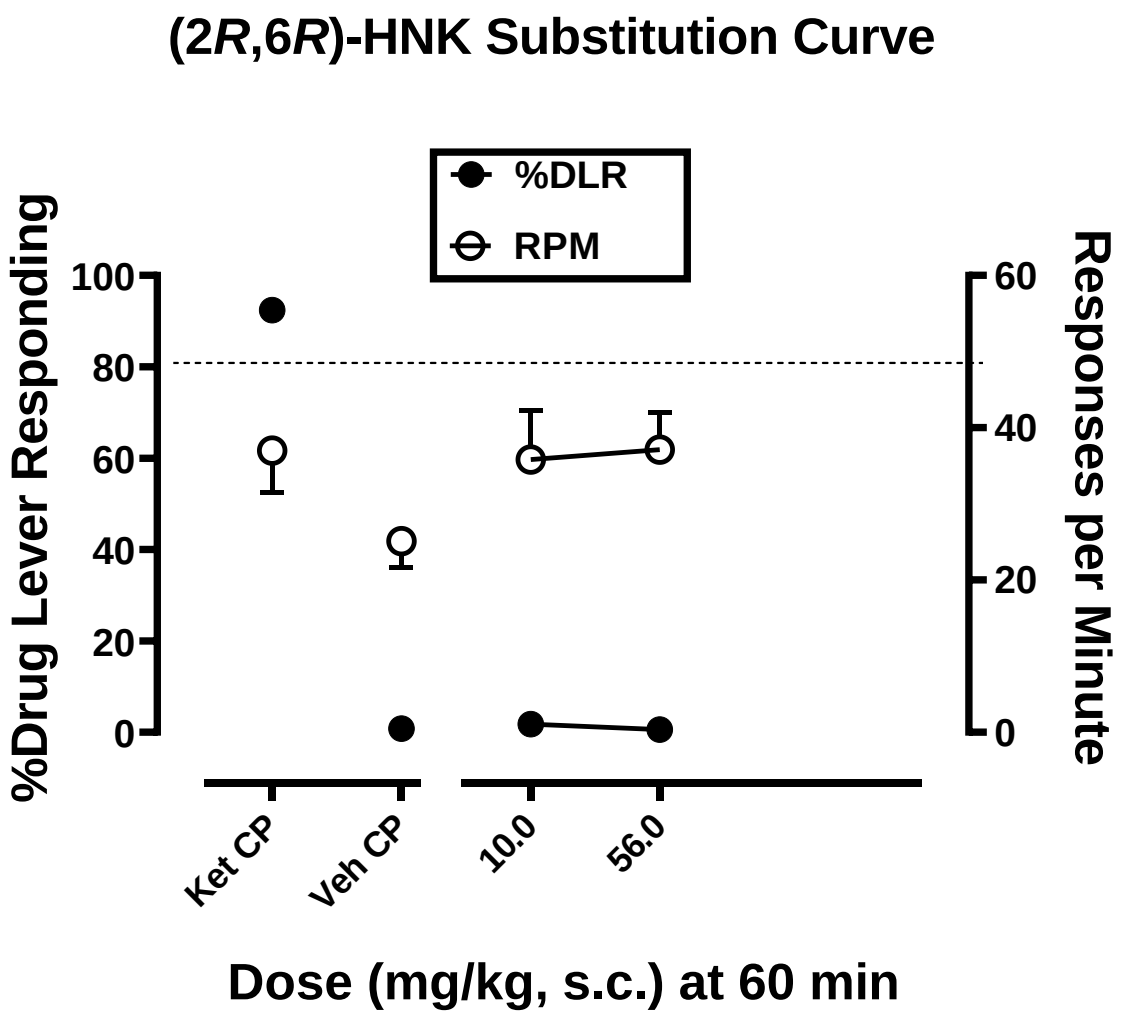

Figure 11. (2*R*,6*R*)-hydroxynorketamine generalization curve. The figure shows mean percent drug lever responding (± SEM) and mean responses per minute (± SEM) for (2*R*,6*R*)-hydroxynorketamine (n = 6). See Figure 7 for more details.

Experiment 3: Spontaneous alternation in the Y-maze

Figure 12 shows the effects of (*R*,*S*)-ketamine treatment on mean % alternation in the Y-maze. One-way ANOVA indicated that subjects treated with (*R*,*S*)-ketamine had a significantly lower % alternation in the Y-maze when compared to vehicle ($F(2, 33) = 4.58$, $p = 0.018$). A Dunnett's multiple comparisons test indicated that spontaneous alternation in the Y-maze was significantly lower when subjects were treated with 32.0 mg/kg (*R*,*S*)-ketamine ($p = 0.033$), but not 10.0 mg/kg (*R*,*S*)-ketamine ($p = 0.959$). One-way ANOVA indicated (*R*)-ketamine (Figure 13), at the tested doses, did not produce any significant changes in % spontaneous alternation ($F(2, 33) = 1.48$, $p = 0.24$). One-way ANOVA indicated treatment with (*S*)-ketamine (Figure 14) significantly reduced % alternation in a Y-maze when compared to vehicle (2, 33) = 7.34, $p = 0.002$. A Dunnett's multiple comparisons test indicated that 32.0 mg/kg (*S*)-ketamine, but not 16.0 mg/kg (*S*)-ketamine, produced a significant decrease as compared to vehicle ($p = 0.036$). One-way ANOVA (2*R*,6*R*)-hydroxynorketamine (Figure 15), at the tested doses, did not produce any significant changes in % spontaneous alternation ($F(3, 44) = 0.84$, $p = 0.48$)

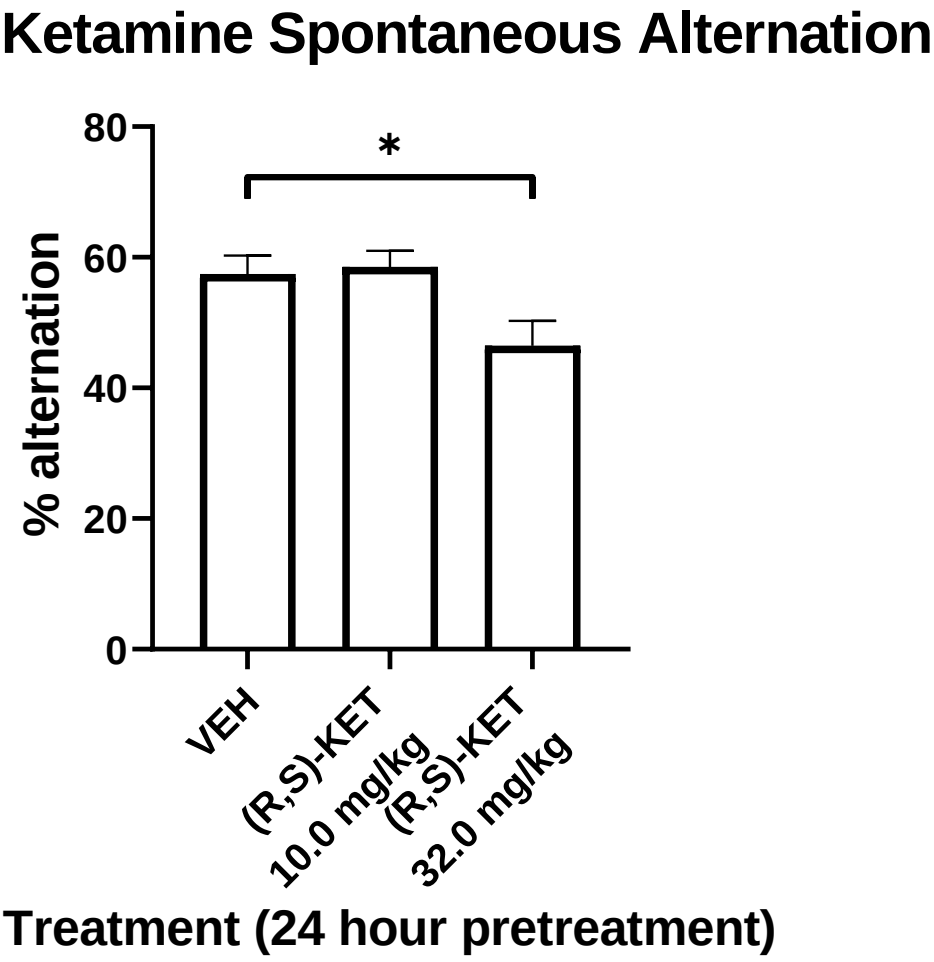

Figure 12. Effects of (*R*,*S*)-ketamine treatment on mean % alternation in Y-maze (n = 12 per group). All data are expressed as means + standard error of the mean (+SEM). The alpha level was set to 0.05 and for all graphs: * = $p < 0.05$. A between-subject design was used for experiment 3.

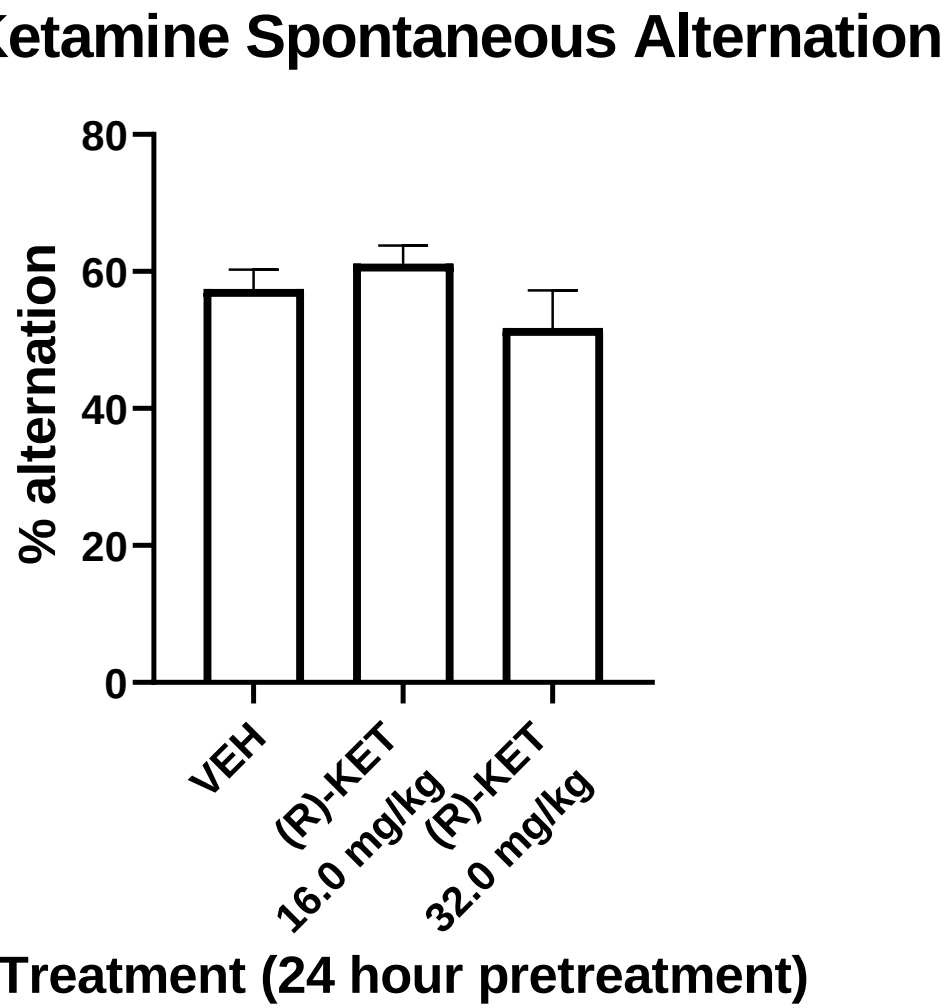

Figure 13. Effects of (*R*)-ketamine treatment on mean % alternation in Y-maze (n = 12 per group). All data are expressed as means + standard error of the mean (+SEM).

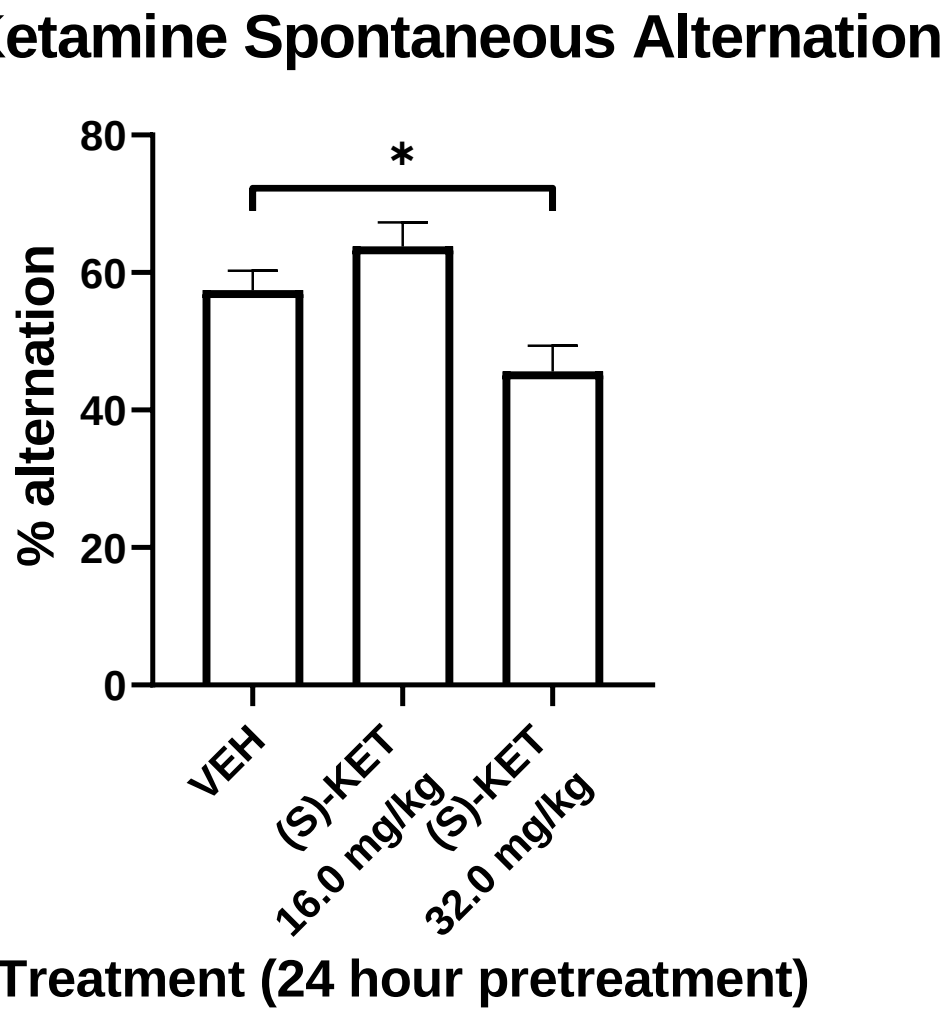

Figure 14. Effects of (*S*)-ketamine treatment on mean % alternation in Y-maze (n = 12 per group). All data are expressed as means + standard error of the mean (+SEM); * = $p < 0.05$.

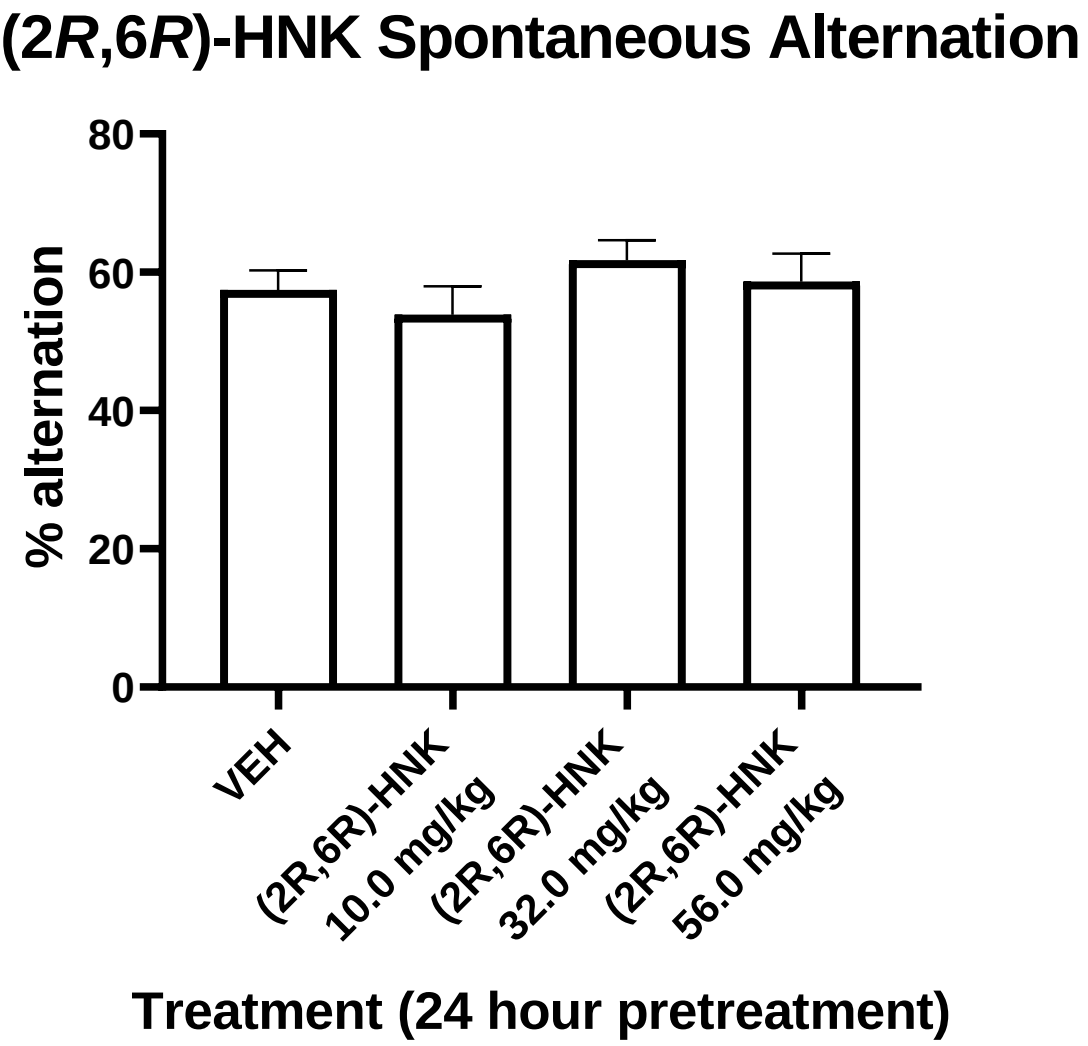

Figure 15. Effects of (2*R*,6*R*)-hydroxynorketamine treatment on mean % alternation in Y-maze (n = 12 per group). All data are expressed as means + standard error of the mean (+SEM).

Discussion

The present study tested (*R*,*S*)-ketamine, the isomers (*R*)-ketamine and (*S*)-ketamine, and the metabolite (2*R*,6*R*)-hydroxynorketamine in C57bl/6 mice in three preclinical behavioral assays; DRL task, drug discrimination, and spontaneous alternation in a Y-maze. These assays provided a screen for antidepressant-like effects, discriminative stimulus effects (subjective effects), and spatial memory (cognitive effects), respectively. In the DRL task, it was hypothesized that each drug, (*R*,*S*)-ketamine, (*R*)-ketamine, (*S*)-ketamine, and (2*R*,6*R*)-hydroxynorketamine, would produce antidepressant-like effects. In the two-lever drug discrimination task, C57BL/6 mice were hypothesized to discriminate between 5.0 mg/kg (*R*,*S*)-ketamine and saline. Also, it was hypothesized that the isomers (*R*)-ketamine and (*S*)-ketamine, but *not* the (2*R*,6*R*)-hydroxynorketamine metabolite would share discriminative stimulus properties with (*R*,*S*)-ketamine (i.e. (2*R*,6*R*)-hydroxynorketamine would *not substitute* for (*R*,*S*)-ketamine). Finally, in the spontaneous alternation Y-maze assay, the mice were hypothesized to exhibit reduced spontaneous alternation when tested with (*R*,*S*)-ketamine and the isomer (*S*)-ketamine, but not when treated with the isomer (*R*)-ketamine or the metabolite (2*R*,6*R*)-hydroxynorketamine. Table 2 shows a summary of the results from each of these studies, which will be fully discussed in the sections below.

Table 2. Summary of results

TREATMENT	**AIM 1: DRL**	**AIM 2: DRUG DISCRIMINATION**	**AIM 3: Y-MAZE**
(*R*,*S*)-KETAMINE	Significant antidepressant-like effect (32.0 mg/kg)	Full substitution	Significantly reduced spontaneous alternation (32.0 mg/kg)
(*R*)-KETAMINE	No effect at the tested doses	Full substitution	No effect at the tested doses
(*S*)-KETAMINE	Significant antidepressant-like effect (17.8 mg/kg and 32.0 mg/kg)	Full substitution	Significantly reduced spontaneous alternation (32.0 mg/kg)
(2*R*,6*R*)-HYDROXYNORKETAMINE	Significant antidepressant-like effect	No substitution	No effect at the tested doses

Note. DRL = Differential-reinforcement-of-low-rate. Significant antidepressant-like effect = significant increases in mean reinforcers and a significant decrease in mean responses. Full substitution = 80% or greater responding on the (*R*,*S*)-ketamine lever

Differential-reinforcement-of-low-rate (DRL) task

The DRL task is a preclinical *in vivo* assay for screening novel compounds to determine possible antidepressant-like therapeutic effects in humans. As expected, the DRL task results with (*R*,*S*)-ketamine in the present study with C57BL/6 mice confirmed the antidepressant-like effects of (*R*,*S*)-ketamine seen with other preclinical assays (FST and TST) in both mice and rats (Ma et al., 2013; Wang et al., 2015). One important difference in the present study's results is that the antidepressant-like effects of (*R*,*S*)-ketamine were not evident until a higher dose of 32 mg/kg was tested (see Figure 3) which is unlike other studies. For example, at least seven studies have demonstrated significant antidepressant-like effects with (*R*, *S*)-ketamine at 10.0 mg/kg in the FST and TST (Ma et al., 2013; Zhang et al., 2015; Dong et al., 2017; Fukumoto et al., 2017; Yamaguchi et al., 2018; Yang et al., 2018; Xiong et al., 2019). The task itself might be a factor; the length of the DRL test sessions are much longer than for the FST and TST. The present study used a 60-minute test DRL session; whereas, FST and TST are very short (usually between 5 -15 minutes), that difference between the assays that might have influenced the results. Furthermore, the present study used a 72 second inter-response interval. Perhaps a DRL task with a lower inter-response interval (e.g. 36 seconds) would be more sensitive to drugs with antidepressant-like effects. Contradictory results may be due to intrinsic differences in antidepressant screening assays and future studies should compare different methodologies.

The hypothesis that (*R*)-ketamine would increase reinforcers and decrease responses in the DRL task (i.e., cause an antidepressant-like effect) was based on other studies that have reported significant antidepressant-like effects with (*R*)-ketamine at 10.0 mg/kg in the FST and TST (Yamaguchi et al., 2018; Xiong et al., 2019). However, no antidepressant-like profile was observed for (*R*)-ketamine in the present study as no significant changes in the number of

reinforcers or responses were observed at the tested doses (10.0 mg/kg, 17.8 mg/kg, and 32.0 mg/kg). The present study, however, did demonstrate a significant antidepressant-like effect with (*S*)-ketamine treatment 10 minutes before the DRL task. A significant effect with (*S*)-ketamine and a non-significant effect with (*R*)-ketamine could be due to stereoselective differences in metabolism and elimination. The ketamine isomers are metabolized in a stereoselective manner; the demethylation of (*S*)-ketamine occurs more rapidly than for (*R*)-ketamine (Portmann et al., 2010). Furthermore, systemic clearance of (*S*)-ketamine occurs more rapidly than (*R*)-ketamine (Ihmsen et al., 2001). Metabolism is critical for antidepressant-like effects because blocking the metabolization of ketamine has been shown to block its antidepressant-like effects (Zanos et al., 2016). Therefore, if our DRL task was conducted with a longer pretreatment time (after (*R*)-ketamine has been metabolized), then perhaps significant antidepressant-like effects in the DRL task with (*R*)-ketamine treatment would be observed. However, other studies have indicated (*R*)-ketamine alone to be effective at 30-minute and 24-hour pretreatment times in the FST, which has a shorter test session than the hour-long DRL task (Yang et al., 2016; Yamaguchi et al., 2018). Another possibility is that higher doses of (*R*)-ketamine are required to produce antidepressant-like effects in the DRL task. The present study observed a similar phenomenon with (*R*,*S*)-ketamine in the DRL task, as several studies have reported 10.0 mg/kg (*R*,*S*)-ketamine to be effective in mice in the FST and TST (Ma et al., 2013; Zhang et al., 2015; Dong et al., 2017; Fukumoto et al., 2017; Yamaguchi et al., 2018; Yang et al., 2018; Xiong et al., 2019). While, the present study found no significant effects at 10.0 mg/kg (*R*,*S*)-ketamine, there was a significant effect at 32.0 mg/kg (*R*,*S*)-ketamine. Nonetheless, (*R*)-ketamine is of great interest and future studies should expand on the methodology used in the present study.

(2*R*,6*R*)-Hydroxynorketamine was hypothesized to increase reinforcers and reduce responses in the present study. Results support this hypothesis as (2*R*,6*R*)-hydroxynorketamine was found to produce an antidepressant-like profile in the DRL task. Other studies are in dispute as to whether (2*R*,6*R*)-hydroxynorketamine has antidepressant-like effects; Zanos et al. (2016) reported significant antidepressant-like effects; whereas, Yamaguchi et al. (2018) and Xiong et al. (2019) observed no effect. Furthermore, the methodology used in these studies to test (2*R*,6*R*)-hydroxynorketamine differed. Zanos et al. (2016) demonstrated antidepressant-like effects in both male and female mice using the FST and did not pre-expose mice to a chronic stress regimen. Yamaguchi et al. (2018) and Xiong et al. (2019) used only male mice but used FST and TST and did pre-expose the mice to a chronic stress regimen. Drawing definitive conclusions regarding antidepressant efficacy with (2*R*,6*R*)-hydroxynorketamine is limited by heterogeneous methodologies and findings across different studies.

One major concern is that FST and TST are acute stressors. Testing antidepressant drugs in mice exposed to only an acute stressor (FST or TST) may not accurately reflect human depression because human depression is often associated with chronic stress (Cryan et al., 2005). A social defeat stress model (repeated exposure to an aggressive mouse) may overcome limitations associated with exposure to an acute stressor (Dong et al., 2017; Xiong et al., 2019; Zhang et al., 2015). Another approach for inducing stress is to pre-expose subjects to compounds that have been shown to increase stress, and in two studies that examined the antidepressant-like effects of (*R*,*S*)-ketamine, two different approaches were used. Fukumoto et al. (2017) administered corticosterone (20 mg/kg) daily for twenty-one days before the FST. and Yamaguchi et al. (2018) and Yang et al. (2018) administered a single injection of lipopolysaccharide (0.5 mg/kg) one day before testing (*R*,*S*)-ketamine and (2*R*,6*R*)-

hydroxynorketamine. Both compounds, corticosterone and lipopolysaccharide, disrupt activity in the hypothalamic-pituitary-adrenal axis (Vakharia & Hinson, 2005), similarly to changes associated with human depressive symptoms and outcomes in preclinical studies (Demuyser et al., 2016; Keller et al., 2017). The choices made by researchers on whether to expose subjects to chronic stress may impact dependent variables in these preclinical studies (such as immobility in the FST and TST), and future studies should consider the implications of pre-exposure to stress.

Operant behavior in the DRL task can be impacted by stressful stimuli (Louis et al., 2006). For example, thermal stress has been reported to significantly reduce responses and reduce reinforcers in the DRL task (Louis et al., 2006). Louis et al. (2006) demonstrated these effects when the ambient air temperature was increased to 28°C and 35°C during a DRL task with rats. Furthermore, exposure to thermal stress significantly increased the protein levels of dopamine transporters and D1 receptors in the dorsal hippocampus (a brain region implicated in human depressive symptoms). Dopaminergic neurons and the hippocampus play an important role in stress (Grace, 2012). Indeed, lesioning the hippocampus has been demonstrated to impair performance in a DRL task (Rawlins et al., 1983). Taken together, stress and the neurological underpinnings of stress have been shown to be important for the performance in the DRL task. It remains to be determined whether stressful stimuli also would impact the effects of (*R*,*S*)-ketamine, its isomers, or metabolites in the DRL task.

In the DRL task, it was hypothesized that each drug, (*R*,*S*)-ketamine, (*R*)-ketamine, (*S*)-ketamine, and (2*R*,6*R*)-hydroxynorketamine, would produce antidepressant-like effects. It was found that 32.0 mg/kg (*R*,*S*)-ketamine, 17.8 mg/kg and 32.0 mg/kg (*S*)-ketamine, and 56.0 mg/kg (2*R*,6*R*)-hydroxynorketamine produced an antidepressant-like effect in DRL task. Due to the lack of in-house capability to synthesize a larger supply of (2*R*,6*R*)-hydroxynorketamine, and to

ensure enough drug was available to test in the other assays, we chose to test a single dose in the DRL task. This dose was chosen based on results from a study by Zanos et al. (2016) reported decreased immobility in the FST with (2*R*,6*R*)-hydroxynorketamine with doses ranging from 5.0 to 125 mg/kg. A dose somewhat in the middle of this range was selected as we wanted to avoid doses too high that might produce undesired side effects on operant responding in the DRL task; however, future studies need to test a full dose-response curve with lower and higher doses. Furthermore, results in the present study indicated that (*R*)-ketamine, at the tested doses (10.0 mg/kg, 17.8 mg/kg, and 32.0 mg/kg), produced no significant effect. The lack of effect with (*R*)-ketamine treatment may be due to slower metabolism of (*R*)-ketamine than (*S*)-ketamine (Portmann et al., 2010). Future studies need to test higher doses of the ketamine isomers and longer pretreatment times in the DRL task.

Drug Discrimination

The present study demonstrated that (*R*,*S*)-ketamine at a dose of 5.0 mg/kg was readily acquired as a discriminative stimulus in C57BL/6 mice in a mean of 19.6 sessions with full generalization at the training dose of 5.0 mg/kg and at the 2.5 and 10.0 mg/kg doses (see Figure 6). While the present study is the first to use 5.0 mg/kg (*R*,*S*)-ketamine as a training dose in mice, these results confirm other studies with both rats and mice that (*R*,*S*)-ketamine can be a salient discriminative stimulus (Benvenga et al., 1991; Zanos et al., 2016). Both (*R*)-ketamine and (*S*)-ketamine shared discriminative stimulus properties with 5.0 mg/kg (*R*,*S*)-ketamine. As expected, the more potent (*S*)-ketamine isomer (Zanos et al., 2018) displayed a leftward shift (2-fold) in the dose-response curve as compared to (*R*,*S*)-ketamine and (*R*)-ketamine (see ED50 values in Table 3). The results from testing with the ketamine isomers in the present study are novel in that no previous study used mice.

Table 3. Comparison of drugs tested in 5.0 mg/kg (*R*,*S*)-ketamine drug discrimination

TREATMENT	**ED_{50} (+ 95% C. I.)**	**LOWEST DOSE > 80% DRL**
(*R*,*S*)-KETAMINE	2.03 mg/kg (1.71-2.41 mg/kg)	2.5 mg/kg
(*R*)-KETAMINE	2.69 mg/kg (1.43-5.05 mg/kg)	10.0 mg/kg
(*S*)-KETAMINE	1.07 mg/kg (0.69-1.63 mg/kg)*	2.5 mg/kg
(2*R*,6*R*)-HYDROXYNORKETAMINE (10 MIN)	N/A	----------
(2*R*,6*R*)-HYDROXYNORKETAMINE (60 MIN)	N/A	----------

Note. * Significantly different from (*R*,*S*)-ketamine, $p < 0.05$. C.I. = Confidence Interval. DRL = Differential-reinforcement-of-low-rate

The present results also are consistent with previous findings comparing the ketamine isomers. Brady and Balster (1982) trained rats to discriminate phencyclidine (PCP; an arylcyclohexylamine like ketamine) from saline and reported that (*S*)-ketamine substituted at a lower dose than did (*R*)-ketamine, as it did in the present study. The differences in the effective doses between the ketamine isomers may be due to the same reasons listed above for the DRL task (differences in pharmacokinetics) along with differences in binding affinity and pharmacological targets (see Zanos et al. 2018). To the extent that abuse liability may be associated with the subjective effects of (*R*,*S*)-ketamine, (*R*)-ketamine may have less potential for abuse liability than (*S*)-ketamine. This finding is especially critical because (*S*)-ketamine was recently approved and is currently used to treat patients diagnosed with depression. Other preclinical drug discrimination studies support this concern (Shannon, 1981; Young et al., 1981). Shannon (1981) trained rats to discriminate between 3.0 mg/kg PCP and saline and reported that ketamine and other PCP analogs, which have been identified in illicit street samples, shared discriminative stimulus properties with PCP. As Shannon (1981) summaries at the end of the article, "....to the extent to which discriminative stimuli in animals are predictive of subjective effects in man, these results suggest that several PCP analogs may have an abuse liability similar to that of PCP". Ketamine-like abuse liability and other side-effects provide a strong impetus to further investigate other molecules in hope of safer treatment options.

At the tested doses, 10.0 mg/kg and 56.0 mg/kg, (2*R*,6*R*)-hydroxynorketamine failed to substitute for 5.0 mg/kg (*R*,*S*)-ketamine. Neither dose of (2*R*,6*R*)-hydroxynorketamine substituted at either 10- or 60-minute pretreatment times. These results are similar to other studies. Zanos et al. (2016) reported that 10.0 mg/kg and 50.0 mg/kg (2*R*,6*R*)-hydroxynorketamine failed to substitute for a 10.0 mg/kg training dose of (*R*,*S*)-ketamine in

mice. The present study used a lower training dose than Zanos et al. (2016) because training dose has been demonstrated to be an important determining factor of the properties of discriminative stimuli (see Stolerman et al. 2011). The results in the present study, with a lower training dose, supports the conclusions drawn by Zanos et al. (2016). Namely, that (2*R*,6*R*)-hydroxynorketamine does not appear to share subjective effects with (*R*,*S*)-ketamine. The clinical relevance of these findings is that (2*R*,6*R*)-hydroxynorketamine may lack the subjective effects that play a role in human abuse of (*R*,*S*)-ketamine. Taken together, the drug discrimination experiment in the present study supports the notion that (2*R*,6*R*)-hydroxynorketamine does not appear to share discriminative stimulus properties or subjective effects with (*R*,*S*)-ketamine, and that it may have reduced abuse liability and reduced psychotomimetic effects in humans.

Y-Maze

Spontaneous alternation behavior was defined as entry into all three arms of the Y-maze in three consecutive arm entries. Both (*R*,*S*)-ketamine 32.0 mg/kg (see Figure 12; 46.43%) and the isomer (*S*)-ketamine 32.0 mg/kg (see Figure 14; 45.60%) produced a significant reduction in spontaneous alternation when compared to vehicle (see Figure 12; 57.44%), thus demonstrating their ability to impair spatial memory cognition. In contrast, the (*R*)-ketamine isomer and the HNK metabolite did not produce any changes in spontaneous alternation at the doses tested, suggesting reduced impact on cognitive functioning. To date, no other studies have reported on the effects of (2*R*,6*R*)-hydroxynorketamine in any measure of cognition.

Regarding the isomers of ketamine, the present study is consistent with other preclinical studies in that (*R*,*S*)-ketamine and (*S*)-ketamine leads to cognitive impairment (Garfield et al., 1985; Hou et al., 2013). For example, Garfield et al. (1985) reported that (*R*,*S*)-ketamine and (*S*)-

ketamine, but not (*R*)-ketamine, produced significant deficits in cognition; the maze-running procedure required twice-daily training for three weeks to traverse a maze for reinforcement. lower doses. Garfield et al. (1985) used Swiss-Webster mice and demonstrated that 15.0 mg/kg (*R*,*S*)-ketamine and 15.0 mg/kg (*S*)-ketamine produced cognitive deficits in a maze-running procedure. The present study, however, reported these effects at different doses. Likewise, Hou et al. (2013) reported 100 mg/kg (*R*,*S*)-ketamine treatment, not 25 mg/kg nor 50 mg/kg, decreased spontaneous alternation behavior; a much higher dose than the present study (32.0 mg/kg). Several methodological differences may explain the difference in outcomes related to dose. Treatment was administered subcutaneously in the present study (which would bypass first-pass metabolism); whereas, Hou et al. (2013) administered (*R*,*S*)-ketamine via intraperitoneal injection. Furthermore, Hou et al. (2013) assessed spontaneous alternation 30-minutes after (*R*,*S*)-ketamine treatment. The present study measured behavior after 24 hours. Species differences may also impact sensitivity to drug effects; the present study used C57Bl/6 mice and Hou et al. (2013) used Swiss-Kunming mice. Control conditions also differed; the control group mean spontaneous alternation behavior in the present study was calculated to be 57.44% and Hou et al. (2003) reported a mean greater than 60%. These methodological differences highlight the need for further replication and examination of (*R*,*S*)-ketamine-like side-effects.

Limitations

The present study had several limitations that should be addressed in future studies. A significant antidepressant-like effect with (*R*)-ketamine, in the DRL task, may be revealed by testing higher doses than what was tested in the present study. Based on other studies, (*R*)-ketamine does have antidepressant-like effects at these tested doses in other preclinical assays

(i.e., FST and TST). A longer pretreatment time also may have revealed significant antidepressant-like effects in the DRL task, as (*R*)-ketamine is metabolized more slowly than (*S*)-ketamine and the ketamine metabolites have been reported to play an important role in the antidepressant-like effects of (*R*,*S*)-ketamine (Portmann et al., 2010; Zanos et al., 2016). In addition, the present study used a 72 second inter-response interval in the DRL task. Perhaps a shorter inter-response interval time (e.g. 36 second) might reveal different (i.e. significant) results for (*R*)-ketamine. The mean number of reinforcers during the vehicle condition was very low in the present study, lower than that normally seen with rats on a DRL 72s schedule (see Hillhouse and Porter, 2014). Future studies should examine the effect of inter-response interval time to determine if different results would be obtained with (*R*)-ketamine. Testing a single dose of (2*R*,6*R*)-hydroxynorketamine (56.0 mg/kg) in the DRL task (which did produce an antidepressant-like profile) is another limitation to the current study. The choice to test a single dose of (2*R*,6*R*)-hydroxynorketamine in the DRL task was predicated on results reported in the study by Zanos et al. (2016). Zanos et al. (2016) reported (2*R*,6*R*)-hydroxynorketamine to reduce immobility in the FST (an antidepressant-like effect) at 5.0 mg/kg, 25.0 mg/kg, 75.0 mg/kg, and 125 mg/kg. Given these findings, the chosen dose (56.0 mg/kg) in the present study somewhat in the middle of this range was hypothesized to produce an antidepressant-like effect. Future studies could expand on the present results by testing a full dose-response curve for (2*R*,6*R*)-hydroxynorketamine and also including other ketamine metabolites like (2*S*,6*S*)-hydroxynorketamine. Likewise, it is possible that higher doses of (*R*)-ketamine and (2*R*,6*R*)-hydroxynorketamine may cause significant reductions in % spontaneous alternation in the Y-maze. The present study also would be strengthened by the addition of other cognitive tasks, like the Morris water maze, delayed set-shifting, or delayed alternation, as all of which measure

different aspects of cognitive functioning. In addition, these tasks involve conditioned behavior, which may yield different results, as conditioned behavioral tasks have been demonstrated to be more sensitive to drug effects than unconditioned behavior (Louis et al., 2006).

Conclusions

In conclusion, the present study demonstrated that (*R*,*S*)-ketamine, (*S*)-ketamine, and (2*R*,6*R*)-hydroxynorketamine produced significant antidepressant-like effects in the DRL task, indicating potential treatment options for depression. The significant antidepressant-like effect in the DRL task observed with (2*R*,6*R*)-hydroxynorketamine treatment supports previous reports of antidepressant-like effects (in the FST and TST) in an assay never before used to test (2*R*,6*R*)-hydroxynorketamine. Notably, no significant effect was observed with (*R*)-ketamine in the DRL task. This lack of effect in the DRL task may be due to the slower metabolism of (*R*)-ketamine as compared to (*S*)-ketamine, or differences in doses, species, or methodologies used in the present study. Future studies should vary the pretreatment times and testing higher doses of (*R*)-ketamine in the DRL task. The present study found (*R*)-ketamine to have no effect on mice in the DRL task, however, the promising findings reported by other studies (Shirayama & Hashimoto, 2017; Leal et al., 2020) provide impetus to further examine (*R*)-ketamine.

In the drug discrimination experiment both isomers substituted for (*R*,*S*)-ketamine, but (*S*)-ketamine was more potent than (*R*)-ketamine as the ED value was shifted more than 2-fold to the left. Moreover, (2*R*,6*R*)-hydroxynorketamine did not substitute for (*R*,*S*)-ketamine at the tested doses. Results in the drug discrimination experiment support other studies, which reported that (2*R*,6*R*)-hydroxynorketamine did not substitute for (*R*,*S*)-ketamine at the tested doses and (*S*)-ketamine substitutes at lower doses than (*R*)-ketamine. Given (*R*,*S*)-ketamine is abused, and subjective effects play a role in abuse liability, these results indicate that (*R*)-ketamine and

(2*R*,2*R*)-hydroxynorketamine may have reduced ketamine-like abuse liability in humans, and they may lack other ketamine-like psychotomimetic effects. The results from the Y-maze experiment indicate (*R*)-ketamine and (2*R*,6*R*)-hydroxynorketamine may have less of an effect on cognition than (*R*,*S*)-ketamine and (*S*)-ketamine. (*R*,*S*)-Ketamine and (*S*)-ketamine reduced spontaneous alternation 24-hours after injection; whereas, no effects on spontaneous alternation were observed with (*R*)-ketamine and (2*R*,6*R*)-hydroxynorketamine at the tested doses.

The present study reported novel findings regarding the antidepressant-like effects, subjective effects, and cognitive effects of (*R*,*S*)-ketamine, its isomers, and the metabolite (2*R*,6*R*)-hydroxynorketamine. Beyond replication and modification of methodology used in the present study, future studies should utilize optogenetic-mediated stimulation in combination with patch clamp electrophysiology to examine drug effects in relevant brain slices. Relevant brain regions may include the hippocampus and medial prefrontal cortex; which have been implicated in schizophrenia-like behavior, memory, and cognition (Kesner et al,. 2008; Yassa et al., 2011; Holden et al., 2012). These future studies will lead to a better understanding of the neurophysiological effects of novel antidepressant treatments. Moreover, completion of these studies will lead to greater understanding and a mechanistic insight into neuropsychiatric disorders associated with aberrant network activity, such as schizophrenia and depression. Finally, the results of the present study and many other preclinical studies suggest that clinical research is warranted for the (*R*)-ketamine (arketamine) isomer and the ketamine metabolite, (2*R*,6*R*)-hydroxynorketamine.

References

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders (DSM-5®)*. American Psychiatric Pub.

Anisman, H., & Zacharko, R. M. (1990). Multiple neurochemical and behavioral consequences of stressors: Implications for depression. *Pharmacology & Therapeutics*, *46*(1), 119–136. https://doi.org/10.1016/0163-7258(90)90039-5

Bandelow, B., Reitt, M., Röver, C., Michaelis, S., Görlich, Y., & Wedekind, D. (2015). Efficacy of treatments for anxiety disorders: A meta-analysis. *International Clinical Psychopharmacology*, *30*(4), 183–192. https://doi.org/10.1097/YIC.0000000000000078

Benvenga, M. J., Wing, A. V., & Del Vecchio, R. A. (1991). The discriminative stimulus effect of MK-801 in ketamine-trained rats. *Pharmacology, Biochemistry, and Behavior*, *38*(1), 211–213. https://doi.org/10.1016/0091-3057(91)90613-7

Brady, K. T., & Balster, R. L. (1982). Discriminative stimulus properties of ketamine stereoisomers in phencyclidine-trained rats. *Pharmacology Biochemistry and Behavior*, *17*(2), 291–295. https://doi.org/10.1016/0091-3057(82)90083-1

Brody, D. J. (2018). *Prevalence of Depression Among Adults Aged 20 and Over: United States, 2013–2016*. *303*, 8.

Calev, A., Cohen, R., Tubi, N., Nigal, D., Shapira, B., Kugelmass, S., & Lerer, B. (1991). Disorientation and Bilateral Moderately Suprathreshold Titrated ECT. *Convulsive Therapy*, *7*(2), 99–110.

Carey, L. M., Rice, R. J., & Prus, A. J. (2017). The Neurotensin NTS1 Receptor Agonist PD149163 Produces Antidepressant-Like Effects in the Forced Swim Test: Further Support for Neurotensin

as a Novel Pharmacologic Strategy for Antidepressant Drugs. *Drug Development Research*, *78*(5), 196–202. https://doi.org/10.1002/ddr.21393

Chen, S.-F., Chuang, C.-Y., Chao, C.-C., Yang, Y.-H., Chu, C.-Y., Yao, C.-Y., Su, Y.-C., Huang, Y.-H., & Liao, R.-M. (2019). Task-Dependent Differences in Operant Behaviors of Rats with Acute Exposure to High Ambient Temperature: A Potential Role of Hippocampal Dopamine Reuptake Transporters. *Frontiers in Behavioral Neuroscience*, *13*. https://doi.org/10.3389/fnbeh.2019.00015

Citri, A., & Malenka, R. C. (2008). Synaptic plasticity: Multiple forms, functions, and mechanisms. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *33*(1), 18–41. https://doi.org/10.1038/sj.npp.1301559

Cryan, J. F., Mombereau, C., & Vassout, A. (2005). The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. *Neuroscience and Biobehavioral Reviews*, *29*(4–5), 571–625. https://doi.org/10.1016/j.neubiorev.2005.03.009

Dierckx, B., Heijnen, W. T., Broek, W. W. van den, & Birkenhäger, T. K. (2012). Efficacy of electroconvulsive therapy in bipolar versus unipolar major depression: A meta-analysis. *Bipolar Disorders*, *14*(2), 146–150. https://doi.org/10.1111/j.1399-5618.2012.00997.x

Dong, C., Zhang, J.-C., Yao, W., Ren, Q., Ma, M., Yang, C., Chaki, S., & Hashimoto, K. (2017). Rapid and Sustained Antidepressant Action of the mGlu2/3 Receptor Antagonist MGS0039 in the Social Defeat Stress Model: Comparison with Ketamine. *The International Journal of Neuropsychopharmacology*, *20*(3), 228–236. https://doi.org/10.1093/ijnp/pyw089

Drevets, W. C., Price, J. L., & Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: Implications for neurocircuitry models of depression. *Brain Structure & Function*, *213*(1–2), 93–118. https://doi.org/10.1007/s00429-008-0189-x

Duan, T.-T., Tan, J.-W., Yuan, Q., Cao, J., Zhou, Q.-X., & Xu, L. (2013). Acute ketamine induces hippocampal synaptic depression and spatial memory impairment through dopamine D1/D5 receptors. *Psychopharmacology*, *228*(3), 451–461. https://doi.org/10.1007/s00213-013-3048-2

Duman, C. H., & Duman, R. S. (2015). Spine synapse remodeling in the pathophysiology and treatment of depression. *Neuroscience Letters*, *601*, 20–29. https://doi.org/10.1016/j.neulet.2015.01.022

Duman, R. S., Malberg, J., & Thome, J. (1999). Neural plasticity to stress and antidepressant treatment. *Biological Psychiatry*, *46*(9), 1181–1191. https://doi.org/10.1016/s0006-3223(99)00177-8

Duman, Ronald S., Aghajanian, G. K., Sanacora, G., & Krystal, J. H. (2016). Synaptic plasticity and depression: New insights from stress and rapid-acting antidepressants. *Nature Medicine*, *22*(3), 238–249. https://doi.org/10.1038/nm.4050

Fava, M., & Davidson, K. G. (1996). DEFINITION AND EPIDEMIOLOGY OF TREATMENT-RESISTANT DEPRESSION. *Psychiatric Clinics of North America*, *19*(2), 179–200. https://doi.org/10.1016/S0193-953X(05)70283-5

Fink, M. (2001). Convulsive therapy: A review of the first 55 years. *Journal of Affective Disorders*, *63*(1–3), 1–15. https://doi.org/10.1016/s0165-0327(00)00367-0

Franceschelli, A., Sens, J., Herchick, S., Thelen, C., & Pitychoutis, P. M. (2015). Sex differences in the rapid and the sustained antidepressant-like effects of ketamine in stress-naïve and "depressed" mice exposed to chronic mild stress. *Neuroscience*, *290*, 49–60. https://doi.org/10.1016/j.neuroscience.2015.01.008

Fukumoto, K., Iijima, M., & Chaki, S. (2016). The Antidepressant Effects of an mGlu2/3 Receptor Antagonist and Ketamine Require AMPA Receptor Stimulation in the mPFC and Subsequent

Activation of the 5-HT Neurons in the DRN. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *41*(4), 1046–1056. https://doi.org/10.1038/npp.2015.233

Fukumoto, K., Toki, H., Iijima, M., Hashihayata, T., Yamaguchi, J.-I., Hashimoto, K., & Chaki, S. (2017). Antidepressant Potential of (R)-Ketamine in Rodent Models: Comparison with (S)-Ketamine. *The Journal of Pharmacology and Experimental Therapeutics*, *361*(1), 9–16. https://doi.org/10.1124/jpet.116.239228

Ganguly, K., & Poo, M. (2013). Activity-Dependent Neural Plasticity from Bench to Bedside. *Neuron*, *80*(3), 729–741. https://doi.org/10.1016/j.neuron.2013.10.028

Garfield, J. M., Rocco, J., & Naulty, J. S. (1985). Differential effects of ketamine stereoisomers on maze performance in the mouse. *Anesthesiology*, *63*(6), 681–683. https://doi.org/10.1097/00000542-198512000-00020

George, M. S., & Post, R. M. (2011). Daily Left Prefrontal Repetitive Transcranial Magnetic Stimulation for Acute Treatment of Medication-Resistant Depression. *American Journal of Psychiatry*, *168*(4), 356–364. https://doi.org/10.1176/appi.ajp.2010.10060864

Grace, A. A. (2012). Dopamine System Dysregulation by the Hippocampus: Implications for the Pathophysiology and Treatment of Schizophrenia. *Neuropharmacology*, *62*(3), 1342–1348. https://doi.org/10.1016/j.neuropharm.2011.05.011

Grandjean, J., Azzinnari, D., Seuwen, A., Sigrist, H., Seifritz, E., Pryce, C. R., & Rudin, M. (2016). Chronic psychosocial stress in mice leads to changes in brain functional connectivity and metabolite levels comparable to human depression. *NeuroImage*, *142*, 544–552. https://doi.org/10.1016/j.neuroimage.2016.08.013

Hashimoto, K., & Shirayama, Y. (2018). What Are the Causes for Discrepancies of Antidepressant Actions of (2R,6R)-Hydroxynorketamine? *Biological Psychiatry*, *84*(1), e7–e8. https://doi.org/10.1016/j.biopsych.2017.12.007

Hegde, A. N., Goldberg, A. L., & Schwartz, J. H. (1993). Regulatory subunits of cAMP-dependent protein kinases are degraded after conjugation to ubiquitin: A molecular mechanism underlying long-term synaptic plasticity. *Proceedings of the National Academy of Sciences*, *90*(16), 7436–7440. https://doi.org/10.1073/pnas.90.16.7436

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

Hillhouse, T. M., & Porter, J. H. (2015). A brief history of the development of antidepressant drugs: From monoamines to glutamate. *Experimental and Clinical Psychopharmacology*, *23*(1), 1–21. https://doi.org/10.1037/a0038550

Hillhouse, T. M., Porter, J. H., & Negus, S. S. (2014). Comparison of antidepressant-like and abuse-related effects of phencyclidine in rats. *Drug Development Research*, *75*(8), 479–488. https://doi.org/10.1002/ddr.21228

Hillhouse, T. M., Rice, R., & Porter, J. H. (2019). What role does the (2*R*,6*R*)-hydroxynorketamine metabolite play in the antidepressant-like and abuse-related effects of (R)-ketamine? *British Journal of Pharmacology*, *176*(19), 3886–3888. https://doi.org/10.1111/bph.14785

Holden, H. M., Hoebel, C., Loftis, K., & Gilbert, P. E. (2012). Spatial pattern separation in cognitively normal young and older adults. *Hippocampus*, *22*(9), 1826–1832. https://doi.org/10.1002/hipo.22017

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: Roles in Neuronal Development and Function. *Annual Review of Neuroscience*, *24*(1), 677–736. https://doi.org/10.1146/annurev.neuro.24.1.677

Ihmsen, H., Geisslinger, G., & Schüttler, J. (2001). Stereoselective pharmacokinetics of ketamine: R(−)-Ketamine inhibits the elimination of S(+)-ketamine. *Clinical Pharmacology & Therapeutics*, *70*(5), 431–438. https://doi.org/10.1016/S0009-9236(01)06321-4

International Union of Pure and Applied Chemistry, McNaught, A., & Wilkinson, A. (1997). *Compendium of chemical terminology: IUPAC recommendations* (2nd ed..). Blackwell Science.

Ionescu, D. F., & Papakostas, G. I. (2017). Experimental medication treatment approaches for depression. *Translational Psychiatry*, *7*(3), e1068. https://doi.org/10.1038/tp.2017.33

Janssen Pharmaceutical Companies. (2019). SPRAVATO (S-ketamine) [package insert]. *Titusville, NJ*.

Janssen Research & Development, LLC. (2019). *A Study to Evaluate the Efficacy, Safety, and Tolerability of Flexible Doses of Intranasal Esketamine Plus an Oral Antidepressant in Adult Participants With Treatment-resistant Depression—(ClinicalTrials.gov identifier (NCT number): NCT02418585)*. https://clinicaltrials.gov/ct2/show/study/NCT02418585

Kamaya, H. M. D., & Krishna, P. R. M. D. (1987). Ketamine Addiction. [Letter]. *Anesthesiology*, *67*(5), 861–862.

Kang, H. J., Voleti, B., Hajszan, T., Rajkowska, G., Stockmeier, C. A., Licznerski, P., Lepack, A., Majik, M. S., Jeong, L. S., Banasr, M., Son, H., & Duman, R. S. (2012). Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. *Nature Medicine*, *18*(9), 1413–1417. https://doi.org/10.1038/nm.2886

Kesner, R. P., Hunsaker, M. R., & Warthen, M. W. (2008). The CA3 subregion of the hippocampus is critical for episodic memory processing by means of relational encoding in rats. *Behavioral Neuroscience, 122*(6), 1217–1225. https://doi.org/10.1037/a0013592

Kessler, R. C. (2012). The costs of depression. *The Psychiatric Clinics of North America, 35*(1), 1–14. https://doi.org/10.1016/j.psc.2011.11.005

Khanegheini, A., Meftahi, G. H., Zarrindast, M. R., Afarinesh, M. R., Sahraei, H., Jahromi, G. P., & Shahyad, S. (2019). Involvement of CA1 GABAA Receptors in Ketamine-Induced Impairment of Spatial and Non-Spatial Novelty Detection in Mice. *Neurochemical Journal*, *13*(1), 81–89. https://doi.org/10.1134/S1819712419010094

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. (2019). PubChem 2019 update: Improved access to chemical data. *Nucleic Acids Research*, *47*(Database issue), D1102–D1109. https://doi.org/10.1093/nar/gky1033

Kraeuter, A.-K., Guest, P. C., & Sarnyai, Z. (2019). The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice. *Methods in Molecular Biology (Clifton, N.J.), 1916*, 105–111. https://doi.org/10.1007/978-1-4939-8994-2_10

Krishnan, V., & Nestler, E. J. (2011). Animal Models of Depression: Molecular Perspectives. *Current Topics in Behavioral Neurosciences*, *7*, 121–147. https://doi.org/10.1007/7854_2010_108

Kujala, I., Rosenvinge, B., & Bekkelund, S. I. (2002). Clinical Outcome and Adverse Effects of Electroconvulsive Therapy in Elderly Psychiatric Patients. *Journal of Geriatric Psychiatry and Neurology*, *15*(2), 73–76. https://doi.org/10.1177/089198870201500204

Kültz, D. (2005). Molecular and evolutionary basis of the cellular stress response. *Annual Review of Physiology*, *67*, 225–257. https://doi.org/10.1146/annurev.physiol.67.040403.103635

Liston, C., Miller, M. M., Goldwater, D. S., Radley, J. J., Rocher, A. B., Hof, P. R., Morrison, J. H., & McEwen, B. S. (2006). Stress-Induced Alterations in Prefrontal Cortical Dendritic Morphology Predict Selective Impairments in Perceptual Attentional Set-Shifting. *Journal of Neuroscience*, *26*(30), 7870–7874. https://doi.org/10.1523/JNEUROSCI.1184-06.2006

Liu, W., Ge, T., Leng, Y., Pan, Z., Fan, J., Yang, W., & Cui, R. (2017). The Role of Neural Plasticity in Depression: From Hippocampus to Prefrontal Cortex. *Neural Plasticity*, *2017*, 6871089. https://doi.org/10.1155/2017/6871089

Liu, Y., Lin, D., Wu, B., & Zhou, W. (2016). Ketamine abuse potential and use disorder. *Brain Research Bulletin*, *126*, 68–73. https://doi.org/10.1016/j.brainresbull.2016.05.016

Louis, C., Cohen, C., Depoortère, R., & Griebel, G. (2006). Antidepressant-Like Effects of the Corticotropin-Releasing Factor 1 Receptor Antagonist, SSR125543, and the Vasopressin 1b Receptor Antagonist, SSR149415, in a DRL-72 s Schedule in the Rat. *Neuropsychopharmacology*, *31*(10), 2180–2187. https://doi.org/10.1038/sj.npp.1301036

Ma, X.-C., Dang, Y.-H., Jia, M., Ma, R., Wang, F., Wu, J., Gao, C.-G., & Hashimoto, K. (2013). Long-lasting antidepressant action of ketamine, but not glycogen synthase kinase-3 inhibitor SB216763, in the chronic mild stress model of mice. *PloS One*, *8*(2), e56053. https://doi.org/10.1371/journal.pone.0056053

MacGillivray, S., Arroll, B., Hatcher, S., Ogston, S., Reid, I., Sullivan, F., Williams, B., & Crombie, I. (2003). Efficacy and tolerability of selective serotonin reuptake inhibitors compared with tricyclic antidepressants in depression treated in primary care: Systematic review and meta-analysis. *BMJ (Clinical Research Ed.)*, *326*(7397), 1014. https://doi.org/10.1136/bmj.326.7397.1014

Maeng, S., Zarate, C. A., Du, J., Schloesser, R. J., McCammon, J., Chen, G., & Manji, H. K. (2008). Cellular mechanisms underlying the antidepressant effects of ketamine: Role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biological Psychiatry*, *63*(4), 349–352. https://doi.org/10.1016/j.biopsych.2007.05.028

Marken, P. A., & Munro, J. S. (2000). Selecting a Selective Serotonin Reuptake Inhibitor: Clinically Important Distinguishing Features. *The Primary Care Companion for CNS Disorders*, *2*(6), 205–210. https://doi.org/10.4088/PCC.v02n0602

Miedel, C. J., Patton, J. M., Miedel, A. N., Miedel, E. S., & Levenson, J. M. (2017). Assessment of Spontaneous Alternation, Novel Object Recognition and Limb Clasping in Transgenic Mouse Models of Amyloid-β and Tau Neuropathology. *Journal of Visualized Experiments: JoVE*, *123*. https://doi.org/10.3791/55523

Mineur, Y. S., & Picciotto, M. R. (2010). Nicotine receptors and depression: Revisiting and revising the cholinergic hypothesis. *Trends in Pharmacological Sciences*, *31*(12), 580–586. https://doi.org/10.1016/j.tips.2010.09.004

Moaddel, R., Sanghvi, M., Dossou, K. S. S., Ramamoorthy, A., Green, C., Bupp, J., Swezey, R., O'Loughlin, K., & Wainer, I. W. (2015). The distribution and clearance of (2S,6S)-hydroxynorketamine, an active ketamine metabolite, in Wistar rats. *Pharmacology Research & Perspectives*, *3*(4). https://doi.org/10.1002/prp2.157

Moffa, A., Martin, D., Brunoni, A., Alonzo, A., Blumberger, D., Bennabi, D., Daskalakis, Z., Fregni, F., Padberg, F., Palm, U., Sampaio-Junior, B., & Loo, C. (2019). Transcranial direct current stimulation for acute major depressive episodes: An updated meta-analysis of individual patient data. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, *12*(2), 479. https://doi.org/10.1016/j.brs.2018.12.562

Murray, C. J. L., & Lopez, A. D. (1996). Evidence-Based Health Policy—Lessons from the Global Burden of Disease Study. *Science, 274*(5288), 740–743. https://doi.org/10.1126/science.274.5288.740

National Institute of Mental Health, (NIMH). (2016, December 1). *Depression Basics*. /nimh/nimh/product/Depression-Basics/TR 16-3561

Ochs-Ross, R., Daly, E., Zhang, Y., Lane, R., Lim, P., Foster, K., Hough, D., Manji, H., Drevets, W., Sanacora, G., Adler, C., McShane, R., Gaillard, R., & Singh, J. (2019). Efficacy and safety of esketamine nasal spray plus an oral antidepressant in elderly patients with treatment-resistant depression. *European Neuropsychopharmacology, 29*, S355–S356. https://doi.org/10.1016/j.euroneuro.2018.11.548

O'Donnell, J. M., Marek, G. J., & Seiden, L. S. (2005). Antidepressant effects assessed using behavior maintained under a differential-reinforcement-of-low-rate (DRL) operant schedule. *Neuroscience and Biobehavioral Reviews*, *29*(4–5), 785–798. https://doi.org/10.1016/j.neubiorev.2005.03.018

Papakostas, G. I. (2014). Cognitive symptoms in patients with major depressive disorder and their implications for clinical practice. *The Journal of Clinical Psychiatry*, *75*(1), 8–14. https://doi.org/10.4088/JCP.13r08710

Patel, S., & Holle, L. M. (2019). Spravato for Treatment-Resistant Depression. *Drug Topics; Monmouth Junction*, *163*(5), 40.

Petit-Demouliere, B., Chenu, F., & Bourin, M. (2005). Forced swimming test in mice: A review of antidepressant activity. *Psychopharmacology, 177*(3), 245–255. https://doi.org/10.1007/s00213-004-2048-7

Pham, T. H., Defaix, C., Xu, X., Deng, S.-X., Fabresse, N., Alvarez, J.-C., Landry, D. W., Brachman, R. A., Denny, C. A., & Gardier, A. M. (2018). Common Neurotransmission Recruited in (R,S)-Ketamine and (2R,6R)-Hydroxynorketamine–Induced Sustained Antidepressant-like Effects. *Biological Psychiatry*, *84*(1), e3–e6. https://doi.org/10.1016/j.biopsych.2017.10.020

Philip, N. S., Carpenter, L. L., Tyrka, A. R., & Price, L. H. (2010). Nicotinic acetylcholine receptors and depression: A review of the preclinical and clinical literature. *Psychopharmacology*, *212*(1), 1–12. https://doi.org/10.1007/s00213-010-1932-6

Popovic, D., Vieta, E., Fornaro, M., & Perugi, G. (2015). Cognitive tolerability following successful long term treatment of major depression and anxiety disorders with SSRI antidepressants. *Journal of Affective Disorders*, *173*, 211–215. https://doi.org/10.1016/j.jad.2014.11.008

Porsolt, R. D., Bertin, A., & Jalfre, M. (1977). Behavioral despair in mice: A primary screening test for antidepressants. *Archives Internationales de Pharmacodynamie et de Therapie*, *229*(2), 327–336.

Porsolt, R. D., Le Pichon, M., & Jalfre, M. (1977). Depression: A new animal model sensitive to antidepressant treatments. *Nature*, *266*(5604), 730–732. https://doi.org/10.1038/266730a0

Porter, J. H., Prus, A. J., & Overton, D. A. (2018). Drug Discrimination: Historical Origins, Important Concepts, and Principles. *Current Topics in Behavioral Neurosciences*, *39*, 3–26. https://doi.org/10.1007/7854_2018_40

Porter, J. H., Prus, A. J., Vann, R. E., & Varvel, S. A. (2005). Discriminative stimulus properties of the atypical antipsychotic clozapine and the typical antipsychotic chlorpromazine in a three-choice drug discrimination procedure in rats. *Psychopharmacology*, *178*(1), 67–77. https://doi.org/10.1007/s00213-004-1985-5

Portmann, S., Kwan, H. Y., Theurillat, R., Schmitz, A., Mevissen, M., & Thormann, W. (2010). Enantioselective capillary electrophoresis for identification and characterization of human cytochrome P450 enzymes which metabolize ketamine and norketamine in vitro. *Journal of Chromatography A, 1217*(51), 7942–7948. https://doi.org/10.1016/j.chroma.2010.06.028

PubChem. (2020, February). *Limonene*. https://pubchem.ncbi.nlm.nih.gov/compound/22311

Rajagopal, L., Burgdorf, J. S., Moskal, J. R., & Meltzer, H. Y. (2016). GLYX-13 (rapastinel) ameliorates subchronic phencyclidine- and ketamine-induced declarative memory deficits in mice. *Behavioural Brain Research, 299*, 105–110. https://doi.org/10.1016/j.bbr.2015.10.060

Rawlins, J. N., Winocur, G., & Gray, J. A. (1983). The hippocampus, collateral behavior, and timing. *Behavioral Neuroscience*, *97*(6), 857–872. https://doi.org/10.1037//0735-7044.97.6.857

Roca, M., Vives, M., López-Navarro, E., García-Campayo, J., & Gili, M. (2015). Cognitive impairments and depression: A critical review. *Actas Espanolas de Psiquiatria*, *43*(5), 187–193.

Rudorfer, M. V., Henry, M. E., & Sackeim, H. A. (n.d.). *Electroconvulsive Therapy*. 37.

Rush, A. J., Fava, M., Wisniewski, S. R., Lavori, P. W., Trivedi, M. H., Sackeim, H. A., Thase, M. E., Nierenberg, A. A., Quitkin, F. M., Kashner, T. M., Kupfer, D. J., Rosenbaum, J. F., Alpert, J., Stewart, J. W., McGrath, P. J., Biggs, M. M., Shores-Wilson, K., Lebowitz, B. D., Ritz, L., … STAR*D Investigators Group. (2004). Sequenced treatment alternatives to relieve depression (STAR*D): Rationale and design. *Controlled Clinical Trials, 25*(1), 119–142.

Sałat, K., Siwek, A., Starowicz, G., Librowski, T., Nowak, G., Drabik, U., Gajdosz, R., & Popik, P. (2015a). Antidepressant-like effects of ketamine, norketamine and dehydronorketamine in forced swim test: Role of activity at NMDA receptor. *Neuropharmacology*, *99*, 301–307. https://doi.org/10.1016/j.neuropharm.2015.07.037

Sałat, K., Siwek, A., Starowicz, G., Librowski, T., Nowak, G., Drabik, U., Gajdosz, R., & Popik, P. (2015b). Antidepressant-like effects of ketamine, norketamine and dehydronorketamine in forced swim test: Role of activity at NMDA receptor. *Neuropharmacology*, *99*, 301–307. https://doi.org/10.1016/j.neuropharm.2015.07.037

Sanger, D. J., & Blackman, D. E. (1989). Operant Behavior and the Effects of Centrally Acting Drugs. In A. A. Boulton, G. B. Baker, & A. J. Greenshaw (Eds.), *Psychopharmacology* (pp. 299–348). Humana Press. https://doi.org/10.1385/0-89603-129-2:299

Sassano-Higgins, S., Baron, D., Juarez, G., Esmaili, N., & Gold, M. (2016). A Review of Ketamine Abuse and Diversion. *Depression and Anxiety*, *33*(8), 718–727. https://doi.org/10.1002/da.22536

Shirayama, Y., & Hashimoto, K. (2017). Lack of Antidepressant Effects of (2R,6R)-Hydroxynorketamine in a Rat Learned Helplessness Model: Comparison with (R)-Ketamine. *International Journal of Neuropsychopharmacology*, *21*(1), 84–88. https://doi.org/10.1093/ijnp/pyx108

Shannon HE (1981) Evaluation of PCP Analogs on the Basis of Their Discriminative Stimulus Properties in the rat. Journal of Pharmacology and Experimental Therapeutics. 216 (3): 543-551

Siegel, R. K. (1978). Phencyclidine and ketamine intoxication: A study of four populations of recreational users. *NIDA Research Monograph*, *21*, 119–147.

Slattery, D. A., & Cryan, J. F. (2012). Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nature Protocols; London*, *7*(6), 1009–1014. http://dx.doi.org.proxy.library.vcu.edu/10.1038/nprot.2012.044

Sloman, L. (2008). A new comprehensive evolutionary model of depression and anxiety. *Journal of Affective Disorders*, *106*(3), 219–228. https://doi.org/10.1016/j.jad.2007.07.008

Snigdha, S., Milgram, N. W., Willis, S. L., Albert, M., Weintraub, S., Fortin, N. J., & Cotman, C. W. (2013). A preclinical cognitive test battery to parallel the National Institute of Health Toolbox in humans: Bridging the translational gap. *Neurobiology of Aging*, *34*(7), 1891–1901. https://doi.org/10.1016/j.neurobiolaging.2013.01.018

Solinas, M., Panlilio, L. V., Justinova, Z., Yasar, S., & Goldberg, S. R. (2006). Using drug-discrimination techniques to study the abuse-related effects of psychoactive drugs in rats. *Nature Protocols*, *1*(3), 1194–1206. https://doi.org/10.1038/nprot.2006.167

Steru, L., Chermat, R., Thierry, B., & Simon, P. (1985). The tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacology*, *85*(3), 367–370. https://doi.org/10.1007/bf00428203

Stolerman, I. P., Childs, E., Ford, M. M., & Grant, K. A. (2011). The role of training dose in drug discrimination: A review. *Behavioural Pharmacology*, *22*(5–6), 415–429. https://doi.org/10.1097/FBP.0b013e328349ab37

Swedberg, M. (2016). Critical role for drug discrimination studies in the assessment of abuse liability of novel compounds. *Journal of Pharmacological and Toxicological Methods*, *81*, 338. https://doi.org/10.1016/j.vascn.2016.02.011

Thierry, B., Steru, L., Chermat, R., & Simon, P. (1984). Searching—waiting strategy: A candidate for an evolutionary model of depression? *Behavioral and Neural Biology*, *41*(2), 180–189. https://doi.org/10.1016/S0163-1047(84)90555-7

Thomas, C. (2018, February 18). *Development of (2R,6R)-Hydroxynorketamine*. National Center for Advancing Translational Sciences. https://ncats.nih.gov/chemtech/projects/active/ketamine

Uher, R., Payne, J. L., Pavlova, B., & Perlis, R. H. (2014). Major Depressive Disorder in Dsm-5: Implications for Clinical Practice and Research of Changes from DSM-IV. *Depression and Anxiety*, *31*(6), 459–471. https://doi.org/10.1002/da.22217

Verma, A., & Moghaddam, B. (1996). NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alternation performance in rats: Modulation by dopamine. *Journal of Neuroscience*, *16*(1), 373–379. https://doi.org/10.1523/JNEUROSCI.16-01-00373.1996

Wang, J. H., Fu, Y., Wilson, F. A. W., & Ma, Y. Y. (2006). Ketamine affects memory consolidation: Differential effects in T-maze and passive avoidance paradigms in mice. *Neuroscience*, *140*(3), 993–1002. https://doi.org/10.1016/j.neuroscience.2006.02.062

Wang, N., Yu, H.-Y., Shen, X.-F., Gao, Z.-Q., Yang, C., Yang, J.-J., & Zhang, G.-F. (2015). The rapid antidepressant effect of ketamine in rats is associated with down-regulation of pro-inflammatory cytokines in the hippocampus. *Upsala Journal of Medical Sciences*, *120*(4), 241–248. https://doi.org/10.3109/03009734.2015.1060281

Warner-Schmidt, J. L., & Duman, R. S. (2006). Hippocampal neurogenesis: Opposing effects of stress and antidepressant treatment. *Hippocampus*, *16*(3), 239–249. https://doi.org/10.1002/hipo.20156

Williams, J. W., Mulrow, C. D., Chiquette, E., Noël, P. H., Aguilar, C., & Cornell, J. (2000). A systematic review of newer pharmacotherapies for depression in adults: Evidence report summary. *Annals of Internal Medicine*, *132*(9), 743–756.

World Health Organization [WHO]. (n.d.). *Depression*. Retrieved November 15, 2019, from https://www.who.int/news-room/fact-sheets/detail/depression

Xiong, Z., Fujita, Y., Zhang, K., Pu, Y., Chang, L., Ma, M., Chen, J., & Hashimoto, K. (2019). Beneficial effects of (R)-ketamine, but not its metabolite (2R,6R)-hydroxynorketamine, in the

depression-like phenotype, inflammatory bone markers, and bone mineral density in a chronic social defeat stress model. *Behavioural Brain Research*, *368*, 111904. https://doi.org/10.1016/j.bbr.2019.111904

Yamaguchi, J.-I., Toki, H., Qu, Y., Yang, C., Koike, H., Hashimoto, K., Mizuno-Yasuhira, A., & Chaki, S. (2018). (2R,6R)-Hydroxynorketamine is not essential for the antidepressant actions of (R)-ketamine in mice. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *43*(9), 1900–1907. https://doi.org/10.1038/s41386-018-0084-y

Yang, B., Zhang, J.-C., Han, M., Yao, W., Yang, C., Ren, Q., Ma, M., Chen, Q.-X., & Hashimoto, K. (2016). Comparison of R-ketamine and rapastinel antidepressant effects in the social defeat stress model of depression. *Psychopharmacology*, *233*(19–20), 3647–3657. https://doi.org/10.1007/s00213-016-4399-2

Yang, C., Han, M., Zhang, J., Ren, Q., & Hashimoto, K. (2016). Loss of parvalbumin-immunoreactivity in mouse brain regions after repeated intermittent administration of esketamine, but not R-ketamine. *Psychiatry Research*, *239*, 281–283. https://doi.org/10.1016/j.psychres.2016.03.034

Yang, C., Kobayashi, S., Nakao, K., Dong, C., Han, M., Qu, Y., Ren, Q., Zhang, J., Ma, M., Toki, H., Yamaguchi, J., Chaki, S., Shirayama, Y., Nakazawa, K., Manabe, T., & Hashimoto, K. (2018). AMPA Receptor Activation–Independent Antidepressant Actions of Ketamine Metabolite (S)-Norketamine. *Biological Psychiatry*, *84*(8), 591–600. https://doi.org/10.1016/j.biopsych.2018.05.007

Yang, C., Qu, Y., Abe, M., Nozawa, D., Chaki, S., & Hashimoto, K. (2017). (R)-Ketamine Shows Greater Potency and Longer Lasting Antidepressant Effects Than Its Metabolite (2R,6R)-

Hydroxynorketamine. *Biological Psychiatry*, *82*(5), e43–e44. https://doi.org/10.1016/j.biopsych.2016.12.020

Yassa, M. A., Lacy, J. W., Stark, S. M., Albert, M. S., Gallagher, M., & Stark, C. E. L. (2011). Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults. *Hippocampus*, *21*(9), 968–979. https://doi.org/10.1002/hipo.20808

Young AM, Herling S, Winger GD, Woods JH. (1981) Comparison of discriminative and reinforcing effects of ketamine and related compounds in the rhesus monkey. NIDA Res Monogr. Feb;34:173-179

Young, R. (2009). Drug Discrimination. In J. J. Buccafusco (Ed.), *Methods of Behavior Analysis in Neuroscience* (2nd ed.). CRC Press/Taylor & Francis. http://www.ncbi.nlm.nih.gov/books/NBK5225/

Zanos, P., Highland, J. N., Stewart, B. W., Georgiou, P., Jenne, C. E., Lovett, J., Morris, P. J., Thomas, C. J., Moaddel, R., Zarate, C. A., & Gould, T. D. (2019). (2R,6R)-hydroxynorketamine exerts mGlu2 receptor-dependent antidepressant actions. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(13), 6441–6450. https://doi.org/10.1073/pnas.1819540116

Zanos, P., Moaddel, R., Morris, P. J., Georgiou, P., Fischell, J., Elmer, G. I., Alkondon, M., Yuan, P., Pribut, H. J., Singh, N. S., Dossou, K. S. S., Fang, Y., Huang, X.-P., Mayo, C. L., Wainer, I. W., Albuquerque, E. X., Thompson, S. M., Thomas, C. J., Zarate, C. A., & Gould, T. D. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature*, *533*(7604), 481–486. https://doi.org/10.1038/nature17998

Zanos, P., Moaddel, R., Morris, P. J., Riggs, L. M., Highland, J. N., Georgiou, P., Pereira, E. F. R., Albuquerque, E. X., Thomas, C. J., Zarate, C. A., & Gould, T. D. (2018). Ketamine and

Ketamine Metabolite Pharmacology: Insights into Therapeutic Mechanisms. *Pharmacological Reviews*, *70*(3), 621–660. https://doi.org/10.1124/pr.117.015198

Zhang, J., Yao, W., Dong, C., Yang, C., Ren, Q., Ma, M., Han, M., & Hashimoto, K. (2015). Comparison of ketamine, 7,8-dihydroxyflavone, and ANA-12 antidepressant effects in the social defeat stress model of depression. *Psychopharmacology*, *232*(23), 4325–4335. https://doi.org/10.1007/s00213-015-4062-3

Curriculum vitae Remington J Rice, Ph.D.

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Academic and professional career

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Select publications

Hillhouse, T. M., Rice, R., & Porter, J. H. (2019). What role does the (2*R*,6*R*)-hydroxynorketamine metabolite play in the antidepressant-like and abuse-related effects of (*R*)-ketamine? *British Journal of Pharmacology*, *176*(19), 3886–3888. https://doi.org/10.1111/bph.14785

Carey, L. M., Rice, R. J., & Prus, A. J. (2017). The Neurotensin NTS1 Receptor Agonist PD149163 Produces Antidepressant-Like Effects in the Forced Swim Test: Further

Support for Neurotensin as a Novel Pharmacologic Strategy for Antidepressant Drugs. *Drug Development Research*, *78*(5), 196–202. https://doi.org/10.1002/ddr.21393

Rice, R. (2013). Juan Huarte. In C. Pernaski, N. Fraire, E. DePetro, C. Brown, S. Wagner, H. Whitaker, E. Smith, T. Clark, L. Carey, M. Moore, B. Palmer, K. England and J. Fancher (Eds.), Approaches to a History of Western Psychology - an e-textbook. (2nd ed.). Published online; please contact Professor Whitaker hwhitake@nmu.edu for access.

Conference presentations

Rice, R., Zhang, F., Goswamee, P., McQuiston A., Porter, J (2019) Effects of Ketamine and (2R-6R)-hydroxynorketamine (HNK) on Cognition: A Role for the Nucleus Reuniens? Society for Neuroscience Chicago Il

Rice, R., Messer, Z., Whitehouse, S., Ottem, E., Adam Prus, A. (2016) Effects of Optogenetic Activation and Pharmacological Modulation of Dopaminergic Neurons. Society for Neuroscience, San Diego, CA

Carey, L., Rice, R., Prus AJ (2014) The Neurotensin NTS1 Receptor Agonist PD149163 Exhibits Anti-Depressant like Effects in a Forced Swim Test. Society for Neuroscience, Washington, D.C.

Memberships in professional societies

Society for Neuroscience

European Behavioral Pharmacological Society

American Psychological Association