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The Effects of Repeated Ketamine Administration During Adolescence on Anxiety and Depressive-like Behaviors Induced by a Predator Odor

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by

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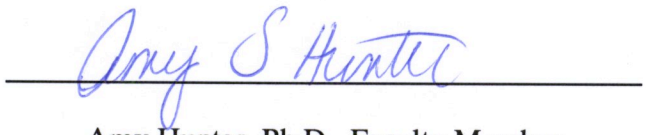

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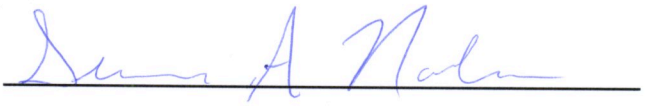

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Abstract

Disorders of anxiety and depression are major public health problems evident by their increasing prevalence and lack of effective treatments. These disorders are highly comorbid and share several debilitating symptoms. Previous research has implicated ketamine in the treatment of depression because of its rapid effects. Ketamine is a noncompetitive NMDA receptor antagonist that stimulates glutamate transmission. The efficacy of non-competitive NMDA receptor antagonists in anxiety treatment, however, lacks consistent findings. The goal of the present study was to examine the effects of repeated ketamine administration during rat adolescence on anxiety and depressive-like behaviors induced by a predator odor.

The use of animal models of anxiety is essential to understanding the neuropathology of anxiety and depression and developing new drug treatments. A common non-invasive animal model of anxiety involves exposing the animal to the odor of a natural predator. Ketamine was administered from PND 45-51, which is considered a conservative age range for adolescence (Spear, 2000). On PND 65, half of the rats were exposed to the odor of a domestic cat, which was presented via cat collars and hand towels attached to the home cage. All animals were then subjected to the sucrose preference test (SPT) and the elevated zero maze (EZM) in order to measure the presence of depressive and anxiety-like behaviors, respectively. There was a drug x odor interaction effect trending toward significance in the habitation phase of the SPT, indicating that the ketamine reversed the reduction in sucrose preference due to the predator odor. There were no significant results from the test phase of the SPT. The elevated zero maze was conducted on two consecutive days. Results for day 2 of EZM testing revealed a significant main effect of odor indicating that the rats exposed to the predator odor made significantly fewer entries into the open quadrants compared to the rats exposed to the control odor. The results demonstrate that ketamine only influenced depressive-like behaviors, thus indicating dissociation between the drug's effect on models of depression and anxiety. Overall, the results provide some support for the preventative effects of ketamine on depressive-like behaviors.

The Effects of Repeated Ketamine Administration During Adolescence on Anxiety and Depressive-like Behaviors Induced by a Predator Odor

Disorders of anxiety share features of excessive fear and anxiety as well as related behavioral disturbances. Generalized anxiety disorder (GAD) is defined as excessive anxiety and worry, occurring more days than not for at least 6 months, about a number of events or activities, such as work or school performance (Diagnostic and Statistical Manual of Mental Disorders, 5th ed.; DSM-5; American Psychiatric Association, 2013). Approximately 19.1 million, or about 13.3%, American adults ranging in age from 18 to 54 have an anxiety disorder (Bener, Ghuloum, & Abou-Saleh, 2012). The World Health Organization projects disorders of anxiety to become one of the top two most burdensome disorders in the world by the year 2020 (Yang et al., 2012). Although effective treatments exist, many individuals can suffer for years after treatment onset. Between 20 and 50% of patients do not respond to drug treatment depending upon the specific disorder (Nordquist, Steckler, Wettstein, Mackie, & Spooren, 2008). When left untreated, anxiety disorders can lead to long-term disability (Kroenke, Spitzer, Williams, Monahan, & Lowe, 2007) and substantial social burden (Bener & Ghuloum, 2010). Furthermore, chronic stress can lead to premature death (Kroenke et al., 2007) due to an increased risk for upper respiratory infection, accelerated progression of coronary artery disease, and a more severe course for autoimmune disorders (Miller, Chen, & Zhou, 2007).

Depression is a major public health problem evidenced by its high ranking among the global burden of diseases (Bener et al., 2012). Major depressive disorder (MDD) afflicts approximately 17-20% of the population and is a leading cause of disability and economic hardship (Garcia et al., 2009). Among those suffering from MDD, 75-80% have recurrent episodes (Mueller et al., 1999) and 10-30% do not reach complete symptom remission (Mann,

2005). MDD is defined as displaying five or more of the following symptoms for a period of two weeks nearly every day: depressed mood, markedly diminished interest in almost all activities, significant weight loss or decrease in appetite, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or excessive or inappropriate guilt, diminished ability to think or concentrate, or recurrent thoughts of death (5th ed., DSM-5; American Psychiatric Association, 2013). Additionally, depression is one of the most costly psychopathologies and is a leading cause of morbidity and mortality worldwide (Garcia et al., 2009). While the underlying pathology associated with depression is still unknown, some researchers believe it could result from molecular and cellular anomalies that interact with environmental and genetic factors (Krishnan & Nestler, 2008).

The current pharmacotherapies available for treating depression include tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin–noradrenergic reuptake inhibitors, and other atypical antidepressant drugs such as monoamine oxidase inhibitors (MAOIs; Nemeroff, 2007). Unfortunately, the efficacy of these antidepressants is often unreliable, and many of them produce unwanted side effects including fatigue, sleep disturbance, tremor, sweating, loss of appetite, weight gain, and sexual dysfunction (Kikuchi, Uchida, Suzuki, Watanabe, & Kashima, 2011). The most serious limitation of current antidepressants is that they require a few weeks to months to induce a therapeutic response, and only about one third of patients respond to their first prescribed antidepressant (Li et al., 2010). This is problematic because depression is associated with high rates of suicide and is projected to become the second leading cause of death worldwide by the year 2020 (Rosenzweig-Lipson et al., 2007). Thus, there is an ever-increasing need for more effective and better-tolerated antidepressants.

Anxiety and Depression Comorbidity

The comorbidity of anxiety disorders and depressive disorders has been widely investigated. More than 80% of patients suffering from anxiety will have a comorbid mental illness during their lifetime. The prevalence of having a current comorbid axis I disorder is 50%, with anxiety and depressive disorders being the most common comorbid disorders (Brown, Campbell, Lehman, Grisham, & Mancill, 2001). Anxiety and depression share many common symptoms including irritability, loss of sleep and appetite, lethargy, and disruptions in social and cognitive functioning. However, each disorder has one unique symptom that allows for differentiation between the two disorders. Anxiety disorders can be diagnosed by the presence of unrealistic and excessive worry and/or fear (McGrandles & Duffy, 2012), and depression can be diagnosed by the core symptom of anhedonia (Pohl, Olmstead, Wynne-Edwards, Harkness, and Menard, 2007). People suffering from both anxiety and depression experience more disability and distress compared to people suffering from only one of these disorders (Cairney, Corna, Veldhuizen, Herrmann, & Streiner, 2008).

There exists some inconsistencies regarding the comorbidity rate mainly due to varying symptoms among patients. In 2004, the American Psychiatric Association reported that approximately 90% of patients with GAD have at least one other mental disorder, with 50% also having a major depressive episode by age 18 (4th ed., DSM-5; American Psychiatric Association, 1994). Hek and colleagues (2011) conducted a study investigating the number of people with anxiety disorders who also suffer from current depression. They found that of all the people with a current anxiety disorder, 17.9% currently had comorbid depression. In contrast, only 2.8% of people without an anxiety disorder had current depression. Comorbidity between depression and an anxiety disorder is associated with greater psychiatric symptom severity (Cyranowski et al., 2012; Klein Hofmeijer-Sevink et al., 2012). In a study conducted by Cyranowski and colleagues,

data from 915 women aged 42 to 52 with a comorbid history of MDD and anxiety reported greater symptom severity, diminished social support, and more distressing life events in the past year compared to women with either MDD or anxiety alone.

Anxiety and Its Underlying Neural Mechanisms

Stress is an important risk factor for the development of certain psychopathologies, including anxiety disorder (Nestler et al., 2002). Scientists have been trying to understand the biological mechanisms involved in chronic stress that cause adverse psychological outcomes. One possible mechanism that has been gaining widespread support is the hypothalamic-pituitary-adrenal (HPA) axis. This system exists within all mammals and becomes activated in the presence of mental and physical stressors. When an animal of prey detects a nearby predator, neurons in the paraventricular nucleus of the hypothalamus secrete corticotropin-releasing hormone. This secretion causes the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary, concluding in the secretion of glucocorticoids (cortisol in humans and corticosterone in rodents) from the adrenal glands into the circulatory system (Kalynchuk, Gregus, Boudreau, & Perrot-Sinal, 2004). Due to ethical issues that arise from human research, animal models are indispensable for investigating the biological bases of anxiety disorders and therefore developing better pharmacological and behavioral therapies (Steimer, 2011). Research has shown that a 30-minute exposure to predator odor activates HPA axis output in adult rats (Masini, Sauer, & Campeau, 2005).

When stress is present, the hypothalamus releases hormones that create a threat to the animal's state of equilibrium. During this period, the animal experiences physiological changes including increased heart rate, blood pressure, and muscle tension. Although anxiety typically has a negative connotation, a small degree of anxiety can be beneficial: it can enhance

performance on a test or heighten an individual's awareness to protect him or herself from danger (McGrandles & Duffy, 2012). This physiological mechanism has survival value because it prepares the animal for flight or fight. When the danger is no longer threatening, these elevated levels subside and the organism returns to a pre-stress level of functioning. Thus, normal HPA axis activation is necessary for survival because it helps the body sustain a state of equilibrium (Kalynchuk et al., 2004). If successful adaptation to stress does not occur, the body maintains this state of hyperarousal and chemical imbalance (Miller et al., 2007). Sustaining this emergency state can have deleterious physiological effects and damage normal brain functioning (Kalynchuk et al., 2004). For example, research has found that exposing a rat to high levels of corticosterone (CORT) reduces dendritic branching of CA3 pyramidal neurons, decreases hippocampal neurogenesis, and decreases hippocampal glucocorticoid receptor mRNA expression (Fuchs & Gould, 2000; Vyas, Mitra, Rao, & Chattarji, 2002). Altered synaptic transmission in the hippocampus has been implicated in both GAD and post-traumatic stress disorder (Chen & Etkin, 2013).

There is a consistent body of literature discerning the action of cortisol in response to stress. Studies have demonstrated that the HPA axis becomes activated and leads to a subsequent increase in ACTH and cortisol immediately following a stressful event. As time passes, cortisol secretion rebounds to below normal levels in an attempt to counteract the initial activation (Miller et al., 2007). Changes in cortisol concentrations affect important physiological processes including immunity, growth, reproduction, and metabolism (Kallen et al., 2008). The time-dependent pattern of cortisol is consistent with studies conducted by several researchers (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Miller, Cohen, & Ritchey, 2002). Cortisol has widespread regulatory influences due to its involvement in learning, memory, and emotion in the

central nervous system (CNS). Additionally, it regulates glucose storage and usage in the metabolic system, and regulates the maturation of lymphocytes and the magnitude of inflammatory responses in the immune system. Scientists have demonstrated that cortisol is the primary biological mechanism through which chronic stressors negatively affect the body. Accumulated stress plays a role in the onset of mental health disorders such as depression and schizophrenia as well as physiological illnesses such as cancer, arthritis, diabetes, and obesity. Researchers believe that stress triggers an increase in cortisol secretion, thereby exposing bodily tissue to an overabundance of the hormone. When this exposure is chronic or sustained, there is subsequent tissue damage and dysregulation of biological systems (Kalynchuk et al., 2004; Miller et al., 2007; Campeau, Nyhuis, Sasse, Day, & Masini, 2008; Wright, Muir, & Perrot, 2013).

The amygdala is the brain structure most frequently implicated in the temporary expression of fear (Pietersen et al., 2006). Current research is investigating methods of fear extinction in animals with the goal that such treatments might be effective in facilitating exposure-based therapy for individuals with clinical anxiety disorders (Davis, Myers, Chhatwal, & Ressler, 2006). Human imaging studies utilizing functional magnetic resonance imaging and positron emission tomography (PET) have demonstrated increased blood flow to the amygdala in the presence of conditioned and unconditioned fearful stimuli. If the amygdala is damaged or inactivated, the animal will not acquire or express conditioned fear responses (Davis et al., 2006). The particular amygdalar subregions involved in this biological system have yet to be established, although many studies have implicated the role of the central nucleus of the amygdala (CeA) in the expression of autonomic responses. The basolateral nucleus (BA) sends Ɣ-aminobutyric acid (GABA) interneurons to the CeA which then send interneurons to the

hypothalamus, brain stem, and basal forebrain regions that directly control autonomic, hormonal and behavioral responses to emotional stimuli (Forster, Novick, Scholl, & Watt, 2012). Thomas, Burock, Knudsen, Deterding, and Yadin (2013) examined single unit activity in the lateral septum (LS) and CeA in the elevated plus maze (EPM). The EPM is a measure of behavioral anxiety that consists of two enclosed arms and two open arms, thus forming a cross. An animal that spends more time in the enclosed arms compared to the open arms is said to be demonstrating anxiety-like behavior. They found that when the animals were exposed to the EPM, there was a decrease in CeA firing and an increase in LS firing when standing in the open arms. These results suggest that when placed in the open arms, a compensatory process takes place that suppresses fear in order for the rat to employ adaptive behavior.

The monoamine neurotransmitters, dopamine (DA), serotonin (5-HT), and norepinephrine (NE), have been linked to disorders of anxiety. Drugs that alter monoaminergic transmission tend to be effective treatments for anxiety disorders. Several studies have shown 5-HT (Inoue, Koyama, & Yamashita, 1993), DA, and NE activation immediately following foot shock, restraint, and fear conditioning (Emoto et al., 1993; McIntyre, Hatfield, & McGaugh, 2002; Yokoyama et al., 2005). The dorsal raphe nucleus gives rise to 5-HT that innervates the amygdala, while the locus coeruleus and ventral tegmental area give rise to NE and DA, which then innervate the amygdala (Forster et al., 2012). This research stimulated the development of anxiolytics that work by enhancing GABAergic transmission (Wierońska, Nowack, & Pilc, 2010). Although these GABA-enhancing drugs (benzodiazepines and antidepressants) are effective, some patients do not respond to this treatment, which has led researchers to uncover alternative target receptors for treating anxiety.

The *N*-methyl-D-aspartate (NMDA) receptor has recently been considered as a target for

treating anxiety because the underlying pathologies of many neurodegenerative and psychiatric disorders are linked to glutamate imbalance. Because glutamate is the main excitatory neurotransmitter in the CNS, its function is to maintain normal brain functioning involving cognition, memory, and learning. Glutamate binds to the NMDA receptor and has high concentrations in the amygdala. Due to the high comorbidity rate between anxiety and depression, NMDA receptor antagonists also have been considered for treating depression. Additionally, the questionable efficacy of MAOIs necessitates a treatment that acts on a different neural pathway. MAOIs block the reuptake or breakdown of monoamines, thereby acting on the neuromodulatory systems (Duman & Voleti, 2012). In contrast, NMDA receptor antagonists work to increase glutamate transmission and the induction of synaptogenesis (Li et al., 2010).

Depression and Its Underlying Neural Mechanisms

The major brain regions involved in regulating mood include the prefrontal cortex (PFC), the hippocampus, and the amygdala. Many clinical studies have shown that stress and depression are associated with atrophy of neurons and glia, which lead to a reduced size and function of the limbic regions, including the PFC and hippocampus (Li et al., 2010). Additionally, magnetic resonance imaging and PET studies consistently report that decreased PFC and hippocampus volume sizes precipitate illness and are associated with a longer duration of ailment (Savitz & Drevets, 2009). The decreased size of the PFC is due to a reduction in pyramidal neuron volume as well as a decrease in the number of GABAergic interneurons and glia (Rajkowska, O'Dwyer, Teleki, Stockmeier, & Miguel-Hidalgo, 2007). These findings support the claim that depression is a neurodegenerative disorder, although neuronal decline can be ameliorated by treatment and stress alleviation (Duman & Voleti, 2012). The amygdala, on the other hand, experiences a somewhat different alteration in depression. Instead of neuronal atrophy, studies of the amygdala

have shown neuronal hypertrophy, including increased dendrite complexity in the BA (Roozendaal, McEwen, & Chattarji, 2009). This hyperactive state can lead to increased anxiety, fear, and emotion (Sotres-Bayon & Quirk, 2010).

One theory regarding the underlying pathophysiology of MDD involves brain-derived neurotrophic factor (BDNF). BDNF is the most abundant neurotrophin in the CNS among mammals and the most studied neurotrophin in the pathophysiology of MDD (Machado-Vieira et al., 2009). Neurotrophins are a multigene family of polypeptide growth factors that exert their effects by binding to receptors on the surface of responsive cells (O'Hanlon, Mabry & Ekekezie, 2014). Postmortem studies have found that depressed patients have reduced hippocampal and cortical BDNF levels, which is consistent with the down-regulation of BDNF in the presence of stress (Altar, 1999). Evidence from animal studies indicates that environmental stressors lead to the onset of depression by suppressing BDNF gene expression in the hippocampus. Bai and colleagues (2012) investigated whether stressors that alter hippocampal BDNF expression and subsequent depressive behavior vary with different stressors. The researchers utilized two classical chronic stress paradigms: maternal deprivation (MD) and chronic unpredictable stress (CUS) to test the role of BDNF in early life stress in Sprague-Dawley rats. They found that BDNF protein expression tended to be the lowest in the MD group and that the MD and CUS groups floated for a significantly longer time in the forced swim test (FST) compared to controls. In conclusion, the down-regulation of the BDNF gene in the hippocampus indicates that different stressor-induced depressive phenotypes may result from dissimilar molecular foundations.

Most antidepressants work by increasing the level of biogenic amines including NE, DA, and 5-HT via blocking reuptake or inhibiting degradation (Ates-Alagoz & Adejare, 2013). Although these medications are able to successfully stabilize mood for many individuals, only

one third of all depressed patients have considerable improvement with a standard antidepressant after two months. Additionally, many patients have to deal with undesirable side effects (Rush et al., 2006). Antidepressants also produce therapeutic effects by reducing inflammatory responses. Based on this information, researchers posit that the etiology of depression is associated with an elevated expression of proinflammatory cytokines (Yang et al., 2012). Developing a more effective antidepressant remains a challenge because the target receptor has yet to be identified. Even more challenging is trying to figure out why an antidepressant will exert a therapeutic effect in one individual but not in a different individual. While NMDA receptor antagonists have been shown to produce rapid, antidepressant effects, the mechanisms underlying antidepressant action are more complicated than NMDA receptor blockage and have yet to be discovered.

Although the pathophysiology of depression remains unknown, several studies have demonstrated that the ionotropic glutamate NMDA receptor plays a major role in the etiology of depression (Javitt, 2004). Dysfunction of the glutamate neurotransmitter system is due to altered levels of glutamate in the blood, cerebral spinal fluid, and brains in patients with MDD and bipolar disorder (Hashimoto, Sawa, & Iyo, 2007). Over-stimulation of NMDA receptors leads to an excessive Ca^{2+} influx that is implicated in many neurodegenerative diseases (Ates-Alagoz & Adejare, 2013). Several studies have identified the NMDA receptor as a target for fast-acting antidepressants. Ketamine has been implicated in treating depression because it is a noncompetitive antagonist of the NMDA calcium pore. Recent research has found that acute and chronic doses of ketamine exerted prolonged antidepressant-like effects evidenced by decreased immobility in the FST, an animal measure of depression, as well as increased NMDA receptor density in the hippocampus (Tizabi, Bhatti, Manaye, Da Sa, & Akinfiresoye, 2012). Further evidence for the glutamate theory is derived from preclinical studies that have shown that

NMDA receptor antagonists such as MK-801, CPP, AP7 and neramexane exert anxiolytic and antidepressant effects in rats when injected into specific brain regions (Kos, Legutko, Danysz, Samoriski, & Popik, 2006; Molchanov & Guimarães, 2002; Menard & Treit, 2000).

Ketamine is a noncompetitive NMDA receptor antagonist that stimulates glutamate transmission (Autry et al., 2011). Because the effects of ketamine are dose dependent, this drug has a variety of uses. At higher doses, ketamine is used as a general anesthetic in medical and veterinary clinics. Since the 1960s, ketamine has been classified as a "dissociative anesthetic" because of its sedative and hallucinogenic properties. In addition, it is commonly used as an anesthetic in pediatric surgery because of its bronchodilating effects and is part of the anesthetic procedures commonly used in electroconvulsive shock therapy in the cases of severe depression (Engin, Treit, & Dickson, 2009). At lower, sub-anesthetic doses, ketamine is able to mimic the effects of an antidepressant (Zarate et al., 2006). It was originally considered for use as an antidepressant due to its unique ability to block the NMDA receptor. Unlike other NMDA antagonists, ketamine has slower open-channel blocking, a "trapping block" type of channel closure, and a nonselective blockade of voltage-dependent calcium channels along with opioid, monoaminergic, and muscarinic receptors (Garcia et al., 2009). Although the evidence for ketamine's antidepressant-like effects are compelling, there is a lack of double-blind, placebo-controlled studies. Additional studies must be conducted to assess the severity of ketamine's psychoactive and euphoric effects at clinical doses (Popik, Kos, Sowa-Kućma, & Nowak, 2008).

Previous research investigating the effectiveness of non-competitive NMDA receptor antagonists has revealed inconsistent results. Recent research, however, has been providing robust evidence for ketamine's anxiolytic effects. For example, Pietersen and colleagues (2006) investigated how acute NMDA receptor blockage with ketamine affects frequency and duration

of freezing as well as associated neural changes in the amygdala, PFC, and hippocampus in fear-conditioned rats. The results indicated that ketamine normalized stress-related behaviors in areas associated with fear and anxiety. Amann and colleagues (2009) found that a chronic, low dose of ketamine reduced freezing behavior in a context fear-conditioning paradigm in ketamine-treated mice as compared with saline-treated mice. Parallel with these findings are those from an experiment conducted by Loss, Córdova, and Oliveira (2012) who found that ketamine administration 15 and 60 minutes after pilocarpine administration (a cholinergic agonist) significantly increased time spent in the open arms of the EPM compared to the ketamine-only groups.

As previously mentioned, a major limitation of current antidepressant treatment is that monoaminergic-based drugs require two to three weeks to exert a therapeutic effect (Maeng et al., 2008). The clinical use of ketamine has gained broad attention because of its rapid (within two hours) therapeutic effects, as well as effects that last for a significant amount of time after a single dose in treatment-resistant patients (Zarate et al., 2006). It has been known for over 15 years that NMDA receptor antagonists produce antidepressant effects after a single administration, evident by measures such as the FST and the tail suspension test (TST; Popik et al., 2008). Ketamine's antidepressant effects after acute administration have been well established. Li and colleagues (2012) found that acute treatment with a non-competitive NMDA channel blocker and the NR2B antagonist, Ro25-6981, tends to improve anhedonia and anxiogenic behaviors induced by chronic mild stress. In order to provide more compelling evidence for human application, future research must investigate the efficacy of long-term treatment paradigms.

Drug Administration in Adolescence

Early life experiences can have a profound effect on an individual's psychological functioning as an adult. A great deal of research seeks to understand how drug administration during adolescence affects human behavior and neurobiology during adulthood. The primary focus of these studies has been on nicotine and ethanol, as both are widely abused during the teenage years (Spear & Varlinskaya, 2005). Age-dependent alcohol studies have demonstrated how adolescent alcohol abuse increases the risk for, or vulnerability to, certain disorders in adulthood. Although ethanol and nicotine are the most commonly abused drugs in adolescence, other drugs including marijuana, 'club drugs', and inhalants are also frequently abused by adolescents (Wiley, Evans, Grainger, & Nicholson, 2008). Ketamine is one of the most dangerous club drugs because of its toxic effect on the CNS (Warren et al., 2006). Wiley, Evans, Grainger, and Nicholson (2011), investigated the acute and repeated effects of ketamine and other 'club drugs' in female adolescent and adult rats. They found that ketamine did not alter activity acutely but did increase ambulatory activity when administered sub-chronically. A few years later, Parise and colleagues (2013) found that ketamine administration in adolescence [postnatal day (PND) 35] reversed the effects of CUS-induced depressive-like behaviors in the FST. In conclusion, these studies illustrate the potential for development of psychopathologies in adulthood as a function of drug administration during adolescence.

The present study will be utilizing an animal model of anxiety to expose rats to the stressor of predator odor. As discussed by Spear (2000), the age span of adolescence in nonhuman animals is often debated among researchers in the field. Conclusions regarding the boundaries of adolescence have not been reached because of variations in gender and growth rate within individual species. Additionally, limited research on biosociobehavioral function during this time period has been conducted. Most research on adolescence focuses on the

neuroendocrinology of puberty and the catalysts of these changes. A conservative age range for adolescence in rats is PND 28-42, which was derived from measures such as timing of growth spurt, loss of amino acid influx to the PFC, and the timing rats emerge from the protected burrow in their natural habitat. The margins of this conservative age range do not imply that animals younger or older than this range are not experiencing some adolescent transitioning. For example, females may experience ontogenetic changes reflective of adolescence as early as 20 days. Additionally, males can display signs of puberty as late as 55 days. Using a broader range of adolescence is wise, especially in experiments designed to expose the animals to a treatment for the entire ontogenetic window. The commonly used age range for rat adulthood is PND 70-90, which follows a late adolescence age range from PND 35-55 (Broadwater & Spear, 2013).

Animal Models of Anxiety

Exposure to Predator Odor Paradigm

Animal models of anxiety are essential to understanding the neuropathology of anxiety and depression and developing new drug treatments. There are several ways to model human anxiety disorders in rodents including repeated CORT injections (Kalynchuk et al., 2004), chronic psychosocial stress models (Slattery et al., 2012) and repeated footshock (Kassai & Gyertyán, 2012). Although these methods are effective models of anxiety, another approach is to utilize natural fear-inducing stimuli that have greater ethological relevance. One currently popular model is the use of predator odors to provoke defensive behaviors. Laboratory rats, in particular, are instinctively fearful of live cats and their fur/skin odor. Although the type of predator odor and defensive mechanism employed are species-specific, defensive behaviors occur and have survival value for all mammals when faced with a potentially dangerous

environmental threat.

Exposure to a predator odor is a paradigm used to understand the neurobiology of anxiety in animal models. Predator odors have an advantage over other stressor models in that they cause no pain or physical injury to the animal. In addition, the behavioral and endocrine response to the odor is innate, meaning the rats do not need to be trained to respond to the stimulus (Masini et al., 2005). The predator odor exposure paradigm uses the principles of unconditioned fear to assess the frequency of defensive behaviors to the odor of a predator. There are several ways to conduct a predator odor paradigm for rodents including exposure to 2,4,5-trimethlythiazoline (TMT), ferret skin/fur odor, and cat skin/fur odor.

TMT

TMT is the synthetic version of a stimulus naturally present in the feces of red foxes *(Vulpes vulpes;* Fendt, Endres, Lowry, Apfelbach, & McGregor, 2005). A major advantage of TMT over alternative methods is the quantifiable nature of this synthetically derived odor. Unlike cat and ferret odor, the stimulus intensity (concentration and volume) can be easily controlled and emulated. Several studies have demonstrated the ability of TMT to induce physiological changes in rodents, such as induction of serum corticosterone (Morrow, Elsworth, & Roth, 2002) and behavioral modifications including changes in food consumption (Endres, Apfelbach, & Fendt, 2005), avoidance, freezing, and risk assessment behaviors (Hacquemand, Choffat, Jacquot, & Brand, 2013). For example, in a study conducted by Hebb and colleagues (2004), mice exposed to TMT displayed significant defensive behaviors including freezing and anxiogenic behaviors in the light-dark test. However, one major disadvantage of this method is that not all laboratories that employ TMT exposure are able to induce fear using this synthetic derivative (Endres & Fendt, 2007; Wallace & Rosen, 2000; Blanchard et al., 2003). In addition,

it has been noted that using odors derived from feces are not as effective as using odor from fur because feces can be found long after a predator has departed, thereby reducing their utility as an indicator of the presence of a predator. In addition, predators tend to defecate in areas where they do not hunt, thereby eliminating the threat of an impending attack. Another disadvantage of using TMT is that it produces less clear and robust effects than that of cat/fur odor. For example, Morrow, Redmond, Roth, and Elsworth (2000) found that TMT exposure did not alter any motor behaviors (immobility, grooming, rearing), however, all of these behaviors were significantly affected by a tone-conditioned footshock.

Ferret Odor

Another predatory odor that can be used to evoke anxiety in the rat is ferret odor. The ferret *(Putorius putorius furo)* belongs to the carnivorous family, Mustelidae, and is a natural predator of rats (Apfelbach, 1978). The odor is generally obtained by placing a cotton hand towel in a cage with one male and one female adult ferret for one month. The towel is then cut into 5cm x 5cm square pieces and stored in an -80°C freezer until the day of testing (Campeau et al., 2008). Ferret odors activate certain brain structures in rodents including the olfactory system, the PFC, the amygdala, and certain areas of the hypothalamus that play a role in the defensive circuit (Staples, 2010). Additionally, they have been shown to elicit endocrine responses and behavioral changes including increased hiding and risk assessment (Masini et al., 2005). McIntyre, Kent, Hayley, Merali, and Anisman (1999) demonstrated that ferret odor alters an animal's physiology state due to increases in plasma CORT and ACTH levels. Additionally, both stressors (restraint and ferret odor) altered NE, DA, and 5-HT mean levels in brain regions associated with stress. Masini and colleagues (2005) found that ferret odor successfully evoked HPA axis activation as evidenced by elevated CORT and ACTH levels. Additionally, rats exposed to ferret odor in a

defensive withdrawal paradigm exhibited more avoidance and risk assessment behaviors compared to rats exposed to the control odor. Finally, brain activation was evident after a single 30-min odor exposure in the LS and bed nucleus of the stria terminalis (BNST).

Cat Fur Odor

Lastly, studies have demonstrated the efficacy of using cat odor obtained directly from a domestic cat in the form of fabric rubbed against the fur. Previous research by McGregor and Dielenberg (1999) has demonstrated that in the presence of a live cat or cat odor, the rat will display specific risk assessment behaviors that allow the rat to investigate the impending threat. "Stretch-attend postures" include stretched attention and flat-back posture, as well as any behaviors that orient the rat in the direction of the threat (Kalynchuk et al., 2004). This behavior can be seen as somewhat ambivalent because the animal is conflicted between wanting to explore the stimulus and wanting to flee from the stimulus (van der Poel, 1979). The "head out" behavior is characterized by scanning the environment from the safety of a confined space (Siviy & Harrison, 2008). In addition, non-defensive behaviors, or behaviors that are not involved in readying the body for flight or fight, are inhibited during a potential predator attack. These behaviors include grooming, feeding, and play behavior (Staples, 2010).

A single exposure to cat odor activates the medial hypothalamic defensive circuit (Canteras, 2002), including inputs from the olfactory system, the medial hypothalamus, the dorsal premammillary nucleus, the anterior hypothalamic nucleus, and the dorsomedial part of the ventral medial hypothalamus (Staples, 2010). Many mammals utilize the olfactory system in order to detect predators, although cat odor is processed primarily through the anterior olfactory nucleus, accessory olfactory bulb, and the medial amygdala. Cat odor is processed as a "kairomone" which is a chemical emitted by one type of species and detected by a different type

of species, allowing the latter species to prepare for immediate danger (Dicke & Grostal, 2001). Another region associated with anxiogenic-like behaviors is the BNST. Exposure to cat odor or a live cat increases amino acid protein expression in the BNST (Dielenberg, Hunt, & McGregor, 2001).

Advantages of the Cat Odor

Rodents have evolved a sensitivity to feline cues that has led to the development of specific defensive behaviors toward these cues. The innate defensive response to cat odor is so strong that a rat will react to the odor even if it has never encountered a live cat. Using cat odor is advantageous over other predator exposure methods for a number of reasons. First, exposure to cat odor is consistent with the type of stimulus that would be encountered in the animal's natural environment and induces innate, ethologically-relevant, unconditioned defensive behaviors (Wright et al., 2013). The rodent's responses can be characterized as "phylogenetically prepared" and will effectively map the brain regions involved in anxiety. In addition, reactions to irrelevant stimuli such as pain and those not found in a rat's natural environment are limited (Staples, 2010). Second, using predator odor as a stressor stimulus allows for behavioral monitoring during the exposure, including quantifying the duration and frequency of certain assessments, which is not always possible with alternative stressor models including footshock and restraint (Wright et al., 2013). Third, the behaviors induced by cat odor resemble certain symptoms of human anxiety disorders including scanning and retreat (Dielenberg & McGregor, 2001). The cat odor induces responses that specifically parallel GAD, with increased vigilance and risk assessment. These responses are also indicative of anxious symptoms because they are attenuated by the administration of benzodiazepines (Staples, 2010). Finally, using cat odor to repel rats has stimulated research in both domestic and agricultural contexts (Dielenberg &

McGregor, 2001).

The presence of predators or predator odors in the wild is a threatening experience for animals because there is a high probability they will get wounded or even killed (Muñoz-Abellán, Andero, Nadal, & Armario, 2008). Thus, rats exposed to a cat demonstrate behavioral changes reflective of heightened anxiety (Blanchard & Blanchard, 1989). When tested on the EPM after odor exposure, rats display anxiogenic behaviors such as a reduction of time spent in the open arms. Fear of the open arms stems from a natural aversion to heights and open spaces. It has been demonstrated that rats exposed to cat odor as opposed to a live cat show similar signs of anxiety (McGregor & Dielenberg, 1999). According to McGregor and colleagues (2002), the type of odor used affects the type of response produced. In their experiment, rats displayed anxiogenic behavior in the EPM and suppressed locomotion in a novel environment immediately after exposure to a collar worn by a cat but not after exposure to TMT.

Exposure Apparatus

Hide Box

Currently, the most replicated predator exposure method is presenting the odor in a rectangular apparatus with a "hide box" (Takahashi, Hubbard, Lee, Dar, & Sipes, 2007; Siviy & Harrison, 2008; McGregor & Dielenberg, 1999; Kalynchuk et al., 2004; Campeau et al., 2008). The hide box is a compartment at one end of the chamber where the rat can hide and poke its head out. One of the purposes of the hide box is for the rat to retreat to a confined area immediately following detection of the odor. From the hide box, the rat can safely scan the environment from a "head out" posture. This behavior is a sensitive measure of risk assessment and an innate defensive mechanism occurring in a rat's natural environment (Dielenberg,

Carrive, & McGregor, 2001).

Home Cage

Although the literature involving the use of a hide box is extensive, the major limitation to this method is that the rat is likely to develop contextual fear to the apparatus. Using a testing chamber only allows for observation of anxiogenic responses in one context. In contrast, the current study will present the predator odor via worn cat collars and a towel in the home cage. Presenting the odor in the home cage allows for observation of anxiety that will potentially generalize to other contexts. Typically, presentation of the odor in the home cage is done via an impregnated cloth hung above or on top of the cage (Sütt et al., 2008; House, Vyas, & Sapolsky, 2011). Several human anxiety disorders present a range of physical and psychological symptoms that are not restrictive to certain settings (McGrandles & Duffy, 2012). Therefore, the animal model of anxiety used in this study is more indicative of the many manifestations of human anxiety disorders.

Based on previous research using predator exposures, there are several questions that remain unanswered which the current experiment will attempt to address. First, will repeated exposure to a predator odor alter behavior in a test of anxiety *and* a test of anhedonia, the core symptom of human depression? Exposure to a predator odor is a stress paradigm typically used to measure anxiety-like behaviors. However, due to the high comorbidity rate between anxiety and depression, it is reasonable to hypothesize that a chronic schedule of stress might also induce depressive-like behaviors. Second, will pretreatment with ketamine influence the presence of depressive and anxiety-like behaviors in rats? Ketamine administered in adolescence has been shown to attenuate depressive-like behaviors, but there is limited research regarding its effects on anxiogenic behaviors. Finally, will pretreatment with ketamine have a protective effect against

the stressor of being exposed to predator odor? The current study predicts that rats exposed to the predator odor will be more likely to display depressive-like behaviors and repeated ketamine administration will tend to reverse the effects of stress during adulthood.

This study investigated the effects of chronic ketamine administration on anxiety and depressive-like behaviors after exposure to a predator odor in Sprague-Dawley rats. The main goal of the experiment was to examine how repeated exposure to a stressor affects long-lasting behavioral responsiveness in novel situations such as the elevated zero maze (EZM) and the sucrose preference test (SPT), and whether that can be reversed by ketamine administration.

Methods

Subjects

Twenty-four rats were used for this study. All rats were fed on an *ad libitum* (in accordance with desire) schedule. The rats were obtained at approximately 38 days old. All animals were subjected to gentle handling once a day for three consecutive days prior to the experiment. They were double housed in the Jubilee Hall vivarium on a 12/12h light/dark cycle. Approval of the Seton Hall Institutional Animal Care and Use Committee was obtained before the start of any experimental procedures.

Apparatus

Predator Odor Exposure

Predator odor exposure took place in the rats' home cages, as previously described in House et al. (2011). The home cage was a clear Plexiglas chamber with four walls [44 cm (L) x 24 cm (W) x 22 cm (H)] with corn cob bedding and a wire grate top for access to rat chow and water. There was a combined method of predator exposure: a worn cat collar and a towel impregnated with cat odor. Worn cat collars were "Breakaway" safety stretch cat collar (Great Choice, China) and were obtained from two domestic, female cats that wore the collars for approximately four weeks. Upon removal from the cats, the collars were placed in separate airtight plastic containers and were stored in a freezer at 0° C. On the day of testing, the collars were cut into 9-cm pieces with one piece being used in each home cage. A binder clip was placed in each home cage one inch away from the water bottle spout. The collar pieces were attached to the binder clips 12cm above the floor of the home cage. Only the pieces of collar that came in direct contact with the cat's fur were used. At the start of the trials, the collar was "warmed up" by placing it on top of a heater for approximately two minutes. The cat collar was

always handled with latex gloves. Four collars were used for this experiment: two cat odor collars and two control collars.

A cotton hand towel (60 cm x 40 cm) was also used to present the cat odor. The towels were placed in the cat's sleeping spot for one night and rubbed against the cat's body the following morning. Upon acquisition, the towels were placed in separate airtight plastic containers and stored in a freezer at 0° C. Twelve towels were used for this experiment: six towels impregnated with cat odor and six control towels. The experimental and control towels were always handled with latex gloves.

Sucrose Preference Test

The SPT apparatus is a metal cage with a wooden top [22 cm (L) x 27 cm (W) x 30 cm (H)]. A wooden apparatus that holds the bottles in place was situated next to the front of the metal cage. The SPT measures the consumption of sweet food or drink to assess anhedonia (Abelaira et al., 2013), the core symptom of human depression (Kumar, Kuhad, & Chopra, 2011). An advantage of the SPT is that unlike other behavioral measures, it is not dependent on motor activity. Although the task's procedures can vary, the most common way to employ the SPT is by allowing the rats to choose between a bottle containing sucrose and a bottle containing tap water (Strekalova & Steinbusch, 2010). The rats are typically subjected to an acclimatization period (Dagyte et al., 2011) where they are trained to consume the sucrose solution (Wang, Zhang, Hui, Zhang, & Hu, 2013) and then deprived of food and water prior to testing so as to motivate them to drink.

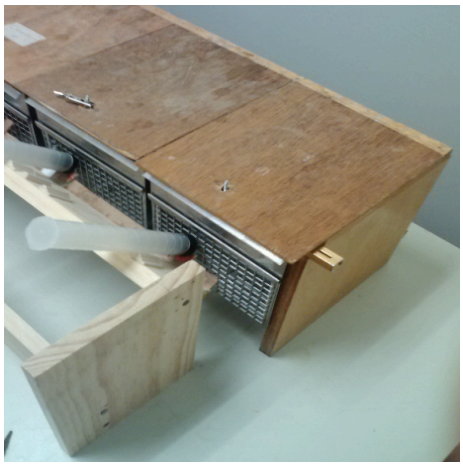

Figure 1. Photograph of the Sucrose Preference Test used in this study

Elevated Zero Maze

The apparatus was comprised of a black, annular platform (100 cm diameter, 10 cm width) elevated to 40 cm above ground level. Two quadrants are enclosed by black cardboard walls (29 cm high) and the other two quadrants remain open. Exploration in the EZM is an unconditioned test of anxiety-like behavior (Sütt et al., 2008), originally described by Shepherd, Grewal, Fletcher, Bill, and Dourish (1994). Rats have a natural aversion to heights and open spaces and therefore will display fear reactions such as freezing and increased plasma CORT levels when placed in the open arms. As a consequence of the aversive nature of the open arms, the rats will spend a greater amount of time, on average, in the closed arms (Shepherd et al., 1994). The zero maze has a major advantage over the EPM: there is no center region. In the EPM, the amount of time spent in the center square of the "plus" shape is difficult to interpret because the behavior is neither indicative of anxiety nor locomotion. Because the zero maze is circular in shape, the center platform is eliminated along with any ambiguity in interpretation (Cook, Williams, & Flaherty, 2001). The zero maze has been pharmacologically validated with anxiolytic drugs. For example, Braun, Skelton, Vorhees, and Williams (2011) found that 1mg/kg of diazepam significantly increased time spent in the open arms of the maze.

Figure 2. Photograph of the Elevated Zero Maze used in this study

Drugs

Ketamine was obtained from JHP Pharmaceuticals, LLC, Rochester, MI 48307 (NDC 420023-138-10). One group of rats received 25 mg/kg of ketamine daily (*n* =12), intraperitoneally (ip), prepared in saline at a volume of 1 mL/100 g while another group of rats (*n* = 12) received daily injections of 25 mg/kg of saline (ip).

Procedure

During rat adolescence on PND 45, twelve of the twenty-four rats received a 25mg/kg injection of ketamine for a total of seven consecutive days. The other twelve rats received a 25mg/kg injection of saline, also referred to as vehicle. On PND 51-62, the animals were subjected to object recognition testing that was a part of a separate study. During young adulthood on PND 65, animals were divided into four groups for the cat odor exposure based on the previous ketamine pretreatment: ketamine pretreatment + cat odor (*n* = 6), ketamine pretreatment + control odor (*n* = 6), vehicle pretreatment + cat odor (*n* = 6), and vehicle pretreatment + control odor (*n* = 6). The animals were then carried in their home cages to the testing room. The rats remained double-housed (two rats per cage) during the entirety of the

experiment. Each cage mate received a different drug administration but both rats received the same type of odor exposure. Without removing the rats from their home cages, the collar piece was attached to the binder clip and the cloth was draped over the top of the cage. Twelve experimental rats were exposed to the predator odor and twelve control rats were exposed to the control odor in the home cage for a 30-min period. The collar piece and cloth were removed immediately after the 30-min session and the rats were carried back to the housing room. This procedure was repeated twice at 24-hour intervals for a total of three predator or control odor exposures. Odor exposure sessions were conducted in a separate testing room to avoid exposing other rats to the cat odor.

One day after the last predator exposure, the SPT began. The predator odors were not present during the SPT because the goal of this task was to assess the presence of depressive-like behaviors that might have developed as a result of the odor exposure. The SPT was conducted during adulthood from PND 68 to PND 71, a total of four days due to staggering the procedures across the four groups. The task was composed of habituation, deprivation and testing (Kumar et al., 2011). During habituation, the animals were trained to consume a 3% sucrose solution in the test apparatus for a 2-h period. Animals were presented simultaneously with two bottles, one containing 3% sucrose solution and the other containing tap water (Bhagya et al., 2011). The volume of sucrose and water intake was measured at the end of the two hours. After habituation, rats were deprived of water for 14h. Immediately after deprivation, the rats were put back into the sucrose chamber and preference for 3% sucrose was assessed during a 2-h test session. During this test session, rats were able to freely choose between two bottles: 100 ml of 3% sucrose solution and 100 ml of tap water. The habituation and test phases took place in an apparatus separate from the home cage. To prevent the possibility of side preference in drinking

behavior, the bottles were reversed at the midway point of both phases. The amount of liquid remaining in each bottle was measured at the end of the test phase. The preference for sucrose was calculated as a percentage of the consumed sucrose solution from the total amount of liquid drank from the formula:

$$SP = \frac{\text{sucrose intake (g)}}{[\text{sucrose intake (g)} + \text{water intake (g)}]} \times 100$$

The SPT was conducted before the EZM to avoid any confounding stress that the latter measure might induce.

EZM testing began on the following day, during adulthood on PND 72 and 73. After each rat was placed in the apparatus, an observer stood 2m from the maze to record behavioral responses. Behavior of rats was recorded for one 5-min session and the following measures were taken: number of open quadrant entries, amount of time spent in the open quadrant(s), number of closed quadrant entries, and amount of time spent in the closed quadrant(s). Behavior was also videotaped with a camera suspended above the apparatus. Percentage scores were calculated for open-quadrant time (i.e., time in open quadrant/total time in any quadrant x 100) and open-quadrant entries (i.e., number of entries into the open quadrants/total number of entries into any quadrant x 100) similar to previous studies (Engin et al., 2009; Pellow, Chopin, File, & Briley, 1985; Engin, Stellbrink, Treit, & Dickson, 2008). Percent time spent in open quadrants is the most accepted scoring variable used to reflect decreased anxiety (Lister, 1987; Shepherd et al., 1994). A higher percentage of open quadrant time or open quadrant entries, in the absence of changes in general activity, was taken as indicators of anxiolysis. Number of closed quadrant entries and total number of quadrant entries both served as measures of general activity in the zero maze. In an attempt to control for a possible lack of movement around the maze due to novelty, animals were exposed to the EZM on two consecutive days. The SPT and EZM were

conducted during adulthood to investigate whether the adolescent ketamine treatment had long-lasting effects (Table 1).

Table 1: Timeline of Experimental Procedures

PND		**Description of Procedures**
45-51	Ketamine injections	Animals received 25 mg/kg (ip) of ketamine (*n* = 12) or saline (*n* = 12) once daily
51-62	Object recognition	Object recognition testing (separate study; data not shown)
63-64	Rest	Rest
65	Odor exposure (30 min)	Four groups: ketamine pretreatment + cat odor (*n* = 6), ketamine pretreatment + control (*n* = 6), vehicle pretreatment + cat odor (*n* = 6), and vehicle pretreatment + control (*n* = 6)
66	Odor exposure (30 min)	Same as day 65
67	Odor exposure (30 min)	Same as day 65
68	SPT: Habituation	Trained to consume 3% sucrose in the apparatus for 2h
69 or 70	SPT: Deprivation	Deprived of water for 14h
70 or 71	SPT: Test	Assessed preference for sucrose in the apparatus for 2h
72	EZM: Day 1	One 5-min session
73	EZM: Day 2	One 5-min session

Statistical Analysis

The behavioral responses recorded in the EZM and SPT were analyzed with analyses of variance (ANOVA). Seven 2 (drug administration) x 2 (predator odor) between-groups ANOVA

were conducted. Partial η^2 was reported as a measure of effect size for each variable (Richardson, 2011). Cohen (1988) suggested that small, medium, and large effects would correspond to values of η^2 of .0099, .0588, and .1379, respectively. A p value of <0.05 indicated statistical significance throughout.

Results

Sucrose Preference Test

Habituation

Preference for 3% sucrose was measured during the habituation and test phases. A two-way between-groups ANOVA did not reveal a significant effect of drug administration, $F(1, 23) = .495$, $p = .490$, partial $\eta^2 = .024$; type of odor, $F(1, 23) = 1.065$, $p = .314$, partial $\eta^2 = .051$; or drug x odor interaction, $F(1, 23) = 3.497$, $p = .076$, partial $\eta^2 = .149$ during habituation. However, the drug x odor interaction effect was trending toward significance with a large effect size. This interaction appeared to be the result of a reduction in sucrose preference after predator odor exposure in the vehicle group but not the ketamine group (Figure 3). Absolute intakes were calculated for each condition, indicating that, on average, rats consumed more sucrose than water (Table 2).

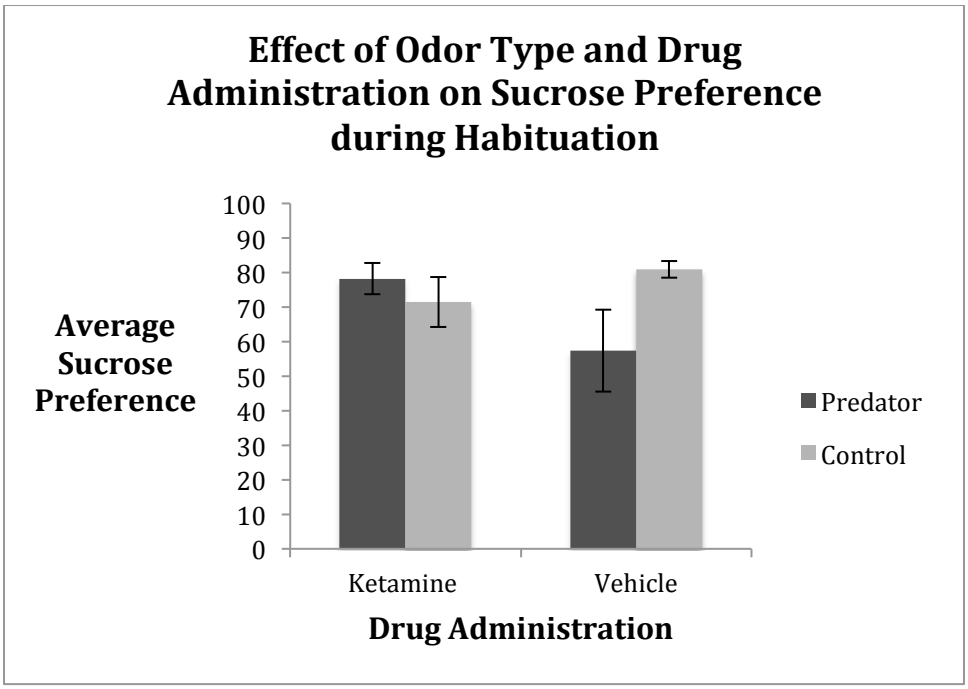

Figure 3. Average sucrose preference during the habituation phase of rats administered ketamine or vehicle. Dark gray bars represent the preference of rats exposed to predator odor;

light gray bars represent the preference of rats exposed to control odor. Error bars represent standard error of the mean.

Table 2. Absolute Sucrose and Water Intakes for Habituation

	Sucrose Intake	**Water Intake**
Ketamine + Predator Odor	95.7±1.6ml	16.9±0.2ml
Ketamine + Control Odor	72.7±1.5ml	28.6±0.4ml
Vehicle + Predator Odor	59.9±2.0ml	35.8±0.4ml
Vehicle + Control Odor	79.2±1.0ml	26.9±0.1ml

Test

After a 14h period of water deprivation, preference for 3% sucrose was measured again during the test phase. A two-way between-groups ANOVA did not reveal a significant effect of drug administration, $F(1, 23) = .691$, $p = .416$, partial $\eta^2 = .033$; type of odor, $F(1, 23) = .021$, $p = .886$, partial $\eta^2 = .001$; or drug x odor interaction, $F(1, 23) = 1.784$, $p = .197$, partial $\eta^2 = .082$ (Figure 4). Absolute intakes revealed that, on average, rats consumed more sucrose than water (Table 3). Additionally, absolute intakes for the treatment groups were higher during the test phase compared to the habituation phase.

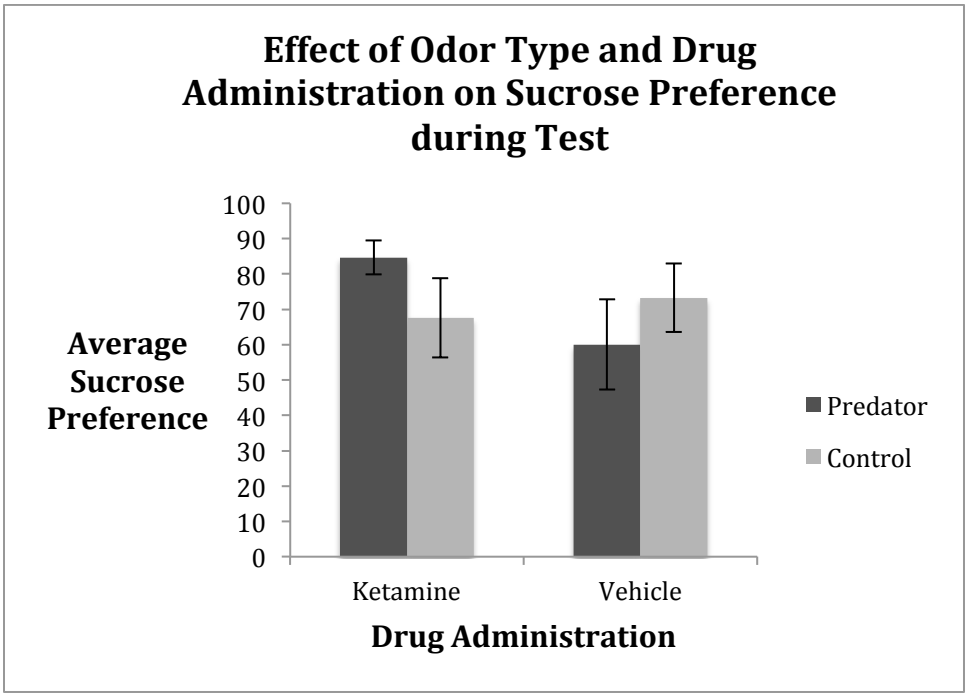

Figure 4. Average sucrose preference during the test phase of rats administered ketamine or vehicle. Dark gray bars represent the preference of rats exposed to predator odor; light gray bars represent the preference of rats exposed to control odor. Error bars represent standard error of the mean.

Table 3. Absolute Sucrose and Water Intakes for Test

	Sucrose Intake	**Water Intake**
Ketamine + Predator Odor	33.3±1.4ml	7.2±0.9ml
Ketamine + Control Odor	36.1±2.8ml	11.4±1.3ml
Vehicle + Predator Odor	30.3±2.6ml	13.1±1.9ml
Vehicle + Control Odor	45.5±2.3ml	10±1.5ml

Change Scores

To address the variability in sucrose consumption during habituation across rats, change scores in sucrose preference percentage were used to calculate changes in preference from

habituation to test. A two-way between-groups ANOVA did not reveal a significant difference of drug administration, $F(1, 23) = .425$, $p = .522$, partial $\eta^2 = .021$; type of odor, $F(1, 23) = 1.124$, $p = .302$, partial $\eta^2 = .053$; or drug x odor interaction, $F(1, 23) = .763$, $p = .393$, partial $\eta^2 = .037$ (Figure 5).

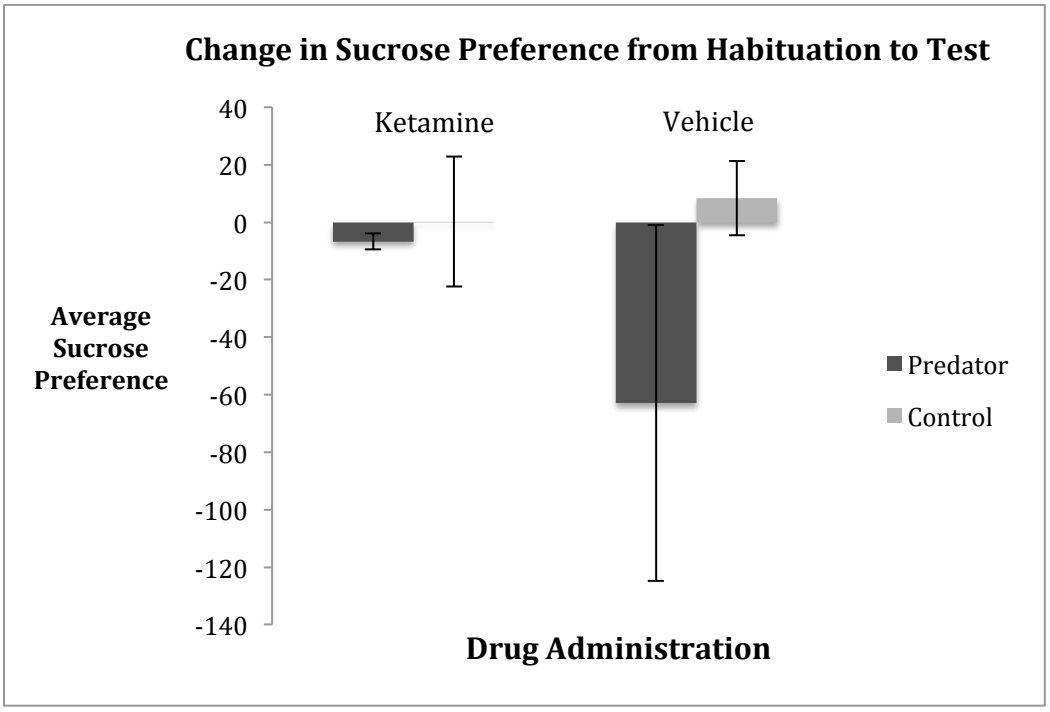

Figure 5. Change in average sucrose preference from habituation to test of rats administered ketamine or vehicle. Dark gray bars represent the preference of rats exposed to predator odor; light gray bars represent the preference of rats exposed to control odor. Error bars represent standard error of the mean.

Elevated Zero Maze, Day 1

Percentage of Time in Open Quadrants

A two-way between-groups ANOVA did not reveal a significant effect of drug administration, $F(1, 23) = .652$, $p = .429$ partial $\eta^2 = .032$; type of odor, $F(1, 23) = .005$, $p = .942$,

partial η^2 = .000; or drug x odor interaction, $F(1, 23) = .317$, $p = .580$, partial η^2 = .016 on the first day of EZM exposure (Figure 6).

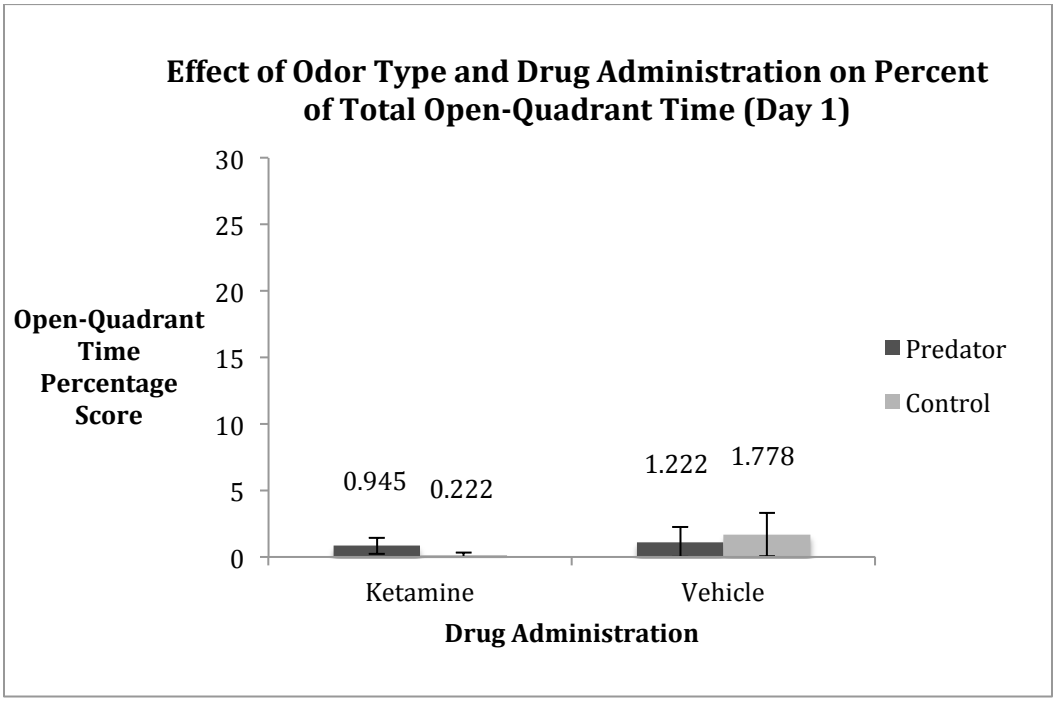

Figure 6. Percent of total EZM session spent in the open quadrants. Dark gray bars represent the percentage score of rats exposed to predator odor; light gray bars represent the percentage score of rats exposed to control odor. Error bars represent standard error of the mean.

Number of Open Quadrant Entries

A two-way between-groups ANOVA did not reveal a significant effect of drug administration, $F(1, 23) = .217$, $p = .646$ partial η^2 = .011; type of odor, $F(1, 23) = .217$, $p = .646$, partial η^2 = .011; or drug x odor interaction, $F(1, 23) = .217$, $p = .646$, partial η^2 = .011 (Figure 7).

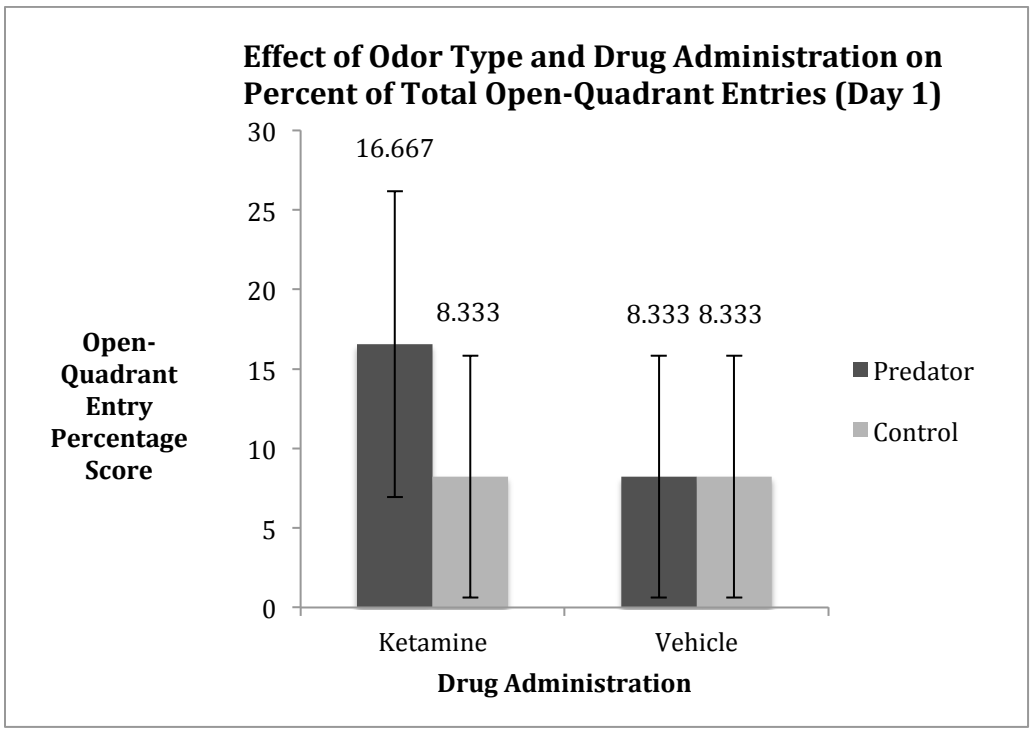

Figure 7. Percent of total EZM entries into the open quadrants. Dark gray bars represent the entry percentage of rats exposed to predator odor; light gray bars represent entry percentage of rats exposed to control odor. Error bars represent standard error of the mean.

Elevated Zero Maze, Day 2

Percentage of Time in Open Quadrants

Due to the finding that rats only rarely left the closed quadrants of the EZM on the first day of testing, rats were again placed into the apparatus on the following day. This was done to determine whether their lack of exploration on day 1 was the result of being placed into a novel environment and would attenuate with a second exposure to the apparatus. A two-way between-groups ANOVA did not reveal a significant effect of drug administration, $F(1, 23) = .624$, $p = .439$, partial $\eta^2 = .030$; type of odor, $F(1, 23) = 4.076$, $p = .057$, partial $\eta^2 = .169$; or drug x odor interaction, $F(1, 23) = .624$, $p = .439$, partial $\eta^2 = .030$ (Figure 8). However, the main effect of odor was trending toward significance with a large effect size. Animals exposed to the control

odor tended to spend more time in the open quadrants (M = .389, SD = .681) than the animals exposed to the predator odor (M = .000, SD = .000).

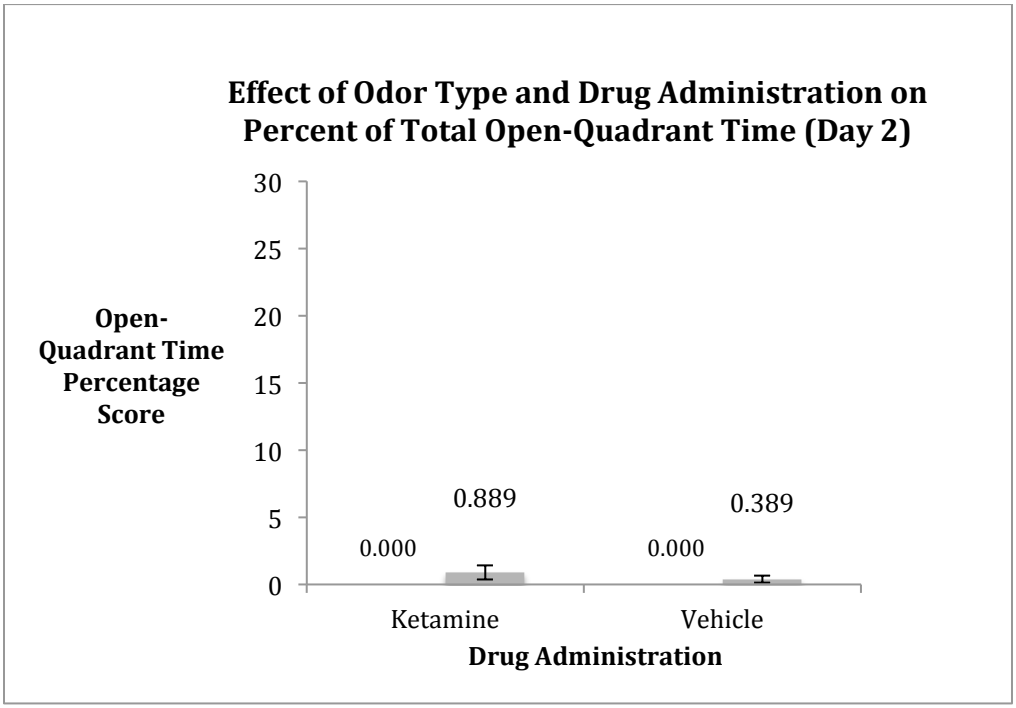

Figure 8. Percent of total EZM session spent in the open quadrants. Error bars represent standard error of the mean.

Number of Open Quadrant Entries

A two-way between-groups ANOVA revealed a significant effect of odor, $F(1, 23) = 5.00$, $p = .04$, partial $\eta^2 = .200$, indicating that the animals exposed to the control odor (M = 16.67, SD = 23.57) made significantly more entries into the open quadrants than the animals exposed to the predator odor (M = .00, SD = .00). This was a large effect. There was no significant effect of drug, $F(1, 23) = .000$, $p = 1.00$, partial $\eta^2 = .000$ or significant drug x odor interaction, $F(1, 23) = .000$, $p = 1.000$, partial $\eta^2 = .000$ (Figure 9).

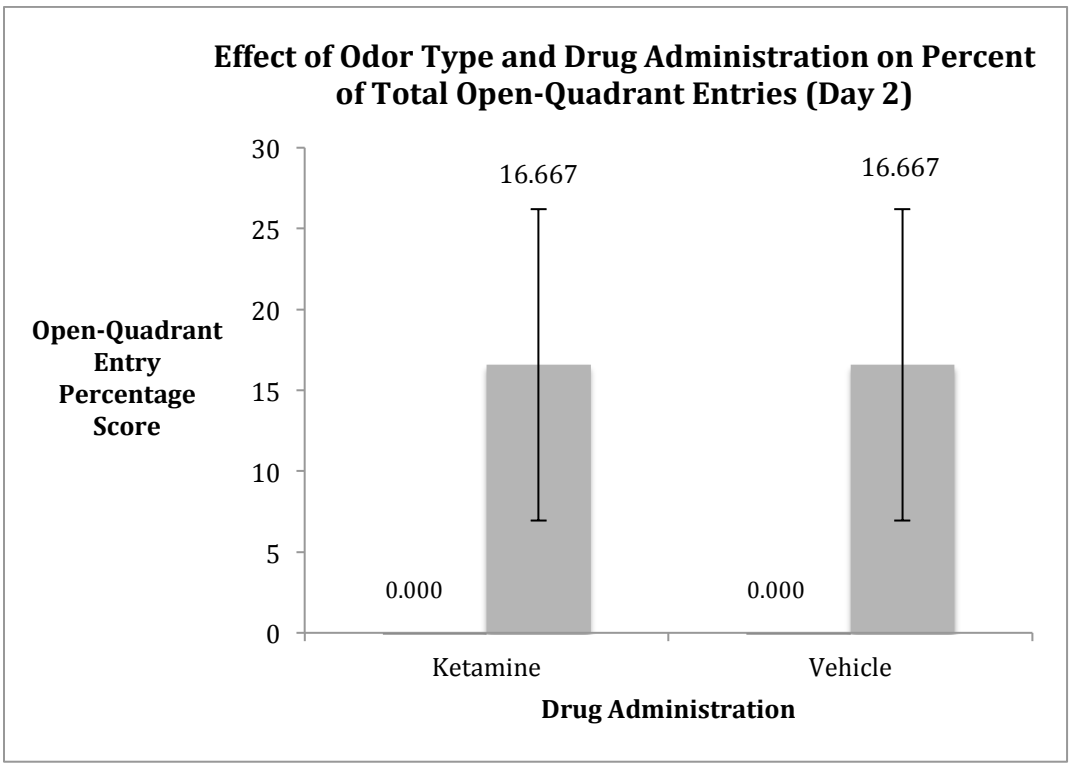

Figure 9. Percent of total EZM entries into the open quadrants. Error bars represent standard error of the mean.

Discussion

The purpose of this study was to investigate the effects of chronic ketamine administration on anxiety and depressive-like behaviors induced by a predator odor. The results indicated a drug x odor interaction effect in the habituation phase of the SPT that was trending toward significance with a large effect size. This interaction appeared to be the result of ketamine tending to reverse the decrease in sucrose preference as a result of the predator odor exposure. However, neither ketamine exposure nor exposure to predator odor significantly influenced the average sucrose preference during the test phase. Additionally, these variables did not significantly affect the change in sucrose preference from habituation to test.

Findings from the first day of EZM did not reveal any significant effects of drug, odor, or their interaction. However, the second day of EZM testing revealed a significant main effect of odor type on anxiety. Animals exposed to the predator odor made significantly fewer entries into the open quadrants than the animals exposed to the control odor. Additionally, the main effect of odor for the percentage of time spent in the open quadrants was trending toward significance. The large effect size suggests that animals exposed to the predator odor tended to spend less time in the open quadrants compared to animals exposed to the control odor.

Although predator odor is typically used exclusively in a model of anxiety, the current study investigated whether repeated exposure would also induce depressive-like behaviors. Previous research suggests that the onset of depression is due in part to the accumulation of stressful life events, and that stressful life events are associated with an increase in depressive symptoms (Mazure, 1998; Stroud, Davila, & Moyer, 2008; Abela & Skitch, 2006). Analyses of the four groups revealed a drug x odor interaction trending toward significance during the habituation phase of the SPT; this suggests that adolescent ketamine administration tended to

reverse the decrease in sucrose preference due to the predator odor exposure. Although this finding is not statistically significant, the large effect size suggests that the effect is meaningful. This finding is in line with research from Xi and colleagues (2011) in which escitalopram treatment reversed the effect of chronic unpredictable mild stress (CUMS) in the SPT.

The present results reveal the long-lasting effects of ketamine administration on sucrose preference, which was measured almost 20 days after drug administration. This is consistent with work from Ma and colleagues (2013) who found that a single administration of ketamine tended to increase preference for sucrose 24 hours, 4, 6, and 8 days later. It is important to note that the drug was no longer in the animals' systems during the predator exposure. Therefore, it is possible that any effect of ketamine was due to neural changes that happened as a consequence of repeated ketamine administration during adolescence. This is consistent with research from Parise and colleagues (2013) who found that ketamine administration in adolescence tended to reverse the effects of CUS-induced depressive-like behaviors in the FST in adulthood.

In contrast to the habituation phase, the test phase did not yield any statistically significant or trending effects. Although the reasons for this are unclear, it is noteworthy that the only manipulation that occurred after the habituation phase was the 14h water deprivation. It is possible there was some degree of neophobia on the habituation day that went away on test day. This is evident when comparing the sucrose preference for the vehicle + predator odor group over time. The mean sucrose preference for this particular group of rats increased slightly from habituation to test, although not significant. Perhaps the predator odor very subtly affected the initial reaction to the new environment, which is why it was only evident during the first day in the sucrose chamber. There is no doubt this is a small effect in terms of the animals' behavior, but it could indicate their anxiety about a novel liquid. Finally, the absolute intakes for the

treatment groups were higher in the test phase compared to the habituation phase indicating that the deprivation period was sufficient.

Another possibility for why the habituation phase showed a stronger effect is because the odor exposure occurred one day prior to this phase. In contrast, the odor exposure occurred three days prior to the test phase. Analysis of the change in sucrose preference from habituation to test did not reveal any significant findings. The reason the change scores were calculated is because perhaps there was too much individual variability, which masked any significant group differences. Small sample sizes (n = 6 rats per group) in the present study could be responsible for much of this variability. A power analysis using G*Power software was conducted to determine the probability of detecting an effect when it exists. G*Power indicated that 51 rats were an appropriate sample size for this experiment based on a specified statistical power of .8, an alpha level of .05, and a partial η^2 of .14. Due to limited resources, this sample could not be achieved and thus contributed to the lack of power to detect any differences.

One area of concern in the current experiment is that a 3% sucrose solution was used compared to much of the literature that uses a 1% solution. The reason for this deviation from standard protocol is because pilot testing with 1% and 2% sucrose revealed minimal average consumption over a two-hour period ($M_{1\%}$ = 5.00g and $M_{2\%}$ = 7.75g). Therefore, a 3% solution ($M_{3\%}$ = 9.35g) was used to ensure the rats would be motivated to drink the sucrose. It is possible that the 3% solution was too concentrated which is why the rats in both the habituation and test phases drank more sucrose than water. This was not a completely novel paradigm as other studies have also used 3% solutions or higher (Diaz et al., 2013; Choi et al., 2013; Kosten & Meisch, 2013). Finally, the total amount of consumption could not be attributed to side

preference because this was accounted for by switching the location of the bottles halfway through the habituation and test phases.

The EZM was used to measure anxiogenic responses after exposure to the predator odor. There were no significant differences in percent of time spent in the open quadrants and percent of open quadrant entries between the four groups on the first day of EZM testing. Overall, the animals were reluctant to move as evident by an overwhelming 79% of rats that never left the closed quadrants. The present results are not consistent with other findings that demonstrate substantial movement in and out of the quadrants. For example, Soares and colleagues (2013) investigated the effects of early protein malnutrition and environmental stimulation on behavioral parameters in rats submitted to two five-minute sessions in the EPM. They found that malnourished (MN) rats displayed higher mean percentages of time in the open arms and made more entries, on average, into the open arms compared to well-nourished rats. The MN rats spent approximately 30% of the time in the open arms and made 30% of their total entries into the open arms, compared to the current study in which the vehicle + predator odor group spent approximately 1% of the time in the open quadrants and made 7% of their total entries into the open quadrants.

A potential contributing factor for the lack of movement on day 1 of EZM testing is the possibility of a floor effect. This lack of movement was unanticipated; pilot testing with the same strain of rat demonstrated a great deal of entries and time spent in the open quadrants. Another possibility is that the novelty of the environment induced anxiety which led to less exploration, on average, among all the rats. This concern was addressed by conducting a second day of testing with this measure. Differences in rat strain between studies could have been a contributing factor (Hogg, 1996). Ferguson and Grey (2005) found evidence for strain and sex

differences among three types of rats in the EPM. Spontaneously hypertensive rats were significantly more active in the EPM compared to Sprague-Dawley and Wistar rats. Additionally, at PND 70, female rats of all strains displayed less time, on average, in the closed quadrants than their male counterparts. It is clear that different rat strains have a major impact on the outcome of this animal measure of anxiety. Perhaps there might have been more activity in the EZM had the Sprague-Dawley rats been female as opposed to male rats.

Despite the lack of significant effects during the first day of EZM testing, there was a significant effect of odor type on the percent of open quadrant entries on the second day. Rats exposed to the control odor made significantly more entries into the open quadrants than the rats exposed to the predator odor. This is consistent with my hypothesis that the predator odor would induce anxiety-like behavior, thus preventing the animals from leaving the safety of the closed quadrants. This finding is in accordance with previous research conducted by Sütt and colleagues (2008) who investigated the role of the endocannabinoid system in cat odor-induced anxiety in rats. Exploratory behavior of rats in the EZM showed a dramatic mean reduction of open-quadrant entries, line crossings, and time spent in the open quadrants after cat odor exposure. Additionally, Campos, Piorino, Ferreira, and Guimarães (2013) investigated whether the behavioral changes induced by predator exposure are associated with changes in the medial prefrontal cortex nitrergic system (mPFC). They found that the animals exposed to a live cat displayed a decreased mean percentage of open arm entries in the EPM and an increased average number of nitric oxide synthase positive neurons in the mPFC.

The effect of odor type on percentage of time spent in the open quadrants was trending toward significance. Rats exposed to the control odor spent more time, on average, in the open quadrants compared to the rats exposed to the predator odor. This is consistent with my

hypothesis that the control odor groups would not tend to display anxiogenic behaviors and therefore would be more likely to actively explore the environment. Of particular importance is the fact that the odor exposure occurred a week prior to EZM testing. The presence of a main effect of odor a week later demonstrates the long-lasting effect of this stress paradigm in the EZM. Although much of the literature with predator odor tends to examine immediate behavioral and physiological effects, there is one study, in particular, that examined delayed behavioral effects to various types of odor. Muñoz-Abellán and colleagues (2008) found that rats exposed to cat fur odor tended to show signs of anxiety-like behavior in the EPM one week after initial stress exposure. This did not occur for rats exposed to alternative stressors including cat urine or immobilization in wooden boards.

Although there were significant effects of odor on the second day of EZM testing, it is possible that greater exposure to the odor may have produced more robust effects that would have also been evident on the first day of testing. The goal of the present study was to expose the animals to a chronic schedule of the predator odor in order to investigate its long-lasting effects. Therefore, the odor was presented three times over three consecutive days, which is considered a chronic schedule. Much of the literature on predator exposure employs only one exposure session and assesses the presence of anxiety-like behaviors during the exposure (Siviy & Harrison, 2008; Kalynchuk et al., 2004) or within 24-h of the exposure (Sütt et al., 2008; Takahashi et al., 2007). While this research is able to examine the immediate effects of the predator odor, it cannot draw conclusions regarding effects lasting for longer than a few days. The present study was able to demonstrate the long-lasting effects of the predator odor exposure five days later on the second day of EZM testing.

There are numerous ways to induce stress in a rat including repeated CORT injections (Kalynchuk et al., 2004), chronic psychosocial stress models (Slattery et al., 2012), and repeated footshock (Kassai & Gyertyán, 2012). It is important to note that the current study used a less invasive method so as to reduce the amount of harm to the animals. Several concerns can be considered with regard to using the predator odor method compared to more invasive methods. First, it is possible that the rats learned to avoid the area with the aversive collar piece after initial contact. This is problematic because it reduces the amount of time the rat is exposed to the threatening stimulus. For example, Dielenberg and McGregor (2001) reported that the rats in their study spent only 7s out of 1200s sniffing the cat odor stimulus, which was not long enough to induce amino acid expression in the olfactory bulb. Second, because the odor was presented via a collar piece and a towel, it had the disadvantage of being diffused throughout the room. A more contained method of odor exposure is using TMT, which is typically presented via a filter paper soaked with the odor. Additionally, this method has the advantage of being able to use a new filter paper for each animal, thereby ensuring a potent odor with every use (Hacquemand et al., 2013).

The current study wanted to investigate whether the anxiogenic effect of the predator odor would generalize to a context in which the rat did not experience the odor. The results demonstrated that the odor exposure in the home cage did, in fact, generalize to the EZM. This is in accordance with an experiment conducted by Dielenberg and McGregor (2001) who found a transfer of defensive behaviors from the avoidance apparatus to the EPM in rats exposed to cat odor but not rats exposed to TMT or triethylamine. Evidence for the effectiveness of the predator odor method came from inspection of the collars after each exposure. Upon removing collar pieces from the home cages, careful observation revealed that the control collars had a

significant amount of chew marks. The cat collars, on the other hand, were not chewed and looked like they were completely avoided. This is consistent with Sütt and colleagues (2008) findings which revealed that none of the animals touched the cat odor-impregnated cloth during predator odor exposure.

As previously mentioned, there is a high comorbidity rate between disorders of anxiety and depression. Although there exists a great deal of inconsistencies regarding the actual percentage of this comorbidity, there is agreement that this co-occurring pattern of illnesses is fairly common. The findings from the current animal model of anxiety provide minimal evidence for the overlap between the characteristics of these two disorders as a result of exposure to the stressor of predator odor. Although the odor induced marginal effects on sucrose consumption during SPT habituation and the second day of EZM testing, these effects were not robust. In addition, adolescent exposure to ketamine affected SPT results but not EZM, indicating dissociation between ketamine's effects on models of depression and anxiety.

Despite the effects that were inconsistent with previous studies, there is still a productive line of research for future experiments. The next step in the line of research would be to validate this ketamine pretreatment with other measures of depression. Because ketamine showed a trending effect on sucrose preference, it is worth investigating if this effect can be found in other animal models of depression such as the FST or TST. Although pretreatment with ketamine did not significantly influence anxiety-like behaviors, an effect of this drug might be present in an alternative measure of anxiety, such as the social interaction test. Therefore, future studies should incorporate multiple measures of anxiety and depression, which are lacking from the current experiment. Due to the unanticipated lack of movement on the first day of EZM testing, future research should include more exposure to the maze. Although much of the literature employs a

single 5-min exposure, future studies could increase the amount of time spent in the maze as well as the number of maze exposures.

In summary, the present results provide some evidence for the preventative effects of ketamine on depressive-like behaviors. The repeated schedule of ketamine in adolescence appeared to have subtle but prolonged effects that were evident in adulthood. Of particular significance is the fact that ketamine tended to prevent the presence of depressive-like behaviors in the SPT. The role of ketamine in predator odor-induced fear conditioning suggests the possibility that various glutamate receptor antagonists may be effective agents for reducing the symptoms of depression. Recreational and prescription drugs are widely abused during adolescence, which can result in altered psychological functioning during adulthood. This raises the question of whether psychiatric medication administered during adolescence can prevent the presence of certain disorders later in life. The present results tell us that pretreatment with ketamine should be considered as a potential pharmacotherapy for clinical depression. Further research regarding the pharmacological modulation of the response to cat odor is necessary in identifying new drugs that are useful in treating human anxiety and depressive disorders.

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