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The Effect of Strain and Drug Combinations on Ketamine's Locomotor Activating Effects in Rats

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Table of Contents

List of Tables

List of Figures

Abbreviations Table:

5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine, serotonin
6-OHDA	6-hydroxydopamine
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ADHD	Attention deficit hyperactivity disorder
CPP	Conditioned place preference
DA	Dopaminergic
DCS	D-cycloserine
DSP	Desipramine
DRL	Differential reinforcement of low rates of responding
FST	Forced swim test
HAM-D	Hamilton Depression Rating Scale
HNK	Hydroxynorketamine
IACUC	Institutional Animal Care and Use Committee
iGluRs	Ionotropic glutamate-activated Receptors
IM	Intramuscular
IN	Intranasal
IV	Intravenous
IV-SA	Intravenous self-administration
L-DOPA	Levodopa
LTP	Long term potentiation
MAA	Monoaminergic antidepressants
MADRS	Montgomery-Asberg Depression Rating Scale
MAOIs	Monoamine oxidase inhibitors

MDD	Major depressive disorder
MORs	μ-Opioid receptors
mPFC	Medial prefrontal cortex
mTOR	Mammalian target of rapamycin
NAc	Nucleus accumbens
nAChR	Nicotinic acetylcholine receptors
NET	Norepinephrine reuptake transporter
NMDA	N-methyl-*D*-aspartate
NTX	Naltrexone
OFA	Open field activity
OFT	Open field test
PCP	Phencyclidine
PhMRI	Pharmacological magnetic resonance imaging studies
REMS	Risk Evaluation and Mitigation Strategy
SD	Sprague Dawley
SERT	Serotonin reuptake transporter
SHR	Spontaneously hypertensive rat
SSRIs	Selective serotonin reuptake inhibitors
SNRIs	Serotonin and norepinephrine reuptake inhibitors
SC	Subcutaneous
TCAs	Tricyclic antidepressants
TRD	Treatment resistant depression
VTA	Ventral tegmental area
WIS	Wistar rats
WKY	Wistar Kyoto rats

Abstract:

Rationale: Initially developed as an anesthetic, ketamine is now used to treat a variety of disorders, including treatment resistant depression (TRD). While ketamine appears to be a breakthrough therapy for TRD, it has many use-limiting side effects, including abuse liability, psychotomimetic effects, and sedation. Utilizing ketamine in combination with other medications, such as the monoaminergic antidepressant desipramine (DSP), opioid antagonist naltrexone (NTX), and the NMDA receptor partial agonist D-cycloserine (DCS) may reduce side-effects resulting from ketamine therapy.

Objectives: The goal of this project was to study ketamine's adverse effects using locomotor activation in an open field as a behavioral correlate for dopamine elevation in the brain, a characteristic linked to abuse-related effects. The locomotor activating effects of ketamine in an open field were characterized in Sprague-Dawley (SD, control), Wistar (WIS, control) and Wistar Kyoto (WKY, stress-vulnerable) rats. Secondly, we examined the effects of ketamine when administered following pretreatment with DSP, DCS and NTX to determine their potential to attenuate ketamine's locomotor activating effects.

Methods: The effects of ketamine (3 - 56 mg/kg, IP) on activity were determined in an open field during 30-min sessions in male WIS rats and both male and female SD and WKY rats. Distance traveled (meters) was recorded using overhead cameras interfaced with AnyMaze software. To evaluate the impact of pretreatment drugs on ketamine-induced locomotor activation, 10 and 30 mg/kg of ketamine were re-evaluated in combination with DSP (0.3- 3 mg/kg, IP), DCS (30-300 mg/kg, SC) and NTX (1-30 mg/kg, SC).

Results: Ketamine dose-dependently increased distance traveled at one or more doses in all three strains in male rats and across both sexes in the SD and WKY rats. In all comparisons, distance traveled by WKY rats was 3- to 4-fold lower than distances traveled by SD and WIS rats. Despite a significant main effect of strain, there was no interaction between strain and treatment supporting a similar increase in activity produced by ketamine in all three strains. A significant effect of sex was demonstrated by locomotor activation occurring at both 10 and 30 mg/kg in females, versus activity only increasing following 30 mg/kg in males. In the locomotor assay, DSP alone produced significant activity decreases in SD females, while DCS had no significant effect on activity in either sex. When administered in combination with ketamine, neither DSP nor DCS produced any alteration in the distance traveled following ketamine administration. NTX also produced a significant reduction in distance traveled in both SD and WKY female rats, but had no effect on male activity. When administered in combination with ketamine, 1 and 10 mg/kg NTX produced a trend for attenuation of ketamine's activating effects in SD males and females; but, the decrease in activity was only significant in WKY females at 10 mg/kg NTX. However, this decrease was reversed by the higher NTX dose, 30 mg/kg.

Conclusions: Consistent with previous literature, ketamine induced locomotor activation in SD and WIS rats. WKY showed a similar response to ketamine, despite the dramatically different distances traveled. Furthermore, the effect of ketamine was more pronounced in female rats in both strains. Overall, the dose combinations with DSP and DCS did not significantly decrease ketamine's activating effects. NTX administered alone suppressed activity. This non-selective suppression of behavior likely accounted for the decreases in ketamine-induced locomotor stimulation that were observed following 1 and 10 mg/kg NTX. It is unclear what is causing the resurgence of locomotor activation following pretreatment with 30 mg/kg NTX, but it has been demonstrated in two different strains and in both sexes and warrants further investigation.

Introduction:

Major depressive disorder (MDD), otherwise known as clinical depression, is currently the second leading cause of hardship and loss associated with disease and disability (Malhi & Mann, 2018). By 2030, MDD is projected to rank as the number one cause (Ferrari et al., 2013). The fifth edition of *Diagnostic and Statistical Manual of Mental Disorders* (DSM V) states that an individual would be diagnosed with MDD if the patient experienced five of the following: at least two weeks of persistent low mood or anxiety, a lack of energy, changes in appetite, concentration difficulties, anhedonia, feelings of worthlessness, and reports of psychomotor agitation or poor sleep quality (American Psychiatric Association, 2022). However, the symptoms experienced by individual patients with MDD differ. The psychosocial functioning of some individuals can be impacted due to experiencing many symptoms, while others may have fewer symptoms, but are still psychosocially impaired (Christensen et al., 2020). The pathophysiology of clinical depression has been difficult to elucidate due to the clinical and etiological heterogeneity of MDD (Hasler, 2010; Matveychuk et al., 2020). While the monoamine hypothesis of depression suggests a functional imbalance of dopamine, norepinephrine, and/or serotonin in the central nervous system (CNS), further investigation has failed to provide cogent evidence of the primary dysfunction in specific circuitry of the monoamine systems (Delgado, 2000; Matveychuk et al., 2020). Overall, this hypothesis does not fully explain the pathophysiology underlying depression and has serious clinical limitations (Hasler, 2010; Matveychuk et al., 2020).

Typically, treatment for patients with MDD includes cognitive therapy, psychotherapy, and antidepressant medications (DeRubeis et al., 2008). Due to the psychological, social, and biological factors that give rise to the different neurochemical imbalances and different symptoms of MDD, the antidepressant medication prescribed can differ for each patient. Historically, antidepressant medications have concentrated on enhancing monoaminergic neurotransmission, particularly serotonin and norepinephrine, which include the following drug classes: selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (Prozac), selective serotonin and norepinephrine inhibitors (SNRIs) like duloxetine (Cymbalta), tetracyclic antidepressants such as mirtazapine (Remeron), tricyclic antidepressants (TCAs)

that act as norepinephrine/serotonin reuptake inhibitors like desipramine (Norpramin) and monoamine oxidase inhibitors (MAOIs) such as phenelzine (Nardil) (Khawam et al., 2006).

Despite this disorder ranking as a leading cause of disability worldwide, antidepressant treatment resistance still exists as a clinically notable problem (Mathew et al., 2012). The available drugs for MDD that target monoaminergic systems have a delayed onset of action and limitations in efficacy (Mathew et al., 2012; Matveychuk et al., 2020). On average, the therapeutic onset of action requires two to three weeks to begin to see systemic efficacy, however, it can take upwards to two months to relieve clinical symptoms (Kverno & Mangano, 2021; Matveychuk et al., 2020). Additionally, following treatment with monoaminergic antidepressants (MAA), significant clinical responses are generally seen in only fifty percent of patients (Souery et al., 1999; Thase & Rush, 1997). After two or more treatments of first-line antidepressants, the patients with MDD that will not achieve reduced symptoms are considered to have treatment-resistant depression (TRD) (Kverno & Mangano, 2021; Pandarakalam, 2018). TRD is generally defined as receiving a substandard response to two adequate trials of two different classes of depressants, at the ideal dosage for the proper duration of time (Souery et al., 1999; Thase & Rush, 1997). A multitude of factors are involved in TRD, such as, but not limited to: assessing the motivation of the patient, the variable routines of day to day life may affect dosing, the re-evaluation of current treatment, and the re-evaluation of personal history, and simultaneous non-psychiatric drug usage, i.e. beta blockers, immunosuppressants, and anticholinergics (Pandarakalam, 2018).

With every subsequent trial of medication, the odds of MDD remission decreases (Kverno & Mangano, 2021). Due to the lack of understanding of the etiology of clinical depression, and the remaining debate on the precise role of deficiencies in monoaminergic systems in MDD, other available treatment options are being investigated as a treatment for TRD (Hasler, 2010; Kverno & Mangano, 2021). There is ample evidence that antidepressants with primary pharmacological targets within the glutamate system could offer an improved therapeutic benefit (Mathew et al., 2012). While subtypes of MDD are more likely to be less responsive to first-line MAAs, ketamine's efficacy in alleviating TRD and its anti-suicidal action have been demonstrated repeatedly (Kverno & Mangano, 2021; Matveychuk et

al., 2020). Importantly, ketamine's onset of action is much more rapid than the MAAs, and this noncompetitive NMDA antagonist has the ability to provide rapid antidepressant properties as early as several hours after administration (Matveychuk et al., 2020). This is an ideal approach for the treatment of MDD, as many patients may not be able to tolerate the severe limitations of MAAs delay in effects. The importance of rapid onset is even greater if these patients are experiencing serious symptoms, including suicidal ideations.

Mechanism of Action of Ketamine

Ketamine is a chiral arylcyclohexylamine. The formulation marketed in the US is a racemic mixture of two stereoisomers, the (*R)* and (*S)* enantiomers (Mathew et al., 2012; Matveychuk et al., 2020). In regards to pharmacokinetic and pharmacodynamic characteristics, the two enantiomers can significantly differ from one another (Matveychuk et al., 2020). Even the uptake and metabolization of ketamine into different tissues has been deemed enantioselective (Muller et al., 2016). The *(S)*-ketamine enantiomer has been found to have a higher clearance rate, and is considered the more potent anesthetic and analgesic enantiomer in comparison to *(R)*-ketamine enantiomer, reflecting three to four times higher affinity of *(S)*-ketamine for the NMDA binding site (Muller et al., 2016). Additionally, *(S)*-ketamine has approximately twice the analgesic potency in comparison to the clinically used racemic mixture (Muller et al., 2016). In rodent models, *(S)*-ketamine produced a greater increase in dopamine tone, and activity in the medial prefrontal cortex (mPFC), as well as greater locomotor activation, ataxia, and head weaving than *(R)*-ketamine at the same dose (Bonaventura et al., 2021; Edwards & Mather, 2001; Janssen Inc., 2020; Janssen Research & Development, 2019; Masaki et al., 2019; Nishizawa et al., 2000; Shim, 2022).

Ketamine, a noncompetitive antagonist of the NMDA receptor, acts through an open channel blocking mechanism, binding within the ion channel and preventing ion flow through the channel (Zorumski et al., 2016). While some molecules of ketamine can pass through the membrane and bind in the channel, more commonly, ketamine needs the channels to open to facilitate access and bind to its channel binding site (Zorumski et al., 2016). Once bound, ketamine shows a higher occurrence of channel

trapping than some other channel blockers (Zorumski et al., 2016). This trapping is where the drug binds to a site within the ion channel, blocks the flow of ions through the open channel, and remains in the channel when the channel closes, causing the channel to be immediately blocked the next time the receptor is activated (Zorumski et al., 2016). This increased occurrence of channel trapping is thought to contribute to the increased risk for adverse effects with ketamine. NMDA receptors are in the family of postsynaptic ionotropic glutamate-activated receptors (iGluRs) and serve as major players in excitatory synaptic transmission (Yu & Lau, 2018). The NMDA receptor is unique in that it requires binding of a co-agonist, most commonly glycine, in order to activate the receptor (Yu & Lau, 2018). As heterotetramers, the NMDA receptors are comprised of four protein subunits including two glycine-binding (GluN1) subunits and two glutamate-binding (GluN2) subunits, which form a tetrameric GluN1/GluN2 receptor (Yu & Lau, 2018). Current flow through NMDA receptors is blocked by the binding of Mg^{2+} in the channel at resting membrane potential (Kampa et al., 2004). Depolarization must occur to relieve the voltage-dependent Mg^{2+} blockade from the receptor prior to the opening of the channel (Kampa et al., 2004). Once ligand binding has occurred concurrent with depolarization of the neuron and relief of the Mg^{2+} block, the ion channel opens permitting Ca^{2+} and Na^{+} to flow into and K^{+} to flow out of the cell (Kampa et al., 2004). Calcium in particular plays an important role in the intracellular signaling of the mammalian target of rapamycin (mTOR) pathway and stimulation of synaptogenesis, as well as the excitotoxicity of neurons if present in excess (Choudhury et al., 2021).

In preclinical rodent-based studies, ketamine has been demonstrated to reverse depression-like behaviors as well as neuropathological changes, which have been linked to MDD in humans, including reversal of dendritic atrophy and enhanced synaptic connectivity (Li et al., 2010; Rajagopal et al., 2016). While ketamine is expected to block excitatory glutamate neurotransmission via NMDA receptor inhibition, an acute increase of glutamate transmission within the synapses of the prefrontal cortex was determined in healthy volunteers using a positron emission tomography with [18F]fluorodeoxyglucose (Zanos & Gould, 2018). These neurological changes result in the reversal of MDD-associated pathological changes and have been suggested to be due to a paradoxical increase in glutamate following

ketamine administration which can be explained by the disinhibition hypothesis (Zanos & Gould, 2018). The disinhibition hypothesis proposes that ketamine produces preferential inhibition of NMDA receptors on GABAergic interneurons (Zanos & Gould, 2018). Therefore, selectively blocking these inhibitory neurons would result in an overall decrease in inhibition of glutamate releasing neurons, which would lead to an enhancement of glutamate levels (Zanos & Gould, 2018). However, numerous studies suggest additional or alternative mechanisms are involved in mediating the antidepressant properties of ketamine (Zanos & Gould, 2018). For example, one hypothesis supports a role for ketamine hydroxynorketamine (HNK) metabolites (Zanos & Gould, 2018). This hypothesis suggests that ketamine exerts NMDAR inhibition-independent antidepressant actions through the action of (*2R,6R*)-HNK and (*2S,6S*)-HNK metabolites (Zanos & Gould, 2018). Following administration, ketamine is metabolized to HNKs and these metabolites act to promote α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)-mediated synaptic potentiation (Zanos & Gould, 2018). An additional hypothesis for ketamine's antidepressant effects is a role for opioid system activation (Williams et al., 2018). Ketamine interacts with a number of receptors including mu, delta, and kappa opioid receptors in addition to the NMDA receptors (Niesters et al., 2014; Williams et al., 2018). The opioid hypothesis suggests that the opioid system may play a role in ketamine's antidepressant effect (Hess et al., 2022). This hypothesis was first derived from a clinical study in which Williams et al. (2018) reported the pretreatment of the mixed opioid antagonist, naltrexone, attenuated ketamine's antidepressant and anti-suicidal effects (Williams et al., 2018). Subsequent preclinical studies have also reported similar results (M. E. Klein et al., 2020; F. Zhang et al., 2021). Nonetheless, the mechanisms are not mutually exclusive and possibly act in combination when exerting the antidepressant actions of ketamine, leading to the strengthening of excitatory synapses necessary for antidepressant effects (Zanos & Gould, 2018).

Clinical Use of Ketamine

First developed in 1962, ketamine was approved for use in human and veterinary medicine as a dissociative anesthetic by the FDA in 1970 (Domino, 2010; Jelen & Stone, 2021; Mathew et al., 2012; Matveychuk et al., 2020). Ketamine was developed as a preferred replacement to its parent compound, phencyclidine (PCP) (Mathew et al., 2012). Ketamine has been noted for less frequent induction of delirium and potentially diminished psychomimetic side effects compared to PCP, likely due to ketamine's much shorter duration of action (Mathew et al., 2012). Ketamine's anesthetic state is characterized by normal skeletal muscle tone and cardiovascular and respiratory stimulation, with minimal respiratory depression (Mathew et al., 2012). Due to these characterizations, at times, it is a preferred anesthetic method for short-term and emergent medical procedures because of its ability to preserve breathing and airway reflexes while simultaneously stimulating the cardiovascular system (Kurdi et al., 2014; M. M. Morgan et al., 2021). This avoids dangerous irregularities in heart rate and blood pressure, such as hypotension and bradycardia (Kurdi et al., 2014).

While ketamine had been used clinically for decades, it was not until 2000 when the first placebo-controlled, double-blinded trial occurred to assess the treatment effects of an N-methyl-D-aspartate (NMDA) receptor antagonist in patients with depression (Berman et al., 2000). This clinical study demonstrated that a single, low dose infusion of ketamine (0.5 mg/kg via IV) produced rapid and prolonged antidepressant effects in patients with MDD within 72 hours post ketamine infusion (Berman et al., 2000). Since Berman et al.'s (2000) discovery, the rapid antidepressant effects in ketamine have been repeatedly demonstrated in human patients (Bahji et al., 2021; Berman et al., 2000; Daly et al., 2018; Muller et al., 2016; Zanos & Gould, 2018). Reports on ketamine improving motivation-related symptoms in depression demonstrated an increase in fronto-striatal functional connectivity in TRD participants toward the levels observed in healthy volunteers (Mkrtchian et al., 2021). Therefore, ketamine normalized fronto-striatal connectivity in TRD participants (Mkrtchian et al., 2021). Overall, the growing preclinical and clinical research suggests that glutamate systems play an important role in the

pathophysiology of major depression and manipulating glutamate neurotransmission is a promising target for antidepressant medications (Berman et al., 2000).

While intravenous (IV) administration would be the anticipated dosing, due to its one hundred percent bioavailability, the impractical logistics and excess costs of IV administration initiated the search for alternative delivery systems from clinicians and researchers (Bahji et al., 2021). A practical approach was the intranasal (IN) administration of *(S)*-ketamine (Bahji et al., 2021). It is absorbed quickly through the vascular bed within the IN cavity and maximum blood concentration is reached within ten to fifteen minutes of administration (Swainson et al., 2019). In fact, IN administration of *(S)*-ketamine is beneficial as it does not undergo extensive hepatic first-pass metabolism, while *(S)*-ketamine administered orally does (Bahji et al., 2021; Swainson et al., 2019). Oral administration exerts a significant effect on ketamine's bioavailability, since a large amount of the drug that is absorbed may be lost before reaching the bloodstream, due to biotransformation in the GI system followed by loss due to first-pass metabolism in the liver (Bahji et al., 2021; Kubota et al., 2013; Novel Psychoactive Substances, 2013; Wu et al., 1995). The bioavailability reported for oral administration was less than sixteen percent (Swainson et al., 2019). Through various routes of administration, IN administration was found to have a forty five percent higher rate than by oral administration, sublingual administration, and rectal administration, demonstrating positive results for IN *(S)*-ketamine treatment in TRD (Swainson et al., 2019). In 2019, the (*S)*-isomer of ketamine was approved for use in TRD in the United States (esketamine nasal spray, Spravato®) and in Europe (Ceramella et al., 2022). Esketamine has been demonstrated to produce rapid antidepressant and anti-suicidal efficacy sustained well beyond its half-life (Swainson et al., 2019). As a safety precaution, Spravato® administration is only available through the Risk Evaluation and Mitigation Strategy (REMS) restricted distribution system (Commissioner, 2020). The patient may only self-administer the nasal spray under the direct supervision of a healthcare provider, due to the risk of adverse outcomes resulting from sedation and dissociation caused by Spravato®(Commissioner, 2020). This also helps to prevent misuse and abuse of Spravato®. In addition to the REMS distribution requirement,

Spravato® is only approved for use in conjunction with an oral antidepressant, therefore patients must be on concurrent, traditional oral monoaminergic medications even though these TRD patients have not previously responded to traditional MAAs (Commissioner, 2020).

Potential Adverse Effects of Ketamine:

Unfortunately, ketamine has a variety of potential adverse effects which may be observed at the same subanesthetic doses commonly utilized for the treatment of depression in humans and to demonstrate antidepressant-like effects in preclinical models (Matveychuk et al., 2020). In humans, most of the potential adverse effects occur acutely during the IV infusion period or the first 30 to 60 min following IN administration and subside shortly thereafter (Matveychuk et al., 2020). The severity of potential adverse effects may also vary with the route of administration, as well as the length of time administration (Matveychuk et al., 2020). One primary physiological effect of concern is an acute and temporary increase in blood pressure, which is usually asymptomatic (Matveychuk et al., 2020). Therefore, blood pressure should always be measured prior to the administration of ketamine, and monitored after its administration until it returns to the normal reference range (Matveychuk et al., 2020). The psychoactive use-limiting side effects of ketamine include abuse liability, dissociative effects, psychotomimetic effects, cognitive disruption, motor impairment, and sedation (Matveychuk et al., 2020; C. J. A. Morgan et al., 2004, 2014; Strous et al., 2022). Spravato carries specific warnings about potential psychotomimetic effects as well as creating feelings of euphoria and a risk for abuse (patient information provided at www.spravato.com).

As mentioned above, ketamine can create feelings of euphoria and has a known abuse potential (Liu et al., 2016). While originally unscheduled, recreational use of ketamine, available in both powder and liquid form, began in the late 1970s and 1980s, and peaked in the 1990's, leading to the FDA classifying ketamine as a schedule III drug in 1999 (Commissioner, 2020; Liu et al., 2016). Due to its potential adverse effects, ketamine faces challenges for clinical use, especially for long-term repeated use, which is important for treating both depression and pain (C. J. A. Morgan et al., 2004, 2014; Strous et al.,

2022). These use-limiting side effects include abuse liability, dissociative effects, cognitive disruption, motor impairment, and sedation (C. J. A. Morgan et al., 2004, 2014; Strous et al., 2022). In rodent studies of abuse-related effects, ketamine served as a stimulus that exhibited reinforcing-like properties in intravenous self-administration (IV-SA), as well as positive motivational effects in conditioned place preference (CPP) (Guo et al., 2016; Liu et al., 2016; Venniro et al., 2015).

Downstream changes in dopamine levels in reward circuitry are extremely relevant to fully understand ketamine's abuse-related effects (Mkrtchian et al., 2021). The four major dopaminergic (DA) pathways in the brain are mesocortical, mesolimbic, nigrostriatal, and tuberoinfundibular (Habibi, Mitra, 2017). There are many decades of behavioral and biochemical research investigating which pathway preside over locomotor activation (Andén, 1972, 1974; Andén & Stock, 1973; Pijnenburg et al., 1973, 1975; Pijnenburg & van Rossum, 1973; Stevens et al., 1974). The preponderance of studies supports primary roles for the mesolimbic and nigrostriatal pathways (Abbott, 2018; Caligiuri & Ellwanger, 2000; Schmidt et al., 2001; Téllez et al., 2008; Treadway & Zald, 2011). Stimulation of locomotor activity is believed to stem from activation of α1 adrenergic receptors and D2 dopamine receptors within the ventral-midbrain (Baik, 2013; Goertz, 2015). The confined, stereotyped behavior of locomotion has been considered to be a property of nigrostriatal rather than mesolimbic DA neurons (Joyce et al., 1981; Kelly et al., 1975; Severino et al., 2020; Vidal & Sans, 2004; Weintraub et al., 2005). In both animals and humans, dopamine neurotransmission in the nucleus accumbens (NAc) has been most notably related to effort and the process of activational motivation (Abbott, 2018; Salamone & Correa, 2012; Treadway et al., 2012). Elevations in DA in the NAc have been associated with reinforcement in behavioral self-administration, as well as an increase in locomotor activation (Baik, 2013; Goertz, 2015; Willuhn et al., 2010).

The possibility of the mesolimbic DA system mediating locomotor activity gained much support after the observation of a bilateral injection of dopamine into the NAc in a rodent model (Pijenenburg, 1976; Pijnenburg & van Rossum, 1973). The injection of low doses of dopamine directly into the NAc produced a significant increase in locomotion, but the bilateral administration of dopamine in the

nigrostriatal did not produce hyperactivity, nor any stereotypical behavior (Costall & Naylor and R.M, 1974; Costall & Naylor, 1974; Pijenenburg, 1976; Pijnenburg & van Rossum, 1973). Due to the activation of DA structures in the NAc and the olfactory tubercle, distinct areas of the mesolimbic dopamine system, these results provide further evidence of the role the mesolimbic pathway has on locomotion (Pijenenburg, 1976; Pijnenburg & van Rossum, 1973). Additionally, the role of mesocorticolimbic dopamine systems in motivation was witnessed in endogenous dopamine receptor stimulation in the NAc (Faure et al., 2008). The blocking of local dopamine ceased glutamate disruptions in different sites in the medial shell (Faure et al., 2008). Dopamine is therefore needed to block AMPA glutamate signals in the NAc, which indicates that dopamine participates in mesocorticolimbic signals that generate motivation (Faure et al., 2008). This neuroanatomical research demonstrates dopamine and glutamate interactions in drug-induced locomotor stimulation within the mesolimbic system (Meyer et al., 2009). Lastly, dopamine in the mesolimbic has shown to be a critical substrate in the brain for unconditioned locomotor activating properties (Gold et al., 1988). An evaluation of the mesolimbic dopamine system, prior to conditioning and post-conditioning, utilized rodent models who received 6-hydroxydopamine (6-OHDA) lesions of the NAc (Gold et al., 1988). The 6-OHDA lesions of the NAc interfered with the effect of amphetamine, suggesting the involvement of the mesolimbic pathway (Gold et al., 1988). The mesolimbic pathway was found to be involved in both the conditioned locomotor responses and the unconditioned locomotor responses with stimulant drugs, due to the behavioral plasticity that resulted from neurocircuitry regulation through both the rodent models environment and experience (Gold et al., 1988). Conditioned locomotion relied on the interaction between presynaptic NAc dopamine and the occupation of postsynaptic NAc dopamine receptors (Gold et al., 1988). This suggests that intact dopamine loads in the VTA and terminates in the NAc, demonstrating that the NAc is involved in various behavioral activity, and affects the influencing abilities of the limbic system on motor activity due to its connection between the limbic system and motor system (Gold et al., 1988). This study displays not only the requirement of an intact presynaptic mesolimbic dopamine system, but even more so, the location of the neurochemical plasticity for these conditioned effects is actually within the mesolimbic

pathway itself (Gold et al., 1988). Overall, exhibiting locomotor hyperactivity behavior stems from dopamine receptor stimulation in the mesolimbic pathway, and how the mesolimbic pathway is believed to play a key role in conditioned locomotor activation in an open field (Gold et al., 1988). Overall, we see overlap between the effects of dopamine increases in locomotor activation and in the reward systems (Abbott, 2018; Caligiuri & Ellwanger, 2000; Schmidt et al., 2001; Téllez et al., 2008; Treadway & Zald, 2011) supporting the use of activity in an open field to provide information which can be correlated with reward system activation and abuse-related behavioral effects.

The Effect of Strain and Sex on Behavior in an Open Field:

Open field activity (OFA) has been extensively utilized to assess both locomotor activity levels and patterns in rat models under baseline conditions, following drug administration as well as other experimental manipulations of interest (Tatem et al., 2014). OFA is a useful assay because it can capture both decreases or increases in locomotor activity (Tatem et al., 2014). Measuring the animal's activity can be done through methods that are based on the frequency of photobeam breaks (activity counts) using infrared photobeam detectors (St. Albans, VT), or distance traveled as assessed by video-tracking systems, utilizing a video camera and software that is designed to not only measure, but map the actual movement of the subject, such as Anymaze software (Stoelting, Co. Wood Dale, IL) (C. J. M. I. Klein et al., 2022; Paulus et al., 1999; C. Zhang et al., 2020). OFA has been used as a critical assay in identifying and establishing specific genetic analyses, such as genetic differences and/or manipulations, that lead to phenotypic differences in rodent models (Burnet et al., 1988; Paulus et al., 1999; Seibenhener & Wooten, 2015). OFA has been utilized to detect genetic variations in performance differences in basal levels of activity as well as the effects of manipulations which could include the induction of pathological models to recreate human and animal diseases, such as a brain or nerve injury (Bennett et al., 2016; Janvier Labs, 2019; Wang et al., 2019). Our laboratory's particular interest is the use of OFA to detect increases in locomotor activity which is typically produced, at one or more doses, by drugs which are commonly abused, such as ketamine. According to the Psychomotor Stimulant Theory of Addiction, rewarding

effects of drugs are often correlated with an increase in locomotion and OFA behavior (Wise & Bozarth, 1987; Zafar, 2017). Similarly, CNS depressant drugs with known abuse liability, like opioids, have shown biphasic dose response curves, which demonstrate increases in locomotor activity in rodents at low to intermediate doses, and suppression of locomotor activity at higher doses (Correa et al., 2003; Nilges et al., 2019). At subanesthetic doses, ketamine is a locomotor stimulant, as shown by an increase in distances traveled (Irifune, 1991; Trujillo et al., 2011). At high doses, ketamine can induce a disruption in general behavior, and overall, result in a decrease in distance traveled, despite a continuance in stimulation of the CNS (Irifune, 1991; Wise & Bozarth, 1987). Specifically, intraperitoneal (IP) injections of 30 mg/kg and 50 mg/kg of ketamine produced peak locomotor activity, while 150 mg/kg doses of ketamine, due to its anesthetic properties, significantly inhibited locomotor activity (Hetzler & Swain Wautlet, 1985; Irifune, 1991). In combination with 0.10 mg/kg haloperidol, a dopamine receptor antagonist, the locomotor stimulating effects of both low and intermediate doses of ketamine are inhibited (Hetzler & Swain Wautlet, 1985). In addition, published literature demonstrates changes in overall activity of dopaminergic neurons in the CNS correlate to changes in locomotor activity, which suggests that the dopaminergic effects of ketamine are in fact directly correlated with locomotor activity (Beninger, 1983). Thus, there is literature that supports that ketamine has dopaminergic properties which contribute to the effect ketamine has on increasing locomotor activity and also in the production of its abuse-related effects.

In addition to locomotor activity, OFA can be used to measure thigmotaxis, to determine novel environment exploration, as well as to assess anxiety-like behavior and willingness to explore (X. Y. Zhang et al., 2021). Thigmotaxis is a rodent's innate behavior to stay close to the walls and avoid the middle of an open field (X. Y. Zhang et al., 2021). Assessment of levels of anxiety-like behavior including changes in thigmotaxis are typically performed in the presence of a stressor, for example, a very bright overhead light. Under these conditions, certain drugs like 5-HT1A receptor agonists that provide anxiolytic-like effects, and benzodiazepines, would reduce the stress-induced inhibition of exploratory behavior and overall, reduce thigmotaxis in an open field (Prut & Belzung, 2003).

The open field test (OFT) is also commonly used to explore sex differences (Knight et al., 2021). Differences noted in baseline levels of activity seen in an OFT may be related to sex, or specific sex hormones, such as estrogen or testosterone (Lightfoot et al., 2008; Rosenfeld, 2017). Indications in many studies suggest male rats are less active than females in the OFT (Belviranli et al., 2012; Blizard et al., 1975; Burke et al., 2016; Tejani-Butt et al., 2003; van Hest et al., 1987; Will et al., 2003). In Belviranli et al., 2012, female WIS rats demonstrated an increase in locomotor activity in OFT in comparison to the males (Belviranli et al., 2012). Similarly, Van Hest et al, 1987 and Will et al, 2003 found the WKY females demonstrated an increase in locomotor activity in comparison to males. Lastly, the SD females were significantly more active compared to the SD males (Burke et al., 2016; van Hest et al., 1987; Will et al., 2003).

Previously published literature have shown differences in the locomotor response to ketamine and other NMDA receptors based on sex, with ketamine being more potent in females than in males (Crawford et al., 2020; McDougall et al., 2017, 2019; Páleníček et al., 2011; Wiley et al., 2011; Wilson et al., 2007). Crawford et al. found that ketamine dramatically increased locomotion in adolescent and adult female rats, while only small stimulatory effects were demonstrated in male adolescent and adult rats (Crawford et al., 2020). Similarly, McDougall et al. discovered that ketamine produced high levels of locomotor activity in female rats, with male adolescent and adult rats exhibiting lesser amounts of ketamine-induced locomotor activity (McDougall et al., 2019). It was reasoned that the large variance in locomotor activity in male and female rats was due to differences in dorsal striatal ketamine and norketamine levels (McDougall et al., 2019). In addition, the sex-associated differences were not noted in preadolescent rats (McDougall et al., 2017, 2019). Results from Wilson et al., indicated that in rodent models, ketamine produced hyperactivity in females and males at 22 days old, but at 35 days old this was only seen in the females (Wilson et al., 2007). A similar finding was seen in Wiley et al, overall demonstrating that gonadal hormone levels are important modulators of behavioral response to ketamine in locomotor activity (McDougall et al., 2017, 2019; Wiley et al., 2007, 2011; Wilson et al., 2007). In addition, with repeated administration, ketamine produced sensitization in adolescent and adult female

rats which differed from the responses seen in males (Wiley et al., 2007, 2011). Overall, these studies suggest that sex strongly influences the effects of ketamine on activity and the production of locomotor activation.

Wistar:

The WIS outbred strain is one of the most popular laboratory rats used in medical research. The WIS is most commonly known for the participation in aging studies, due to its longevity and high rate of spontaneous tumors (Janvier Labs, 2023). The WIS was developed in 1906 at the Wistar Institute, and was the original rat mated to engineer the Long Evans rat and the WKY rat (Okamoto & Aoki, 1963; Sengupta, 2013). Overall, the WIS has been described as a rodent model with multi-purpose characteristics (Kent Scientific Corporation, 2023). The WIS has been mentioned to be a slower learner than the Long Evans breed, but is a calm, easy-to-handle, albino strain (Janvier Labs, 2023; JBBS, 2023). The WIS is known for easier-handling due to its smaller body size, which is in fact, smaller than the SD rats (McCormick, 2017). Gradually, the WIS rats are becoming one of the most used laboratory rats world-wide (Sengupta, 2013) and therefore provide well-characterized control strain with extensive published data.

Sprague Dawley:

The SD rats, an outbred model, were first developed in the 1920s by Robert S. Dawley (Brower et al., 2015). Dawley bred laboratory derived and wild stocks of the WIS rats to hybrids (Brower et al., 2015). The SD is one of the most commonly used control strains in almost all types of medical research, including pharmacology and toxicology (Gileta et al., 2022; Taconic Models, 2023). The SD is known for its elongated head, albino coat, and large litter sizes (Janvier Labs, 2023; Taconic Models, 2023). The SD rat generates timely pregnancies, with excellent maternal characteristics (Taconic Models, 2023). The most significant advantage is that the SD has a docile-like disposition, and is among one of the easier rats to handle (Inotiv, 2023; Janvier Labs, 2023). Importantly, SD rats are one of the most highly used rat

strains in behavioral pharmacology research, including studies of the abuse-related effects of drugs. There is a vast literature on these animals directly pertinent to the current studies and this laboratory has a long history of use of SD rats. Overall, there is extensive historical data with which to compare our current findings.

Wistar Kyoto:

The Wistar Kyoto (WKY), an inbred rat strain, displays heightened stress responses and stress vulnerability in comparison to other strains of rats (Lahmame & Armario, 1996; Lemos et al., 2011; O' Mahony et al., 2013). The WKY strain was originally developed at the Kyoto School of Medicine from the same Wistar-based strain as the spontaneously hypertensive rat (SHR) strain (Abbott, 2018; Okamoto & Aoki, 1963). However, the WKY rats do not develop hypertension and therefore serve as the normotensive controls for the SHR strain (Okamoto & Aoki, 1963). Because the SHR strain also exhibits behaviors which mimic aspects of attention deficit hyperactivity disorder (ADHD), the WKY strain has also been used as a control for studies evaluating SHR in behavioral assays (Aleksandrova et al., 2019; Kin et al., 2017; López-Rubalcava & Lucki, 2000; Marusich et al., 2011; Tejani-Butt et al., 2003). More recently, due to the behavioral and neurochemical parallels to MDD patients, the WKY has been suggested as a rodent model of clinical depression (Burke et al., 2016; López-Rubalcava & Lucki, 2000; Paré & Redei, 1993; Tejani-Butt et al., 2003). The WKY parallels to MDD-associated abnormal neurobiology include altered serotonergic, noradrenergic, and dopaminergic systems, a decrease in the number and soma size of orexin A neurons (which regulate feeding, reward, thermogenesis, and wakefulness), and congenital impairment in hippocampal neurogenesis (Abbott, 2018; Allard et al., 2004, 2007; J. Jiao et al., 2011; Kin et al., 2017; Lin & Huang, 2022). Research has demonstrated that in the mesolimbic pathways, the WKY rat has dopamine system hypofunction, determined using both ex vivo methods, as well as in vivo microdialysis (Heal et al., 2008; X. Jiao et al., 2006; Morganstern & Tejani-Butt, 2010; Yaroslavsky et al., 2006). Published literature has shown a dysfunction of dopamine neurotransmission in the WKY in comparison to Wistars (WIS), a commonly used control strain, with

WKY having lower striatal dopamine transporters (DAT), as well as lower D1, D2 and D3 dopaminergic (DA) receptors in comparison to WIS (Abbott, 2018; Novick et al., 2008). Additionally, previous research has found lower brain tissue concentrations of DA, 5-HIAA, and 5-HT in comparison to Sprague-Dawley (SD) rodents, a commonly used control strain (Abbott, 2018; Burke et al., 2016; De La Garza & Mahoney, 2004; Scholl et al., 2019).

In a range of behavioral studies, the WKY rats have exhibited depressive-like behaviors (Berton et al., 1997; Will et al., 2003). These behavioral and physiological aspects demonstrated include an increased immobility in the forced swim test (an assay used to detect antidepressant-like effects), greater social avoidance, altered sleep patterns, upregulated basal serum corticosterone levels and increased anxiety-like behavior (Berger & Starzec, 1988; Lemos et al., 2011; Marusich et al., 2011; Nam et al., 2014; Nandam et al., 2020; Paré, 1994, 2000; Rittenhouse et al., 2002; Solberg et al., 2001).
Also, most relevant to the current studies, in comparison to other strains, the WKY are considered hypoactive in the open field test (OFT) and, in response to environmental stressors, show decreased levels of general activity (Nam et al., 2014; Sestakova et al., 2013). In stressful situations, the WKY rats show a higher tendency to freeze, and display reduced activity in OFT (Berger & Starzec, 1988; Berton et al., 1997; Lemos et al., 2011; López-Rubalcava & Lucki, 2000; Pardon et al., 2002; Tejani-Butt et al., 2003). In comparison to five other rodent models: The Brown Norway, Fischer 344, Lewis (Lew), Spontaneously Hypertensive Rat (SHR), and Wistar Furth, the WKY had the lowest tendency to approach an aversive stimulus, the highest avoidance of an aversive stimulus, the lowest indication of social interaction, the highest passivity in non-social tests, and the lowest locomotor activity in novel environments (Berton et al., 1997). Additionally, the WKY spent the least amount of time mobile in the elevated plus maze, as well as the lowest number of entries into both the open arms (an indication of anxiety-like behavior) and the enclosed arms (an indication of decreased motor activity) (Berton et al., 1997). Also, the WKY had the lowest exploration time in the closed-arm exploration of the elevated plus maze in comparison to both the Lew and SD rats, and overall, exhibited a reduction in behavioral activity

in all tests (Pardon et al., 2002). These findings display a reduction in arousal and attention, as well as a high level of passivity in response to environmental stimuli in all testing situations (Pardon et al., 2002). When compared against the SD strain, both male and female WKY exhibited significantly lower locomotor activity and increased thigmotaxis (Burke et al., 2016; Pardon et al., 2002; van Hest et al., 1987). The latter was demonstrated by a reduced time in the center zone of the open field compared to the SD (Burke et al., 2016). In the OFT, the WKY had significantly longer response latency scores for leaving the placement segment, as well as a longer response latency to enter the different segments with all four feet compared to the SD and WIS (Tejani-Butt et al., 2003). Consistently amongst the literature, a sex difference has been noted in baseline activity levels (Burke et al., 2016; Chelaru et al., 2012; Hyde & Jerussi, 1983; Paré & Redei, 1993). Specifically, the WKY males have been found to have a decreased level of activity compared to the WKY females, a characteristic seen across the majority of rodent strains (van Hest et al., 1987; Will et al., 2003). While the WKY females were more active in an open field in comparison to the WKY males, they still remained significantly less active compared to SD females (Burke et al., 2016). Even when the males were administered an antidepressant treatment, such as DSP, their activity levels were still significantly reduced compared to the females (Tejani-Butt et al., 2003).

Ketamine in Drug Combinations:

Due to the previously mentioned limitations, there are many mechanisms that could potentially improve ketamine's therapeutic profile and attempt to overcome or minimize the clinical limitations. Drug combinations are one approach which could minimize ketamine's adverse effects, and even concurrently enhance its therapeutic effects. With much literature reiterating the clinical relevance of ketamine, it is of interest to study different drug combinations which might improve ketamine's therapeutic use. Ultimately, identifying beneficial drug combinations which decrease ketamine's abuse-related effects could be particularly useful to the overall clinical outcome, as well as could suggest different cellular mechanisms that contribute to ketamine's acute abuse-related behavioral effects.

Traditional Monoaminergic Antidepressant - Desipramine:

Desipramine (DSP), a secondary amine tricyclic antidepressant was first approved by the FDA in 1964 for the therapy of depression ("Desipramine," 2018; Maan et al., 2023). Tricyclic antidepressants prevent the monoamines norepinephrine and serotonin from being transported back into the presynaptic terminal once they have been released (Riggs & Gould, 2021). Therefore, TCAs prolong the actions of these neurotransmitters by blocking their reuptake from the synaptic cleft (Riggs & Gould, 2021). DSP is also proposed to downregulate serotonin receptors and beta-adrenergic receptors, have effects on α1 blocking, as well as antihistamine and anticholinergic effects (Dean, 2017a, 2017b; Euwema & Swanson, 2022; Maan et al., 2023). Proposed theories of secondary amine tricyclic antidepressants are believed to have a more profound blockade of norepinephrine in comparison to tertiary amine tricyclic antidepressants that have a more profound blockade at serotonin receptors (Maan et al., 2023).

In regards to its toxicity profile, potential DSP-associated adverse effects include: cardiac abnormalities, blurred vision, urinary retention, orthostatic hypotension, sexual dysfunction and suicide (Maan et al., 2023; Riediger et al., 2017). However, in regards to the class of tricyclic antidepressants, DSP is the least likely to cause any of these side effects (Caldwell et al., 2016; "Desipramine," 2018; Maan et al., 2023). In fact, the combination of low-dose ketamine and DSP has been deemed successful in alleviating symptoms in TRD patients, with tricyclic antidepressants in preclinical studies showing an enhancement of the antidepressant effects of ketamine (Scheuing et al., 2015; Thangathurai et al., 2010). Additionally, DSP has been shown to modulate the inhibitory effects of ketamine on the function of NET and SERT (Zhao & Sun, 2008). Spravato®, the S-isomer of ketamine used for TRD and MDD, is specifically approved in combination with MAAs (Janssen Inc., 2020). Most of the approved antidepressant medications for MDD act through monoaminergic mechanisms (Iadarola et al., 2015). While many antidepressant combinations are deemed safe, there is uncertainty about any enhanced or synergistic effects, both positive and negative, that results from any drug combination (DUNNER, 2014).

NMDA Receptor Partial Agonist - D-Cycloserine:

Ever since the centrally active mechanism as a partial NMDA-agonist was discovered, D-cycloserine (DCS) has been studied extensively in neuropsychiatric studies (Schade & Paulus, 2015). Originally, DCS was FDA approved in 1964 as an anti-tuberculosis drug, however, at higher doses, DCS acts as an NMDAR antagonist, and was first reported in 1959 for its potential antidepressant effects (Crane, 1959; Dong et al., 2021). DCS binds to glycine binding sites on GluN2A, GluN2B, and GluN2D subunits of the NMDA receptors (Newport et al., 2015). Additionally, at the glycine binding site on GluN2C subunits, DCS acts as a full agonist (Newport et al., 2015). The rationale for the use of DCS in depression is due to the speculation of an overactive glutamate system contributing to the pathology (Schade & Paulus, 2015).

DCS has a more favorable safety profile than ketamine, and in two double-blind studies in patients with TRD, DCS demonstrated anti-suicidal efficacy at a dose greater than 500 mg in MDD (Chen et al., 2019; Dong et al., 2021; Heresco-Levy et al., 2013). Alone, DCS produced antidepressant effects in a subset of patients with depression (Chen et al., 2019). In preclinical models with schizophrenia, DCS has been shown to reverse some acute behavioral effects of ketamine and PCP, and reverse anesthetic effects of ketamine (Goff, 2017; Irifune, 1991). In rat hippocampal slices, DCS influenced long-term potentiation (LTP) (Watanabe et al., 1992). Therefore, it is possible that DCS could decrease ketamine's adverse effects while potentiating ketamine's antidepressant effects by producing similar therapeutic effects.

Opioid Antagonist - Naltrexone:

In 1963, naltrexone (NTX), a competitive μ-opioid receptor (MOR) antagonist that is used to treat alcohol use disorder and opioid dependence was developed (Singh & Saadabadi, 2023). However, it was not patented until 1967, and it was not FDA approved for medical use in the United States until 1984 (Srivastava & Gold, 2018). For many years, there was little to no mention of the use of NTX (Srivastava & Gold, 2018). Much of the interest in NTX did not peak until 1972, when Congress passed the Drug

Abuse Office and Treatment Act in hopes to develop treatments for heroin-substance abuse (Srivastava & Gold, 2018). NTX blocks the effect of opioids, preventing opioid intoxication in opioid users (Singh & Saadabadi, 2023). Due to its appeal as an alternative option to methadone, which was known for euphoric, sedative, and potentially fatal side effects, NTX became an eligible option for opioid-use disorder (Srivastava & Gold, 2018). Currently, researchers still believe that NTX is one of the most viable options in addressing and treating patients with opioid-use disorder (Peterkin et al., 2022; Singh & Saadabadi, 2023).

While NTX primarily blocks μ-opioid receptors, it is also known as a weaker antagonist at kappa and delta-opioid receptors (Jiang et al., 2021; Singh & Saadabadi, 2023). Recent clinical and preclinical studies demonstrate that NTX attenuates the maximum systemic antidepressant effect of ketamine, seeing as synaptic signaling in the mPFC plays a significant role in the treatment and pathophysiology of clinical depression (Jiang et al., 2021). In 2018, Williams et. al., released a preliminary report in human patients that opioid receptors are necessary for the acute antidepressant effects of ketamine (Williams et al., 2018). This was determined utilizing clinician-administered scales [Hamilton Depression Rating Scale (HAM-D) and Montgomery-Asberg Depression Rating Scale (MADRS)] of related measures of depression, which demonstrated that the pretreatment with NTX attenuated ketamine's antidepressant effect in patients with TRD (Williams et al., 2018). In 2021, Zhang et. al., demonstrated NTX blocked the antidepressant effects of ketamine in preclinical models of antidepressant-like activity in mice (F. Zhang et al., 2021). While that may be the case, the same doses of NTX that blocked the antidepressant-like effects of ketamine in mice, in fact, failed to reverse ketamine's locomotor activating effects (F. Zhang et al., 2021). These effects are believed to be due to the enhanced dopamine release in the brain, which is one of the most commonly used behavioral indicators of an increase of CNS dopamine (F. Zhang et al., 2021). These studies gave rise to our interest in pursuing NTX as a pretreatment drug combination in this project, to determine if the lack of blockade of ketamine's locomotor activating effects could be extended to another rodent species.

Study Aims and Hypotheses:

The overarching goal of the current experiments was to identify a drug that when combined with ketamine would attenuate ketamine-induced locomotor activation. In order to address this goal, we tested the effects of ketamine alone and in combination with DSP, DCS, and NTX on activity in an open field through the following two Aims:

Aim 1: Characterize the effect of ketamine on activity in an open field to determine the impact of strain and sex. Data generated in this Aim was used to guide dose combination testing (Aim 2) as well as determine select patient populations which may be more or less sensitive to ketamine's effects.

Aim 2: Investigate the effect of drugs, with differing cellular mechanisms, on ketamine's locomotor activating effects. Compounds representing three drug classes with potential for modifying ketamine's behavioral effects were administered in combination with doses of ketamine shown to increase locomotor activity. The ideal outcome was to identify a compound which could reverse or attenuate ketamine-induced activation at one or more doses. First, we tested the hypothesis that acute pretreatment with the traditional tricyclic antidepressant DSP will decrease ketamine's locomotor activation. Next, we investigated the hypothesis that acute pretreatment with the glutamatergic partial agonist DCS will attenuate ketamine's locomotor activation. Lastly, we tested the hypothesis that acute pretreatment with the opioid antagonist NTX will not block ketamine-induced locomotor activation.

Methods and Materials:

Subjects:

Open field activity (OFA) behavior was assessed in a total of 24 adult Wistar rats (WIS, Male = 24), 32 adult Sprague Dawley rats (SD, Male = 16; Female = 16), and 24 adult Wistar Kyoto rats (WKY, Male = 12; Female = 12) over the course of all experiments. Specific experimental group sizes are delineated in the results section. All rats completed minimally the ketamine dose response curve. Subsets

of SD rats also completed combination testing with two or three pretreatment drugs. A subset of 8 WKY rats also completed preliminary testing of NTX/KET combinations.

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

Apparatus:

All locomotor testing was conducted in an 43cm x 43cm x 30cm open field chamber with activity monitored and recorded through overhead digital cameras. The floor was covered by a matte black piece of plexiglass (¼" thick) to facilitate tracking of the albino rats by the camera system. The walls of the plexiglass chamber were covered with light gray paper externally to visually isolate the subjects from any external stimuli. The cameras were connected to a computer equipped with AnyMaze software (Version 4.99) that recorded and analyzed total distance traveled (in meters) within the open field. All subjects were acclimated to the laboratory setting and handling for approximately 2 weeks prior to starting any behavioral testing.

Procedure:

On each test day, the animals were brought to the laboratory to acclimate for 30 minutes before any behavioral testing was conducted. The animals were tested at most twice per week, with a minimum of 72 hours between sessions to avoid any drug carryover effects, as well as minimizing any sensitization to ketamine's effects and contact time with the open field (Wiley et al., 2007, 2011). Prior to any drug

administration, animals had undergone three habituation sessions in their assigned open field chambers. During the first two habituation sessions, the animals were placed in their assigned chamber for 30 minutes while their activity was recorded. On the third habituation session, the animals received an injection of saline intraperitoneally (IP) immediately prior to placement in the chamber, which was consistent with the procedure followed during test sessions.

The time frame for testing sessions (30 minutes) was chosen due to activity levels being the highest within the first 30 minutes in OFA (Piazza et al., 1989; Swain et al., 2018). Each animal was tested in the same chamber across all test sessions, at approximately the same time of day, with separate chambers designated for males and females. After each session, chambers were cleaned in an effort to reduce any odors that might impact behavior. The chamber floors were removed, washed with detergent, rinsed and dried, while the interior walls of the chamber were cleaned with a 30% ethanol in water spray, and wiped dry between the testing of each subject.

A counterbalanced approach was used when administering drug doses and/or dose combinations within each dose response curve determination in order to minimize any order effect. On combination test days, all animals were administered saline (vehicle) or a pretreatment drug (DSP, DCS, or NTX) and then returned to their home cage following the pretreatment administration (see Table 1A below). Once the appropriate pretreatment time had elapsed, the animals received either an injection of saline or ketamine and then were immediately placed into the open field chamber following the second injection.

As previously stated, ketamine has various routes of administration with the rate of absorption being the fastest in intravenous (IV) > intraperitoneal (IP) > intramuscular (IM) > subcutaneous (SC) > oral (Levin-Arama et al., 2016). The IP method of administration is typically used when IV administration is too difficult or it is not feasible. With IP administration in rodents, it is possible to safely administer large volumes of fluid without the need to anesthetize the animals beforehand (Turner et al., 2011). However, while IP administration has rapid absorption, drugs administered by this route are subject to hepatic first-pass metabolism. Drugs which have high hepatic extraction (undergo extensive

metabolism in the liver) will undergo biotransformation by the liver before being distributed throughout the body resulting in a significant decrease in drug bioavailability (Lukas et al., 1971). With the SC method of administration, the injections are absorbed at a slower rate as compared to IV and IP administration, which provides a sustained effect, and avoids the first pass effect in the liver; however, the slower rate of absorption may lower the maximum blood levels achieved (Turner et al., 2011). The SC space is a large enough space for large volumes of fluid in small mammals. These two routes of administration, which were utilized in our experiments, avoid technical difficulties which can sometimes be seen with IV administration. The specific drug/drug combination dose response curves and their testing order are listed below. Following habituation, subjects were tested in the following order:

1. Aim 1: Ketamine (KET) dose response curves were completed first in all subjects. During these ketamine only testing days, the animals were given IP injections immediately prior to being placed in their open field chambers.
2. Aim 2, Experiment 1:
    1. DSP dose response curve. Subjects were administered a dose of DSP 30 min prior to a saline injection immediately before being placed into the open field.
    2. Ketamine + DSP tests. On days where combination treatments were used, pretreatments of DSP were administered 30 minutes prior to injection of saline or ketamine and immediately placed in the chamber.
3. Aim 2, Experiment 2:
    1. DCS dose response curve. Subjects were administered a dose of DCS 20 min prior to a saline injection and then placed into the open field.
    2. Ketamine + DCS tests. Subjects were administered a dose of DCS 20 min prior to an injection of ketamine or saline immediately prior to placement in the chamber.
4. Aim 2, Experiment 3:
    1. NTX dose response curve. Subjects were administered a dose of NTX 20 min prior to an IP injection of saline immediately prior to placement in the chamber.

2. Ketamine + NTX tests. Pretreatments of NTX were administered 20 minutes prior to an injection of saline or ketamine and immediately placed in the chamber.

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

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

Drugs: Ketamine HCl (Ketaved®, Vedco, Inc. St. Joseph, MO) was obtained from Patterson Veterinary Supply and diluted in sterile saline to provide the individual doses based on an injection volume of 1 ml/kg. DSP HCl (Sigma Aldrich, Co, St. Louis, MO) was dissolved in saline to provide a solution of 3 mg/ml. This solution was sterilized by filtration (0.22 micropore filter) and further diluted to 1 and 0.3 mg/ml to provide appropriate injection concentrations based on 1 ml/kg. DCS (Sigma Aldrich, Co, St. Louis, MO) was dissolved in sterile water to a maximum concentration of 100 mg/ml and sterilized by filtration. It was further diluted with sterile water for the 30 mg/kg dose. Both 30 and 100 mg/kg doses used 1 ml/kg injection volumes and the 300 mg/kg dose used a 3 ml/kg injection volume. NTX HCl (Mallinkrodt, Inc., St. Louis, MO) was diluted in saline to a concentration of 20 mg/ml and sterile filtered. This was further diluted with sterile saline to provide the 1 and 10 mg/kg doses at 1 ml/kg

injection volumes. The 30 mg/kg NTX dose was achieved by using a 1.5 ml/kg injection volume. All doses are based on the salt weights of the drug except DCS.

Data Analysis:

Total distance traveled (in meters) was measured in the OFT as a measure of activity. Dependent measures were expressed as the mean of the test group plus or minus the standard error of the mean (±SEM). For expressing data as a percent of the control, each subject's test measure (distance traveled) was divided by their corresponding values under saline conditions. These percent of control data where then averaged and presented as mean values (±SEM) in the graphs. The time spent in the center was reviewed. However, the changes in center time were associated with overt sedation and were not a reliable measure of anti-anxiety-like effects. Therefore, the data are not shown.

For Aim 1 investigating the effect of strain (WIS, SD and WKY) on ketamine's effects on locomotor activity in male rats, a two-way ANOVA (Treatment X Strain) was performed using both raw and % of control data. Due to a lack of a significant interaction, no post hoc analyses were performed. For comparison of group means between WKY and SD male and female rats, initially a three-way ANOVA was performed (ketamine Dose X Strain X Sex). Because there was no significant three-way interaction, no post hoc comparisons were performed and the data were divided based on strain. This within strain comparison of the effects of ketamine on male versus female rats utilized two-way ANOVAs followed by Sidak's multiple comparisons test for identification of specific doses of ketamine producing significant changes in distance traveled relative to vehicle. Finally, one-way ANOVAs were performed on data for each group based on sex and strain followed by Dunnett's post hoc comparisons to identify individual doses producing significant changes in distance traveled relative to the vehicle control.

In Aim 2 experiments, the single drug testing conditions (DSP, DCS and NTX dose response curves), the data were evaluated using two-way ANOVA with the main effect of sex as between-subject factors and dose as within-subject factors followed by Sidak's multiple comparisons test where

appropriate. To evaluate whether or not different treatment combinations were significantly different from controls, the data were evaluated using a two-way repeated measure analysis of variance (ANOVA) evaluating factors of Sex x Dose condition. Significant main effects were explored for individual differences using Sidak's multiple comparison post hoc analysis. Finally, the analysis of NTX pretreatment on 10 mg/kg ketamine effects on locomotor activity in male and female WKY rats was done using one-way repeated measure ANOVA within each sex followed by Dunnett's multiple comparisons post hoc analysis. For this study, differences were considered significant if the p-value was equal to or less than 0.05.

Results:

Aim 1: The effects of strain and sex

Adult male rats from all three strains, WIS (n=24), SD (n=12), and WKY (n=12), completed testing of the effects of KET on activity in an open field. Figure 1A presents the mean distance traveled over a 30-minute session following administration of vehicle (saline) or varying doses of ketamine (3 mg/kg to 56 mg/kg). A two-way (Strain x Treatment) ANOVA comparing distance traveled showed a significant main effect of Strain [$F(2,45) = 25.43$, $p<0.0001$] and a significant main effect of Treatment [$F(4,180) = 9.843$, $p<0.0001$], but no significant interaction ($p=0.2901$). Ketamine produced a dose-dependent change in distance traveled with one or more doses significantly increasing distance traveled across the three strains. Additionally, as seen in the graph, the main effect of strain can be attributed to the WKY rats displaying lower distances traveled. .

Figure 1B assesses the effects of Treatment and Strain on the distance traveled, with the data normalized and presented as a percent of the saline control values. The two-way (Strain x Treatment) ANOVA comparing the normalized data showed a significant main effect of Strain [$F(2,45) = 3.975$, $p<0.0257$] and a significant main effect of Treatment [$F(4,180) = 11.61$, $p<0.0001$], but no significant interaction ($p=0.0697$). When evaluating distance traveled relative to saline distances, it is now observed

that ketamine produced similar relative increases in distance traveled at intermediate doses in the WKY rats (see Table 6 for 1-way ANOVA results within sex and strain).

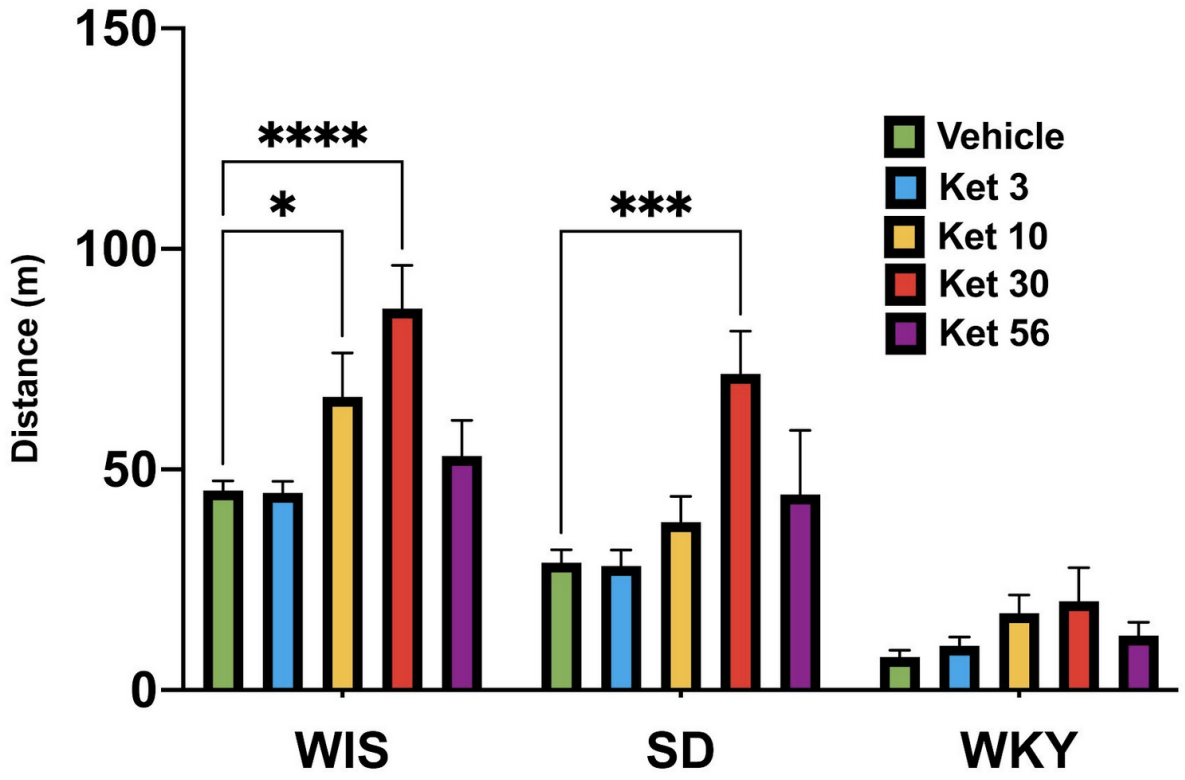

Figure 1A: The effects of ketamine on distance traveled in WIS (Wistar; n = 24, M = 24), SD (Sprague Dawley; n = 12, M = 12), and WKY (Wistar Kyoto; n = 12, M = 12), rats following administration of saline (VEH) or varying doses of ketamine. * denotes a significant effect of ketamine dose realtive to Vehicle at $p \leq 0.05$, *** $p \leq 0.001$, and **** $p \leq 0.0001$ based on 1-way ANOVA within strain comparisons (see Table 6).

Table 1B: Mean distance traveled (meters) in the open field following saline (VEH) or varying doses of ketamine in adult male WIS (Wistar; n = 24, M = 24), SD (Sprague Dawley; n = 12, M = 12), and WKY (Wistar Kyoto; n = 12, M = 12).

Strain	VEH	KET 3 mg/kg	KET 10 mg/kg	KET 30 mg/kg	KET 56 mg/kg
WIS	45.27	44.71	66.39	86.49	53.06
SD	28.85	28.12	38.00	71.64	44.33
WKY	7.507	10.08	17.40	20.15	12.36

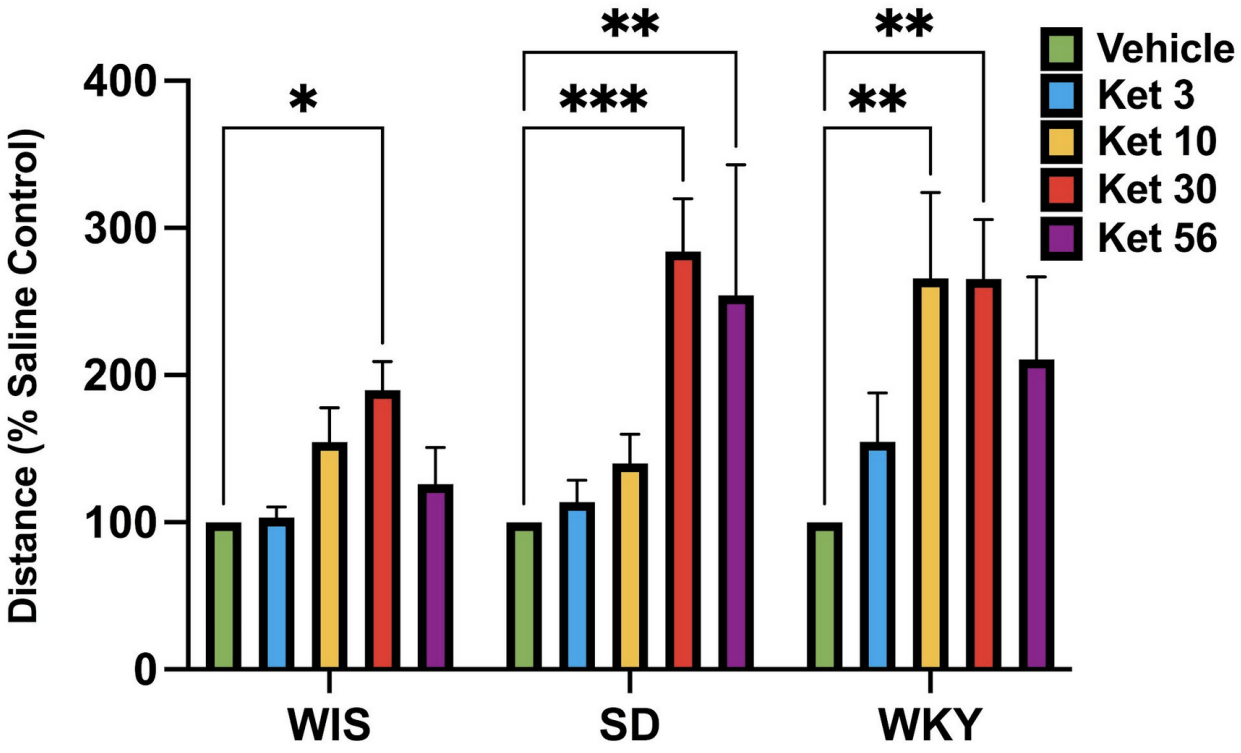

Figure 1B: The effects of ketamine dose and Strain on the distance traveled when expressed as a percent of the saline control for WIS (Wistar; n = 24, M = 24), SD (Sprague Dawley; n = 12, M = 12), and WKY (WKY; n = 12, M = 12), following saline (VEH) or varying doses of ketamine. * denotes a significant effect of ketamine dose realtive to Vehicle at $p \leq 0.05$, *** $p \leq 0.01$, and **** $p \leq 0.001$ based on 1-way ANOVA within strain comparisons (see Table 6).

Figure 2 and Tables 2A (SD) and 2B (WKY) display the mean distance traveled with data presented separately based on strain. These data illustrate that SD rats traveled longer distances than WKY rats under all treatment conditions. SD rats traveled minimally three-fold greater distances following saline and each dose of ketamine relative to the corresponding distance in the WKY. A two-way (Strain x Treatment) ANOVA comparing distance traveled showed a significant main effect of Strain [$F(1,38) = 85.53$, $p<0.0001$] and a significant main effect of Treatment [$F(4,152) = 10.84$, $p<0.0001$], with a significant Strain x Treatment interaction ($p=0.0027$). Ketamine produced a dose-dependent change in the SD distance traveled at intermediate doses with the 10 mg/kg dose ($p=0.007$) and 30 mg/kg dose ($p<0.0001$) of ketamine significantly increasing distance traveled in comparison to distances following saline (vehicle) administration. In order to investigate the Sex x Strain differences and interactions, a three-way ANOVA was conducted, the graph is in the subsequent Figures: 3A, 3B, and Table 3C. Figure 3A displays the mean distance traveled when assessing the interaction between Sex x Treatment x Strain between the control group, SD (n=16, Female = 8; Male = 8), and the stress vulnerable model, WKY (n=24, Female = 12; Male = 12), treated with varying doses of ketamine or saline (vehicle). The three-way ANOVA comparing distance traveled showed a significant main effect of Treatment [$F(4,72)=9.677$, $p<0.001$], Strain [$F(1,18)=111.4$, $p<0.0001$], and Sex [$F(1,18)=17.17$, $p=0.0006$]. Additionally, a significant main interaction of Treatment x Strain [$F(4,72)=3.794$, $p=0.0074$], Treatment x Sex [$F(4,72)=4.238$, $p=0.0039$], Strain x Sex [$F(1,18)=9.881$, $p=0.0056$], but no significant interaction between Sex x Treatment x Strain ($p=0.0527$). Therefore, sex had a significant effect on ketamine effects as well as strain, however when considering each group based on sex AND strain (e.g., WKY females, WKY males, SD females and SD males), there is no difference in the relative response to ketamine. Figure 3B assesses the interaction of effects between Sex x Treatment x Strain in the distance traveled when expressed as a percent of the saline control distance. This three-way ANOVA showed a significant main effect of only Treatment [$F(4,55) = 6.581$, $p=0.002$], with no significant interaction ($p=0.9159$). Table 3C demonstrates further that regardless of Sex and Strain in Figure 3B, that VEH vs. KET 10

(p=0.0107) and VEH vs. KET 30 (p<0.0001) is significant. When comparing data expressed as a percent of the saline control, it is now visible that ketamine exerts a similar effect on distance traveled, regardless of strain.

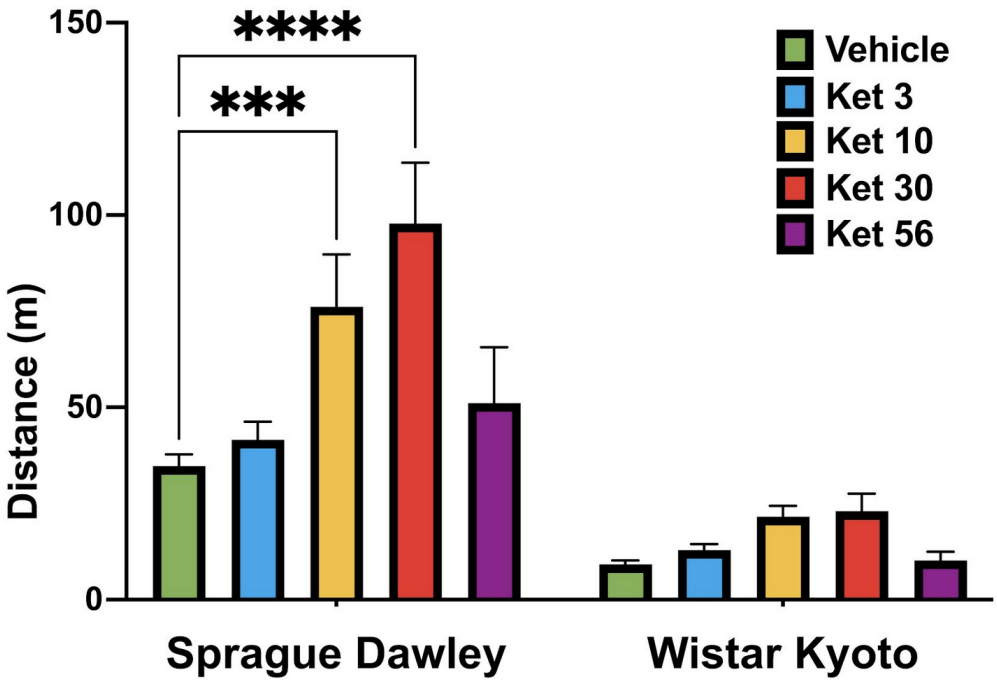

Figure 2: Effects of different doses of ketamine on activity in an open field with data separated based on strain. Each bar represents the mean distance traveled (in meters) ±SEM for Sprague Dawley (n=16) for male (n=8) and female (n = 8) and Wistar Kyoto (n=24) for male (n=12) and female (n=12). *** denotes the mean distance traveled is significantly different from VEH at $p \leq 0.001$ and **** $p \leq 0.0001$.

Table 2A: Mean distance traveled in the open field and corresponding SEMs following saline (VEH) or varying doses of ketamine in Sprague Dawley rats (n=16) for male (n=8) and female (n=8).

Ketamine Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	34.75	3.00
3	41.57	4.25
10	76.14	13.46
30	97.74	15.26
56	51.08	15.01

Table 2B: Mean distance traveled in the open field and corresponding SEMs following saline (VEH) or varying doses of ketamine in Wistar Kyoto rats (n=24) for male (n=12) and female (n=12).

Ketamine Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	9.17	1.05
3	12.92	1.55
10	21.57	2.81
30	22.98	4.55
56	10.19	2.23

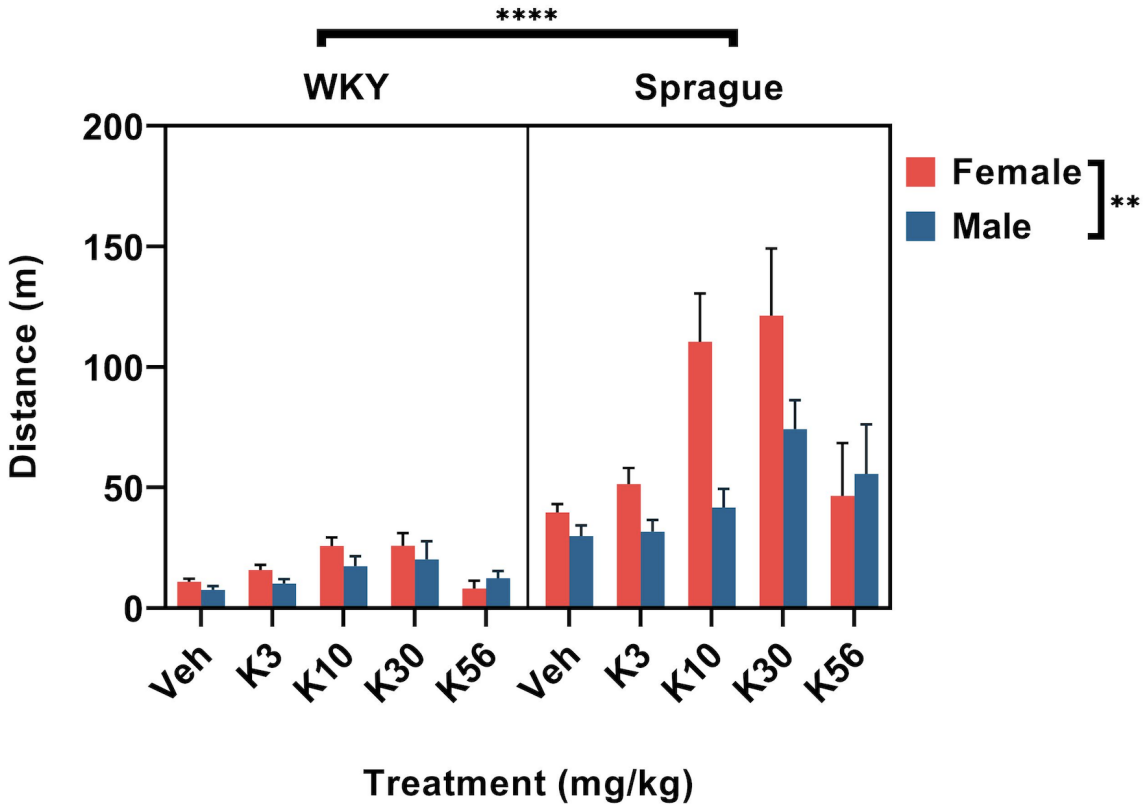

Figure 3A: Assessing the effects and the interactions between Sex, Treatment, and Strain on the distance traveled in an open field. Shown are the mean (±SEM) distances traveled by Sprague Dawley (n = 16) (F = 8, M = 8), and Wistar Kyoto rats (n = 24) (F = 12, M = 12), following administration of saline (VEH) or varying doses of ketamine. ** denotes a significant main effect of sex at $p \leq 0.01$ and a significant main effect of strain at **** $p \leq 0.0001$.

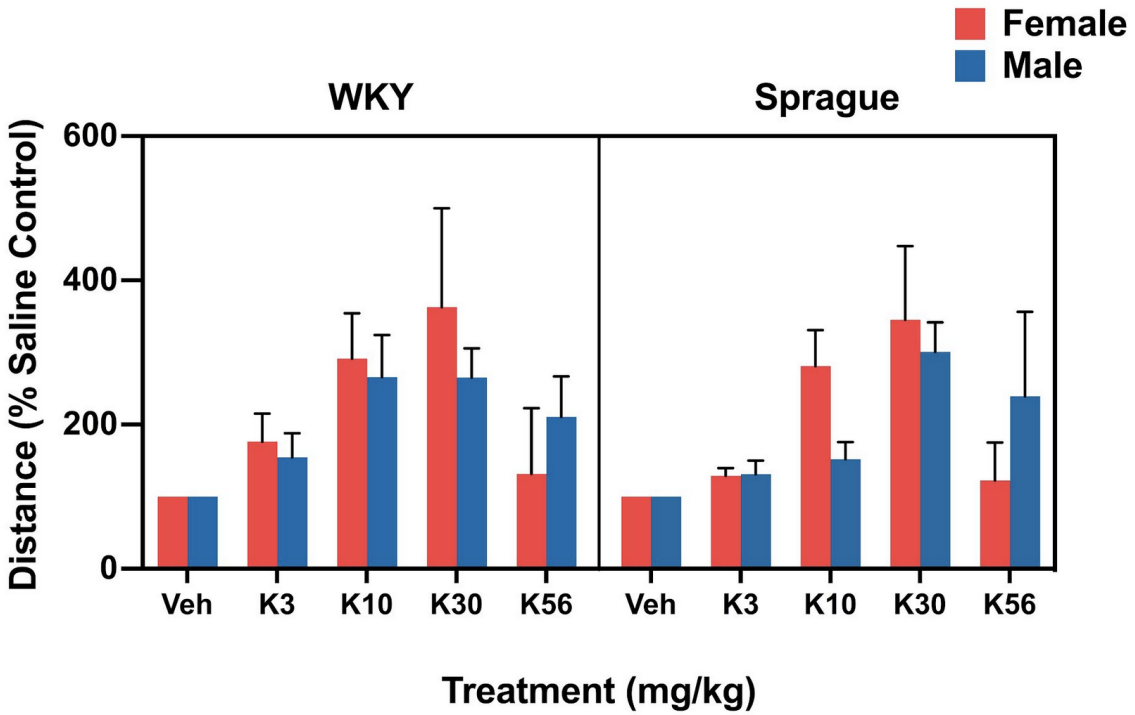

Figure 3B: Assessing the effects and the interactions between Sex, Treatment, and Strain on the distance traveled in an open field. Shown are the mean (±SEM) distances traveled by Sprague Dawley (n = 16) (F = 8, M = 8), and Wistar Kyoto rats (n = 24) (F = 12, M = 12) when expressed as a percent of the saline control following administration of saline (VEH) or varying doses of ketamine.

Table 3: Post-hoc review using Dunnett's multiple comparisons demonstrated that regardless of sex and strain, 10 and 30 mg/kg of ketamine produced a significant increase in distance traveled.

Ketamine Dose (mg/kg, IP)	(SD) Distance Traveled (% Saline Control)	(WKY) Distance Traveled (% Saline Control)	Combined Distance Traveled (% Saline Control)	Adjusted P Value
VEH	100	100	100	
VEH vs. K3	165.5	128.8	147.2	0.7125
VEH vs. K10	278.5	214.8	246.6	0.0107
VEH vs. K30	313.9	320.9	317.4	<0.0001
VEH vs. K56	171	179.9	175.5	0.3223

Figure 4 and Tables 4A (female) and 4B (male) display the mean distance traveled by SD rats with data presented separately for females and males. These data illustrate that there was a trend for female rats to travel longer distances than males under all treatment conditions except for the 56 mg/kg dose of ketamine. A two-way (Sex x Treatment) ANOVA comparing distance traveled showed a significant main effect of Sex [$F(1,14) = 8.092$, $p=0.0130$] and a significant main effect of Treatment [$F(3,42) = 13.50$, $p<0.0001$], but no significant interaction ($p=0.4816$). Ketamine produced a dose-dependent increase in the females' distance traveled at intermediate doses of 10 mg/kg dose ($p=0.0409$) and 30 mg/kg dose ($p<0.0001$) of ketamine. In contrast, no dose of ketamine tested significantly altered the distance traveled compared to vehicle in male SD rats.

A similar analysis was performed in the WKY rats to determine if sex differences would also be observed in the highly stressed vulnerable model. Data in Figure 5 and Tables 5A (female) and 5B (male), show the mean distance traveled, with data presented separately for males and females, demonstrate the presence of a significant sex-dependent difference in the WKY rats as well. These data illustrate that the WKY female rats only slightly traveled longer distances than the males under all treatment conditions of low and intermediate doses. A two-way (Sex x Treatment) ANOVA comparing distance traveled showed a significant main effect of only Treatment [$F(4,88) = 7.367$, $p<0.0001$], but no significant interaction ($p=0.3939$). Ketamine produced a dose-dependent change in the females distance traveled with intermediate doses. A Sidak's multiple comparisons test showed the differences were significant at 10 mg/kg dose ($p=0.0096$) and 30 mg/kg dose ($p=0.0092$) of ketamine for the females, and significance at 30 mg/kg dose ($p=0.0376$) in the males.

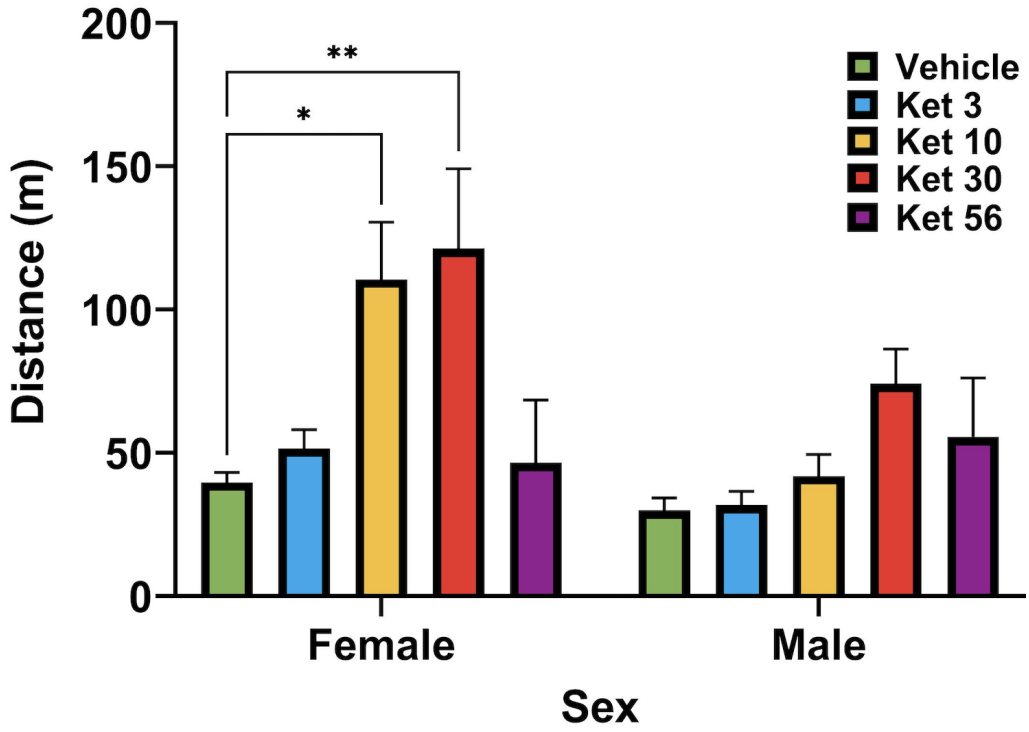

Figure 4: Effects of different doses of ketamine on activity in Sprague Dawley rats in an open field with data separated based on sex. Each bar represents the mean distance traveled (±SEM) for male (n=8) and female (n=8) rats. * denotes the mean distance traveled is significantly different from the corresponding VEH point at $p \leq 0.05$ and ** $p \leq 0.01$.

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

Ketamine Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	39.61	3.54
3	51.44	6.66
10	110.52	20.00
30	121.29	27.83
56	46.52	21.87

Table 4B: Mean distance traveled in the open field and corresponding SEMs following saline (VEH) or varying doses of ketamine in male Sprague Dawley rats (n=8).

Ketamine Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	29.89	4.40
3	34.78	3.66
10	41.60	6.11
30	80.42	10.18
56	55.64	20.50

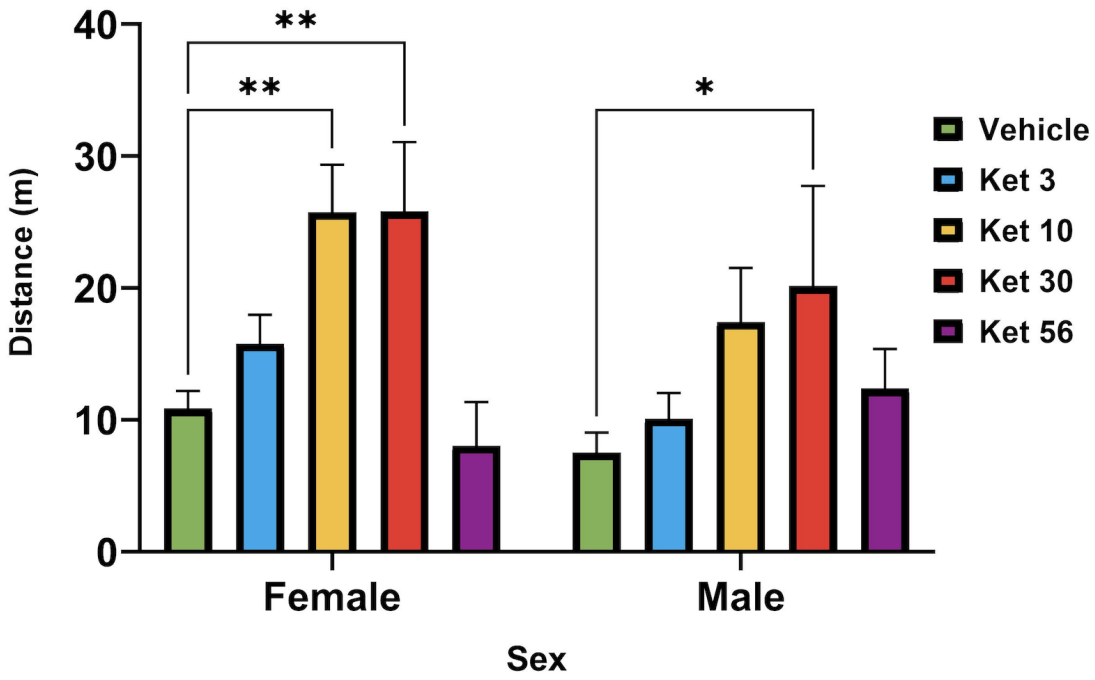

Figure 5: Effects of different doses of ketamine on activity in Wistar Kyoto rats in an open field with data separated based on sex. Each bar represents the mean distance traveled (±SEM) for male (n=12) and female (n=12) Wistar Kyoto rats. * denotes the mean distance traveled is significantly different from VEH at $p \leq 0.05$ and ** $p \leq 0.01$.

Table 5A: Mean distance traveled in the open field and corresponding SEMs following saline (VEH) or varying doses of ketamine in female Wistar Kyoto rats (n=12).

Ketamine Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	10.84	1.34
3	15.77	2.19
10	25.75	3.59
30	25.81	5.24
56	8.02	3.32

Table 5B: Mean distance traveled in the open field and corresponding SEMs following saline (VEH) or varying doses of ketamine in male Wistar Kyoto rats (n=12).

Ketamine Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	7.51	1.52
3	10.08	1.95
10	17.40	4.11
30	20.15	7.60
56	12.36	3.00

Table 6: Shown are results of one-way repeated measures ANOVA comparing doses of ketamine producing significant increases in distance traveled relative to vehicle across strain and sex. Doses producing locomotor activation as denoted by p values ≤ 0.05 determined using Dunnett's multiple comparisons.

Strain	Sex	Ket 3	Ket 10	Ket 30	Ket 56
Wistar	Male	ns	ns	p=0.0005	ns
Sprague Dawley	Male	ns	ns	p=0.0046	ns
	Female	ns	p=0.0238	p=0.0497	ns
Wistar Kyoto	Male	ns	ns	ns/ p=0.006*	ns
	Female	p=0.0443	p=0.0259	ns	ns

ns = no significant change; * denotes

Table 6 presents a summary of the results of one-way repeated measures ANOVA comparing distance traveled following vehicle and different doses of ketamine within each strain and sex tested. There was a significant effect of ketamine in all groups except the WKY males when comparing the raw data [WIS males $F(2.949, 67.83) = 7.335$, $p=0.0003$; SD males $F(1.836, 20.19) = 4.623$, $p=0.0246$; SD females $F(1.884, 13.19) = 4.047$, $p=0.0445$; WKY males $F(1.801, 19.81) = 1.986$, $p=0.1665$; WKY females $F(1.941, 13.59) = 4.965$, $p=0.0248$]. When values for the male WKY rats was converted to a percent of the saline control to account for variability in baseline activity, a significant effect of ketamine was detected [$F(2.424, 26.66) = 3.456$, $p=0.0385$]. For each strain/sex group, one or more doses of ketamine significantly increased distance traveled as identified by Dunnett's multiple comparisons. Individual p values for significant effects are shown in Table 6.

Aim 2: Effect of Drug Combinations on Ketamine's Effects

DESIPRAMINE:

A similar analysis of ketamine's effects on locomotor activity was performed using data from a subset of 16 SD subjects (8 male, 8 female) that examined the effects of pretreatment with DSP on ketamine-induced changes in locomotor activity. The data include the effects of DSP alone [Figure 6 and Tables 6A (female) and 6B (male)] and in combination with ketamine [Figures 7 and 8, Tables 7A and 8A (female) and 7B and 8B (male)] alter locomotor activity. For all tests, DSP was administered IP 30 min prior to the start of the session, followed by IP saline (DSP dose-effect curve) or 10 or 30 mg/kg of ketamine immediately prior to placement in the open field.

When administered alone, as shown in Figure 6 and Tables 6A (female) and 6B (male), DSP produced a dose-dependent decrease in distance traveled. Analysis of these data using a two-way repeated measures ANOVA revealed a significant main effect of Dose [$F(3, 42) = 4.976$, $p=0.0048$]. A Sidak's multiple comparisons test confirmed the 3 mg/kg dose of DSP significantly decreased activity relative to the vehicle control ($p=0.0008$) in the female subjects. In the male subjects, DSP produced only modest decreases in the distance traveled.

Data in Figure 7 and Tables 7A (female) and 7B (male), represent the effect of different DSP pretreatment doses in combination with 10 mg/kg of ketamine. A two-way (Sex x Treatment) ANOVA comparing distance traveled showed a significant main effect of Sex [$F(1,14) = 28.60$, $p=0.0001$], a significant main effect of Treatment [$F(4,56) = 6.040$, $p=0.0004$], and a significant interaction of Sex x Treatment [$F(4,56) = 2.740$, $p=0.0375$]. At this dose of ketamine, only the female rats demonstrated a significant increase in distance traveled. No dose of DSP altered the ketamine-induced activation. In male rats, there was no effect of DSP to alter activity levels when combined with 10 mg/kg of ketamine.

Figure 8 and Tables 8A (female) and 8B (male) show data following administration of the same pretreatment doses of DSP, now combined with 30 mg/kg of ketamine. Examination of the data using a two-way (Sex x Treatment) ANOVA failed to detect a significant main effect of Sex ($p=0.2504$), but detected a main effect of Treatment [$F(4,56) = 11.35$, $p<0.0001$]. At this dose of ketamine, both males

and females displayed a significant increase in distance traveled relative to the VEH + VEH condition. However, no dose of DSP altered the ketamine-induced increase in activity.

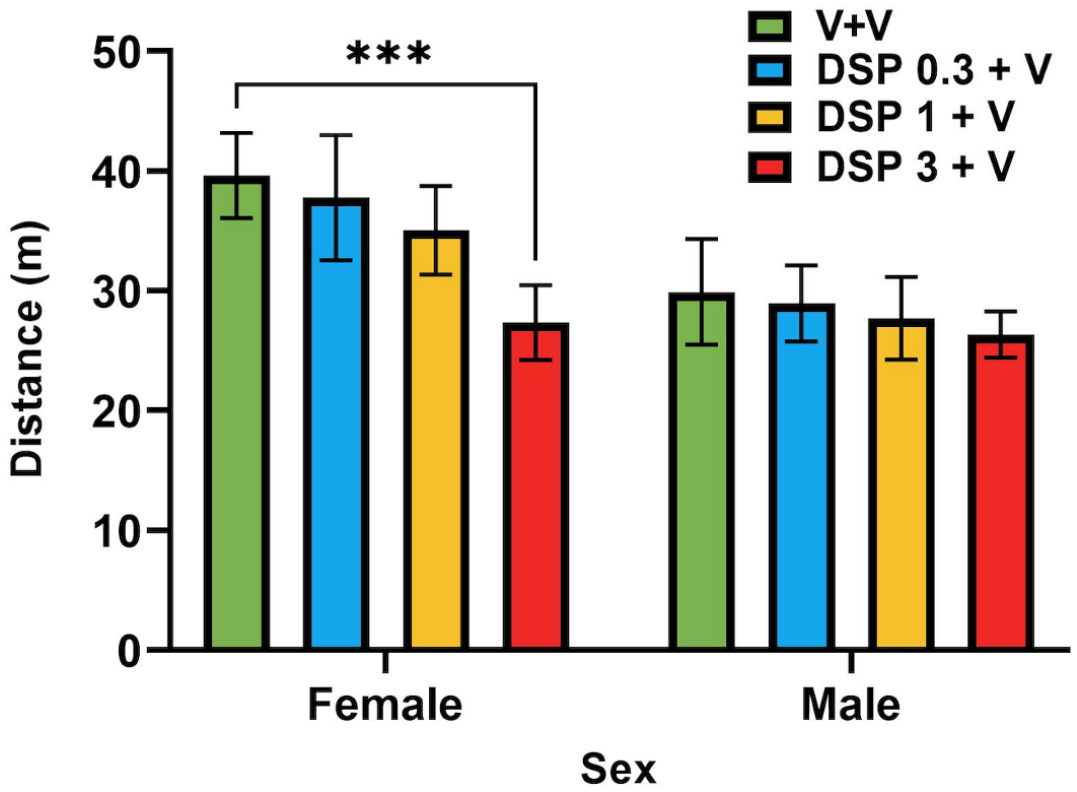

Figure 6: Effects of saline or different doses of ketamine in combination with DSP pretreatment on activity in an open field for 16 Sprague Dawley rats (Male = 8; Female = 8). Each bar represents the mean distance traveled (±SEM). *** denotes the mean distance traveled was significantly different from VEH + VEH at $p \leq 0.001$

Table 6A: Effects of DSP alone on distance traveled in 8 female Sprague Dawley rats.

DSP Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	39.61	3.54
DSP 0.3	37.76	5.22
DSP 1	35.04	3.71
DSP 3	27.34	3.11

Table 6B: Effects of DSP alone on distance traveled in 8 male Sprague Dawley rats.

DSP Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	29.89	4.40
DSP 0.3	28.94	3.17
DSP 1	27.68	3.45
DSP 3	26.34	1.93

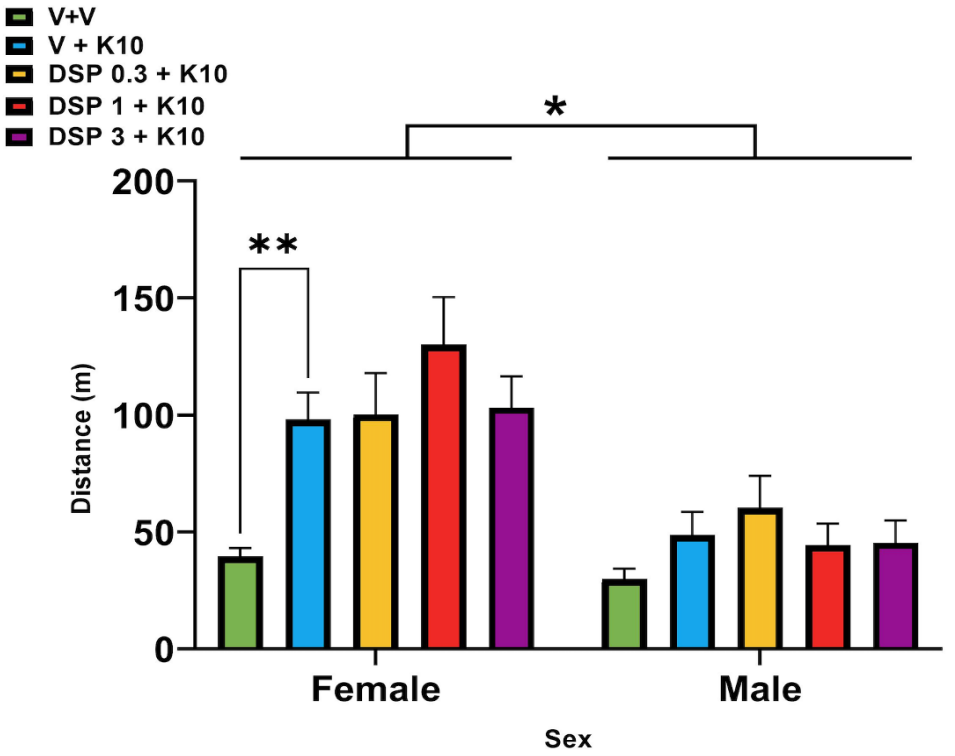

Figure 7: Effects of saline or 10 mg/kg of ketamine in combination with DSP pretreatment on activity in an open field for 16 Sprague Dawley rats (Male = 8; Female = 8). Each bar represents the mean distance traveled (in meters) ±SEM. * denotes a significant main effect of strain. ** $p \leq 0.01$ denotes the mean distance traveled is significantly different from VEH + KET.

Table 7A: Effects of DSP combined with 10 mg/kg of ketamine on distance traveled in 8 female Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM
VEH	39.61	3.54
VEH + K10	98.11	11.51
DSP 0.3 + K10	100.3	17.60
DSP 1 + K10	130.12	20.25
DSP 3 + K10	103.12	13.40

Table 7B: Effects of DSP combined with 10 mg/kg of ketamine on distance traveled in 8 male Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM
VEH	29.89	4.40
VEH + K10	45.52	8.67
DSP 0.3 + K10	60.38	13.66
DSP 1 + K10	44.48	9.09
DSP 3 + K10	45.33	9.61

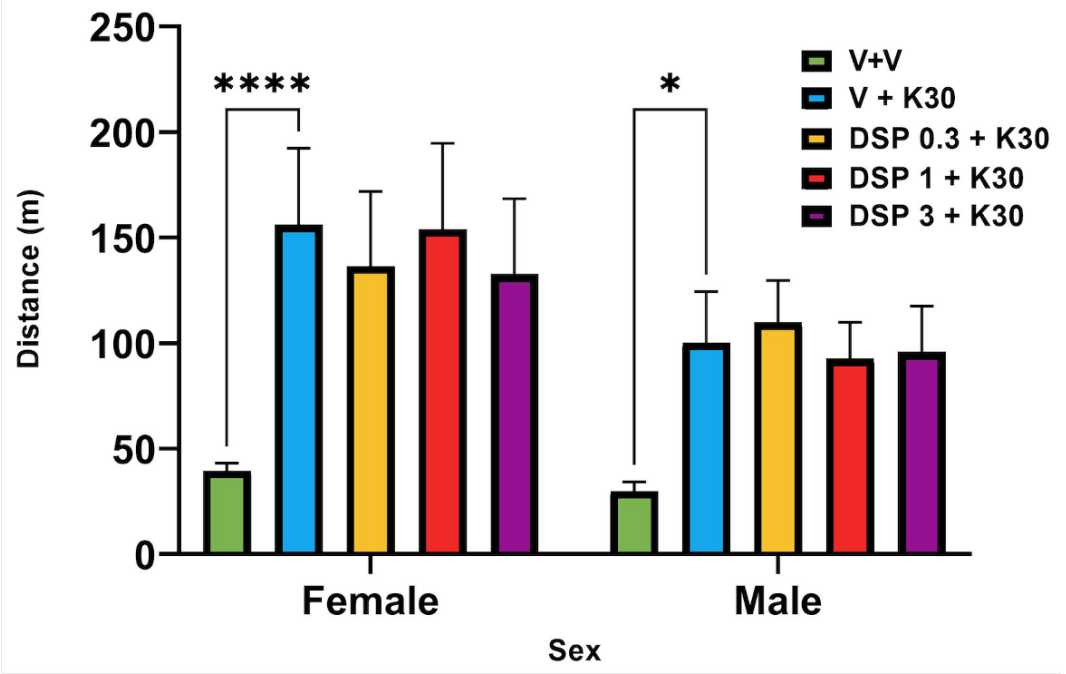

Figure 8: Effects of saline or 30 mg/kg of ketamine in combination with DSP pretreatment on activity in an open field for 16 Sprague Dawley rats (Male = 8; Female = 8). Each bar represents the mean distance traveled (in meters) ±SEM. * denotes the mean distance traveled is significantly different from VEH + KET 30 at $p \leq 0.05$ and **** $p \leq 0.0001$.

Table 8A: Effects of DSP combined with 30 mg/kg of ketamine on distance traveled in 8 female Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM
VEH	39.61	3.54
VEH + K30	156.12	36.17
DSP 0.3 + K30	136.51	35.40
DSP 1 + K30	154.02	40.64
DSP 3 + K30	132.86	35.54

Table 8B: Effects of DSP combined with 10 mg/kg of ketamine on distance traveled in 8 male Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM
VEH	29.89	4.40
VEH + K30	88.12	15.30
DSP 0.3 + K30	109.73	19.91
DSP 1 + K30	92.67	17.28
DSP 3 + K30	96.07	21.43

D-CYCLOSERINE:

An investigation of ketamine's effects on locomotor activity was performed using data from a subset of 16 SD subjects (8 male, 8 female) that examined the effects of pretreatment with DCS on ketamine-induced changes in locomotor activity. The data included the effects of DCS alone [Figure 9 and Tables 9A (female) and 9B (male)] and in combination with intermediate ketamine doses of 10 mg/kg and 30 mg/kg [(Figures 10 and 11, Tables 10A and 11A (female) and 10B and 10B (male)]. For all tests, DCS was administered SC 20 min prior to the start of the session, followed by IP saline (DCS dose-effect curve) or 10 or 30 mg/kg of ketamine immediately prior to placement in the open field.

When administered alone, as shown in Figure 9 and Tables 9A (female) and 9B (male), 30 mg/kg and 300 mg/kg of DCS had no effect on distance traveled across the dose range tested. Analysis of these data using two-way repeated measures ANOVA failed to detect a significant main effect of Sex ($p=0.4333$), Treatment ($p=0.4356$), nor an interaction ($p=0.3946$).

For data shown in Figure 10 and Tables 10A (female) and 10B (male), various DCS pretreatment doses were administered in combination with 10 mg/kg of ketamine. A two-way (Sex x Treatment) ANOVA comparing distance traveled showed a significant main effect of Treatment [$F(4,48) = 3.962$, $p=0.0074$], but failed to detect a significant main effect of Sex ($p=0.4556$), or a significant interaction of Sex x Treatment ($p=0.8082$).

For data in Figure 11 and Tables 11A (female) and 11B (male), the test dose combinations with 30 mg/kg of ketamine utilizing the two-way (Sex x Treatment) ANOVA failed to detect a significant main effect of Sex ($p=0.1618$), but detected a main effect of Treatment [$F(4,48) = 11.72$, $p<0.0001$]. A Sidak's multiple comparisons test showed the differences were significant in the females at VEH + KET 30 vs. VEH + VEH ($p<0.0001$) and in the males at VEH + KET 30 vs. VEH + VEH ($p=0.0287$).

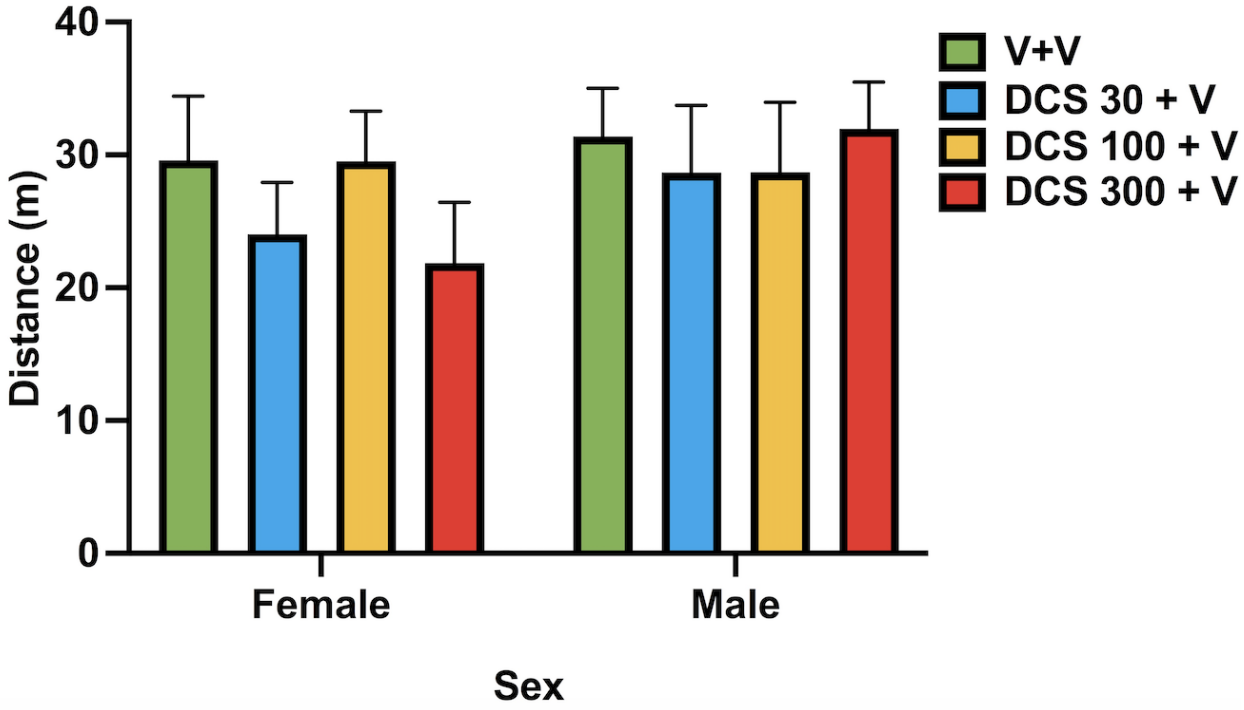

Figure 9: Effects of different doses of DCS followed by vehicle (V; saline) on activity in an open field for 16 adult Sprague Dawley rats (Male = 8; Female = 8). Each bar represents the mean distance traveled (±SEM).

Table 9A: Effects of DCS alone on distance traveled in an open field in 8 female adult Sprague Dawley rats.

DCS Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	29.58	4.85
DCS 30	24.02	3.90
DCS 100	29.52	3.76
DCS 300	21.86	4.56

Table 9B: Effects of DCS alone on distance traveled in an open field in 8 male adult Sprague Dawley rats.

DCS Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	31.37	3.65
DCS 30	28.66	5.06
DCS 100	28.69	5.27
DCS 300	31.95	3.53

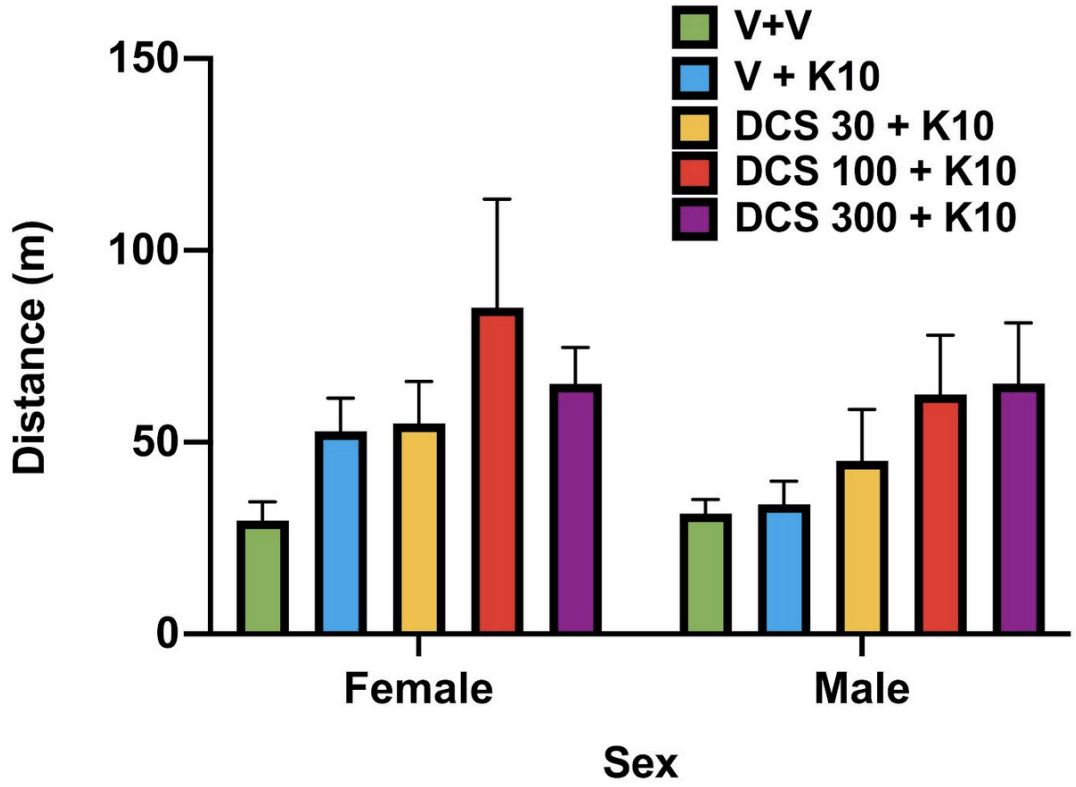

Figure 10: Effects of 10 mg/kg of ketamine following pretreatment with varying doses of DCS (DCS; 30 mg/kg - 300 mg/kg) on distance traveled in an open field for 16 adult Sprague Dawley rats (Male = 8; Female = 8). Each bar represents the mean distance traveled (in meters) ±SEM.

Table 10A: Effects of different DCS + 10 mg/kg of ketamine dosing conditions on distance traveled in an open field in 8 female adult Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM
VEH	29.58	4.85
VEH + K10	42.02	7.32
DCS 30 + K10	54.85	11.00
DCS 100 + K10	85.12	28.24
DCS 300 + K10	65.23	9.46

Table 10B: Effects of different DCS + 10 mg/kg of ketamine dosing conditions on distance traveled in an open field in 8 male adult Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM
VEH	31.37	3.65
VEH + K10	66.23	15.10
DCS 30 + K10	45.13	13.40
DCS 100 + K10	62.48	15.44
DCS 300 + K10	65.25	15.81

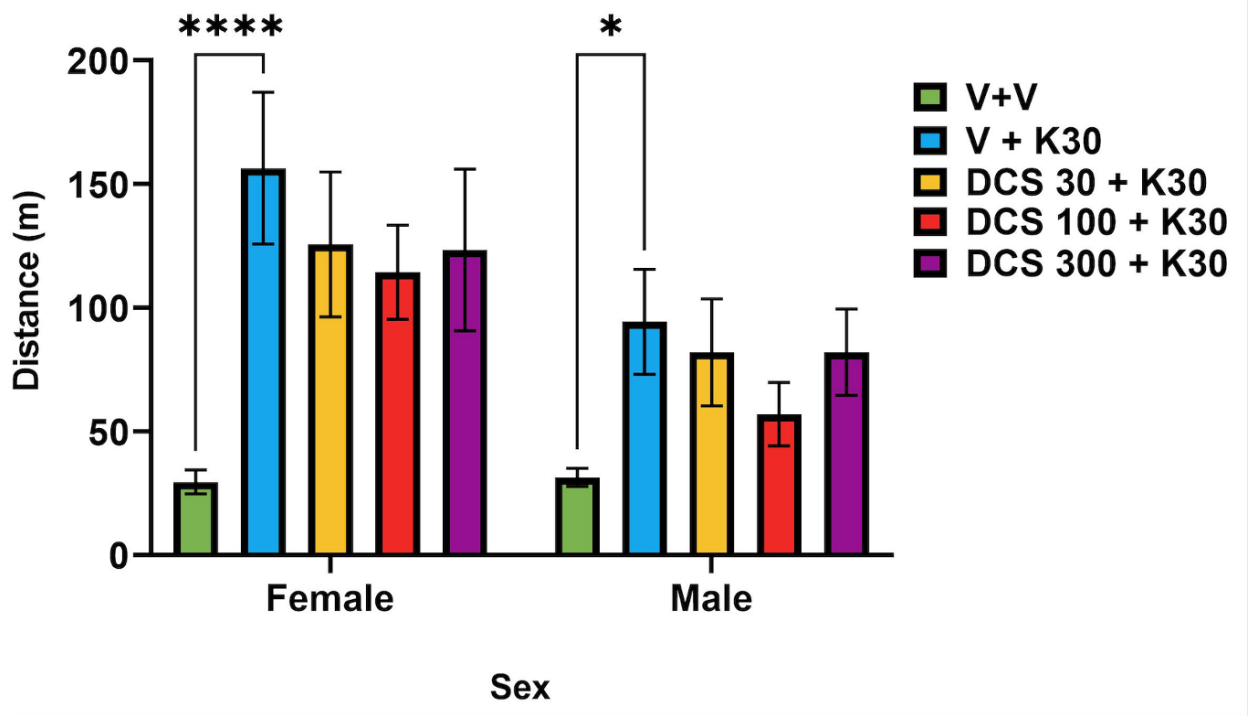

Figure 11: Effects of 30 mg/kg of ketamine following pretreatment with varying doses of DCS (30 mg/kg - 300 mg/kg) on distance traveled in an open field for 16 adult Sprague Dawley rats (Male = 8; Female = 8). Each bar represents the mean distance traveled (in meters) ±SEM. * denotes the mean distance traveled is significantly different from VEH + KET 30 at $p \leq 0.05$ and **** $p \leq 0.0001$.

Table 11A: Effects of different DCS + 30 mg/kg of ketamine dosing conditions on distance traveled in an open field in 8 female adult Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM
VEH	29.58	4.85
VEH + K30	369.72	87.83
DCS 30 + K30	125.65	29.32
DCS 100 + K30	114.36	19.04
DCS 300 + K30	123.39	32.74

Table 11B: Effects of different DCS + 30 mg/kg of ketamine dosing conditions on distance traveled in an open field in 8 male adult Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM
VEH	31.37	3.65
VEH + K30	421.4	106.64
DCS 30 + K30	81.99	21.66
DCS 100 + K30	57.00	12.82
DCS 300 + K30	82.02	17.49

Naltrexone effects in male and female SD Rats:

A similar analysis of the effect of pretreatment with different doses of NTX (1, 10, and 30 mg/kg) on ketamine's locomotor activating effects was performed using data from 16 SD subjects (8 male, 8 female). Figure 12 and tables 12A (female) and 12B (male) display the effects of different doses of NTX administered SC 20 min prior to the start of the session, followed by saline administration immediately prior to placement in the open field. The effects of the different NTX doses in combination with ketamine (10 or 30 mg/kg) on locomotor activity are presented in Figures 13 and 14, Tables 13A and 14A (female) and 13B and 14B (male).

When administered alone, as shown in Figure 12 and Tables 12A (female) and 12B (male), NTX produced a dose-dependent decrease in distance traveled in the females, but only a decreasing trend in the males. Analysis of these data using a two-way repeated measures ANOVA detected a significant main effect of Treatment [$F(3,66) = 5.573$, $p=0.0018$], but failed to detect a significant main effect of Sex ($p=0.0961$), or an interaction of Sex x Treatment ($p=0.7427$). A Sidak's multiple comparisons test showed the distances were significantly different from the VEH + VEH condition in the females at NTX 10 + VEH ($p=0.0309$) and NTX 30 + VEH ($p=0.0100$).

For data in Figure 13 and Tables 13A (female) and 13B (male), various NTX pretreatment doses were used in combination with 10 mg/kg of ketamine. Two-way (Sex x Treatment) ANOVA comparing distance traveled showed a significant main effect of Sex [$F(1,18) = 5.452$, $p=0.0313$], and Treatment [$F(4,72) = 7.902$, $p<0.0001$], but failed to detect a significant interaction of Sex x Treatment ($p=0.2422$). A Sidak's multiple comparisons test showed the differences were significant in the females at VEH + KET 10 vs. VEH + VEH ($p<0.0001$).

For data in Figure 14 and Tables 14A (female) and 14B (male), the test dose combinations with 30 mg/kg of ketamine utilizing the two-way (Sex x Treatment) ANOVA comparing distance traveled showed a significant main effect of Sex [$F(1,14) = 6.144$, $p=0.0265$], and Treatment [$F(4,56) = 10.53$, $p<0.0001$], but failed to detect a significant interaction of Sex x Treatment ($p=0.3314$). A Sidak's multiple

comparisons test showed the differences were significant in the females at VEH + KET 30 vs. VEH + VEH ($p<0.0001$) and in the males at VEH + KET 30 vs. VEH + VEH ($p=0.0251$).

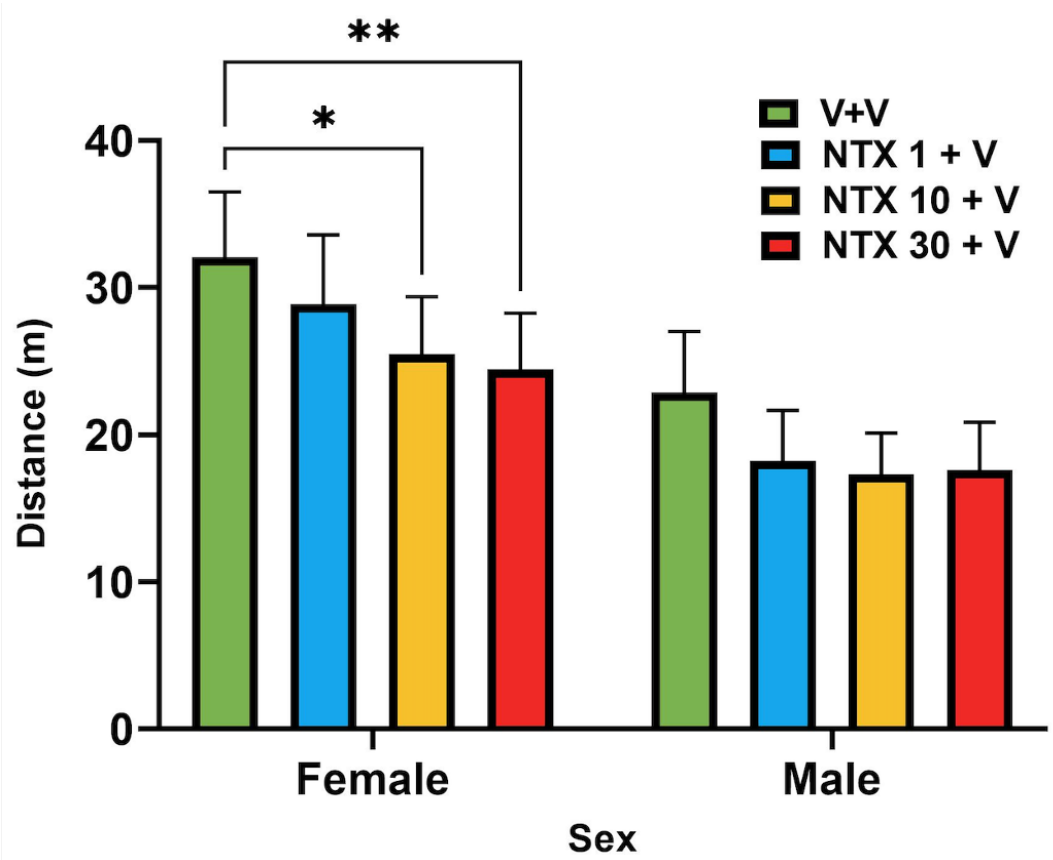

Figure 12: Effects of different doses of NTX (1 mg/kg - 30 mg/kg) alone on activity in an open field for 16 (Male = 8; Female = 8) adult Sprague Dawley rats. Each bar represents the mean distance traveled (in meters) ±SEM. * denotes the mean distance traveled is significantly different from VEH + VEH at $p \leq 0.05$ and ** $p \leq 0.01$.

Table 12A: Effects of NTX alone on distance traveled in 8 female adult Sprague Dawley rats.

NTX Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	39.61	3.54
NTX 1	204.38	67.24
NTX 10	25.46	3.93
NTX 30	24.43	3.84

Table 12B: Effects of NTX alone on distance traveled in 8 male adult Sprague Dawley rats.

NTX Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	29.89	4.40
NTX 1	90.48	16.70
NTX 10	17.3	2.80
NTX 30	17.61	3.25

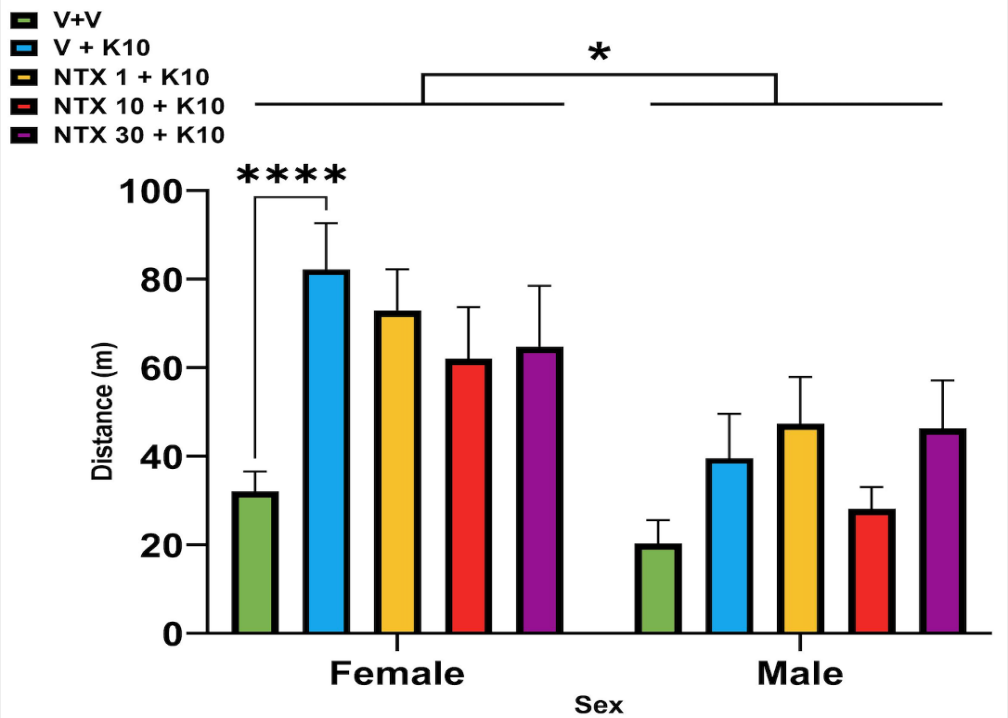

Figure 13: Effects of 10 mg/kg of ketamine following pretreatment with varying doses of NTX (1 mg/kg - 30 mg/kg) on distance traveled in an open field for 16 adult Sprague Dawley rats (Male = 8; Female = 8). Each bar represents the mean distance traveled (in meters) ±SEM. * denotes a significant main effect of sex on distance traveled. **** $p \leq 0.0001$ denotes the mean distance traveled is significantly different from VEH + KET 10.

Table 13A: Effects of different NTX + 10 mg/kg of ketamine dosing conditions on distance traveled in an open field in 8 female adult Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM
VEH	39.61	3.54
VEH + K10	98.11	11.51
NTX 1 + K10	72.92	9.3
NTX 10 + K10	61.97	11.68
NTX 30 + K10	64.77	13.69

Table 13B: Effects of different NTX + 10 mg/kg of ketamine dosing conditions on distance traveled in an open field in 8 male adult Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM
VEH	29.89	4.40
VEH + K10	45.52	8.67
NTX 1 + K10	47.34	10.55
NTX 10 + K10	28.14	4.90
NTX 30 + K10	46.39	10.81

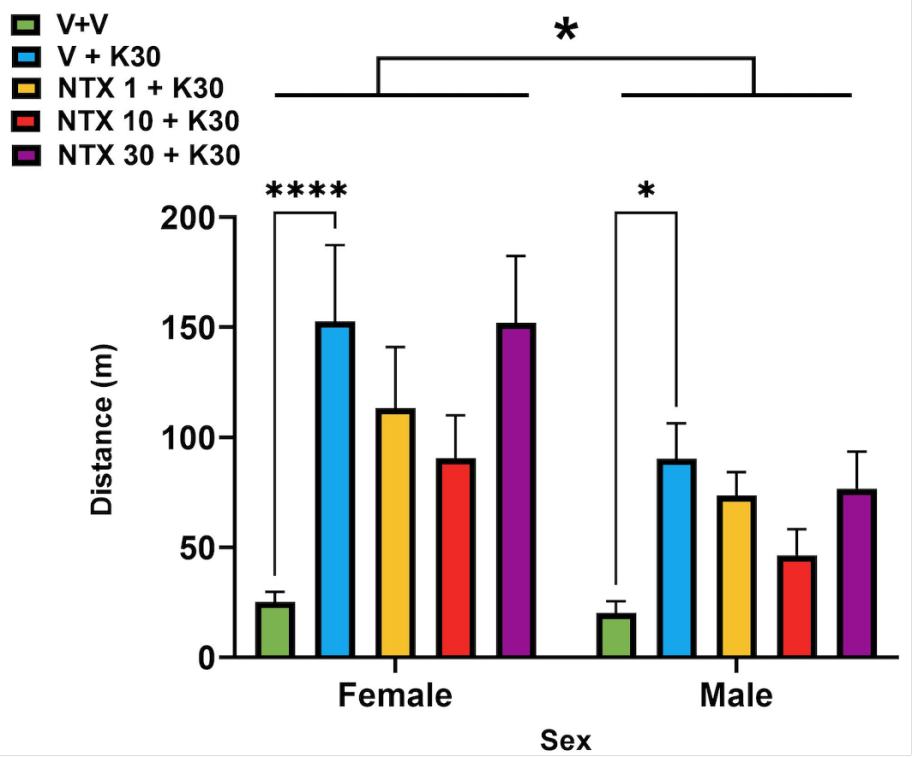

Figure 14: Effects of 30 mg/kg of ketamine following pretreatment with varying doses of NTX (1 mg/kg - 30 mg/kg) on distance traveled in an open field for 16 adult Sprague Dawley rats (Male = 8; Female = 8). Each bar represents the mean distance traveled (in meters) ±SEM. * denotes a significant main effect of sex on distance traveled. For within sex comparisons, * denotes the mean distance traveled is significantly different from VEH + KET 30 at $p \leq 0.05$ and **** $p \leq 0.0001$.

Table 14A: Effects of different NTX + 30 mg/kg of ketamine dosing conditions on distance traveled in an open field in 8 female adult Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM
VEH	39.61	3.54
VEH + K30	156.12	36.17
NTX 1 + K30	113.25	27.72
NTX 10 + K30	90.60	19.40
NTX 30 + K30	152.13	30.22

Table 14B: Effects of different NTX + 30 mg/kg of ketamine dosing conditions on distance traveled in an open field in 8 male adult Sprague Dawley rats.

	Distance Traveled (m)	Distance SEM
VEH	29.89	4.40
VEH + K30	88.12	15.30
NTX 1 + K30	73.51	10.63
NTX 10 + K30	46.50	11.73
NTX 30 + K30	76.73	16.78

Naltrexone effects in male and female WKY Rats:

A similar analysis of ketamine's effects on locomotor activity was performed using data from 8 WKY subjects (4 male, 4 female). The results for NTX administration alone and in combination with ketamine are presented in Figures 15 through 18 and Tables 15A through 16B. These preliminary data include NTX pretreatments with 1, 10, or 30 mg/kg in combination with an intermediate ketamine dose of 10 mg/kg. The tables provide mean values for distance traveled for each dosing condition, separated by sex, for NTX alone, or across the four NTX pretreatment doses in combination with the ketamine dose.

When administered alone, as shown in Figure 15 and Tables 15A, analysis of these data using a two-way repeated measures ANOVA detected a significant interaction between Sex and Treatment [$F(3,9) = 4.949$, $p=0.0268$], as well as a significant main effect of Treatment [$F(3,9) = 4.726$, $p=5.544$], but failed to detect a main effect of Sex ($p=0.0532$). A Sidak's multiple comparisons test showed the differences were significant in the females at VEH + VEH vs. NTX 1 + VEH ($p=0.0014$), VEH + VEH vs. NTX 10 + VEH ($p=0.0014$), as well as VEH + VEH vs. NTX 30 + VEH ($p=0.0014$). No significant effects were found in the males.

For data in Figure 16 and Tables 16A (female) and 16B (male), various NTX pretreatment doses were used in combination with 10 mg/kg of ketamine. A two-way (Sex x Treatment) ANOVA comparing distance traveled failed to detect a significant main effect of Sex ($p=0.0650$) and Treatment ($p=0.0813$), as well as failed to detect a significant interaction of Sex x Treatment ($p=0.0666$). However, when performing Sidak's multiple comparisons, the test identified significance in the females at VEH + KET 10 vs. NTX 10 + KET 10 ($p=0.0073$).

For data in Figure 17 and 18, the data was then separated based on sex and underwent one-way repeated measures ANOVA (Figure 17, females) (Figure 18, males) to detect significant effects within each sex. In the female group, the one-way repeated measures ANOVA showed a significant main effect of NTX on distance traveled when combined with 10 mg/kg of ketamine [$F(1.901, 5.702) = 7.284$, $p=0.0276$]. Dunnett's multiple comparisons test identified significance in the females at VEH + KET 10 vs. VEH + VEH ($p=0.0372$) as well as VEH + KET 10 vs. NTX 10 + KET 10 ($p=0.0062$) condition. In the male

group, the one-way repeated measures ANOVA showed no effect of NTX on distance traveled when combined with 10 mg/kg of ketamine, ($p=0.6068$).

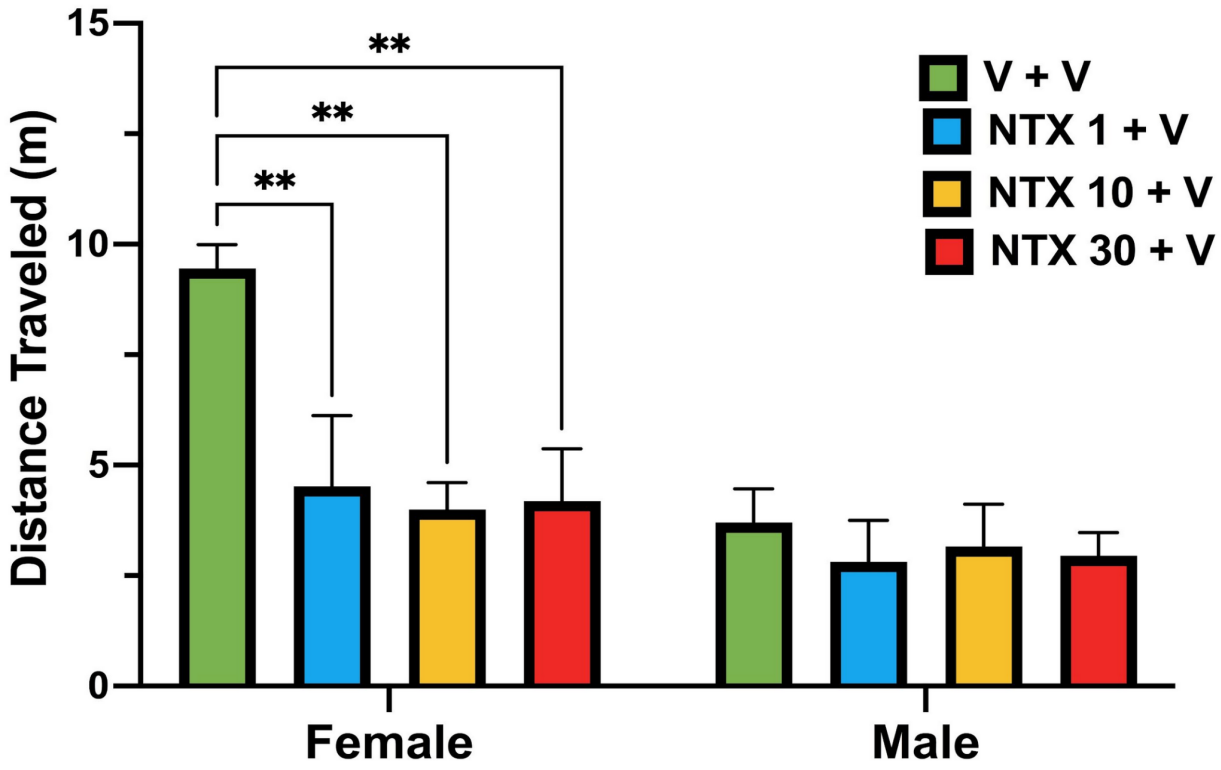

Figure 15: Effects of different doses of NTX (1 mg/kg - 30 mg/kg) alone on activity in an open field for 8 (Male = 4; Female = 4) adult Wistar Kyoto rats. Each bar represents the mean distance traveled (in meters) ±SEM.

Table 15A: Effects of NTX alone on distance traveled in 8 (M=4, F=4) adult Wistar Kyoto rats.

NTX Dose (mg/kg, IP)	Distance Traveled (m)	Distance SEM
VEH	6.58	1.17
NTX 1	3.66	0.92
NTX 10	3.58	0.55
NTX 30	3.83	0.64

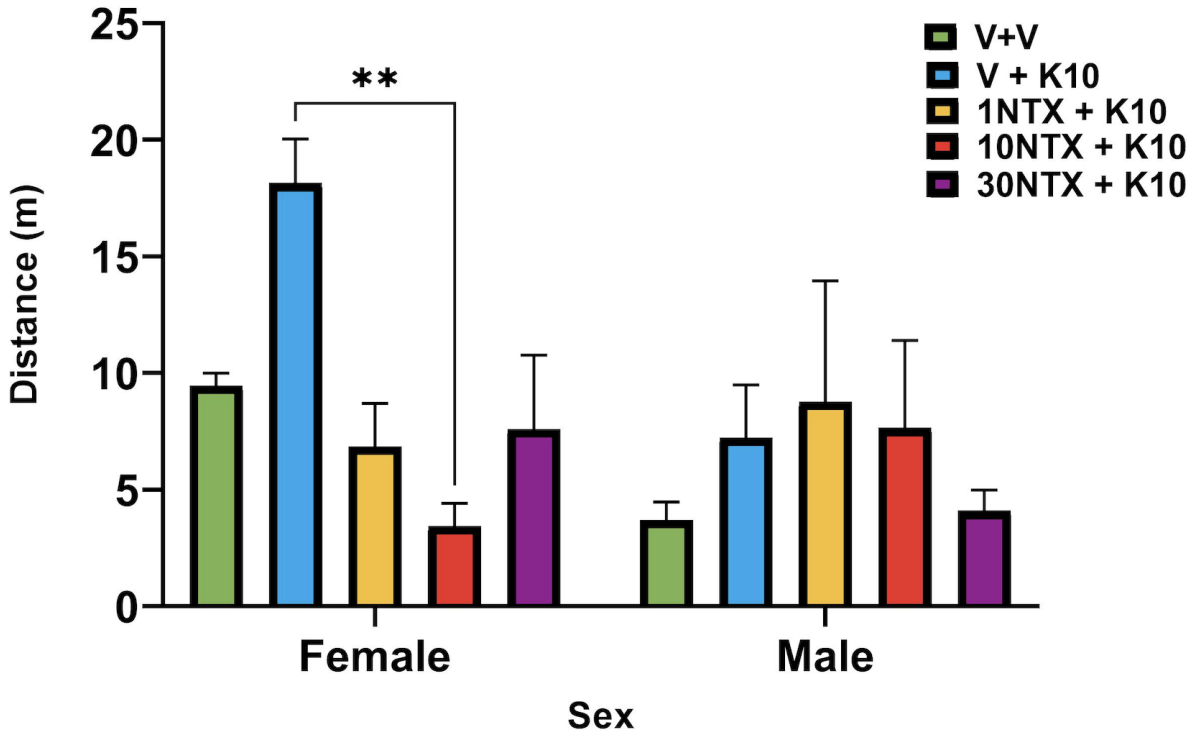

Figure 16: Effects of 10 mg/kg of ketamine following pretreatment with varying doses of NTX (1 mg/kg - 30 mg/kg) on distance traveled in an open field for 8 adult Wistar Kyoto rats (Male = 4; Female = 4). Each bar represents the mean distance traveled (in meters) ±SEM. ** denotes the mean distance traveled is significantly different from VEH + KET 10 at $p \leq 0.01$.

Table 16A: Effects of different NTX + 10 mg/kg of ketamine dosing conditions on distance traveled in an open field in 4 female adult Wistar Kyoto rats.

	Distance Traveled (m)	Distance SEM
VEH	9.45	0.54
VEH + K10	18.15	1.88
NTX 1 + K10	6.84	1.86
NTX 10 + K10	3.44	0.98
NTX 30 + K10	7.60	3.17

Table 16B: Effects of different NTX + 10 mg/kg of ketamine dosing conditions on distance traveled in an open field in 4 male adult Wistar Kyoto rats.

	Distance Traveled (m)	Distance SEM
VEH	3.70	0.77
VEH + K10	7.23	2.26
NTX 1 + K10	8.77	5.19
NTX 10 + K10	7.65	3.74
NTX 30 + K10	4.10	0.88

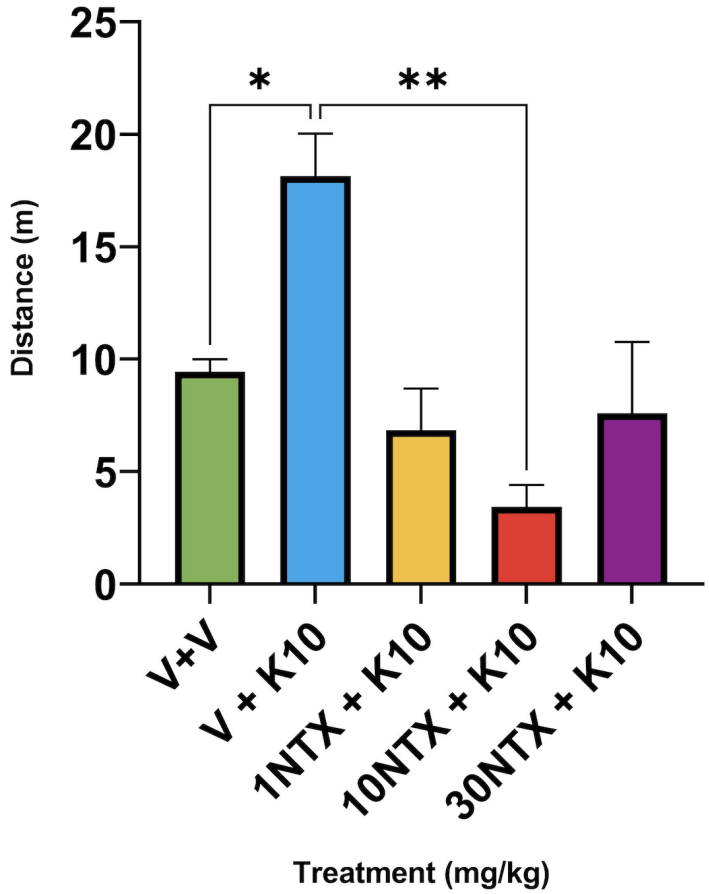

Figure 17: Effects of 10 mg/kg of ketamine following pretreatment with varying doses of NTX (1 mg/kg - 30 mg/kg) on distance traveled in an open field for 4 adult Wistar Kyoto rats (Female = 4). Each bar represents the mean distance traveled (in meters) ±SEM. * denotes the mean distance traveled is significantly different from VEH+K10 at $p \leq 0.05$ and ** $p \leq 0.01$.

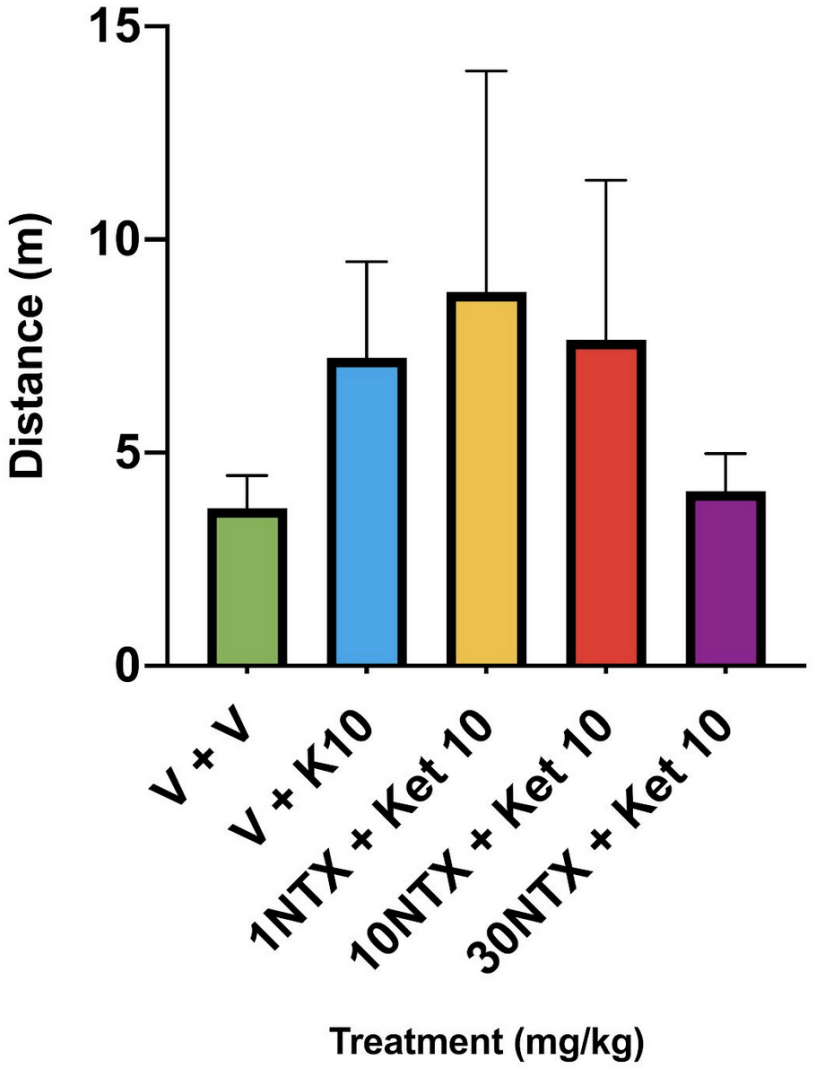

Figure 18: Effects of 10 mg/kg of ketamine following pretreatment with varying doses of NTX (1 mg/kg - 30 mg/kg) on distance traveled in an open field for 4 adult Wistar Kyoto rats (Male = 4). Each bar represents the mean distance traveled (in meters) ±SEM.

Discussion:

Ketamine represents an important therapeutic with ever increasing clinical use for sedation/anesthesia, pain management, and most recently, the treatment of MDD. Ketamine is also being investigated for a variety of other neuropsychiatric disorders including post-traumatic stress disorder, anxiety disorders, chronic neurodegenerative disorders and even substance use disorders. Unfortunately, ketamine produces a variety of undesirable effects which become use-limiting, particularly in the treatment of chronic disorders requiring long term therapy. One approach to improving NMDA/glutamate-based therapies is to develop a novel compound that lacks ketamine's adverse effects. An alternative approach is to continue to utilize ketamine, but combine it with drugs which may decrease its side effects, and therefore improve its overall therapeutic index. Of the different use-limiting effects produced by ketamine, the abuse-related effects are of particular interest to this laboratory. Thus, the overarching goal of this project was to identify a drug/drug class which might have potential to attenuate ketamine's adverse effects. While long-term self-administration studies are planned, for the initial series of experiments, we selected to investigate the effects of three drug classes on ketamine's locomotor activating effects, a behavioral assay associated with increases in dopamine in mesolimbic and nigrostriatal pathways.

Locomotor activation, an increase in activity level as measured by an increase in distance traveled, has been linked to dopamine release in the brain (Chen, 2023; Kokkinou et al., 2018). As discussed previously, most drugs that are abused by humans will produce locomotor activation at one or more doses reflecting dopamine release in the brain, including regions associated with reward and motivation. Therefore, locomotor activation is a commonly used behavioral measure which relates to dopamine release and the abuse-related effects of drugs. Previous studies have demonstrated that acute or chronic ketamine increases locomotor activity which corresponds with rising dopamine levels in the cortex, NAc, and striatum (Chen, 2023; Kokkinou et al., 2018). Thus, locomotor activity provides a high throughput screening approach to assess our drug combinations for the attenuation of ketamine's locomotor activating effects and by extension, theoretically, its abuse-related effects.

The impact of strain and sex on ketamine's locomotor activating effects:

Prior to initiating combination studies, we wanted to more fully characterize ketamine-induced changes in locomotor activity in different subject populations reflecting different potential human populations. This included studying ketamine's effects in two "normal", control strains (WIS and SD rats) as well as its effects in a stress vulnerable rodent model (WKY rats) proposed to mimic aspects of neurobiology and behavior consistent with MDD in humans. In addition, we were interested in the impact of sex on ketamine's locomotor activating effects. Previous studies in this laboratory and others have demonstrated that at low doses of ketamine, 3 mg/kg, or saline-like doses of ketamine, minimal to no change in activity was produced (Irifune, 1991; McDougall et al., 2017; Usun et al., 2013; Wiley et al., 2008, 2011; Yamamoto et al., 2016). At intermediate doses, 10 mg/kg or 30 mg/kg of ketamine, induced an immediate behavioral activation, characterized by hyperlocomotion, potentially due to previously reported ketamine-induced increases in dopaminergic activity (Beninger, 1983; Hetzler & Swain Wautlet, 1985; Lindefors et al., 1997; Razoux, 2007). Lastly, at 56 mg/kg of ketamine, or high doses of ketamine, distance traveled was characterized by a decrease back to vehicle levels due to ketamine's anesthetic and/or CNS depressant effects (Kurdi et al., 2014; Marland et al., 2013).

Overall, the current results are consistent with the predicted outcomes as seen in Figures 1A-5. Regardless of strain or sex tested, when compared to distance traveled following saline administration in all subjects (n=56), intermediate doses of 10 mg/kg and/or 30 mg/kg significantly increased locomotor activity. The effect was most pronounced following 10 mg/kg in females of SD and WKY, and males of WIS and WKY, and 30 mg/kg of ketamine in the WIS, SD and WKY males, where distance traveled dramatically increased by two- to three-fold. This could be explained by an increase in dopaminergic activity, either directly induced by ketamine or indirectly (Can et al., 2016; Hancock & Stamford, 1999; Irifune, 1991; Uchihashi et al., 1992; Usun et al., 2013).

At 56 mg/kg dose of ketamine, data suggests that activity levels have returned to baseline levels. However, when looking at individual subject results, we saw that this high dose actually produced an

activating effect in a subset of subjects, which in turn, led to an increase in distance traveled. In contrast, in other subjects, this high dose produced a strong sedative effect and limited the total distance traveled. Previously published literature reported rodent models injected with moderate to high doses of ketamine exhibited hypoactivity (Hetzler & Swain Wautlet, 1985; Irifune, 1991). This is suspected to be due to the anesthetic effects produced by the drug, through a blockade of NMDA receptors and decreased neuronal excitation (Hetzler & Swain Wautlet, 1985; Irifune, 1991). The variability in response across subjects, some being stimulated and some being sedated, explains why the mean activity level at 56 mg/kg dose was not significantly different compared to saline levels of activity as seen in Figures 1A-5.

In the current study, we initially compared ketamine's effects in male rats from three different strains, WKY, SD and WIS. There was a significant main effect of Strain and that was primarily due to the low level of activity exhibited by the WKY across all treatment conditions. Rats from all three strains showed a trend for increased levels of activity across one or more doses of ketamine. However, analysis showed that only in the control strains, WIS and SD rats, was the increase in distance traveled significant. While this might suggest that ketamine does not cause locomotor activation in WKY rats, interpretation of the results was somewhat complicated by the distinct baseline differences in activity reflected in the different distances traveled following vehicle (saline) administration. In fact, the mean distances traveled were four- (vs. SD) to six-fold (vs WIS) lower in the WKY male rats following saline. When the data were expressed as a percent of the saline control values (Figure 1B), thereby taking into account the differences in baseline behavior, compared to the raw data (Figure 1A), the difference in response to ketamine across strain was no longer present. A significant main effect of Strain was still present, but now it was due to the lower level of response in the WIS rats. Importantly, now all three strains show a similar response to ketamine with locomotor activation occurring at one or more of the intermediate doses (10 and/or 30 mg/kg) in each strain.

In further characterization of ketamine's locomotor activating effects, we determined a ketamine dose response curve in both male and female rats of the SD and WKY strains. Once again, we see a major difference in activity across strain (Figure 2) with WKY rats showing three- to five-fold lower distances

traveled compared to SD rats across all treatment conditions including vehicle. Also similar to the data in the male only rats, while we can detect ketamine-induced locomotor activation in the SD rats, ketamine produced no significant changes in distance traveled by WKY rats when assessing the raw data. It is only when expressed as a percent of the vehicle control that once again, we can identify doses of ketamine causing locomotor activation in WKY rats (Figure 3B and Table 3). The data were further divided based on sex (Figure 3A). Analysis found a significant main effect of Strain and Sex. When testing the female rats, there was a trend for increased levels of activity across multiple doses of ketamine as well as under vehicle control conditions compared to the male rats. When the raw data were normalized based on distance traveled following saline, we were able to identify that ketamine produced significant locomotor activation regardless of Sex or Strain at 10 and 30 mg/kg.

In comparison to previously published literature, the results are consistent with behavioral studies in rodents that have shown differences in the locomotor response to ketamine and other NMDA receptor based on sex, with ketamine being more potent in females than in males (Crawford et al., 2020; McDougall et al., 2017, 2019; Páleníček et al., 2011; Wiley et al., 2011; Wilson et al., 2007). The sex differences observed with ketamine are believed to be pharmacodynamic differences in NMDA receptor numbers and/or subunit composition across sex (Saland & Kabbaj, 2018). Also, it could be based on the inherent biological differences in baseline behavior between males and females that are not specific to the glutamate system (Radford et al., 2020). On the other hand, the increase in locomotor activity could be due to pharmacokinetic factors, such as different rates of metabolism, which result in higher blood level values in females (Páleníček et al., 2011; Saland & Kabbaj, 2018).

The results of Aim 1 characterizing ketamine's effects on locomotor activity demonstrate that while the three strains are genetically and phenotypically different, and the two sexes are biologically and behaviorally different, overall, ketamine is exerting a similar effect, regardless of Strain and Sex. Ketamine causes a dose-dependent increase in activity at one or more intermediate subanesthetic doses suggesting a concurrent increase in dopamine in mesolimbic and nigrostriatal pathways.

Desipramine's effects on locomotor activity and ketamine-induced locomotor activation:

DSP, and its parent compound, imipramine, have extensive literature regarding behavioral effects that focuses on repeated, sub chronic dosing. Additionally, studies often observed the effects of DSP in rodent models of depression, or attention deficit hyperactivity disorder, which would have an altered baseline activity due to the model. Few studies have actually evaluated the effect on locomotor activity following acute dosing in a "normal" rodent model. Based on the studies with comparable conditions (Estrada-Camarena et al., 2004; Umehara et al., 2013), we expected to see DSP produce a dose-dependent decrease in distance traveled. DSP, as with other drugs that enhance serotonin levels, may disrupt dopaminergic neurotransmission in rodents, and may interfere with the reward system (Estrada-Camarena et al., 2004; Lamanna et al., 2021; Umehara et al., 2013; Zagrodzka et al., 1987). Based on the observation that an increase in ketamine-induced locomotor activity is due to an increase in DA, it was hypothesized that DSP would decrease ketamine's locomotor activating effects in OFT. If this effect were due to pharmacodynamic interactions between the two drugs, we would expect DSP to decrease locomotor activation by ketamine at DSP doses which did not themselves suppress activity. Under conditions of the pretreatment with DSP followed by saline (DSP was administered alone) immediately before placement in the open field, we saw a significant dose dependent decrease in activity following administration of the 3 mg/kg dose in females, with a modest decreasing trend in the males (Figure 6; compare Tables 6A and 6B). Overall, there was a trend for sex-associated differences in locomotor activity that was apparent in the subset of animals completing the combination testing (Figure 6; compare Tables 6A and 6B; Figure 7; compare Tables 7A and 7B and Figure 8; compare Tables 8A and 8B). In the study combining DSP and KET 10, a significant main effect of sex was observed. The female rats displayed a two- to threefold increase in activity following KET 10 administration regardless of DSP or vehicle pretreatment. In comparison, and consistent with prior testing in SD males, KET 10 failed to significantly alter activity relative to the vehicle condition, nor did DSP pretreatment alter activity levels when compared to KET 10 pretreated with vehicle. While the sex-dependent effect was lost in the DSP + KET 30 dose response curve as now, a significant main effect of Treatment was produced in both the

females and the males where VEH + VEH condition was significantly different from VEH + KET 30, showing ketamine induced locomotor activation in both males and females. However, as with the KET 10 data, DSP did not alter KET 30's locomotor activating effects. The relevant information gathered from these data is that DSP, which may be administered in combination with ketamine in the treatment of depression, neither attenuates nor enhances ketamine-induced locomotor activation and therefore suggests it does not alter ketamine-induced increases in dopamine in the brain. However, clinically DSP is administered daily and requires two weeks or longer to demonstrate therapeutic efficacy. Therefore, it is possible that under repeated dosing conditions, DSP may alter ketamine's locomotor activating effects.

D-Cycloserine's effects on locomotor activity and ketamine-induced locomotor activation:

DCS, is a NMDA receptor glycine-site partial agonist. In the presence of high levels of glycine, it may function as an antagonist at the receptor, but in the presence of diminished NMDA receptor activity it may function as an agonist at the receptor. This makes DCS of great interest in treating disorders where the NMDA receptor activation occurring is out of balance, either too high or too low. A study reported DCS was able to produce a significant antidepressant effect at 1000 mg/d when combined with MAAs (Heresco-Levy et al., 2013). This antidepressant effect did so without producing ketamine-like psychotomimetic and dissociative effects (Heresco-Levy et al., 2013). Additionally, in combination, DCS was found to improve ketamine-exacerbated schizophrenia (Heresco-Levy & Javitt, 2004). Lastly, DCS has been reported to reverse ketamine's anesthetic effects, which is most pertinent to the study at hand (Irifune et al., 1992). Therefore, while DCS may not completely antagonize ketamine's effects, we believe it may attenuate ketamine's locomotor activation.

Under conditions of the pretreatment with DCS followed by saline (DCS was administered alone) immediately before placement in the open field, we saw no significant change in activity in DCS in males or female rats (Figure 9; compare Tables 9A and 9B). These findings are in agreement with previously published literature in which chronic and acute pretreatment of DCS had no influence on acute locomotor behavior after amphetamine administration (El-Ghundi et al., 2010). When DCS was combined with KET

10, no significant main effect of Sex or Treatment was observed. In the subset of subjects completing DCS + KET testing, neither the males nor the females showed a significant locomotor activation following KET 10 administration. There was a trend for locomotor activity to actually increase following DCS 100 and DCS 300 pretreatment in both sexes, but the effect was not significant. These results are similar to a study which found that DCS induced potentiated locomotor stimulation produced by NMDA antagonists (uncompetitive = MK-801 and competitive = D-CPPene) combined with clonidine in mice (Carlsson et al., 1994). Ultimately, further testing of DCS in combination with ketamine is warranted to clarify the relationship between DCS and locomotor activity. In the DSP + KET 30 data, a significant main effect of Treatment was witnessed in both the females and the males in comparison to the VEH + VEH. Distance traveled increased 3- (male) to 5-fold (female) following KET 30 administration. DCS pretreatment produced only modest, non-significant decreases in KET 30-induced locomotor activation. Therefore, the relevant information gathered from these data is that DCS, which may be administered in combination with ketamine in the treatment of depression, does not appear to attenuate nor enhance ketamine-induced locomotor activation and therefore suggests it does not alter ketamine-induced increases in dopamine in the brain.

Naltrexone's effects on locomotor activity and ketamine-induced locomotor activation:

In 2018, the first piece of evidence suggesting a role for opioid receptor activation in ketamine's acute antidepressant effects in human patients was published (Williams et al., 2018). Thus, NTX, an opioid receptor antagonist, in combination with ketamine, may also be able to block other behavioral effects of ketamine, including the locomotor stimulating effects.

At mu and kappa opioid receptors, ketamine demonstrates low binding affinity and moderate efficacy (Zanos & Gould, 2018). In 2021, a study demonstrated that NTX could block the antidepressant-like effects of ketamine in mice in differential reinforcement of low rates of responding (DRL) (F. Zhang et al., 2021). However, other studies demonstrate that 10 mg/kg dose of NTX failed to block the antidepressant-like effects of 10 mg/kg of ketamine in mice in the forced swim test (FST) (K. Zhang &

Hashimoto, 2019). It is a possibility that the findings differ due to the different routes of administration, as that affects bioavailability, as well as different clinical and preclinical models. To our knowledge, no studies have been performed to determine whether or not opioid receptors play any role in either the locomotor activating or the reinforcing effects of ketamine. Therefore, we hypothesized that the acute pretreatment with the opioid antagonist, NTX, would not decrease ketamine's locomotor activation.

When tested alone, in the SD rats, NTX produced a significant decrease in distance traveled in the females at the 10 and 30 mg/kg dose of NTX (Figure 12; Table 12A). However, there was no significant effect on distance traveled in the males. Similar to our other treatment groups, KET 10 produced locomotor activating effects in the female rats only [Figure 13 and Tables 13A (female) and 13B (male)] with distance traveled increasing by over 400%. The same dose of ketamine in the male rats produced a modest, but nonsignificant increase in activity. Following various NTX pretreatment doses, modest but insignificant decreases in activity, relative to the VEH + KET 10, were seen in both males and females, suggesting limited efficacy of NTX to alter ketamine's effects. An interesting observation was the biphasic curve in the male rats, where the NTX 10 + KET 10 combination showed a moderate nonsignificant decrease, followed by an increase in locomotor activity for the NTX30 + KET 10 combination (Figure 13). VEH + KET 30 produced a dramatic increase in activity in both sexes (Figure 14) relative to VEH + VEH conditions. Pretreatment with NTX doses showed a trend for production of biphasic curves, with the NTX 10 combination showing the most dramatic decrease in distance traveled, but returning to vehicle baseline levels in the NTX 30 combination (Figure 14). This unusual effect, combined with unexpected results in other behavioral assays in the laboratory investigating KET/NTX combinations (personal communication F. Zhang), sparked an interest in determining if this effect would also occur in the stress vulnerable WKY rats.

As a follow up, the study of NTX and KET combination was also initiated in a subset of WKY rats (N=8; Male = 4, Female = 4). At this time, testing has only been completed for NTX in combination with KET 10 (Figure 16; compare Tables 16A and 16B).When tested alone, in the WKY rats, NTX produced a dramatic decrease in distance traveled in the females at all three doses, 1 mg/kg, 10 mg/kg,

and 30 mg/kg dose of NTX, whereas there was no change in activity from baseline in the males, (Figure 15; Table 15A). When various NTX pretreatment doses were used in combination with 10 mg/kg of ketamine, we again see the interesting observation in the females where the NTX 10 pretreatment dose significantly reduces ketamine-induced locomotor activation, followed by a return of locomotor activation following NTX 30 pretreatment (Figures 16 and 17; tables 16A and 16B). It is unclear what is causing this effect, it is possible that kappa antagonism produces opposite effects to those of mu antagonism, which might help explain this u-shaped curve. Regardless it has been demonstrated in two different strains and in both sexes, and warrants further investigation. Studies in WKY rats using KET 30 in combination with NTX are ongoing.

Conclusion

In conclusion, the observed effects of ketamine alone were consistent with both published literature and previous work completed in this laboratory. This included locomotor activation at intermediate doses of ketamine with a sex-dependent difference in sensitivity to these activating effects. When the data were expressed as a percent of the saline control values (Figure 1B), thereby taking into account the differences in baseline behavior, compared to the raw data (Figure 1A), the difference in response to ketamine across strain was no longer present. The overarching goal was to explore the possibility of decreasing locomotor activating effects utilizing our three test compounds, DSP, DCS and NTX in combination with ketamine. The effects of DSP on ketamine-induced locomotion were modest and inconsistent. Additionally, DSP suppressed activity when administered alone. DCS alone produced no significant dose dependent decrease in activity. However, it failed to alter ketamine-induced locomotor activation. Lastly, NTX administered alone suppressed activity at the intermediate and high dose. This non-selective suppression of behavior likely accounted for the moderate decreases in ketamine-induced locomotor stimulation that was observed. While so far, our test compounds have failed to demonstrate clinically useful effects, future testing with alternate MAAs (e.g., an SSRI) as well as testing ketamine following repeated administration of an MAA, may provide a better therapeutic outcome.

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