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The Short and Long-Term Effects of Chronic Ketamine during Adolescence on Object Recognition Memory in Rats

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by

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Abstract

Prior research has demonstrated that ketamine causes deficits in object recognition and location memory following both acute and chronic administration (Pitsikas & Boultadakis, 2009; Venãncio et al., 2011). Although it is well established that abuse of many different kinds of drugs during the critical developmental period of adolescence can lead to impairments in cognition later in adulthood (Gilpin et al., 2012; Wagner et al., 2010), no research has investigated the effects of chronic ketamine administration during adolescence and its effects on behavior in adulthood. With recent research establishing that chronic ketamine during adolescence produces late-onset alterations in electrophysiology during adulthood (Featherstone et al., 2014), the current study examined the effect of chronic ketamine during adolescence on the object recognition (ORT) and location tasks (OLT). Additionally, the current study sought to clarify how the combination of natural neurodevelopmental processes and NMDA receptor antagonism affected both short- and long-term performance on both the object recognition and location tasks. Therefore, the animals received one injection of 25 mg/kg of ketamine daily for seven consecutive days during adolescence. Following drug administration, the animals were assessed in a locomotor assessment and subsequently in behavioral testing on the object recognition and location tasks. The animals underwent choice phases where they had to discriminate a novel object or location from a familiar object or location following a 15-minute and 24-hour delay during both adolescence and adulthood. Ketamine administration produced impairments in object recognition and location tasks at all time points tested (i.e., adolescence and adulthood, 15m and 24h delays). In addition, performance on the OLT was affected by both age and delay, with superior discrimination occurring in adult rats as compared to adolescent rats and at the 15-minute time point as compared to the 24-hour time point. There was also a trend toward an age by delay interaction on the ORT such that older rats were more impaired than younger rats, but only at the 24-hour delay. Taken together, the current findings expand the breadth of knowledge regarding the effects of ketamine on object recognition and location memory. Specifically, this study suggests that administration during adolescence induces deficits in how rats explore novel objects and locations in adolescence and that these deficits persist into adulthood.

The Short and Long-Term Effects of Chronic Ketamine during Adolescence on Object Recognition Memory in Rats

Ketamine

Ketamine has been referred to as a 'dirty' drug (Morgan & Curran, 2006), primarily because of its complex and varying behavioral and neurochemical effects. Since its inception in the early 1960s as an alternative to the anesthetic, phencyclidine (PCP), ketamine has been used in numerous fields of medicine, and research has indicated new potential directions for the 'dissociative anesthetic' (Meyer & Quenzer, 2005, p. 358). Currently, it is used as an anesthetic in pediatric work with children and remains the most widely used anesthetic in animal medicine (Meyer & Quenzer, 2005, p. 358; Morgan & Curran, 2011). Similarly, ketamine is also used for pain management in both humans and animals and has produced significant pain-alleviating effects in many different conditions (Eichenberger et al., 2008; Schwartzman et al., 2009; Sorensen et al., 1995). Due to its broad range of uses, better understanding the effects of ketamine would allow for advances in many fields of research.

However, ketamine also produces adverse effects, including hallucinations, delusions, and drastic sensory and emotional changes (Morgan & Curran, 2011). These negative effects have led to research on its potential effects as a novel agent in different fields of medicine. Symptomology similar to schizophrenia has been modeled in rats using ketamine to assess the wide range of signs indicative of this illness (Becker et al., 2003); similarly, ketamine induces transient schizophrenia-like symptoms in healthy humans (Morgan, Muetzelfeldt, & Curran, 2004). Additionally, research has demonstrated that ketamine may have implications for another psychiatric disorder, as it has been found to produce significant alleviation of depressive-like symptoms in animal models of depression (Autry et al., 2012; Koike, Iijima, & Chaki; 2011).

Comparable effects have been found in human participants as well, with a single dose rapidly improving depressive symptoms in patients with major depressive disorder and treatment-resistant depression (Berman et al., 2000; Diazgranados et al., 2010).

While the various negative effects of ketamine, ranging from vivid dreams and hallucinations to dissociation and psychosis, have been beneficial to some areas of medicinal research, these effects have also caused ketamine to become an increasingly popular drug of abuse, primarily at dance parties and raves (Corazza et al., 2012; Meyer & Quenzer, 2005, p. 358; Morgan, Monaghan, & Curran, 2004). Reasons for the increase may be due to its short time-to-effect (i.e., 30 seconds intravenously, 5-30 minutes intranasally, and 20 minutes orally), long-lasting duration of action (i.e., up to three hours), and low cost and accessibility, with its illegal use typically coming from the medical-grade drug (Corazza et al., 2012; Morgan et al., 2004). Ketamine has been ranked in popularity among many other common drugs of abuse, including cannabis, cocaine, and ecstasy (Hoare, 2010); it is a top-five drug of abuse in the United Kingdom and is the most abused drug in Hong Kong (Dick & Torrance, 2010; Morgan & Curran, 2011). Importantly, in people aged 16-24, ketamine used doubled between 2007-2008 and 2008-2009 (Hoare, 2010). Similarly, first time use of ketamine in this age group increased 1.8% over a three-year span, surpassing the use of ecstasy, amyl nitrate, amphetamines, and magic mushrooms (Hoare, 2010). In the United States, ketamine has been used by an estimated 2% of 10th and 12th graders (Johnston, O'Malley, Bachman, & Schulenberg, 2010). While not as prevalent in the United States as some countries, ketamine use is on the rise and should still be considered a public health concern, particularly among adolescents and young adults.

Unlike some drugs that have highly addictive qualities, there is contradictory evidence regarding development of dependence on ketamine. It has been argued that ketamine has no

dependence liability (Chakraborty et al., 2011; Britt & McCance-Katz, 2005) and that there is little evidence of physiological withdrawal syndrome (Pal, Berry, Kumar, & Ray, 2002). However, others have indicated that ketamine use can result in physical (Morgan, Rees, & Curran, 2008) and psychological symptoms resembling withdrawal syndrome, including cravings and high tolerance (Jansen, 2000). Regardless, it is well established that chronic abuse of many types of drugs can elicit cognitive impairments (Wood et al., 2013). Research has attempted to clarify the underlying mechanisms that may contribute to these deficits; first, however, a basic knowledge of ketamine's pharmacological properties is necessary.

Neurobiology of Ketamine

Ketamine's main site of action is on glutamate, the major excitatory neurotransmitter in the brain, and is a non-competitive antagonist at the N-methyl-D-aspartate (NMDA) receptor (Meyer & Quenzer, 2005, p. 360). Ketamine also works upon μ-opioid and non-opioid δ receptors, however, affinity for these sites is fairly low (Morgan & Curran, 2011). When administered, it binds to the same NMDA receptor site as PCP, which is located in the calcium channel, and leads to an obstruction of calcium flow through these channels (Britt & McCance-Katz, 2005; Chakraborty, Neogi, & Basu, 2011). The blocking of the calcium channels results in decreased excitatory neurotransmission and has been associated with alterations in memory and cognition (Cotman & Monaghan, 1987). Ketamine has also been shown to play a role in neural mechanisms essential for these processes, such as long-term potentiation and synaptic plasticity (Morgan & Curran, 2011). At the neurochemical level, the blocking of NMDA receptors has been associated with serotonin, dopamine, and norepinephrine neurotransmitter systems (Britt & McCance-Katz, 2005). Ketamine has been found to inhibit the reuptake of these three

neurotransmitters (Smith Larive, & Romanelli, 2002), as well as increase dopamine (DA) release in the prefrontal cortex (PFC) and midbrain (Britt & McCance-Katz, 2005).

Ketamine-Induced Cognitive Impairments

In addition to the well-documented psychological and physiological effects induced by ketamine, research has yielded data regarding its effects on a wide range of cognitive functions in humans and animals. In rodents, research has produced varied findings in different types of memory assessments as well as different stages of memory (i.e., acquisition, consolidation, and retrieval) when a single dose of ketamine is administered. Two studies administered doses ranging from 25-150 mg/kg in mice and found that none of the doses impaired spatial memory on the radial-arm maze or memory acquisition, consolidation, or retrieval in the Y-maze (Ribeiro, Rodrigues, Valentim, & Antunes, 2013; Valentim, Ribeiro, Olsson, & Antunes, 2013). Studies assessing the performance of rats on the Morris water maze have shown impairments in memory acquisition by 15 mg/kg and 100 mg/kg, consolidation by 100 mg/kg (Moosavi, Khales, Rastegar, & Zarifkar, 2012), and retrieval by doses of 15, 30, and 100 mg/kg (Duan et al., 2013; Moosavi et al., 2012). Using similar doses of ketamine, Verma and Moghaddam (1996) found that 20 and 30 mg/kg of ketamine dose-dependently induced impairments on the T-maze, which is an assessment of spatial delayed alternation, indicating deficits in PFC-sensitive working memory.

The effects of subchronic and chronic administration of ketamine on different types of memory assessments have been researched in rats as well. Subchronic administration is, typically, the administration of a drug for a certain time period less than a week (Becker et al., 2003), while chronic administration is typically a week or longer (Gama et al., 2012; Venãncio et al., 2011). Rushforth, Steckler, and Shoaib, (2011) investigated the effects of ketamine at doses

of 10 and 30 mg/kg for 5 consecutive days on the odor span task (OST), which is a working memory task that involves detecting a novel odor from an increasing number of presented odors. They found dose-dependent deficits on the OST that persisted for 14 days following drug cessation, which suggests that ketamine induces persistent deficits in working memory. Utilizing a similar administration schedule of 30 mg/kg for 5 consecutive days, Becker and colleagues (2003) found deficits in the ketamine-treated group on latent inhibition, as rats were unable to ignore irrelevant stimuli. Ketamine, when administered at a dose of 25 mg/kg for 7 consecutive days, has also been found to impair performance on the inhibitory avoidance task, which, during training, presents rats with a shock upon stepping down from a platform. During test, the animals are not presented with a shock and their latency to step off the platform is measured. Gama and colleagues (2012) found that the ketamine-treated rats had significantly lower latency to step off the platform during three different test intervals, indicating impairments in working, short-term, and long-term memory.

Overall, the inconsistent findings in studies with rodents demonstrate that ketamine induces impairments in learning and memory, primarily spatial memory, in rats but not mice (Table 1). Future research needs to further clarify which aspects of memory (e.g., acquisition, consolidation, and retrieval) are affected by which doses and administrations because, currently, the research is conflicting in regards to the dose-dependent effects of ketamine.

Research done with humans has yielded findings consistent with the animal studies investigating the cognitive and behavioral effects of ketamine. In a young adult (*M* age = 19.84 years) sample of ketamine users who used the drug at least once per month for the last two years, Chan and colleagues (2013) found that users had impaired verbal fluency, verbal learning, and cognitive processing speed compared to controls. Interestingly, ketamine has also been found to

cause impairments in healthy humans who had no history of ketamine use. Dose-dependent deficits have been found in verbal and nonverbal (declarative) memory (Newcomer et al., 1999), while intravenous ketamine has produced deficits in free recall and recognition memory (Malhotra et al., 1996). Reductions in the delayed recall of words presented immediately before, but not during, drug infusion, have been found, implying that ketamine interferes with early consolidation processes (Parwani et al., 2005). Collectively, human research has demonstrated that ketamine can produce impairments in several domains of learning and memory.

Table 1. Effects of ketamine on learning and memory in rodents

	Species	Dose of ketamine	Frequency of administration	Behavioral task	Results
Becker et al. (2003)	Male Sprague-Dawley rats	30 mg/kg	1x daily for 5 consecutive days	Elevated plus maze, latent inhibition	No effects on elevated plus maze 2 or 4 weeks after ketamine; 4 weeks following ketamine, latent inhibition was disrupted
Duan et al. (2013)	Male Sprague Dawley rats	30 mg/kg before retrieval phase of Morris Water Maze	Single dose	Morris Water maze, locomotor activity in the open field	Ketamine impaired spatial memory retrieval (less time in target quadrant) and increased locomotor activity
Gama et al. (2012)	Male Wistar rats	25 mg/kg	Chronic – 1x daily for 7 consecutive days	Inhibitory avoidance, locomotor activity in open field	Ketamine increased locomotor activity and induced working (immediately after training), short-term (1.5 h after training), and long-term memory (24 h after training) deficits
Moosavi et al. (2012)	Male Sprague-Dawley rats	15 mg/kg 40 minutes before training; 15 or 100 mg/kg immediately after training (consolidation); retrieval-15 or 100 mg/kg	Single dose	Morris water maze	15 mg/kg impaired memory acquisition and retrieval; 100 mg/kg impaired memory acquisition, consolidation, and retrieval
Ribeiro et al. (2013)	Male C57BL/6 mice	25, 75, or 150 mg/kg	Single dose	Radial-arm maze	No effects on working/reference memory
Rushforth et al. (2013)	Male hooded Lister rats	10 and 30 mg/kg	1x daily for 5 consecutive days followed by 2 day washout period	Odor span task	Ketamine produced a dose-dependent impairment that persisted for 14 days following exposure
Valentim et al. (2013)	Male C57BL/6 mice	40 mg/kg	Single dose	Y-maze	No effects on memory acquisition, consolidation, or recall
Verma & Moghaddam (1996)	Male Sprague Dawley rats	10, 20, or 30 mg/kg	Single dose	T-maze	20 and 30 mg/kg dose-dependently reduced percent correct choice in spatial delayed alternation

Note: All studies utilized an intraperitoneal (ip) injection route of administration.

Table Legend
mg/kg – milligram/kilogram
PND – post-natal day
n – number of animals receiving manipulation/undergoing specific task

Neurobiological Mechanisms Underlying Ketamine's Effect on Cognition

Changes in many brain regions have also been discovered following ketamine administration, including the PFC, cerebellum, hippocampus, striatum, and cerebral cortex (Becker et al., 2003; Canever et al., 2010; Oliveira et al., 2011); however, not all evidence is consistent (Ribeiro et al., 2013; Valentim et al., 2013). At the cellular level, enzyme levels in these regions have been altered by ketamine, including elevated creatine kinase, an enzyme important for energy in brain tissue (Canever et al., 2010). Chronic ketamine administration of 25 mg/kg over 7 days has induced changes in mitochondrial activity lasting up to 6 hours post-administration in the hippocampus, striatum, and PFC (Oliveira et al., 2011), suggesting that ketamine affects the respiratory chain complex and crucial aspects of cell function, such as energy metabolism and compensatory mechanisms that promote cellular and neuronal survival.

On the other hand, there is contradictory evidence regarding the effects of ketamine on some of the previously mentioned brain regions. For example, ketamine has been shown to not affect neurodegeneration in brain areas typically affected by chronic drug use, such as the thalamic nucleus, striatum, and nucleus accumbens (Ribeiro et al., 2013). Similarly, parts of the hippocampus and surrounding regions, including CA1, CA3, the dentate gyrus, all of which have high densities of NMDA receptor-binding sites (Monaghan & Cotman, 1985), have been demonstrated as immune to the neurodegenerative effects of ketamine, indicated by no differences in the number of dead cells compared to controls (Ribeiro et al., 2013; Valentim et al., 2013).

Additionally, certain neurotransmitters have been shown to play a role in cognitive deficits induced by ketamine. Verma and Moghaddam (1996) demonstrated a preferential increase of dopamine release in the PFC compared to the striatum. Becker and colleagues (2003)

found that ketamine treatment led to increases in D2 receptor binding in the hippocampus and a decrease in glutamate receptor binding in the frontal cortex. They found an increase in the density of DA transporters in the striatum and 5-HT (serotonin) transporters in striatum, hippocampus, and frontal cortex.

Overall, there are inconsistencies within the literature regarding brain regions affected by ketamine. Further research is necessary to provide insight into these differences, which may lie within the different methodological approaches used, including the doses, schedules, and routes of administration.

Adolescence

As ketamine is associated with a high prevalence of abuse, especially in adolescents (Wu, Schlenger, & Galvin, 2006), it is important to investigate potential links between ketamine and adolescence, both behaviorally and physiologically. Adolescence has been broadly defined as a gradual transition period from childhood to adulthood; however, it is not defined by any one specific event (Pickles et al., 1998). Therefore, it is difficult to determine the specific beginning and end of this critical developmental stage; however, it is generally agreed that human adolescence begins around age 12 (Dahl, 2004; Spear, 2000) and lasts until about 18 years of age (Eiland & Romeo, 2012; Spear, 2000); however, this range is not universally agreed upon. The age of onset has been proposed as earlier by some, beginning at age 10 (Eiland & Romeo, 2012), while the offset of adolescence is sometimes considered to extend into the mid-twenties (Dahl, 2004). In rats, adolescence has been conservatively defined and considered to begin around postnatal day (PND) 28-30 (Eiland & Romeo, 2012; Spear; 2000) and extend anywhere between PND 42 (Spear, 2000) and 60 (Eiland & Romeo; 2012). Rats between postnatal days 28-42 are often hyperactive and exhibit greater exploration in novel situations than other aged rats (Spear,

Shalaby, & Brick, 1980), similar to the typical behaviors of adolescent humans including high rates of exploration, emotional imbalance, and novelty-seeking (Eldreth, Hardin, Pavletic, & Ernst, 2013). Not only is adolescence a period of physical change, it is a period of neurobiological change as well (Gulley & Juraska, 2013).

Neurodevelopmental and Behavioral Changes during Adolescence

At the neural level, there are many changes that occur during adolescence. The PFC is an area involved in executive functioning (e.g., planning, problem solving, working memory, emotional regulation, and inhibitory control). While many brain regions mature earlier, the PFC undergoes significant changes during adolescence and develops well into this stage of life (Giedd et al., 1996; Gogtay et al., 2004; Lenroot & Giedd, 2006; Sowell et al., 1999) across a variety of species (Spear, 2000). Other changes found in this region include synaptic elimination of glutamatergic inputs (Huttenlocher, 1984; Zecevic, Bourgeois, & Rakic, 1989). More specifically, in rats, cortical binding to NMDA receptors peaks in early adolescence (i.e., PND 28), which is followed by a subsequent loss of about 1/3 of these cortical glutamate receptors during late/post adolescence (i.e., PND 60; Insel, Miller, & Gelhard, 1990). In addition to this decrease of cortical NMDA receptors, sensitivity to neurotoxicity induced by NMDA antagonists increases significantly in late adolescence (i.e., PND 45; Farber et al., 1995). This vulnerability to NMDA antagonist-induced hypofunction was found to be age-dependent, indicating a potentially more potent effect of ketamine during this period compared to rats less than a month old (Farber et al., 1995).

The synaptic pruning and development during adolescence are presumed to be excitatory in nature and thought to result in a major decline in the amount of excitatory neurotransmission within the cortex (Rakic, Bourgeois, & Goldman-Rakic, 1994). Substantial amounts of energy

are required for neural activity in the brain and this activity is typically estimated through measures of oxygen utilization, blood flow, and glucose metabolism (Spear, 2000). The significant restriction of excitatory input to the cortex during adolescence results in deficits in two of these primary estimators; adolescent rats tend to have significantly lowered rates of oxygen consumption and glucose utilization (Tyler & van Harrevald, 1942) compared to adult rats. Similarly, these measures of brain activity show developmental declines during adolescence. This increased metabolic activity during development is particularly evident in the neocortex and forebrain regions (Chugani, 1994). The neocortex has been found to play a significant role in learning and memory; more specifically, it has been implicated in gradual learning over multiple trials and is thought to work as a compensatory mechanism in the absence of normal hippocampal processing (Wiltgen et al., 2006). Similar to the PFC, glutamate receptors in the hippocampus also undergo substantial pruning during adolescence, with a loss of about ¼ of the NMDA receptors in hippocampal pyramidal regions between PND 28 and 60 in rats (Insel, Miller, & Gelhard, 1990). Also, $GABA_B$ synaptic transmission in this region develops relatively late, gradually maturing between PND 35 and 45, while another receptor complex, GABA/BDP, undergoes maturational changes as well, including an increased sensitivity to environmental changes and stressors (Nurse & Lacaille, 1999).

Altogether, manipulations to developing brain regions involved in learning and memory processes, such as the PFC, hippocampus, and neocortex, during adolescence, may produce not only immediate but long-lasting deficits in cognition. These regional changes along with alterations in cellular processes, such as the blocking of calcium flow induced by NMDA receptor antagonists, such as ketamine, and synaptic pruning during development may,

combined, produce the increased vulnerability to drug administration during adolescence that result in persisting cognitive deficits.

Moreover, being that adolescence is a period of great physiological change, behavioral research in adolescent-aged animals has attempted to provide a link to these physiological changes in the brain. However, the research is limited and there have been conflicting results, making it difficult to draw conclusions regarding behavior in adolescent rats. Spear (2000) discusses that younger rats often display worse performance on complex avoidance tasks compared to adult rats, potentially due to higher distractibility and an inability to maintain focus. However, Spear also argues that, on less challenging cognitive tasks, performance of rats may actually be enhanced compared to adult-aged animals, specifically in tasks requiring increased exploration and activity. Additionally, adolescence is an influential and critical developmental stage that is associated with increased risk-taking behaviors in a variety of species. This suggests that organisms within this transitional period may be more susceptible to environmental influences and everyday stressors compared to adults, including experimentation with substances. Importantly, studies investigating adolescence have demonstrated that drug abuse during this period increases vulnerability to drug-induced cognitive impairment in adulthood as well (Pope et al., 2003).

Recognition Memory

Since ketamine acts upon NMDA receptors, which have high densities of binding sites in many regions important for learning and memory (Monaghan & Cotman, 1985), and there is some research that indicates robust ketamine-induced impairments in learning and memory, it is important that future research examines more specific aspects of these cognitive processes that are negatively affected. Recognition memory is a cognitive process that allows both humans and

animals to tell familiar items from novel items (Meunier & Barbeau, 2013). When researchers test recognition memory, subjects must identify whether they saw a particular item at an earlier time (Matlin, 2009, p. 129). Research on recognition memory has clinical implications as it may help the understanding of cognitive deficits underlying disorders such as Alzheimer's and schizophrenia (Meunier & Barbeau, 2013). The object recognition and object location tasks (ORT/OLT) are two assessments used to study this capacity in rats and will be further examined in subsequent sections.

Object Recognition Task

Introduced in 1988, Ennaceur and Delacour developed the ORT in order to study the neurobiology underlying recognition memory in rats. Unlike earlier tasks used to assess recognition memory, such as the delayed matching and non-matching-to-sample tasks, the object recognition task does not require rats to learn a rule since it is based solely on their spontaneous exploratory behavior towards objects (Ennaceur & Delacour, 1988). The primary dependent variable is the amount of time spent exploring the objects and the difference is calculated, resulting in a discrimination ratio. Typically, it is expected that rats will explore the novel object longer than the familiar object in a given test session. There are many variations to the procedure of this task, however, similar methodology to the original task used by Ennaceur and Delacour (1988) and Ennaceur and colleagues (2005), with slight modifications, will be described and utilized in the current study.

For this task, an open box that contains objects made of different materials (i.e., glass, plastic, or metal) is used. The objects weigh enough that they cannot be moved or displaced by the rats. One day before testing, the rats undergo habituation, which is decreased exploratory behavior in response to a continued or repeated environment or stimulus (Leussis & Bolivar,

2006). During habituation, the rats are allowed to explore the empty apparatus. Two testing sessions are used, separated by delay. Each session contains two trials; in the first trial (sample phase), two objects (familiar duplicates) are placed near the rear of the box equidistant from the back corners. During the second trial (choice phase), a novel object replaces one of the duplicates and the original object is placed in the other back corner. At the start of each trial, rats are placed in the center of the front wall, facing opposite the objects. The duration of the sample phase is 5 minutes and the duration of the choice phase is 3 minutes (Ennaceur & Delacour, 1988).

Environmental control measures are taken, including a sound-isolated room that contains enough light to provide constant illumination of the test apparatus as well as the cleaning and replacement of objects between trials to avoid olfactory cues. As far as experimental control, the positioning of the objects in the choice phase is counterbalanced and randomized to avoid order effects. Also, a different pair of objects is used during each session. The counterbalancing and randomization of objects reduces the potential occurrence of place and preference effects; the objects also have no natural significance to the rats and have never been associated with reinforcement or reward (Ennaceur & Delacour, 1988; Ennaceur et al., 2005).

Neural Mechanisms Underlying the Object Recognition Task

Research has clarified the neurobiological mechanisms involved during the ORT. Studies have shown that lesions to the hippocampus, fornix, or medial septal regions generate no effect in object recognition (Ennaceur & Meliani, 1992; Good et al., 2007; Langston & Wood; 2010) and, at delays of 10 seconds and 1 minute between study and test phases, Clark and colleagues (2000) discovered intact object recognition. However, at longer delays (i.e., 10 minutes, 1 and 24 hours), Clark and colleagues (2000) found that rats with lesions to the

hippocampus and surrounding dorsal tissue had significantly impaired object recognition compared to sham-lesioned rats. Temporary inactivation of neurons in the dorsal hippocampus induced by lidocaine in mice has resulted in impaired performance on this task after a 24 hour but not 5 minute delay (Hammond, Tull, & Stackman, 2004), suggesting different mechanisms at play during different stages of memory processes.

The mixed findings regarding the importance of the hippocampus in object recognition memory may be due to the amount of damage to this region, as studies have indicated that lower percentages of hippocampal damage (i.e., less than 75%) do not result in object recognition deficits after delays of 10 minutes, 1, 3, and 4 hours (Ainge et al., 2006; Broadbent, Squire, & Clark, 2004). However, higher damage percentages (i.e., greater than 75%) have resulted in significant impairments after these delays (Ainge et al., 2006; Broadbent et al., 2004), suggesting that complete lesions or damage to the hippocampus will result in deficits in object recognition at delays larger than 5 minutes.

According to Warburton and colleagues (2013), both the perirhinal cortex and the hippocampus are necessary to recognize the novel object if the two objects explored during the sample phase are different. On the contrary, if the two objects explored during the sample phase are matching, the perirhinal cortex is necessary but the hippocampus is not (Barker & Warburton, 2011).

Object Location Task

The procedure of the OLT is similar to that of the ORT; however, instead of replacing a familiar object with a novel object in the choice phase, a familiar object is relocated to a novel location (Ennaceur, Michalikova, Bradford, & Ahmed, 2005). Typically, the rat will explore the novel location more than the familiar one. This task is considered a spatial working memory

task because it forces the rat to remember and differentiate between spatial positions that have or have not been previously occupied by objects (Warburton et al., 2013).

Neural Mechanisms Underlying the Object Location Task

The neural mechanisms underlying successful performance on the OLT differ from those necessary for the ORT. Lesions studies have demonstrated that performance on the OLT depends upon the hippocampus and fornix but not perirhinal cortex or medial PFC (Barker & Warburton, 2011; Ennaceur et al., 1996).

Object Recognition and Age

Performance on both the object recognition and location tasks has been examined in rats of different ages. In 2004, Anderson and colleagues investigated the differences between 18-day-old pups and 90-day-old adult rats on the ORT. They were tested at two different retention intervals (i.e., 1 minute or 2 hours) and found that the younger rats had significantly impaired object recognition at the 2-hour interval, suggesting that the lack of development in the pups compared to adults was the reason for the deficit. To further investigate these findings, Reger and colleagues (2009) used three different age groups of rats: weanlings (i.e., PND 20-23), juvenile (i.e., PND 29-40), and young adulthood (i.e., PND 50+). These three groups were exposed to four different retention intervals on the ORT (i.e., 0.25, 1, 24, or 48 hours). The results of Reger and colleagues (2009) indicated that weanlings showed intact object recognition at the 15-minute and 1 hour intervals but impaired performance at longer time points, while the juvenile and adult rats showed novel object preference up to 24 hours. While these results suggest that very young rats exhibit inferior long-term memory retention compared to that of older rats, it shows that the ORT can be performed successfully, albeit at shorter intervals, by these younger rats.

The significant synaptic pruning of glutamate and NMDA receptors in the hippocampus and surrounding areas during development (Insel et al., 1990), along with development of regions indicated in this task, primarily the perirhinal cortex, may explain the increase in performance as the rats aged. Perhaps regions surrounding the hippocampus, such as the neocortex, which has been indicated as an important compensatory mechanism in learning processes in the absence of the hippocampus (Wiltgen et al., 2006), may be further developed in the older rats, therefore providing stronger synaptic connections to enhance performance and compensate for underdeveloped regions at the longer retention intervals.

Advantages of the Object Recognition and Location Tasks

Both the object recognition and location tasks are useful tools in the study learning and memory and the underlying neurobiological mechanisms (Ennaceur & Delacour, 1988). First of all, they have potential for cross-species generalization because they have been used in rats, (Ennacuer & Delacour, 1988; Goulart et al., 2010; Silvers et al., 2007), mice (Clarke et al., 2000; Hammond et al., 2004), rabbits (Hoffman & Basurto, 2013) and monkeys (Buckmaster et al., 2004; Peissig et al., 2007). Also, neither the ORT nor OLT is restricted to a certain area of research, which allows for the studying of various issues, including learning, memory, preference for novelty, the brain mechanisms underlying recognition, and the effects of drugs on it (Antunes & Biala, 2012). Also, they require no external motivation, reward, or punishment; however, a little training or habituation is required (Silvers et al., 2007). They can also be completed in a relatively short period of time (Silvers et al., 2007) and lack overt stress factors (Reger et al., 2009). The freedom from response contingencies, the lack of training and stress-inducing factors involved make them an optimal task for examining development in rats (Reger et al., 2009) because, depending upon age, young rats may be vulnerable to such factors and unable to learn

certain task rules (Bachevalier & Beauregard, 1993). Perhaps one of the main advantages of these two tasks includes the

NMDA Antagonists and Recognition Memory

Single-dose administration of NMDA antagonists has been found to induce deficits in the object recognition and location tasks (de Lima et al., 2005; Grayson & Neill, 2004; King et al., 2004), including some long-term deficits when administered prior to training as well as immediately after (de Lima et al., 2005). Similar results have been found when using a chronic administration schedule. PCP, when administered twice daily for 7 days at a 2 mg/kg dose followed by a 7-day washout period, resulted in significantly decreased exploration of the novel object (Grayson, Idris, & Neill, 2007). Using a higher dose of 10 mg/kg, Hashimoto, Fujita, Shimizu, and Iyo (2005) administered PCP once daily for 10 days (i.e., days 1-5, 8-12) and found significant deficits on the ORT. Comparable results have also been found in mice when administering the same dose but over the span of 14 days (Nagai et al., 2009).

Ketamine and Object Recognition Memory

Deficits in spatial and non-spatial object recognition memory have been found when ketamine was administered acutely (Boultadakis & Pitsikas, 2011; Goulart et al., 2010; Nikiforuk et al., 2013; Pitsikas & Boultadakis, 2009; Pitskias, Boultadakis, & Sakellaridis, 2008) or chronically (Venâncio, Magalhães, Antunes, & Summavielle, 2011; Table 2). In acute administration studies, ketamine has been administered prior to the sample phase in many different intervals (Goulart et al., 2010; Nikiforuk et al., 2013 Pitsikas & Boultadakis, 2009; Pitsikas et al., 2008). A wide range of doses, administered 20 minutes prior to the sample phase, have impaired performance on both tasks (Pitsikas et al., 2008), as well as caused alterations in exploration time of the objects (Nikiforuk et al., 2013). While Goulart and colleagues (2010)

found no significant impairments induced by ketamine at longer intervals (i.e., 24 hours), Pitsikas and Boultadakis (2009) did, showing that a much higher, anesthetic dose of 100 mg/kg given 24 hours prior to the sample phase reduced the discrimination index on both tasks. On the other hand, ketamine has also been administered immediately (Boultadakis & Pitsikas, 2011; Goulart et al., 2010; Pitsikas et al., 2008) or 6 hours (Goulart et al., 2010) after the sample phase. Similar to administration prior to the sample phase, a wide range of doses have impaired performance on both tasks when given immediately after (Boultadakis & Pitsikas, 2011; Pitsikas et al., 2008). Altogether, these findings suggest that ketamine, when administered acutely in a range of doses and time intervals, can induce impairments on many facets of non-spatial and spatial memory.

Research is limited regarding chronic administration of ketamine and its effects on object recognition memory; however, Venãncio and colleagues (2011) administered ketamine in doses of 5 or 10 mg/kg injected every 12 hours for 14 consecutive days. Following ketamine administration, rats' performance on the object recognition and OLTs was assessed. Venãncio and colleagues (2011) demonstrated that the 5 mg/kg dose caused rats to preferentially explore the familiar object longer than the novel object during the ORT after a 15 minute delay but not 24 hours. This dose also impaired performance on the OLT, as this group of rats was the only one to not explore the novel location significantly longer after the 15-minute delay. These findings further suggest that ketamine induces impairments in non-spatial and spatial working memory, even when administered chronically and provide rationale for the current study.

Table 2. Effects of ketamine on performance in the object recognition and location tasks

	Species	Dose of ketamine	Frequency of administration	Effects on ORT	Effects on OLT
Boultadakis & Pitsikas (2011)	Male Wistar rats	100 mg/kg or 3 mg/kg	Single dose – immediately after sample phase on day 2 (24 hours after sample phase on day 1)	Post-training administration of 100 mg/kg decreased exploration time and impaired novel object recognition	Not tested
Goulart et al. (2010)	Male Wistar rats	4, 8, or 20 mg/kg	Single dose – either immediately after training, 6 hours after training, or 24 hours before training	8 and 20 mg/kg doses immediately after training decreased preference for novel object; 20 mg/kg group showed significantly lower preference for novel object during test compared to training	Not tested
Nikiforuk et al. (2013)	Male Sprague Dawley rats	20 mg/kg	Single dose – 45 minutes before sample phase	Ketamine groups had lower exploration time	Not tested
Pitsikas & Boultadakis (2009)	Male albino Wistar rats	100 mg/kg	Single dose – either 24, 48, or 72 hours before sample phase	100 mg/kg administered 24 hours prior to sample phase reduced discrimination index (standard conditions)	100 mg/kg administered 24 and 48 hours prior to sample phase reduced discrimination index
Pitsikas et al. (2008)	Male Wistar rats	0.3, 1, or 3 mg/kg	Single dose – either 20 minutes before sample phase or immediately after	1 and 3 mg/kg impaired performance when administered 20 minutes before sample phase or immediately after	1 and 3 mg/kg impaired performance when administered 20 minutes before sample phase or immediately after
Venãncio et al. (2011)*	Male Wistar rats	5 or 10 mg/kg	Chronic – 2x daily for 14 consecutive days	5 mg/kg dose resulted in lower discrimination index after 15 minute delay	5 mg/kg dose impaired performance after 15 minute delay

***Note**: All studies utilized an intraperitoneal (ip) injection route of administration except Venãncio et al. (2011; subcutaneous).

The Current Study

The current study will attempt to clarify some of the inconsistencies in the literature regarding ketamine-induced deficits in object recognition and location memory. Despite the widespread use of ketamine by adolescents, not much is known about the immediate and long-term consequences of sustained adolescent ketamine use on neural development and its overt effects on both adolescent and adult cognition. While Gama and colleagues (2012) have

demonstrated that chronic administration of ketamine during adolescence can induce deficits in working, short- and long-term memory retention, the long-term effects of ketamine on object recognition memory have yet to be clarified. Recent research has shown that chronic ketamine administered during adolescence results in delayed-onset alterations in physiology in adulthood (Featherstone et al., 2014). While this is the first study to elucidate long-term effects of chronic ketamine on physiology, no studies, to our knowledge, have investigated the long-term effects of chronic ketamine during adolescence on behavior and cognition.

The present study will investigate not only the short- and long-term effects of chronic ketamine on object recognition memory, but also the effects of age as well. Short-term memory and long-term memory during both adolescence and adulthood will be assessed on spatial (i.e., OLT) and non-spatial (i.e. ORT) memory. Many studies involving ketamine and object recognition memory have utilized adult rats but none have used adolescent rats, yet research has indicated that drug use during the critical period of adolescence can result in significant behavioral effects that can persist into adulthood (Gilpin, Karanikas, & Richardson, 2012; Maldonado-Devincci, Badanich, & Kirstein, 2010). Research has also demonstrated that drug use during adolescence can result in a more robust impairment in cognition, specifically spatial memory, compared to use in adulthood (Sircar et al., 2010).

The previously mentioned neural mechanisms underlying the effects of ketamine point to potential lasting neural modifications when administered during adolescence. The synaptic pruning, development of brain regions, and prevalence of NMDA receptors in areas important for learning and memory processes provide further rationale for the current study. Ketamine administered during this developmental period may inhibit NMDA receptor-dependent learning

during both the object recognition and location tasks not only in the short-term, but may also produce long-term deficits that persist into adulthood.

Method

Subjects

Twenty-four experimentally-naïve Sprague-Dawley rats were used in this study. All rats were fed on an *ad libitum* schedule. The rats were obtained at approximately 36-40 days old and weighed from 116-137g at the start of the study. The rats were given one week for environmental adjustment before drug administration and behavioral testing and were handled daily for approximately 15 minutes. The rats were weighed each day during the experimental period. They were 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

An experimental apparatus similar to that used in previous studies (Ennaceur & Delacour, 1988; Ennaceur et al., 2005; Venãncio et al., 2011) was utilized with slight modifications. The apparatus for the ORT was a tan plastic bin (dimensions: 67.8 x 40.1 x 35.3 cm).

The apparatus for the OLT was a similar tan plastic bin with additional visual cues, including a black bull's-eye and a black and white striped pattern affixed to two adjacent sides of the bin. The purpose of this was to introduce additional cues and differentiate between spatial (i.e., object location) and non-spatial (i.e., object recognition) memory (Kenny, Adoff, Wilkinson, & Gould, 2011). The apparatuses were cleaned with an alcohol cleaning solution following each use.

The objects used in both tasks were made of biologically neutral material, including brick terracotta pots (d = 4.1 cm, h = 3.7 cm), plastic cylinders (d = 2 cm, h = 20 cm), empty clear glass bottles (5 x 5 x 13.5 cm), tin cans (d = 5 cm, h = 8.8 cm), empty aluminum cans (d = 6.0 cm, h = 13.2 cm), empty brown glass bottles (d = 5 cm, h = 22 cm), and empty yellow glass vases (d = 5 cm, h = 22 cm). The objects were heavy enough so that the rats could not displace them and there were multiple identical versions of each object, which allowed for the use of different versions of the same objects in the different phases to avoid object recognition through olfactory cues.

To assess locomotor activity, a clear cubic arena was used (dimensions: 60.96 x 60.96 cm). The floor was a piece of plywood that was lined with black tape for later determination of the number of crossings.

Stopwatches were used to record time spent in the experimental apparatuses. A camera mounted on the ceiling above the apparatuses was used to record all phases of the experiment (i.e., locomotor activity, habituation, sample phases, and choice phases) to a rewritable DVD for later coding.

Drugs

Half of the rats (n =12) received 25 mg/kg of ketamine, intraperitoneally (ip), prepared in saline at a volume of 1 mL/100 g while the other half received 25 mg/kg of saline (ip) injection. All doses were calculated based on the individual rats' weights. All rats received injections once daily for seven consecutive days. This dose, route, and schedule of administration have been found to produce short- and long-term effects in adolescent rats (i.e., PND 52; Gama et al., 2012).

Object Recognition Task

There were four phases of the ORT. First was habituation, where each rat was placed in the empty experimental apparatus for 10 minutes. There were no objects present during habituation so the rats could become habituated to the apparatus itself. The next day, the rats underwent the sample phase, which consisted of two identical objects present, and the rats were allowed to freely explore. Lastly, there were two choice phases, one occurring 15-minutes following the sample phase and one after 24-hours. For the choice phases, there was one familiar object that was previously presented in the sample phase, as well as a novel object, which the rats had never been exposed. Different novel objects were used in both of the choice phases. See Figures 1 and 2 for illustrations of the task during adolescence and adulthood.

Object Location Task

Similar to the ORT, there were four phases of the OLT. The first was habituation, where each rat was placed in the empty experimental apparatus, with additional spatial cues, for 10 minutes. There were no objects present during habituation so the rats could become habituated to the apparatus itself. The next day, the rats underwent the sample phase, which consisted of two identical objects present, and the rats were allowed to freely explore. Lastly, there were two choice phases, one 15 minutes after the sample phase and one 24-hours after. For the choice phases, one of the identical objects (i.e., familiar object) from the sample phase was moved to a novel location. Different novel locations were used in both of the choice phases. See Figures 1 and 2 for illustrations of the task during adolescence and adulthood.

Procedure

Experimental procedures began one week after the rats' arrival in the laboratory. Each day, for 7 consecutive days, the animals received one intraperitoneal injection of 25 mg/kg of

ketamine or saline. Body weight was monitored daily for the duration of the experiment. Thirty minutes after the last injection, locomotor activity was evaluated by placing each rat individually into the open field arena for 15 minutes and the total number of line crossings measured, similar to the methodology of Gama and colleagues (2012).

The order of tasks was counterbalanced between rats such that half of the animals were exposed to the ORT while the other half were exposed to the ORT. Twenty-four hours after the locomotor activity assessment, the habituation phase of the previously determined task was conducted. Each rat was placed in the empty experimental apparatus for 10 minutes (Venãncio et al., 2011) and underwent three days of habituation (Grayson et al., 2014). There were no objects present during habituation so the rats could become habituated to the apparatus itself (Ennaceur & Delacour, 1988).

Rats began the sample phase twenty-four hours later. The sample phase consisted of two identical objects present, equidistant from the walls of the apparatus, and affixed to the floor either by a screw or duct tape. The rats were placed in the middle of the apparatus, facing away from the objects, and allowed to explore for 3 minutes (Venãncio et al., 2011). The total time (seconds) exploring each object was measured. Following the sample phase, the rats were returned to their home cages.

After a delay of 15 minutes, the rats underwent the choice phase, where they were allowed to explore for 3 minutes and were then returned to their home cages (Venãncio et al., 2011). The total time (seconds) exploring each object was measured.

Twenty-four hours later, the rats underwent a second choice phase in which the same familiar object from the sample and first choice phases was presented with an entirely novel object (Venãncio et al., 2011). Similar to the first choice phase, rats were allowed to explore for

3 minutes and then returned to their home cages. The rats were then left undisturbed in their home cages for 48 hours.

Following the 48-hour break, the rats began habituation to the task in which they had not been previously tested. The rats underwent three days of habituation. Twenty-four hours following the third day of habituation, the rats underwent the sample phase.

Following a 15-minute delay, the rats underwent a choice phase. Twenty-four hours later, rats underwent a second choice phase. For each phase, the rats were allowed to explore the objects for 3 minutes and the total time (seconds) exploring the objects was measured. Also, following each phase, the animals were returned to their home cages and left undisturbed until the next phase. In accordance with other research (Gama et al., 2012), the testing done during these two weeks (PND 52-62) was during adolescence.

After a 13-day delay, the rats were again assessed on both the ORT and OLT to evaluate the potential long-term effects of chronic ketamine administered during adolescence. This session of behavioral testing took place in adulthood and tested the possible enduring effects of ketamine on non-spatial and spatial object recognition memory, respectively.

These behavioral tests followed the same procedure as previously described. However, a new set of objects was used for both tasks. These two weeks of behavioral testing were operationalized as 'adulthood' as the testing occurred when the rats were substantially older (i.e., PND 76-86). Animals underwent testing on the two tasks in opposite order which they were tested during weeks 1 and 2. For example, if the rats were tested on object recognition during week 1 and object location during week 2, they were tested on object location during week 3 and object recognition during week 4. See Figure 2 for task design for weeks 3 and 4 (adulthood). See Table 3 for complete procedural timeline.

Data Analysis

All statistical analyses were performed using SPSS statistical software. The statistical level of significance was considered at $p < 0.05$. Partial eta squared (η_p^2) was used as a measure of effect size (Richardson, 2011); a η_p^2 of .0099 revealed a small effect size, .0588 for a medium effect size, and .1379 for a large effect (Cohen, 1988). Follow-up analyses for interactions with trends toward significance were conducted if there was prior rationale to do so (Wilcox, 1987); a trend toward significance was considered as a *p* value that ranged from .05 - .10. Data are presented as mean ± standard error of the mean (SEM). Differences between the weights of the ketamine and saline groups during the experimental period were evaluated using a 2 (drug: ketamine or saline) x 27 (day: 1-27) mixed-design ANOVA. Differences between the mean total number of lines crossed during the locomotor activity assessment for the ketamine and saline groups were analyzed by using an independent-samples *t*-test. Preference of rats' exploration of objects or locations during the sample phases was analyzed using paired-samples *t*-tests. A 2 (drug: ketamine or saline) x 2 (age: adolescence or adulthood) x 2 (delay: 15 minutes or 24 hours) x 2 (order: ORT/OLT, OLT/ORT) mixed-design ANOVA was conducted to evaluate total exploration time. Also, a discrimination ratio (*D*) was calculated: $[D = (N - F)/(N + F)]$. The discrimination ratio represents the difference in exploration time expressed as a ratio of the total time spent exploring the novel and familiar objects/object locations in the choice phases (Boultadakis & Pitsikas, 2011; Ennaceur & Delacour, 1988; Ennaceur et al., 2005; Venãncio et al., 2011). A discrimination ratio closer to 1 indicates a stronger preference for the novel object/object location, whereas a discrimination ratio closer to 0 or -1 indicates a preference for the familiar object/object location (Boultadakis & Pitsikas, 2011; Pitsikas et al., 2008). Discrimination ratios were analyzed using a 2 (drug: ketamine or saline) x 2 (age: adolescence or

adulthood) x 2 (delay: 15 minutes or 24 hours) x 2 (order: ORT/OLT, OLT/ORT) mixed-design ANOVA. Drug and order were analyzed as between-subjects variables while age and delay were analyzed as within-subjects variables.

Figure 1. Positions of objects used for weeks 1 and 2 (adolescence) of behavioral testing

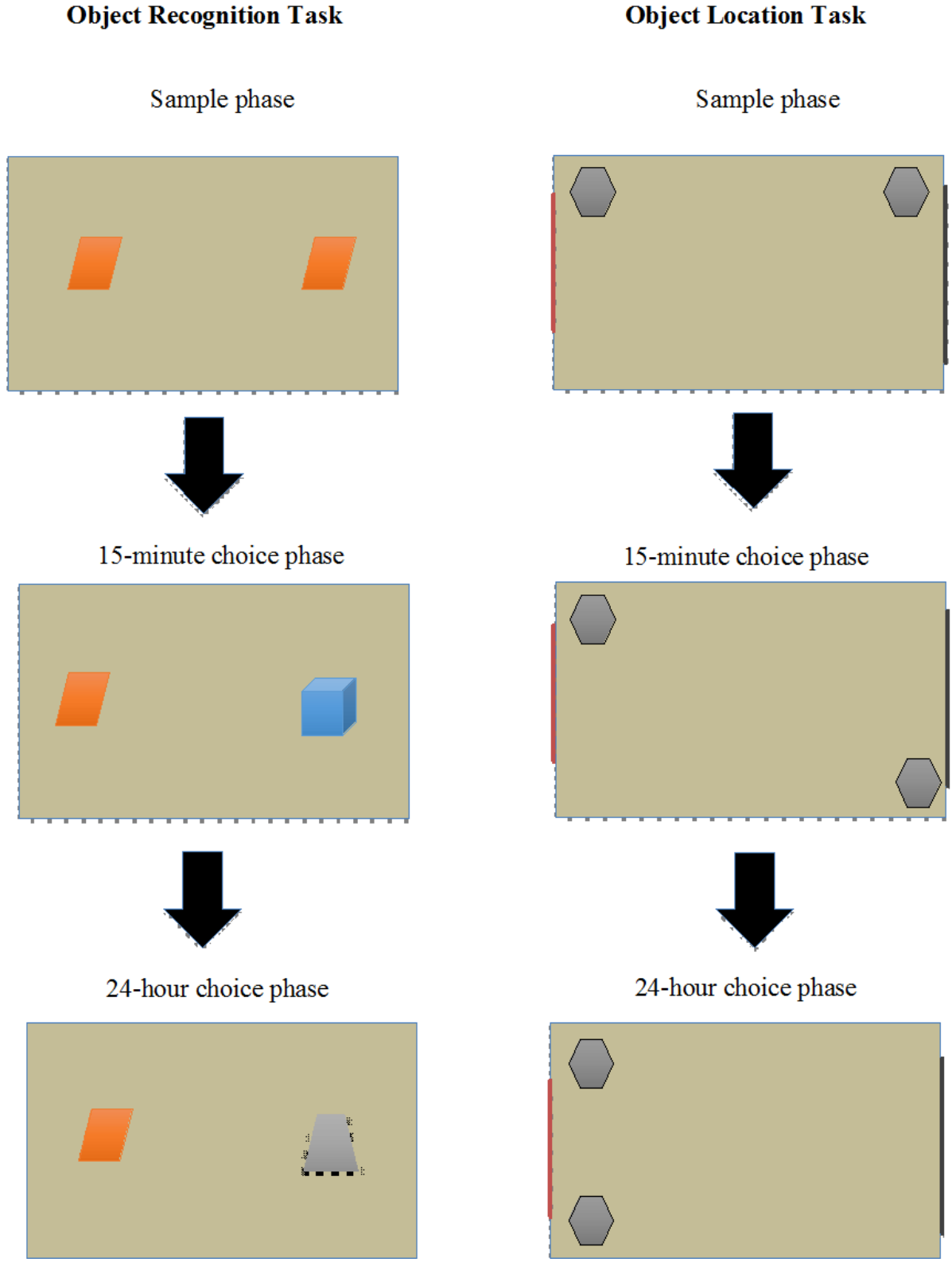

Figure 2. Positions of objects used for weeks 3 and 4 (adulthood) of behavioral testing

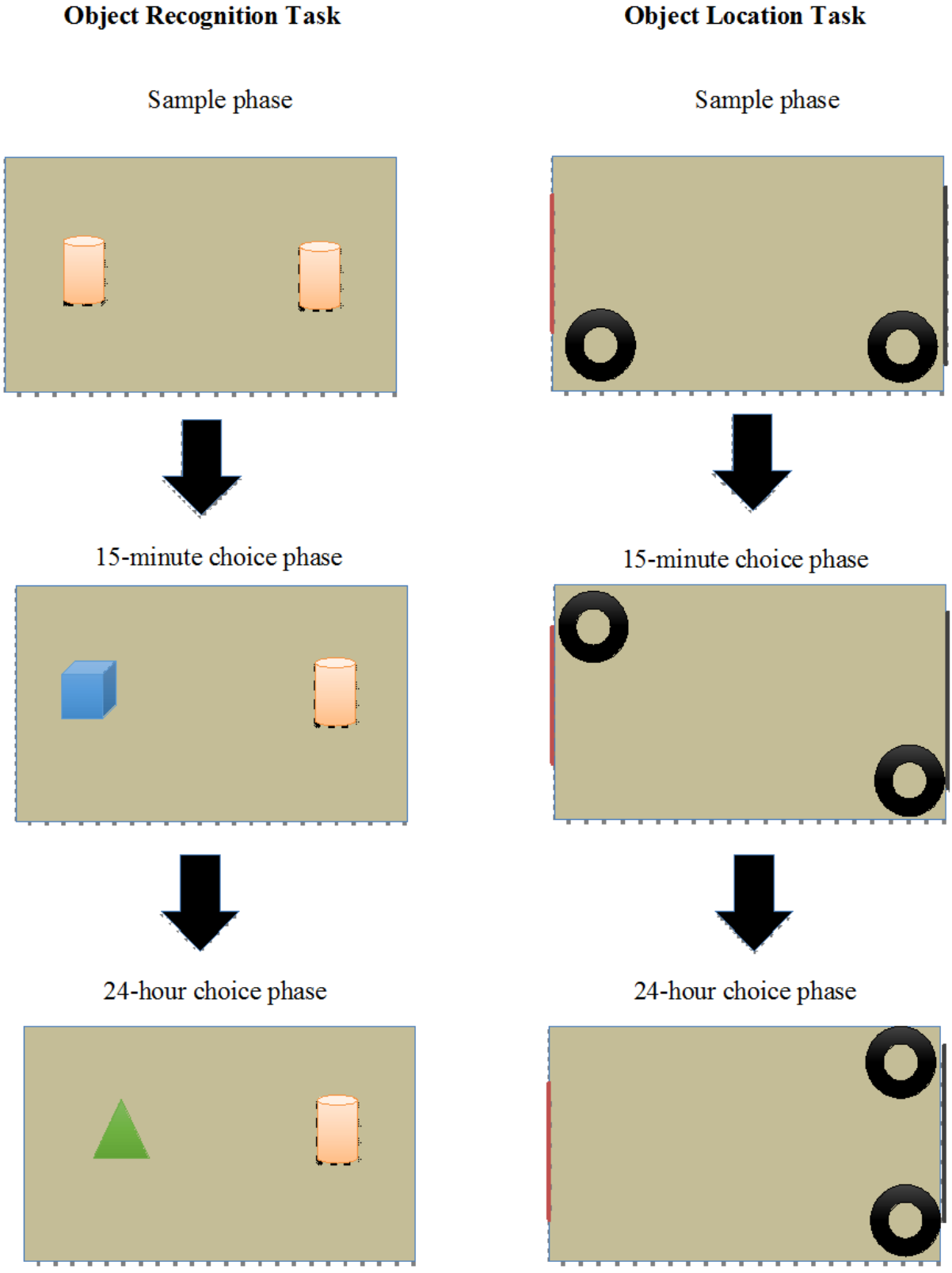

Table 3. Procedural Timeline

PND	
45 - 51	Animals received 25 mg/kg (ip) of ketamine (*n* = 12) or saline (*n* = 12) once daily
51 (*n* = 24) – 30 minutes following the last drug administration	Locomotor activity assessed for 15 minutes
52 – 24 hours following the last drug administration – behavioral testing – object recognition (*n* =12)/object location (*n* = 12)	Habituation – 10 minutes of exploration
53	Habituation – 10 minutes of exploration
54	Habituation – 10 minutes of exploration
55	Sample phase – 3 minutes of exploration
55 – 15 minutes after sample phase	Choice phase – 3 minutes of exploration
56 – 24 hours following choice phase	Choice phase – 3 minutes of exploration
58 – behavioral testing – object recognition (*n* =12)/object location (*n* = 12)*	Habituation – 10 minutes of exploration
59	Habituation – 10 minutes of exploration
60	Habituation – 10 minutes of exploration
61	Sample phase – 3 minutes of exploration
61 – 15 minutes after sample phase	Choice phase – 3 minutes of exploration
62 – 24 hours following choice phase	Choice phase – 3 minutes of exploration
63 – 75	Additional behavioral testing (separate experiment)
76 – behavioral testing – object recognition (*n* =12)/object location (*n* = 12)	Habituation – 10 minutes of exploration
77	Habituation – 10 minutes of exploration
78	Habituation – 10 minutes of exploration
79	Sample phase – 3 minutes of exploration
79 – 15 minutes after sample phase	Choice phase – 3 minutes of exploration
80 – 24 hours following choice phase	Choice phase – 3 minutes of exploration
82 – behavioral testing – object recognition (*n* =12)/object location (*n* = 12)*	Habituation – 10 minutes of exploration
83	Habituation – 10 minutes of exploration
84	Habituation – 10 minutes of exploration
85	Sample phase – 3 minutes of exploration
85 – 15 minutes after sample phase	Choice phase – 3 minutes of exploration
86 – 24 hours following choice phase	Choice phase – 3 minutes of exploration

*Object recognition/location tasks were counterbalanced; animals underwent testing on task in which they had not been previously tested during that session of testing

Results

Weight

A 2 (drug: ketamine or saline) x 27 (day: 1-27) mixed-design ANOVA was used to assess whether there were significant changes in mean body weight during the experimental period. The purpose of this analysis was to assess growth rate throughout the experiment and look for possible effects of drug administration. This analysis revealed a significant main effect of day, $F(26, 572) = 1055.460$, $p = .000$, $\eta_p^2 = .980$, reflecting increased weight over time. Additionally, there was trend toward significance for the main effect of drug, $F(1, 22) = 3.122$, $p = .091$, $\eta_p^2 = .124$, with a medium-large effect size. Follow-up independent-samples *t*-tests revealed a trend toward a significant difference in weight between the ketamine- and saline-treated groups beginning on day 3, $t(22) = -.1933$, $p = .066$, $d = .15$. The ketamine-treated rats had significantly lowered weights between days 4, $t(22) = -2.400$, $p = .025$, $d = .21$, and day 11, $t(22) = -2.476$, $p = .021$, $d = .22$, of the experimental period. The weights between the two groups remained trending toward significance between days 12, $t(22) = -1.950$, $p = .064$, $d = .15$, and 16, $t(22) = -2.020$, $p = .056$, $d = .16$, of the experimental period (Figure 3). There were no significant differences in weight between the ketamine and saline groups during adulthood (i.e., experimental days 18-27; Figure 4).

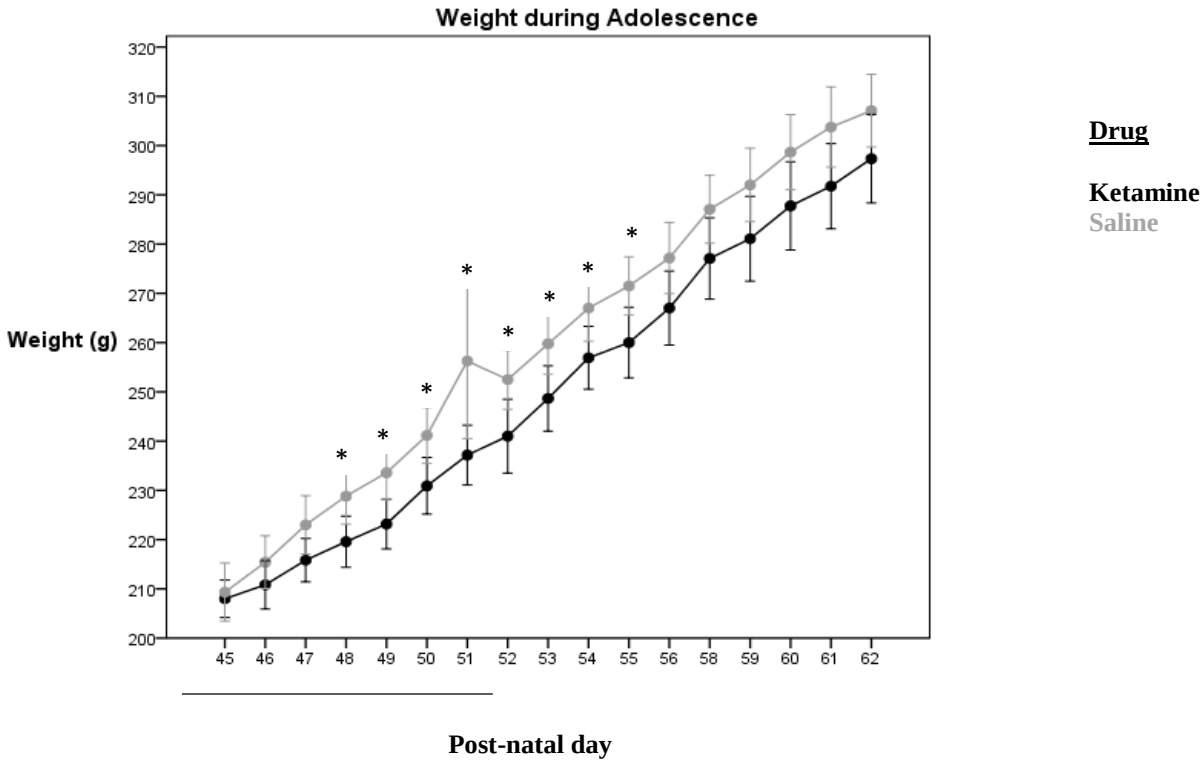

Figure 3. Mean body weight during post-natal days 45-62 (adolescence); error bars represent SEM. Black line represents days of ketamine administration. * $p < .05$ compared to the saline-treated group.

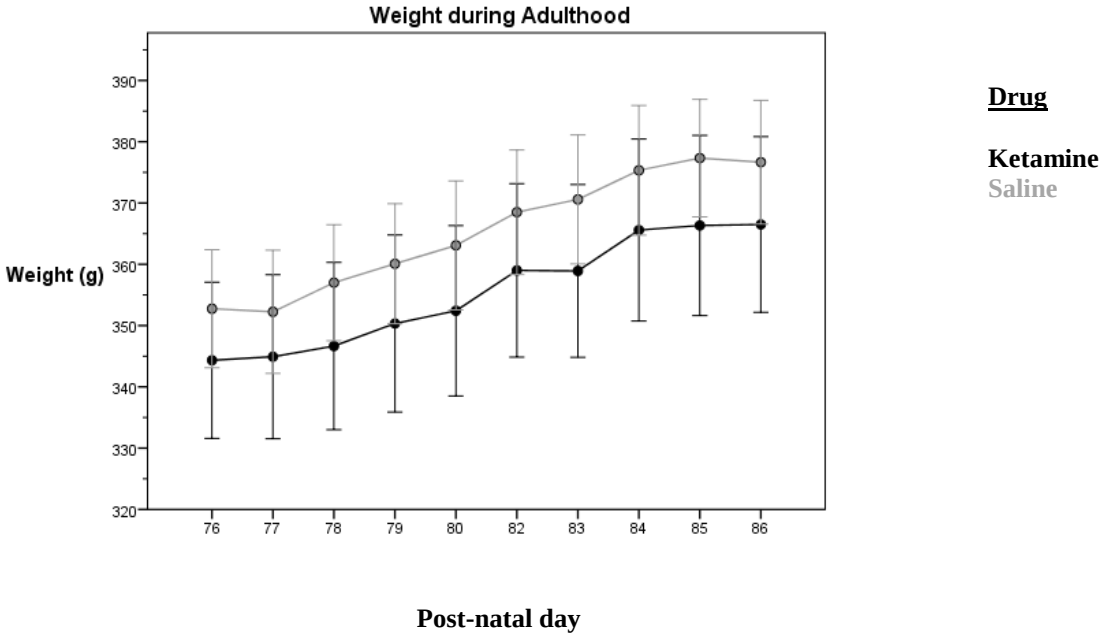

Figure 4. Mean body weight post-natal days 76-86 (adulthood); error bars represent SEM.

Locomotor Activity

An independent-samples *t*-test indicated that, on average, the ketamine-treated rats (*M* = 219.92, *SE* = 23.432) did not cross more lines than the saline-treated rats (*M* = 257.50, *SE* = 14.439) during the locomotor activity assessment, *p* = .175. See Figure 5.

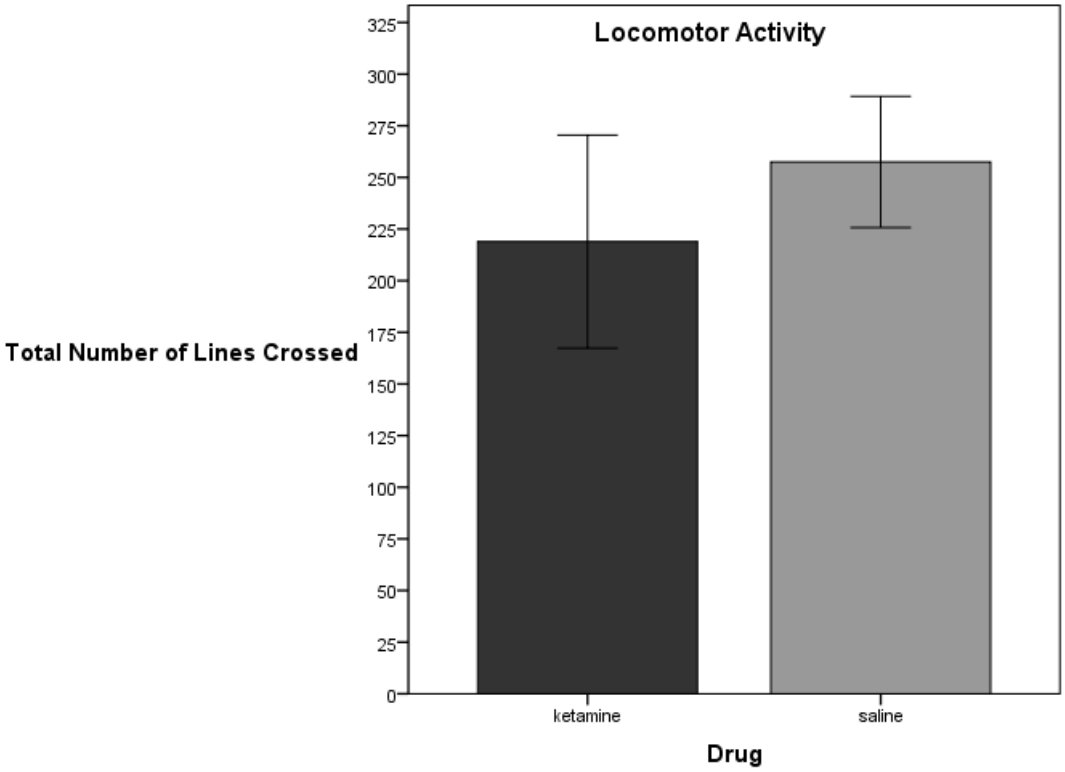

Figure 5. Mean total number of lines crossed during open-field locomotor assessment; error bars represent SEM.

Object Recognition Task

Sample Phase Exploration

During the sample phase of the ORT, ketamine-treated rats (*M* = 57.08 seconds, *SE* = 3.21) spent significantly less time exploring the objects as compared to the saline-treated rats (*M* = 68.50 seconds, *SE* = 3.33) during adolescence, *t*(22) = -2.467, *p* = .022, *d* = .22; however, during adulthood, there was no difference in total exploration time between the two groups, *p* = .204.

To see whether the time spent exploring the objects differed within each drug condition, further paired-samples *t*-tests were conducted. These revealed that the ketamine-treated rats did not differ in their time exploring the left and right objects during adolescence, $p = .547$; however, during adulthood, the ketamine-treated rats spent significantly more time exploring the right object ($M = 29.17$, $SE = 2.03$) as compared to the left ($M = 21.42$, $SE = 1.38$), $t(11) = 2.879$, $p = .015$, $d = .27$. The saline-treated rats did not differ in their time spent exploring the left and right objects during adolescence or adulthood, *p*s > .065.

Choice Phases - Total Exploration Time

A 2 (drug: ketamine or saline) x 2 (age: adolescence or adulthood) x 2 (delay: 15 minutes or 24 hours) mixed-design ANOVA revealed a significant main effect of drug, $F(1, 22) = 6.685$, $p = .017$, $\eta_p^2 = .233$, indicating that the ketamine-treated rats ($M = 45.40$, $SE = 1.578$) spent significantly less time exploring the objects compared to the saline-treated rats ($M = 56.24$, $SE = 2.215$). It also revealed a significant main effect of age, $F(1, 22) = 11.959$, $p = .002$, $\eta_p^2 = .352$, on total exploration time during the ORT, indicating that the rats spent more time exploring the objects during adolescence ($M = 54.73$, $SE = 2.063$) than they did during adulthood ($M = 46.94$, $SE = 2.086$).

Additionally, there was a trend toward significance with a medium effect size for the interaction between delay and drug, $F(1, 22) = 3.554$, $p = .073$, $\eta_p^2 = .139$, indicating that the performance at different time points varied based on drug administration. Follow-up independent samples *t*-tests, with Bonferroni-adjusted alpha levels of .025 per test, revealed significant between-groups differences in total exploration time of the objects. The ketamine-treated ($M = 42.00$, $SE = 2.547$) rats spent less time exploring the objects compared to the saline-treated rats ($M = 56.71$, $SE = 3.514$) during the 15-minute choice phases, $t(46) = -3.389$, $p =$

.001, d = .45, while there was no difference on the total exploration time between the ketamine-treated (M = 48.79, SE = 2.267) and saline-treated (M = 55.83, SE = 2.772) rats during the 24-hour choice phases, $t(46) = -1.967$, p = .055, d = .28. Together, this indicates that the saline-treated rats performed similarly at both the 15-minute and 24-hour delays, while the ketamine-treated rats explored significantly less during the 15-minute delay. This is illustrated in Figure 6.

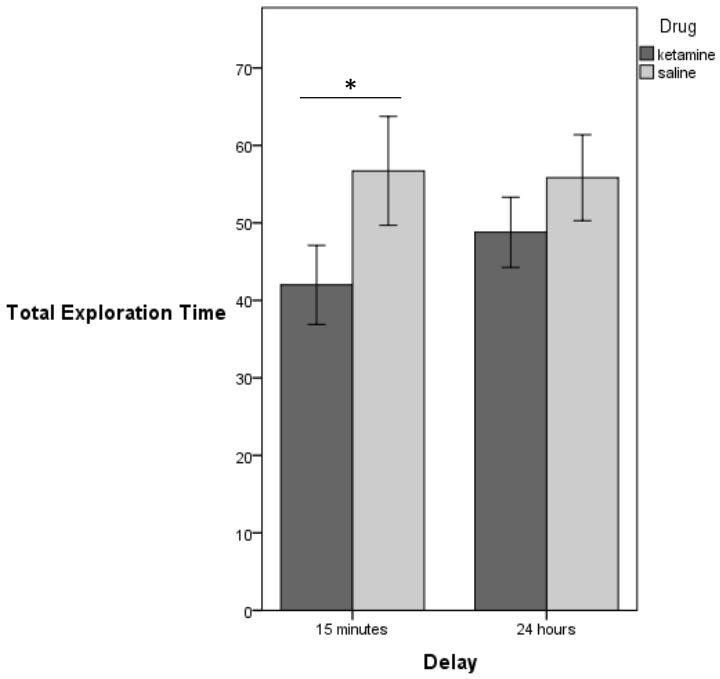

Figure 6. Delay x drug interaction on total exploration time (seconds) during the choice phases of the ORT; error bars represent SEM. * $p < .05$ compared to the saline group.

Choice Phases - Discrimination Ratio

A 2 (Drug: ketamine or saline) x 2 (age: adolescence or adulthood) x 2 (delay: 15 minutes or 24 hours) mixed-design ANOVA revealed a significant main effect of drug, $F(1, 22) = 13.163$, $p = .001$, partial $\eta^2 = .374$, indicating that discrimination ratios for the ketamine-treated rats were significantly lower than those for the saline-treated rats, which suggests that the ketamine-treated rats explored the novel object less than the familiar object compared to the saline-treated rats and indicates impaired memory for the familiar object as a result of ketamine administration. See Figure 7.

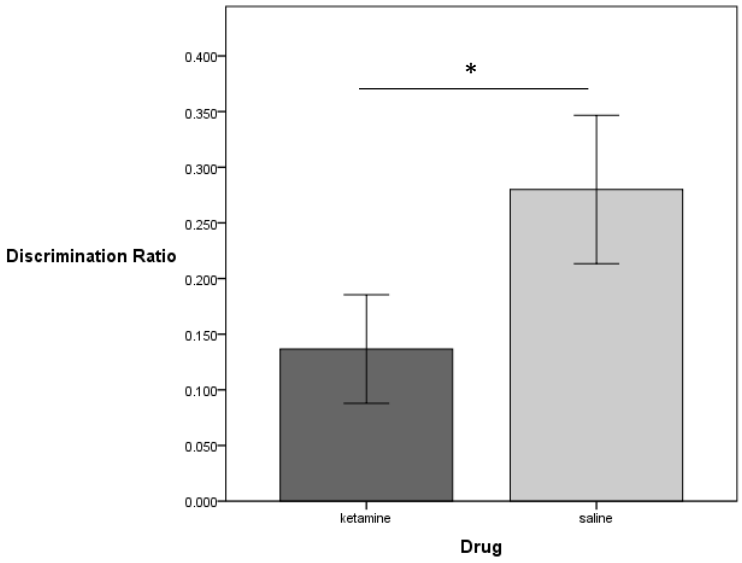

Figure 7. The main effect of drug on discrimination ratio during the choice phases of the ORT; error bars represent SEM. * $p < .05$ compared to saline group.

Additionally, there was a main effect of delay, $F(1, 22) = 32.180$, $p = .000$, partial $\eta^2 = .594$, indicating that the rats' discrimination ratios were significantly higher during the 15-minute delay compared to the 24-hour delay. This indicates that, on average, the rats spent significantly more time exploring the novel object compared to the familiar object during the shorter delay.

Also, this analysis revealed a trend toward a significant interaction between the age of the rats and delay, $F(1, 22) = 4.050$, $p = .057$, partial $\eta^2 = .155$. Follow-up paired-samples *t*-tests revealed no significant difference between adolescence and adulthood during the 15-minute choice phase, $p = .305$; however, there was a significant difference between the discrimination ratios during adolescence ($M = .181$, $SE = .036$) and adulthood ($M = .069$, $SE = .042$) during the 24-hour choice phase, $t(23) = 2.196$, $p = .038$, $d = .17$, indicating that the effect of delay was more pronounced during adulthood than it was during adolescence (Figure 8).

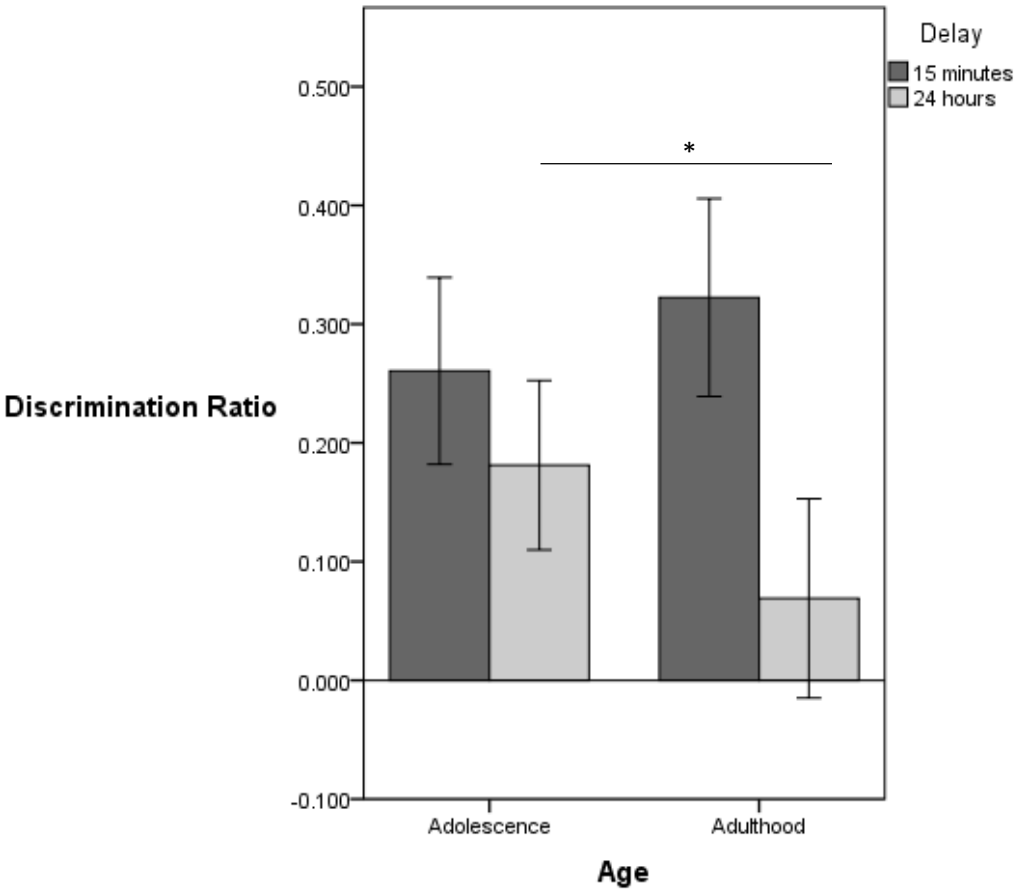

Figure 8. Age x delay interaction on discrimination ratios during the ORT; error bars represent SEM. * $p < .05$ compared to the 24-hour delay during adulthood.

Order Effects in the ORT

A 2 (order: ORT first or OLT first) x 2 (drug: ketamine or saline) x 2 (age: adolescence or adulthood) x 2 (delay: 15 minutes or 24 hours) mixed-design ANOVA was used to assess potential order effects. This analysis revealed a trend toward a significant main effect of order on discrimination ratios during the ORT, $F(1, 20) = 4.172$, $p = .055$, partial $\eta^2 = .173$, but not on total exploration time, $F(1, 20) = 2.149$, $p = .158$, partial $\eta^2 = .097$, indicating that rats that underwent the OLT first during adolescence (ORT first during adulthood; $M = .2468$, $SE = .0304$) spent significantly longer exploring the novel object compared to the rats that underwent the ORT first during adolescence (OLT first during adulthood; $M = .1698$, $SE = .0306$), indicating better discrimination and memory for the familiar object. See Figure 9.

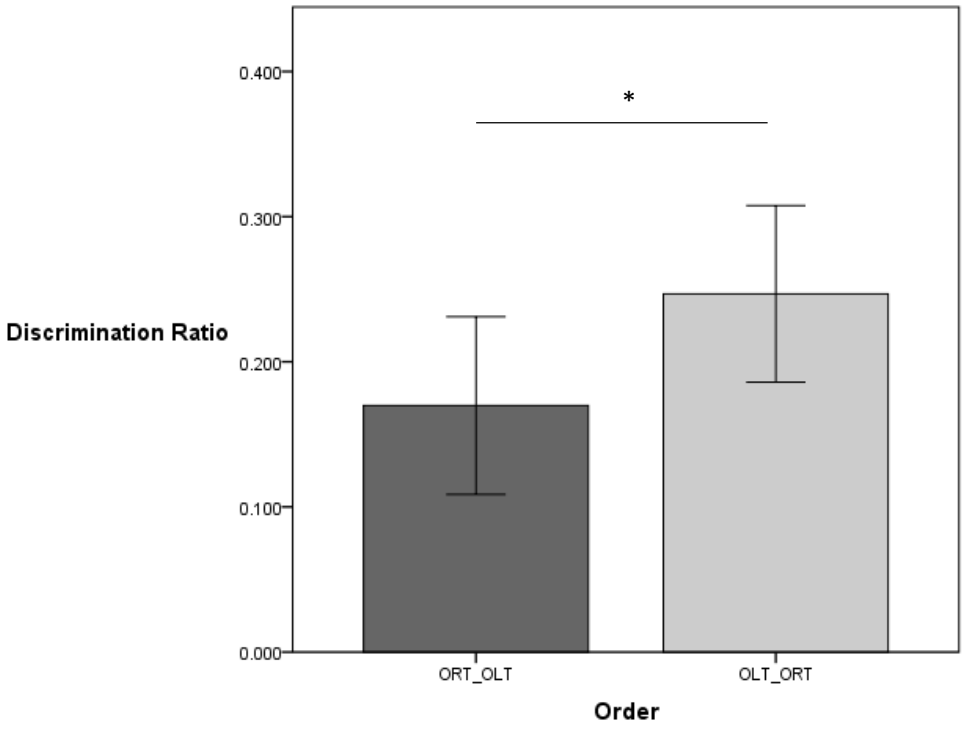

Figure 9. Effect of order on discrimination ratios during the ORT; error bars represent SEM. * $p < .05$ compared to the group that underwent OLT testing first.

Summary of findings for the ORT

Ketamine-treated rats explored the objects less than the saline-treated rats during the ORT, and this was true during both the 15-minute and 24-hour delays. Further within-groups analyses revealed that the ketamine-treated rats explored significantly less during the 15-minute delay as compared to the 24-hour delay, whereas the saline-treated rats performed similarly during both delays. Ketamine was also found to induce deficits in the rats' discrimination ratios, as they were significantly lower than those for the saline-treated rats, indicating poorer memory for the familiar object.

When collapsing across the drug groups, the rats had better discrimination during the 15-minute delay as compared to the 24-hour delay. Additionally, the rats had lower discrimination ratios in adulthood during the 24-hour delay, indicating that the effect of delay was more pronounced during adulthood than it was during adolescence. However, there was an effect of order on discrimination ratios, indicating that the rats that underwent the ORT first during adolescence had poorer discrimination and memory for the familiar object as compared to the rats that underwent the OLT first during adolescence.

Object Location Task

Sample Phase Exploration

During the sample phase of the OLT, the opposite pattern of results as compared to the sample phase of the ORT was found. Specifically, during adolescence, there was no difference in total exploration time between the ketamine- and saline-treated groups, $p = .380$. However, during adulthood, the ketamine-treated rats ($M = 45.33$, $SE = 2.44$) spent significantly less time exploring the objects as compared to the saline-treated rats ($M = 56.58$, $SE = 2.63$) during adolescence, $t(22) = -3.132$, $p = .005$, $d = .31$.

To see whether the time spent exploring the objects differed within each drug condition, further paired-samples *t*-tests were conducted. The ketamine-treated rats did not differ in their time spent exploring the objects during adolescence, $p = .176$; however, during adulthood, the ketamine-treated rats spent significantly more time exploring the right object ($M = 26.08$, $SE = 2.15$) as compared to the left one ($M = 19.25$, $SE = .938$), $t(11) = 3.052$, $p = .011$, $d = .30$. The saline-treated rats did not differ in their time spent exploring the left and right objects during adolescence or adulthood, $ps > .481$.

Choice Phases - Total Exploration Time

2 (Drug: ketamine or saline) x 2 (age: adolescence or adulthood) x 2 (delay: 15 minutes or 24 hours) mixed-design ANOVA revealed a significant interaction between age and delay, $F(1, 22) = 36.672$, $p = .000$, partial $\eta^2 = .625$, indicating that the total exploration time during the two different delays differed based on the age of the rat at time of testing. Follow-up paired-samples *t*-tests revealed that, on average, during adolescence, rats spent more time exploring the objects during the 15-minute choice phase ($M = 31.38$, $SE = 2.058$) compared to the 24-hour choice phase ($M = 25.21$, $SE = 1.542$), $t(23) = 2.968$, $p = .007$, $d = .28$. On the contrary, during

adulthood, rats spent more time exploring the objects during the 24-hour choice phase (M = 38.75, SE = 2.507), on average, compared to the 15-minute choice phase (M = 27.29, SE = 1.417), $t(23) = -4.991$, $p = .000$, $d = .512$. This is illustrated in Figure 10.

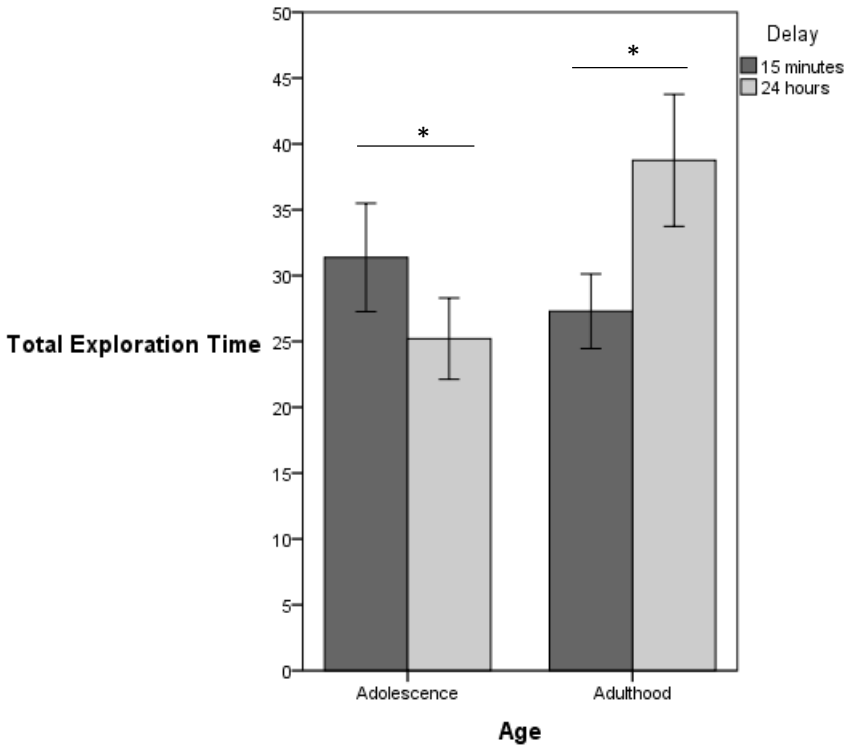

Figure 10. Age x delay interaction on total exploration time (seconds) during the choice phases of the OLT; error bars represent SEM. * $p < .05$ compared to the 24-hour delay during adolescence, # $p < .05$ compared to the 15-minute delay during adulthood.

Choice Phases - Discrimination Ratio

2 (Drug: ketamine or saline) x 2 (age: adolescence or adulthood) x 2 (delay: 15 minutes or 24 hours) mixed-design ANOVA revealed a significant main effect of drug, F (1, 22) = 4.981, p = .036, partial η^2 = .185, indicating that the ketamine-treated rats had impaired object discrimination when compared to the saline-treated rats. This ANOVA also revealed a significant main effect of age, F (1, 22) = 11.547, p = .003, partial η^2 = .344, indicating that the rats had higher discrimination ratios during adulthood as compared to adolescence. Lastly, there

was a main effect of delay, F (1, 22) = 29.074, p = .000, partial η^2 = .569, indicating that the rats had superior object discrimination during the 15-minute choice phases as compared to the 24-hour choice phases. See Table 4.

Group	***N***	Discrimination Ratio			
		Adolescence		**Adulthood**	
		15-minute delay	24-hour delay	15-minute delay	24-hour delay
Ketamine	12	.206	-.080	.086	.119
Saline	12	.280	.007	.282	.180

Table 4. The main effects of age, delay, and drug on discrimination ratio during the choice phases of the OLT. Data displayed are the mean discrimination ratios for the ketamine and saline groups during all time points tested.

Order Effects in the OLT

A 2 (order: ORT first or OLT first) x 2 (drug: ketamine or saline) x 2 (age: adolescence or adulthood) x 2 (delay: 15 minutes or 24 hours) mixed-design ANOVA was used to assess potential order effects. This analysis revealed a significant age by order interaction on total exploration time, F(1, 19) = 10.040, p = .001, partial η^2 = .514, indicating that the total time the rats spent exploring the locations during the two ages varied based on the order in which the underwent the tasks. Follow-up t-tests revealed that rats that underwent testing on the OLT first during adolescence (M = 33.42, SE = 2.034) spent significantly more time exploring the locations during adolescence compared to the rats that underwent the ORT first (M = 23.17, SE = 1.008), t(46) = -4.515, p = .000, d = 0.17. The opposite pattern of results was true during adulthood; the rats that underwent the ORT first (M = 36.08, SE = 2.488) spent significantly

more time exploring the locations compared to the rats that underwent the OLT first (M = 29.96, SE = 2.036), $t(46) = 1.905$, $p = .063$, $d = .55$. See Figure 11.

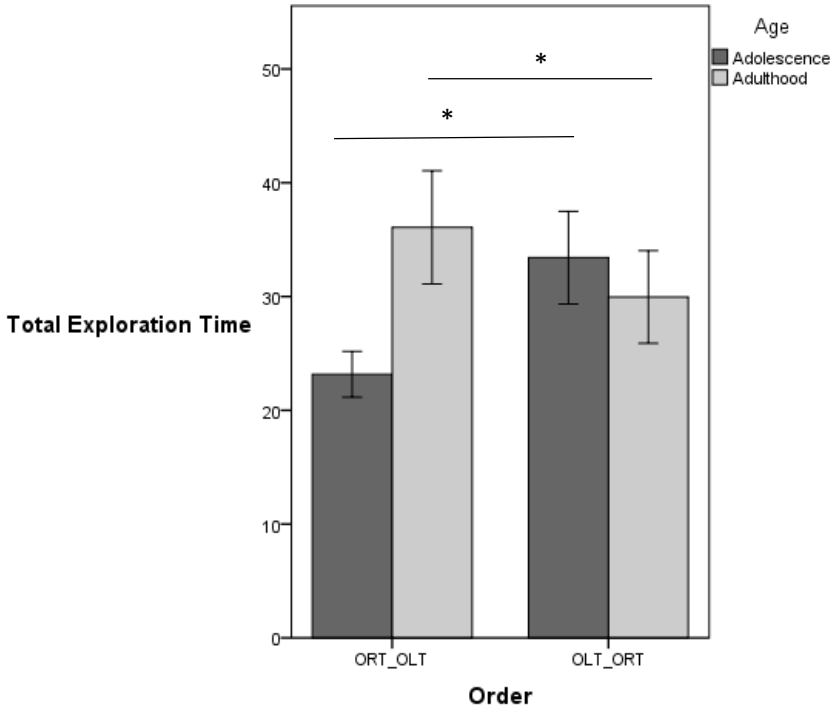

Figure 11. Age x order interaction on total exploration time during the OLT; error bars represent SEM. * $p < .05$ compared to the group that underwent OLT testing first.

Additionally, this ANOVA revealed a significant delay by order interaction on total exploration time, $F(1, 19) = 6.699$, $p = .018$, $\eta^2 = .251$, indicating that the total time the rats spent exploring the locations during the two delays varied based on the order in which they underwent the tasks. Follow-up t-tests revealed that rats that underwent testing on the OLT first during adolescence (M = 32.25, SE = 2.098) spent significantly more time exploring the locations during the 15-minute delay compared to those that underwent the ORT first (M = 26.42, SE = 1.210), $t(46) = -2.408$, $p = .02$, $d = .72$. However, during the 24-hour delay, there was no difference between the rats that underwent testing on the OLT first during adolescence (M =

31.13, *SE* = 2.028) and those that underwent the ORT first (*M* = 32.83, *SE* = 2.911), *t*(46) = .482, *p* = .632, *d* = 0.14.

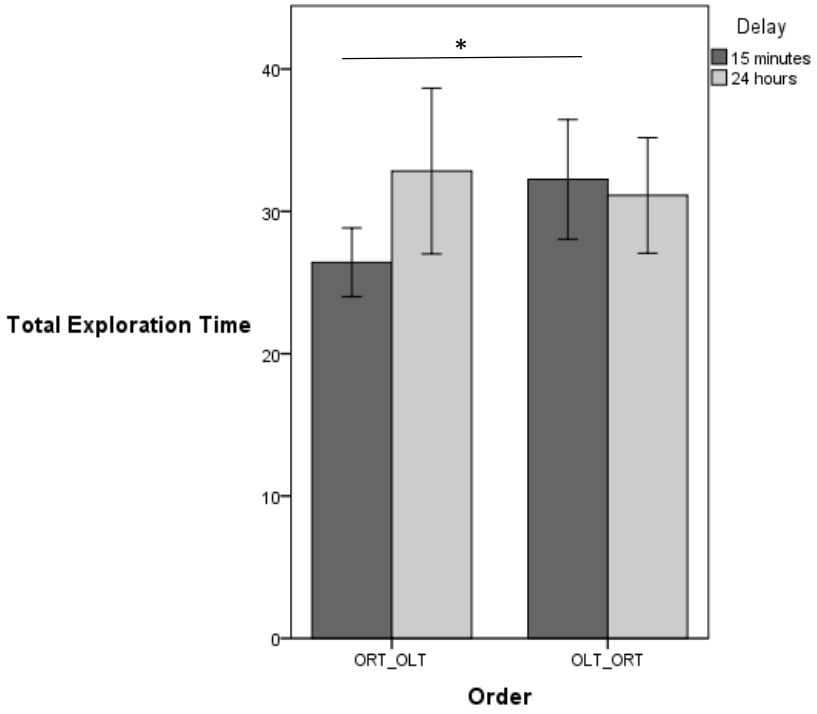

Figure 12. Delay x order interaction on total exploration time during the OLT; error bars represent SEM. * $p < .05$ compared to the group that underwent OLT testing first.

Summary of findings for the OLT

Unlike during the ORT, there were no ketamine-induced effects on total exploration time during the OLT and the ketamine-treated rats did not differ from the saline-treated rats. However, similar to the ORT, there was a ketamine-induced effect on discrimination ratios, suggesting that ketamine administration impaired memory for the familiar location compared to the saline-treated rats.

When collapsing across drug groups, analyses revealed that the total exploration time during the 15-minute and 24-hour delays differed based on the age of the rat at the time of testing. During adolescence, rats spent more time exploring the objects during the 15-minute

delay compared to the 24-hour delay. On the contrary, during adulthood, rats spent more time exploring the objects during the 24-hour choice phase compared to the 15-mintue choice phase.

However, the order in which the tasks were administered had a significant effect on the exploration time during the two ages and delays. Rats that underwent the OLT first during adolescence spent more time exploring the locations during adolescence as compared to the rats that underwent the ORT first. The opposite pattern of results was true during adulthood, where the rats that underwent the ORT first spent significantly more time exploring the locations as compared to the rats that underwent the OLT first.

Additionally, the order of tasks had an effect on total exploration time during the two delays. Rats that underwent testing on the OLT first during adolescence spent more time exploring during the 15-minute delay as compared to those that underwent the ORT first.

Furthermore, discrimination ratios were found to be higher during adulthood as compared to adolescence, suggesting that older rats had better memory for the familiar locations. Lastly, discrimination ratios were higher during the 15-minute delay compared to the 24-hour delay, suggesting that the rats had better memory for the familiar locations during the shorter delay. Importantly, no order effects were found for discrimination ratios.

Discussion

The current study sought to elucidate the effects of chronic ketamine during adolescence on short- and long-term recognition memory in rats. The results demonstrated that the current administration of chronic ketamine during adolescence interacted with typical neurodevelopment to impair short- (i.e., 15 minutes) and long-term (i.e., 24 hours) memory in ORT and OLT both immediately (i.e., adolescence) and long-term (i.e., adulthood). In addition, performance on the OLT was affected by both age and delay, with superior discrimination occurring in adult rats as compared to adolescent rats and at the 15-minute time point as compared to the 24-hour time point, indicating better memory for the familiar location during adulthood and the shorter delay. There was also a trend toward an age by delay interaction on the ORT such that older rats were more impaired than younger rats, but only at the 24-hour delay, indicating poorer memory for the familiar object following the longer delay during adulthood.

Object Recognition Task – Total Exploration Time

Ketamine caused impairments on the ORT, which is a non-spatial memory assessment that requires animals to explore objects during a study phase and differentiate between new and old objects in subsequent test phases (Ennaceur & Delacour., 1988). During adolescence, the ketamine-treated rats spent significantly less time exploring the objects as compared to the saline-treated rats during the sample phase. Similarly, during the choice phases, ketamine caused the rats to spend significantly less time exploring the objects as compared to the saline-treated rats. More specifically, the ketamine-treated rats explored less following the 15-minute delay during both adolescence and adulthood, which suggests that ketamine produces short-term alterations in time spent exploring the objects that are present both transiently and persistently. Although the rats underwent three days of habituation to the apparatus, it may be that the

ketamine-treated rats did not properly habituate to the apparatus or did not produce a lasting representation, which would result in more time exploring the apparatus as if it was novel during the choice phases. Prior research has shown that chronic ketamine produces alterations in habituation, indicated by the lack of decreasing exploratory behavior in an open field arena across sessions compared to control rats (Venãncio et al., 2011). At the neural level, glutamate is known to facilitate memory retention, while NMDA antagonists have been shown to dose-dependently decrease habituation (Rosat et al., 1992**)**, which strengthens the argument that NMDA antagonists, such as ketamine, can modify habituation properties.

Additionally, the finding that the ketamine-treated rats explored significantly less during the 15-minute choice phases contradicts previous research that has investigated the effects of ketamine following similar delays; Venãncio and colleagues (2011) found that chronic administration of 5 or 10 mg/kg doses of ketamine resulted in no differences in total object exploration following 15-minute and 24-hour delays compared to saline controls. On the other hand, an acute 20 mg/kg dose of ketamine administered 24 hours prior to the sample phase has been found to increase total exploration time during the choice phase following a 24-hour delay compared to saline controls, while the same dose produced no differences following a delay of only 90 minutes (Goulart et al., 2010). Being that, in the current study as well as the previously cited studies (Goulart et al., 2010; Venãncio et al., 2011), behavioral testing took place well after the ketamine had cleared, it is clear that there is a residual, long-lasting effect of ketamine that alters total exploration time of the objects. However, the mechanisms underlying this behavioral phenomenon are unclear, and future research will need to explore the potential dose-dependent effects of ketamine on time spent exploring objects, as well as attempt to elucidate the

mechanisms underlying the differences between the ketamine-induced effects following different delays.

There was also a difference in total exploration time during the ORT based on the age of the rats; specifically, the rats spent more time exploring the objects during adolescence compared to adulthood. This increased exploration during adolescence may be due to the novelty of the task. In adolescence, it was the first time that the rats were exposed to the task, while in adulthood, the objects were novel but the task itself was not. This finding is in agreement with some prior research that has examined different ages of rats on total exploration time during the ORT. Silvers and colleagues (2007) demonstrated that adolescent rats more rapidly approach a novel object in a familiar environment, as well as spend more time with a novel object relative to a familiar one. In the same way, adolescent rats display higher levels of overall activity in a novel environment and tasks that require exploration (Spear, 2000). While they used older rats (i.e., almost 2 years of age), Burke and colleagues (2010) demonstrated similar results. They found that the aged rats, compared to adult (i.e., PND 90) rats, spent significantly less time exploring the objects. While the rats used by Burke and colleagues (2010) were significantly older than the rats used in the current study, this research provides further rationale for the idea that, throughout the lifespan of rats, they explore less as they get older; however, the prior literature is not consistent in this regard. No differences in total exploration time were found between four different ages of rats, weanlings (PND 20-23), juveniles (PND 29-40), adults (PND 50), and older adults (PND 90; Heyser & Ferris, 2012; Reger et al., 2009).

Object Recognition Task – Discrimination Ratios

Furthermore, ketamine induced deficits in discrimination ratios, which are an indication of the rats' preference for the novel versus the familiar object while controlling for differences in

total exploration time, during the choice phases of the ORT. As previously mentioned, higher discrimination ratios indicate a stronger preference for exploring the novel object (i.e., > .000), while a lower discrimination ratio signifies a weaker preference for exploring the novel object (Ennaceur & Delacour, 1988; Pitsikas et al., 2008). The current study revealed that ketamine-treated rats had significantly lower discrimination ratios, indicating that these rats preferentially explored the novel object less than the familiar object compared to the saline-treated rats during the choice phases. This drug-induced impairment in object recognition memory is in line with research examining the effects of acute (Pitsikas et al., 2008) as well as chronic (Venãncio et al., 2011) ketamine on discrimination ratios during this task. However, the fact that ketamine produced impairments in adolescence as well as adulthood suggests a long-lasting effect of drug administration on the rats' abilities to differentiate a novel object from a familiar one. While prior research has shown that chronic ketamine during adolescence can produce deficits in short- and long-term memory during adolescence (Gama et al., 2012), the current study provides the first evidence that chronic ketamine during adolescence can result in recognition memory deficits in adulthood. Jablonski and colleagues (2013) recently examined the effect of acute administration of an NMDA antagonist, MK-801, in juvenile (i.e., PND 31) rats prior to training on the object recognition and OLTs and found that MK-801 produced deficits in the object location, but not object recognition, task compared to controls following a 5-minute delay. They suggested that the ORT may become increasingly difficult as the choice-phase delay increases, possibly requiring a greater involvement of NMDA receptors and/or additional brain regions, such as portions of the hippocampus or PFC (Jablonski et al., 2013; Gaskin et al., 2009). Therefore, being that ketamine induced deficits in both short- and long-term recognition memory, it may be that the localization of NMDA receptor impairment is delay-dependent and

underlying regions depend on the time between sample and choice phases. More specifically, the ORT may require the involvement of additional NMDA receptors in the PFC, which plays a role in working memory (Lenroot & Giedd, 2006), during shorter delays, such as 15 minutes, whereas during longer delays, such as 24 hours, more NMDA receptor activation in the hippocampus, which has been shown to be integral for long-term object recognition (Hammond et al., 2004), may be required for better performance. Therefore, the administration of an NMDA antagonist, such as ketamine, would likely inhibit the activation of NMDA receptors in these regions, resulting in the deficits seen in the current study.

In addition to the drug-induced effects on discrimination ratios, there was a trend toward an interaction between age and delay on discrimination ratios during the ORT. This finding revealed that the rats had significantly lower discrimination ratios during the 24-hour choice phase in adulthood compared to the 24-hour choice phase in adolescence. These results are not in line with some previous research investigating the effects of different ages and delays on recognition memory. Reger and colleagues (2009) demonstrated that adult rats (i.e., PND 50+) explored the novel object significantly more following both 15-minute and 24-hour choice phases compared to weanlings (i.e., PND 20-23), and this preference did not decrease until after a 48-hour delay, indicating that adult rats have intact object recognition memory up until 48 hours following exposure to the sample phase. On the other hand, Burke and colleagues (2010) found that aged (i.e., almost 2 years old) rats, compared to significantly younger, adult (i.e., PND 90) rats, spent less time exploring the objects following 15-minute, 2-hour, and 24-hour choice phases.

The current findings may indicate a stronger role of the perirhinal cortex, which is important for novel object recognition during shorter delays (Antunes & Biala, 2012**),** and lower

activation in the hippocampus, which is important for coding object memory and maintaining strong novel object preference after long but not short delays (Hammond et al., 2004**)**. Hammond and colleagues (2004) found that hippocampal-lesioned rats showed no differences in sample phase object exploration time, as well as no alterations in their habituation; however, after a 24-hour delay, these rats exhibited impaired object recognition memory, which supports a delay-dependent role of the hippocampus in object recognition memory. This idea of delay-dependent and compensatory mechanisms has been found in other medial temporal lobe regions during other types of learning, such as contextual fear conditioning. Specifically, Wiltgen and colleagues (2006) found that animals with hippocampal lesions were able to learn at rates comparable to non-lesioned rats during short-delays; however, learning over multiple trials was impaired in the lesioned rats and other mechanisms, such as the neocortex, are thought to activate and compensate during longer delays. Together, these parallel findings across different types of learning suggest a similar, yet dynamic, role of different brain regions and future research investigating these roles more specifically may provide a better understanding of basic processes as well as implications for different illnesses and disorders.

Furthermore, during the ORT, analyses revealed that rats that were first exposed to the OLT during adolescence had higher discrimination ratios compared to those that underwent testing in the ORT first. Generally speaking, this difference in performance may be due solely to exposure to the apparatus. The rats that were first exposed to the OLT were habituated in a similar apparatus to ORT, but with the addition of two spatial cues. This may have elicited additional internal mechanisms, thereby improving performance on the later tasks. See table 5 for a summary of ORT results.

Object Location Task – Total Exploration Time

While the hippocampus may play a role in long-term object recognition memory (Hammond et al., 2004), the OLT is a spatial task that requires animals to explore identical objects during a sample phase and differentiate between new and old locations of the objects in subsequent choice phases, and is considered to be hippocampus-dependent (Barker & Warburton, 2011; Ennaceur & Delacour, 1988; Ennaceur et al., 1996). As previously mentioned, the ketamine-treated rats explored the objects significantly less as compared to the saline-treated rats during the sample phase of the ORT during adolescence; however, during the sample phase of the OLT, the opposite trend of results were found, as the ketamine-treated rats spent less time exploring the objects as compared to the saline-treated rats during adulthood.

Regarding the choice phases of the OLT, the rats spent more time exploring the objects during the 15-minute choice phase compared to the 24-hour choice phase during adolescence, whereas, during adulthood, the opposite pattern of results emerged, where rats spent significantly more time exploring the objects during the 24-hour choice phase compared to the 15-minute choice phase. The findings in adolescence are to be expected, since the task is new to the rats following the 15-minute delay; however, the opposite trend in adulthood is a surprising result. Burke and colleagues (2010) found that aged rats (i.e., 2 years old) do not show an overall habituation to object exploration nor do they show declines in motivation to explore. Contrary to the current findings, they found that older rats had reduced object exploration following longer delays (i.e., 24 hours) and suggested that this age-associated deficit was because of intervening stimuli spontaneously encountered during the long delay periods, which may have shared common features with the objects presented during the choice phase (Burke et al., 2010). This, along with the possibility that older rats may be less able to discriminate different stimuli that

share common features (Burke et al., 2010), may have led to the increased exploration time during the 24-hour choice phase in adulthood. Importantly, the pattern of results for total exploration time of the ketamine- and saline-treated rats during the 24-hour choice phase of the OLT during adulthood is proportional to the pattern of the discrimination ratios, with the two groups spending a similar amount of time exploring both the familiar and novel object locations. Therefore, it may be that, in adulthood, intervening stimuli with similar features experienced during longer delays altered previously-existing representations and, ultimately, caused the rats to spend more time exploring the objects.

Furthermore, during the OLT, there were order effects found during total exploration time, but not discrimination ratios, suggesting the order in which the rats underwent the tasks did not affect their abilities to discriminate a novel location from a familiar one. Rats that underwent testing on the OLT first during adolescence spent more time exploring the objects during adolescence compared to the rats that underwent the ORT first, whereas the opposite pattern of results was true during adulthood. In addition to the rats' exploration during the two ages varying based on the order of tasks, rats that underwent testing on the OLT first during adolescence spent more time exploring the objects during the 15-minute delay compared to those that underwent the ORT first, whereas there was no difference in total exploration time between the two groups during the 24-hour delay.

Object Location Task – Discrimination Ratios

Similar to the findings in the discrimination ratios during the ORT, ketamine-treated rats were found to have significantly lower discrimination ratios compared to saline-treated rats during the OLT. While research pertaining to whether or not object location is sensitive to pharmacological manipulations is not as common as studies involving the ORT (Pitsikas, 2007),

Pitsikas and colleagues (2008) demonstrated that acute subanesthetic doses of 1 and 3 mg/kg, but not 0.3 mg/kg, of ketamine, administered either before or after training, resulted in significantly lowered discrimination ratios. Interestingly, acute administration of much higher doses of ketamine (i.e., 100 mg/kg) has also been found to produce deficits in discrimination ratios that persist up until 72 hours following drug treatment (Pitsikas & Boultadakis, 2009), while, more recently, Jablonski and colleagues (2013) found that acute administration of another NMDA antagonist, MK-801, induced deficits in the discrimination ratios of juvenile rats (i.e., PND 31) during the OLT. While it is difficult to draw conclusions from studies using such varied methodology and pharmacological manipulations, this research further solidifies the role of the hippocampus in successful performance on the OLT, as it is an NMDA receptor-abundant region (Monaghan & Cotman, 1985).

Moreover, the rats in the present study had significantly higher discrimination ratios during the 15-minute choice phases compared to 24-hour choice phases, demonstrating impaired object discrimination at the longer time interval. This is to be expected, considering that it is substantially more difficult to maintain a representation of objects after a 24-hour delay as compared to a 15-minute delay, and is comparable to work done by Ennaceur and Delacour (1988), who found that discrimination ratios were significantly lower following a 24-hour delay compared to shorter delays, such as 1 minute, 1 hour, and 4 hours.

Additionally, during adolescence, the rats had significantly lower discrimination ratios than they did during adulthood, indicating impaired object discrimination during adolescence. This is a somewhat surprising finding, considering younger rats are typically more hyperactive and perform better on tasks that require exploration (Spear, 2000). However, this deficit in adolescence may be due to natural developmental processes. The significant synaptic pruning of

glutamate receptors and NMDA receptors in the hippocampus, as well as the restriction of excitatory input that occurs during adolescence (Spear, 2000) may have produced the age-dependent deficits seen in the spatial OLT in the current study. By the time the rats were tested in adulthood, they were no longer undergoing synaptic pruning and there was no restriction in excitatory input (Spear, 2000). Thus, enhanced synaptic connections and increased excitatory input to essential brain regions may be the underlying cause of the significantly higher discrimination ratios seen during adulthood on the OLT. It is also possible that the enhanced exploration and impaired discrimination in the younger rats was the result of increased locomotor activity. While this variable was measured in adolescence to assess the possible effect of ketamine on locomotion, it was not measured during adulthood for comparison. Therefore, it is possible that the rats in the present study exhibited some degree of hyperactivity, which may have impacted an ability to see drug-induced changes during that age period. See table 6 for a summary of OLT results.

Locomotor Activity

While ketamine induced deficits on performance during the object recognition and location tasks, it did not produce effects on the locomotor activity assessment. Not only was this assessment utilized to examine the effects of drug administration, but also to examine initial locomotor activity differences between the two groups before behavioral testing in the object recognition and location tasks, which require extensive exploration and movement (Ennaceur & Delacour, 1988). In the present study, ketamine- and saline-treated rats did not significantly differ in the total number of lines crossed during the locomotor activity assessment, which indicated no drug-induced alterations in locomotion. The lack of difference between the ketamine- and saline-treated groups on total number of lines crossed suggests that differences

between the two groups in total exploration time during the sample and choice phases of the two tasks are not likely due to a preliminary difference in exploratory behavior. This finding is in agreement with some previous research; however, there are some inconsistencies in the literature regarding the effects of ketamine on locomotor activity. Similar to the current study, Venãncio and colleagues (2011) found that chronic ketamine did not increase locomotor activity compared to controls, while Becker and colleagues (2003) found that 30 mg/kg of ketamine for 5 consecutive days produced no differences following the final injection between the ketamine- and saline-treated rats. However, the lack of an effect of ketamine on locomotor activity in the present study suggests that alterations in object exploration as a consequence of ketamine administration were not due to motor effects.

In contrast to the present findings, Gama and colleagues (2012) found that ketamine significantly increased distance covered at 5, 10, and 15 minute time points in the open-field task following the same drug administration dose, schedule, and delay following the last administration (i.e., 30 minutes) in same-aged rats as the current study. A primary reason for the difference in results between Gama and colleagues (2012) and the current study may be the strain of rat used. While they were the same age, Gama and colleagues used Wistar rats and the current study used Sprague-Dawley rats. Moreover, Hou and colleagues (2013) found that chronic administration of three different doses (i.e., 25, 50, and 100 mg/kg i.p., 7 days) resulted in a significant increase in locomotor activity compared to the saline control group. Additionally, ketamine has demonstrated stimulant-like properties in lower (i.e., 5 mg/kg) doses, as well as different combinational effects when these doses are combined with other drugs on motor activities such as locomotion, stereotypy, and ataxia (Lai, Lee, & Yin, 2013).

Weight

Moreover, body weight was monitored throughout the experimental period to assess growth rate throughout the experimental period and look for variability due to drug administration. Although ketamine-treated rats showed significantly lower body weights during days 4-11, these effects did not persist through adulthood. These findings are in opposition to some previous research that used a chronic ketamine administration (i.e., 7 days, 15mg/kg), which was found to increase body weight and food consumption (Gracia et al., 2009). However, these findings are similar to others (Venãncio et al., 2011), which revealed that ketamine-treated rats had significantly lowered weights compared to the controls throughout the experimental period. These latter findings are in agreement with studies investigating chronic ketamine administration in humans; participants reported side effects such as nausea and vomiting with consequent loss of appetite (Cvrcek, 2008). Additionally, ketamine is known to interfere with gustatory trace in a dose-dependent manner (Traverso et al., 2008), which may also account for the reduced food intake and, ultimately, significantly lowered weight. However, it is important to note that these effects on weight were short lived and it is unlikely that ketamine produced any long-term gastrointestinal changes that could account for the behavioral effects of the drug observed in adulthood.

Limitations

While this is, to our knowledge, the first study to simultaneously examine the effects of chronic ketamine administration during adolescence on both the ORT and OLT, there are some potential confounds. During the sample phases of the object recognition and location tasks, the rats are expected to not display a preference for either object because it is their first exposure (Ennaceur & Delacour, 1988; Ennaceur et al., 2010). However, in the current study, during the sample phases for both the object recognition and location tasks during adulthood, the ketamine-

treated rats spent significantly more time exploring the right object as compared to the left one. As the two objects presented during the sample phases are identical, it is difficult to speculate why this initial preference may be present; however, this finding may be a potential limitation of the current study and may have affected exploration in subsequent phases.

While often considered advantageous, these two tasks also have disadvantages that must be considered (Ennaceur et al., 2010). Ennaceur (2010) discusses that our lack of knowledge about the perceptual capabilities in rats and mice is one potential limitation underlying these tasks. The argument is that we know very little about how these rodents discriminate between a familiar and novel object and, ultimately, how their memory performance is achieved. Ennaceur (2010) points out that the distinction between 'preference for a novel object' and 'novelty detection and encoding' has yet to be determined. 'Preference for a novel object' is when an object is already represented in memory and 'novelty detection and encoding' is an immediate response. Lastly, Ennaceur discusses the involvement of episodic memory and not working memory during these tasks, arguing that they are not working memory tasks because the rat explores objects and returns to its home cage without training and they are not expecting to be tested again. However, the task requires episodic memory as the rats are exposed to objects (i.e., what), context (i.e., where), and during a particular time, date or age (i.e., when) and testing of the temporal (i.e., when) remains very difficult (Ennaceur, 2010).

Aside from the tasks used, there are other potential methodological limitations worth mentioning. In the current study, we are presuming that 7 days of daily ketamine administration produced long-lasting changes in brain structure and function. While it has recently been shown that chronic ketamine during adolescence results in electrophysiological alterations during

adulthood (Featherstone et al., 2014), the current study did not directly assess physiological changes.

One potential interpretation matter with the current results is differentiating between the 15-minute and 24-hour choice phases and ensuring that they are due to memory and not practice. The present findings on the ORT and OLT suggest that it is due to memory and not practice. On both the ORT and OLT, the results demonstrated that the rats had better discrimination ratios during the 15-minute delay, which is to be expected since it is a significantly shorter time to hold a memory trace as compared to 24 hours. If this was not a memory deficit and was due to practice, one would expect better discrimination during the later delay because there is additional exposure to the objects. However, to directly assess this matter, future studies would do well to include an additional control group that undergoes a choice phase solely after 24 hours.

Conclusion

Although there are potential limitations in the current study, there are many implications for future research investigating the effects of ketamine on the object recognition and location tasks. An extensive amount of research, in both animals (Becker et al., 2003) and humans (Newcomer et al., 1998), has revealed that ketamine induces symptoms similar to the positive, negative, and cognitive symptoms of schizophrenia. The object recognition and location tasks are considered to contain an episodic memory-like component (Ennaceur, 2010), which is impaired in individuals with schizophrenia (Gruzelier et al., 1988). Additionally, there is a lot of emerging evidence pointing toward schizophrenia as a developmental illness (Gama et al., 2012), and NMDA hypofunction is considered as a convergence point for the progression and symptoms of it (Snyder & Gao, 2013). Thus, being that chronic administration of NMDA antagonists mimics the symptoms seen in this devastating illness, this line of research may

provide novel ways to investigate potential causes, mechanisms, and interventions through multidisciplinary approaches.

In conclusion, the current study provides evidence of short-term and long-term effects of chronic ketamine administration on both object recognition and object location memory. While recent research has established that chronic ketamine during adolescence produces deficits in short- and long-term memory during adolescence (Gama et al., 2012) as well as late-onset alterations in electrophysiology in adulthood (Featherstone et al., 2014), this is the first study to display cognitive impairments in adulthood following chronic ketamine during adolescence. An extensive amount of research has been done during the critical period of adolescence, as studies have demonstrated that drug use during adolescence can result in robust impairments in cognition (Sircar et al., 2010), as well as increased vulnerability to drug-induced impairment (Pope et al., 2003) and persisting deficits in adulthood (Gilpin et al., 2012; Maldonado-Devincci et al., 2010; Wagner et al., 2012). Additionally, being that adolescence is a prime time for experimentation with substances (Spear, 2000) and developmental delays in certain brain regions can result in higher risk for substance abuse (Fishbein, 2000), it is important that future research provides links between physiological and behavioral evidence during both adolescence and adulthood to further clarify the impact of drugs not only during development, but later in life as well.

Table 5. Summary of main effects and interactions on ORT

	Total exploration time					Discrimination ratio				
		Drug	**Age**	**Delay**	**Order**		**Drug**	**Age**	**Delay**	**Order**
Drug	Ketamine explored less than saline	n/a	ns	Ketamine-treated rats explored less during 15m delay, whereas saline-treated rats explored equally during both 15m and 24h	ns	Ketamine-treated rats had lower discrimination ratios	n/a	ns	ns	ns
Age	Adolescence explored more than adults	ns	n/a	ns	ns	ns	ns	n/a	Rats had lower discrimination ratios during adulthood during 24h delay	ns
Delay	ns	Ketamine-treated rats explored less during 15m delay, whereas saline-treated rats explored equally during both 15m and 24h	ns	n/a	ns	Rats had higher discrimination ratios during 15m as compared to 24h	ns	Rats had lower discrimination ratios during adulthood during 24h delay	n/a	ns
Order	ns	ns	ns	ns	n/a	Rats that underwent ORT first during adolescence had lower discrimination ratios	ns	ns	ns	n/a

Table Legend
ns – not significant
n/a – not applicable

Table 6. Summary of main effects and interactions on OLT

Total exploration time						Discrimination ratio				
		Drug	**Age**	**Delay**	**Order**		**Drug**	**Age**	**Delay**	**Order**
Drug	ns	n/a	ns	ns	ns	Ketamine-treated rats had lower discrimination ratios	n/a	ns	ns	ns
Age	ns	ns	n/a	Adolescent rats explored more during 15m; adult rats explored more during 24h	During adolescence, rats that underwent OLT first during adolescence explored more; during adulthood, rats that underwent ORT first explored more	Adult rats had higher discrimination ratios	ns	n/a	ns	ns
Delay	ns	ns	Adolescent rats explored more during 15m; adult rats explored more during 24h	n/a	During 15m, rats that underwent OLT first during adolescence explored more	Rats had higher discrimination ratios during 15m choice phase	ns	ns	n/a	ns
Order	ns	ns	During adolescence, rats that underwent OLT first during adolescence explored more; during adulthood, rats that underwent ORT first explored more	During 15m, rats that underwent OLT first during adolescence explored more	n/a	ns	ns	ns	ns	n/a

Table Legend
ns – not significant
n/a – not applicable

References

Ainge, J. A., Heron-Maxwell, C., Theofilas, P., Wright, P., de Hoz, L., & Wood, E. R. (2006). The role of the hippocampus in object recognition in rats: examination of the influence of task parameters and lesion size. *Behavioural Brain Research, 167*, 183-195. doi: 10.1016/j.bbr.2005.09.005.

Anderson, M. J., Barnes, G. W., Briggs, J. F., Ashton, K. M., Moody, E. W., Joynes, R. L., & Riccio, D. C. (2004). Effects of ontogeny on performance of rats in a novel object-recognition task. *Psychological Reports*, *94*, 437-43. doi: 10.2466/PR0.94.2.437-443

Antunes, M. & Biala, G. (2012) The novel object recognition memory: neurobiology test procedure, and its modifications. *Cogn Process*, 13, 93-110. Doi: 10.1007/s10339-011-0430-z.

Autry, A. E., Adachi, M., Nosyreva, E., Na, E. S., Los, M. R., Cheng, P.,…Monteggia, L. M. (2012). NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature*, *475*(*7354*), 91-95. doi:10.1038/nature10130

Backevalier, J. & Beauregard, M. (1993). Maturation of medial temporal lobe memory functions in rodents, monkeys, and humans. *Hippocampus*, 3, 191-202.

Barker, G. R. I. & Warburton, E. C. (2011). When is the hippocampus involved in recognition memory? *J Neurosci*, 31, 10721-10731. doi: 10.1523/JNEUROSCI.6413-10.2011

Barron, S., White, A., Swartzwelder, H. S., Bell, R. L., Rodd, Z. A., Slawecki, C. J…Spear, L. P. (2005). Adolescent vulnerabilities to chronic alcohol or nicotine exposure: findings from rodent models. *Alcohol Clin Exp Res.*, *29*(*9*), 1720-5. doi:10.1097/01.alc.0000179220.79356.e5

Becker, A. & Grecksch, G. (2004). Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Test of predictive validity. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *28*, 1267-1277. doi: 10.1016/j.pnpbp.2004.06.019

Becker, A., Peters, B., Schroeder, H., Mann, T., Huether, G., & Grecksch, G. (2003). Ketamine-induced changes in rat behaviour: A possible animal model of schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *27*, 687-700. doi: 10.1016/S0278-5846(03)00080-0

Ben-Shlomo, I., Rosenbaum, A., Hadash, O., & Katz, Y. (2001). Intravenous midazolam significantly enhances the lethal effect of thiopental but not that of ketamine in mice. *Pharmacol Res*, *44*, 509-12. http://dx.doi.org/10.1006/phrs.2001.0900

Berman, R. M., Cappiello, A., Anand, A., Oren, D. A., Heninger, G. R., & Charney, D. S. (2000). Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry*, *47*, 351–4. doi.org/10.1016/S0006-3223(99)00230-9

Boultadakis, A. & Pitsikas, N. (2011). Anesthetic ketamine impairs rats' recall of previous information. *Anesthesiology*, *114*(*6*), 1345-1353. doi: 10.1097/ALN.0b013e318219524e

Britt, G. C. & McCance-Katz, E. F. (2005). A brief overview of the clinical pharmacology of "club drugs." *Substance Use and Misuse*, *40*, 1180-1201. doi.org/10.1081/JA-200066730.

Broadbent, N. J., Squire, L. R., & Clark, R. E. (2004). Spatial memory, recognition memory, and the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 14515-14520. doi.org/10.1073/pnas.0406344101.

Brown, S. A., Tapert, S. F., Granholm, E., & Delis, D. C. (2000). Neurocognitive functioning of adolescents: effects of protracted alcohol use. *Alcohol Clin Exp Res, 24*, 164-171. doi.org/10.1111/j.1530-0277.2000.tb04586.x.

Buckmaster, C. A., Eichenbaum, H., Amaral, D. G., Suzuki, W. A., & Rapp, P. R. (2004). Entorhinal cortex lesions disrupt the relational organization of memory in monkeys. *J Neurosci*, 24, 9811-9825. Doi: 10.1523/jneurosci.1532-04.2004.

Burke, S. N., Wallace, J. L., Nematollahi, S., Uprety, A. R., & Barnes, C. A. (2010).

Canever, L., Oliveira, L., de Luca, R., Correa, P. T. F., Fraga, D., Matos, M. P…Zugno, A. I. (2010). A rodent model of schizophrenia reveals increase in creatine kinase activity with associated behavior changes. *Oxidative Medicine and Cellular Longevity*, *3*(*6*), 421-427. doi.org/10.4161/oxim.3.6.13446.

Chakraborty, K., Neogi, R., & Basu,, D. (2011). Club drugs: review of the 'rave' with a note of concern for the Indian scenario. *The Indian Journal of Medical Research*, *133*(*6*), 594-604.

Chan, K. W., Lee, T. M., Siu, A. M., Wong, D. P., Kam, C. M., Tsang, S. K., & Chan, C. C. (2013). Effects of chronic ketamine use on frontal and medial temporal cognition. *Addict Behav*, *38*(*5*), 2128-32. doi.org/10.1016/j.addbeh.2013.01.014.

Chatterjee, M., Ganguly, S., Srivastava, M., & Palit, G. (2011). Effect of 'chronic' versus 'acute' ketamine administration and its 'withdrawal' effect on behavioral alterations in mice: Implications for experimental psychosis. *Behavioural Brain Research*, *216*, 247-254. doi:10.1016/j.bbr.2010.08.001.

Chugani, H. T. (1994). Development of regional brain glucose metabolism in relation to behavior and plasticity. In: Dawson, G., Fisher, K. W., editors. *Human behavior and the developing brain*, New York, NY: Guildford, 153-75.

Clark, R. E., Zola, S. M., & Squire, L. R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci*, *20*, 8853-8860.

Clark, R. E., Zola, S. M., & Squire, L. R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci*, 20, 8853-8860.

Cohen, J. (1988). *Statistical Power analysis for the behavioral sciences*. New York: Academic Press.

Corazza, O. & Schifano, F. (2010). Ketamine use: a prospective study on the emergence of near-death states among a group of 50 ketamine recreational users. *Subst Use Misuse*, *45*, 916-924. Doi:10.3109/10826080903565321.

Corazza, O. Assi, S., & Schifano. (2013). From "Special K" to "Special M": the evolution of the recreational use of ketamine and methoxetamine. *CNS Neuroscience & Therapeutics*, *19*, 454-460. doi: 10.1111/cns.12063

Cotman, C. W. & Monaghan, D. T. (1987). Chemistry and anatomy of excitatory amino acid systems. In: Meltzer HY, editor. *Psychopharmacology: The third generation of progress*. New York: Raven, pp. 194-210.

Cvrcek, P. (2008). Side effects of ketamine in the long-term treatment of neuropathic pain. *Pain Med.*, *9*, 253-257.

Dahl, R. E. (2004). Adolescent brain development: a period of vulnerabilities and opportunities. *Ann NY Acad Sci, 1021*, 1-22. doi.org/10.1196/annals.1308.001.

De Bellis, M. D., Clark, D. B., Beers, S. R., Soloff, P. H., Boring, A. M., Hall, J…Keshavan, M. S. (2000). Hippocampal volume in adolescent-onset alcohol use disorders. *Am J, 157*(*5*), 737-44. doi: 10.1176/appi.ajp.157.5.737

De Bellis, M. D., Narasimhan, A., Thatcher, D. L., Keshavan, M. S., Soloff, P., & Clark, D. B. (2005). Prefrontal cortex, thalamus, and cerebellar volumes in adolescents and young adults with adolescent onset alcohol use disorders and comorbid mental disorders. *Alcohol Clin Exp Res, 29,* 1590-1600. doi.org/10.1097/01.alc.0000179368.87886.76.

Degenhardt, L., Chiu, W. T., Sampsom, N., Kessler, R. C., Anthony, J. C., Angermeyer, M…Wells, J. E. (2008). Toward a global view of alcohol, tobacco, cannabis, and cocaine use: findings from the WHO World Mental Health Surveys. *PLoS Med.* 5:e141. doi:10.1371/journal.pmed.0050141.

deLima, M. N., Laranja, D. C., Bromberg, E., Roesler, R., & Schroder, N. (2005). Pre- or posttraining administration of the NMDA receptor blocker MK-801 impairs object recognition memory in rats. *Behav Brain Res, 31,* 47-59. doi.org/10.1016/j.bbr.2004.05.016.

Diazgranados, N., Ibrahim, L., Brutsche, N. E., Newberg, A., Kronstein, P., Khalife, S.,…Zarate Jr., C. A. (2010b). A randomized add-on trial of *N*-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. *Arch Gen Psychiatry, 67*(*8*), 793-802. doi:10.4088/JCP.09m05327blu.

Diazgranados, N., Ibrahim, L., Brutsche, N., Ameli, R., Henter, I. D., Luckenbaugh, D. A.,…Zarate Jr., C. A. (2010a). Rapid Resolution of Suicidal Ideation after a Single Infusion of an NMDA Antagonist in Patients with Treatment-Resistant Major Depressive Disorder. *J Clin Psychiatry, 71*(*12*), 1605-1611. doi:10.1001/archgenpsychiatry.2010.90.

Dick, D. & Torrance, C. (2010). MixMag drugs survey. *Mix Mag 225*, 44-53.

Domino, E. F., Chodoff, P., & Corssen, G. (1965). Pharmacologic effects of CI-581, a new dissociative anesthetic, in man. *Clin. Pharmacol. Ther.*, *6*, 279-291.

Drug Enforcement Administration (DEA). (2004). [accessed on July 20, 2013]. Available from: http://www.dea.gov/pubs/inteI/01026/index.html

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

Eichenberger, U., Neff, F., Syveticic, G., Bjrgo, S., Petersen-Felix, S., Arendt-Nielsen, L., & Curatolo, M. (2008). Chronic phantom limb pain: the effects of calcitonin, ketamine, and their combination on pain and sensory thresholds. *Anesth Analg*, *106*, 1265-1273. doi: 10.1213/ane.0b013e3181685014.

Eide, P. K., Rum, E. Stubhaug, A., Bremnes, J., & Breivik, H. (1994). Relief of post-herpetic neuralgia with N-methyl-D-aspartic acid receptor antagonist ketamine: A double-blind, cross-over comparison with morphine and placebo. *Pain*, *58*, 347-354. doi.org/10.1016/0304-3959(94)90129-5.

Eide, P. K., Stubhaug, A., & Stenehjem, A. E. (1995). Central dysesthesia pain after traumatic spinal cord injury is dependent on N-methyl-D-aspartate receptor activation. *Neurosurgery*, *37*, 1080-1087. doi.org/10.1097/00006123-199512000-00007.

Eiland, L. & Romeo, R. D. (2012). Stress and the developing adolescent brain. *Neuroscience*. doi.org/10.1016/j.neuroscience.2012.10.048.

Eldreth, D., Hardin, M. G., Pavletic, N., & Ernst, M. (2013). Adolescent transformations of behavioral and neural processes as potential targets for prevention. *Prev Sci*, *14*(*3*), 257-66. doi.org/10.1007/s11121-012-0322-1.

Ennaceur, A. & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research*, *31*, 47-59. doi.org/10.1016/0166-4328(88)90157-X.

Ennaceur, A. & Meliani, K. (1992). A new one-trial test for neurobiological studies of memory in rats. III. Spatial vs. non-spatial working memory. *Behavioural Brain Research*, *51*, 83-92. doi.org/10.1016/S0166-4328(05)80315-8.

Ennaceur, A. (2010). One-trial object recognition in rats and mice: methodological and theoretical issues. *Behavioral Brain Research, 215*, 244-254. Doi:10.1016/j.bbr.2009.12.036.

Ennaceur, A., Michalikova, S., Bradford, A., & Ahmed, S. (2005). Detailed analysis of the behavior of Lister and Wistar rats in anxiety, object recognition and object location tasks. *Behavioural Brain Research, 159,* 247-266. doi.org/10.1016/j.bbr.2004.11.006.

Ennaceur, A., Neave, N., Aggleton, J. P. (1996). Neurotoxic lesions of the perirhinal cortex do not mimic the behavioural effects of fornix transection in the rat. *Behav Brain Res*, *80*, 9-25. doi.org/10.1016/0166-4328(96)00006-X.

Farber, N. B., Wozniak, D. F., Price, M. T., Labruyere, J., Huss, J., St. Peter, H., & Olney, J. W. (1995). Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: potential relevance to schizophrenia? *Biol Psychiatry*, *38*, 788-796. doi.org/10.1016/0006-3223(95)00046-1.

Featherstone, R. E., Nagy, L. R., Hahn, C. G. & Siegel, S. J. (2014). Juvenile exposure to ketamine causes delayed emergence of EEG abnormalities during adulthood in mice. *Drug and Alcohol Dependence*, 134, 123-127. doi.org/10.1016/j.drugalcdep.2013.09.017.

Fishbein, D. H. (2000). Neuropsychological dysfunction, drug abuse and violence: conceptual framework and preliminary findings. *Crim. Justice Behav.*, 27, 139-159.

Gable, R. S. (2004). Acute toxic effects of club drugs. *J Psychoact Drugs, 36,* 303-13. doi.org/10.1080/02791072.2004.10400031.

Gama, C. S., Canever, L., Panizzutti, B., Gubert, C., Stertz, L., Massuda, R…Zugno, A. I. (2012). Effects of omega-3 dietary supplement in prevention of positive, negative, and cognitive symptoms: a study in adolescent rats with ketamine-induced model of schizophrenia. *Schizophrenia Research, 141,* 162-167. doi.org/10.1016/j.schres.2012.08.002.

Gaskin, S., Gamliel, A., Tardif, M., Cole, E., & Mumby, D. G. (2009). Incidental (unreinforced) and reinforced spatial learning in rats with ventral and dorsal lesions of the hippocampus. *Behavioural Brain Research, 202,* 64–70. Doi:10.1016/j.bbr.2009.03.016.

Giedd, J. N., Snell, J. W., Lange, N., Rajapakse, J. C., Casey, B. J., Kozuch, P. L…Rapoport, J. L. (1996). Quantitative magnetic resonance imaging of human brain development: ages 4-18. *Cereb Cortex, 6*(*4*), 551-60. doi.org/10.1093/cercor/6.4.551.

Gilpin, N. W., Karanikas, C. A., & Richardson, H. N. (2012). Adolescent binge drinking leads to changes in alcohol drinking, anxiety, and amygdalar cortiocotropin releasing factor cells in adulthood in male rats. *PLoS ONE, 7*(*2*) e31466. doi:10.1371/journal.pone.0031466.

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C…Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proc. Natl. Acad. Sci. U.S.A., 101,* 8174-8179. doi.org/10.1073/pnas.0402680101.

Good, M. A., Barnes, P., Staal, V., McGregor, A., & Honey, R. C. (2007). Context-but not familiarity-dependent forms of object recognition are impaired following excitotoxic hippocampal lesions in rats. *Behav Neurosci, 121,* 218-223. doi.org/10.1037/0735-7044.121.1.218.

Goulart, B. K., de Lima, M. N. M., de Farias, C. B., Reolon, G. K., Almeida, V. R., Quevedo, J…Roesler, R. (2010). Ketamine impairs recognition memory consolidation and prevents learning-induced increase in hippocampal brain-derived neurotrophic factor levels. *Neuroscience, 167,* 969-973. doi:10.1016/j.neuroscience.2010.03.032

Gracia, L. S, Comim, C. M., Valvassori, S. S., Reurs, G. Z., Stertz, L., Kapczinski, F…Quevedo, J. (2009). Ketamine treatment reverses behavioral and physiological alterations induced by chronic mild stress in rats. *Prog. Neuropsychopharmacol. Biol. Psychiary, 33,* 450-455. Doi:10.1016/j.pnpbp.2009.01.004.

Grayson, B., Adamson, L., Harte, M., Leger, M., Marsh, S., Piercy, C. & Neill, J. C. (2014). The involvement of distraction in memory deficits induced by NMDAR antagonism: Relevance to cognitive deficits in schizophrenia. *Behavioural Brain Research, 266,* 188-192.

Grayson, B. & Neill, J. C. (2004). The effect of PCP on novel object recognition in the rat. *J Psychopharmacol, 18*(3), A72.

Grayson, B., Idris, N. F., & Neill, J. C. (2007). Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the novel object recognition task in the rat. *Behav Brain Res, 184,* 31-38. doi.org/10.1016/j.bbr.2007.06.012.

Gruzelier, J., Seymour, K. & Wilson, J. (1988). Impairments on neuropsychologic tests of temporohippocampal and frontohippocampal functions and word fluency in remitting schizophrenic and affective disorders. *Arch Gen Psychiatry, 45,* 623-638.

Gulley, J. M. & Juraska, J. M. (2013). The effects of abused drugs on adolescent development of corticolimbic circuitry and behavior. *Neuroscience*. doi.org/10.1016/j.neuroscience.2013.05.026.

Haber, S. N. & Knutson, B. (2010). The reward circuit: Linking primate anatomy and human imaging. *Neuropsychopharmacology, 35,* 4-26. doi.org/10.1038/npp.2009.129.

Hammond, R. S., Tull, L. E., & Stackman, R. W. (2004). On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiology of Learning and Memory, 82,* 26-34. doi.org/10.1016/j.nlm.2004.03.005.

Hammond, R. S., Tull, L. E., & Stackman, R. W. (2004). On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiology of Learning and Memory*, 82, 26-34.

Harnishferger, K. K. (1995). The development of cognitive inhibition: Theories, definitions, research. In F. N. Dempster & C. J. Brainerd (Eds.) *Interference and inhibition in cognition* (pp. 176-206). San Diego: Academic Press.

Hartley, D. E., Elsabagh, S., & File, S. E. (2004). Binge drinking and sex: effects on mood and cognitive function in healthy young volunteers. *Pharmacol Biochem Behav, 78,* 611-619. doi.org/10.1016/j.pbb.2004.04.027

Hashimoto, K., Fujita, Y., Shimizu, E., & Iyo, M. (2005). Phencyclidine- induced cognitive deficits in mice are improved by subsequent subchronic administration of clozapine, but not haloperidol. *Eur J Pharmacol, 519*, 114-117. doi.org/10.1016/j.ejphar.2005.07.002.

Heyser, C. J. & Ferris, J. S. (2012). Object exploration in the developing rat: methodological considerations. *Developmental Psychobiology, 55(4)*, 373-81. Doi 10.1002/dev.214041.

Hicks, B. M., Iacono, W. G., & McGue, M. (2010). Consequences of an adolescent onset and persistent course of alcohol dependence in men: adolescent risk factors and adult outcomes. *Alcohol Clin Exp Res., 34*, 819-833. doi.org/10.1111/j.1530-0277.2010.01154.x.

Hoare, J. & Moon, D. Drug misuse declared: findings from the 2009/10 British Crime Survey England and Wales. Home Office Statistical Board. London: Home Office; 2010.

Hoare, J. Drug misuse declared: findings from the 2008/09 British Crime Survey England and Wales. Home Office Statistical Board. London: Home Office; 2009.

Hoffman, K. L. & Basurto, E. (2013). One-trial object recognition memory in the domestic rabbit (*Oryctolagus cuniculus*) is disrupted by NMDA receptor antagonists, *Behavioural Brain Research.* http://dx.doi.org/10.1016/j.bbr.2013.04.049

Huttenlocher, P. R. (1984). Synapse elimination and plasticity in developing human cerebral cortex. *American Journal of Mental Deficiency, 88(5)*, 488-96.

Insel, T. R., Miller, L. P., & Gelhard, R. E. (1990). The ontogeny of excitatory amino acid receptors in rat forebrain: I. *N*-methyl-D-aspartate and quisqualate receptors. *Neuroscience, 35*, 31-43. doi.org/10.1016/0306-4522(90)90117-M.

Jablonski, S. A., Schreiber, W. B., Westbrook, S. R., Brennan, L. E., & Stanton, M. E. (2013). Determinants of novel object and location recognition during development. *Behavioral Brain, 256,* 140-150. Doi:10.1016/j.bbr.2013.07.055.

Jansen, K. L. (2000). A review of the nonmedical use of ketamine: use, users and consequences. *J Psychoact Drugs*, *32*, 419-33. doi.org/10.1080/02791072.2000.10400244.

Johnston, L. D., O' Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2010). *Monitoring the Future National Results on Adolescent Drug Use: Overview of Key Findings, 2009*. Bethesda, MD: National Institute on Drug Abuse; 2010.

Johnston, L. D., O' Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2011). *Marijuana use continues to rise among U.S. teens, while alcohol use hits historic lows*. Ann Arbor, MI: University of Michigan News Service.

Kenny, J. W., Adoff, M. D., Wilkinson, D. S. & Gould, T. J. (2011) The effects of acute, chronic, and withdrawal from chronic nicotine on novel and spatial object recognition in male C57BL/6J mice. *Psychopharmacology (Berl), 217(3),* 353-365. doi: 10.1007/s00213-011-2283-7.

King, M. V., Slight, A. J., Woolley, M. L., Topham, I. A., Marsden, C. A., & Fone, K. C. F. (2004). 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation – an effect sensitive to NMDA receptor antagonism. *Neuropharmacology, 47,* 195-204. doi.org/10.1016/j.neuropharm.2004.03.012.

Koesters, S. C., Rogers, P. D., Rajasingham, C. R. (2002). MDMA ('ecstasy') and other 'club drugs': The new epidemic. *Pediatr. Clin. North Am. 49,* 415-433. doi.org/10.1016/S0031-3955(01)00012-8.

Koike, H., Iijima, M., & Chaki, S. (2011). Involvement of AMPA receptor in both the rapid and sustained antidepressant-like effects of ketamine in animal models of depression. *Behavioral Brain Research, 234,* 107-111. doi:10.1016/j.bbr.2011.05.035

Koskinen, S. M., Ahveninen, J., Kujala, T., Kaprio, J., O'Donnell, B. F., Osipova, D…Rose, R. J. (2011). A longitudinal twin study of effects of adolescent alcohol abuse on the neurophysiology of attention and orienting. *Alcohol Clin Exp Res, 35*(*7*), 1339-50.

Langston, R. F. & Wood, E. R. (2010). Associative recognition and the hippocampus: differential effects of hippocampal lesions on object-place, object-context, and object-place-context memory. *Hippocampus, 20,* 1139-1153. doi: 10.1002/hipo.20714

Larkin, G. L. & Beautrais, A. L. (2011). A preliminary naturalistic study of low-dose ketamine for depression and suicide ideation in the emergency department. *Int J. Neuropsychopharmacol, 14*(*8*), 1127-31. doi.org/10.1017/S1461145711000629.

Lenroot, R. K. & Giedd, J. N. (2006). Brain development in children and adolescents: insights from anatomical magnetic resonance imaging. *Neurosci. Biobehav. Rev., 30,* 718-729. doi.org/10.1016/j.neubiorev.2006.06.001.

Leussis, M. P. & Bolivar, V. J. (2006). Habituation in rodents: a review of behavior, neurobiology, and genetics. *Neurosci. Biobhev. Rev., 30,* 1045-1064.

Li, N., Liu, R. J., Dwyer, J. M., Banasr, M., Lee, B., Son, H.,…Duman, R. S. (2011). Glutamate *N*-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biological Psychiatry, 69,* 754-761. doi:10.1016/j.biopsych.2010.12.015

Lyvers, M., Czerczyk, C., Follent, A., & Lodge, P. (2009). Disinhibition and reward sensitivity in relation to alcohol consumption by university undergraduates. *Addict Res Theory, 17*, 668-677. doi.org/10.3109/16066350802404158.

Lyvers, M., Duff, H., & Hasking, P. (2011). Risky alcohol use and age of onset of regular alcohol consumption in relation to frontal lobe indices, reward sensitivity and rash impulsiveness. *Addict Res Theory*, *19*, 251-259. doi.org/10.3109/16066359.2010.500751.

Machado-Vieira, R., Yuan, P., Brutsche, N., Diazgranados, N., Luckenbaugh, D., Manji, H. K., & Zarate Jr., C. A. (2009). Brain-derived neurotrophic factor and initial antidepressant response to *N*-Methyl-D-aspartate antagonist. *J Clin Psychiatry*, *70*(*12*), 1-9. doi:10.4088/JCP.08m04659.

Maldonado-Devincci, A. M., Badanich, K. A., & Kirstein, C. L. (2010). Alcohol during adolescence selectively alters immediate and long-term behavior and neurochemistry. *Alcohol*, *44*(*1*), 57-66. doi: 10.1016/j.alcohol.2009.09.035

Malhotra, A. K., Pinals, D. A., Weingartner, H., Sirocco, K., Missar, C. D., Pickar, D., & Breier, A. (1996). NMDA receptor function and human cognition: the effects of ketamine in healthy volunteers. *Neuropsychopharmacology*, *14*(*5*), 301-307. doi.org/10.1016/0893-133X(95)00137-3.

Matlin, M. W. (2009). *Cognition*. Hoboken, NJ. John Wiley & Sons, Inc.

Mercadante, S., Arcuri, E., Tirelli, W., & Casuccio, A. (2000). Analgesic effect of intravenous ketamine in cancer patients on morphine therapy: A randomized, controlled, double-blind, crossover, double-dose study. *J Pain Symptom Manage*, *20*, 246-252. doi.org/10.1016/S0885-3924(00)00194-9.

Meunier, M. & Barbeau, E. (2013). Recognition memory and the medial temporal lobe: from monkey research to human pathology. *Rev Neurol (Paris)*, *168*(*6-7*), 459-69. doi: 10.1016/j.neurol.2013.01.623

Meyer, J. S., & Quenzer, L. F. (2005). *Psychopharmacology Drugs, the Brain and Behavior*. Sunderland, MA: Sinauer Associates, Inc.

Monaghan, D. T. & Cotman, C. W. (1985). Distribution of *N*-Methyl-D-aspartate-sensitive L-[3H]Glutamate-binding sites in rat brain. *The Journal of Neuroscience*, 5(11), 2909-2919.

Moosavi, M., Khales, G. Y., Rastegar, M., & Zarifkar, A. (2012) The effect of sub-anesthetic and anesthetic ketamine on water maze memory acquisition, consolidation, and retrieval. *European Journal of Pharmacology, 677*, 107-110. doi.org/10.1016/j.ejphar.2011.12.021.

Morgan, C. J. A. & Curran, H. V. (2011). Ketamine use: a review. *Addiction, 107*, 27-38. doi:10.1111/j.1360-0443.2011.03576.x

Morgan, C. J. A., Monaghan, L., & Curran, V. (2004). Beyond the K-hole: a 3-year longitudinal investigation of the cognitive and subjective effects of ketamine in recreational users who have substantially reduced their use of the drug. *Addiction*, *99*, 1450-1461. doi.org/10.1111/j.1360-0443.2004.00879.x.

Morgan, C. J., Muetzelfeldt, L., & Curran, H. V. (2010). Consequences of chronic ketamine self-administration upon neurocognitive function and psychological wellbeing: a 1-year longitudinal study. *Addiction, 105(1),* 121-33. doi: 10.1111/j.1360-0443.2009.02761.x

Morgan, C. J., Rees, H., & Curran, H. V. (2008). Attentional bias to incentive stimuli in frequent ketamine users. *Psychol Med, 38*, 1331-40. doi.org/10.1017/S0033291707002450.

Murrough, J. W. (2012). Ketamine as a Novel Antidepressant: From Synapse to Behavior. *Clinical Pharmacology & Therapeutics*, *91*(*2*), 303-309. doi:10.1038/clpt.2011.244.

Nagai, T., Murai, R., Matsui, K., Kamei, H., Noda, Y. Furukawa, H., Nabeshima, T. (2009). Ariprazole ameliorates phencyclidine-induced impairment of recognition memory through dopamine D1 and serotonin 5-HT1A receptors. *Psychopharmacology (Berl)*, *202*(*1-3*), 315-28. doi.org/10.1007/s00213-008-1240-6.

National Institute on Drug Abuse (NIDA). (2003). *NIDA Community Drug Alert Bulletin-Club Drugs*. [Online] [accessed on July 20, 2013]. Available from http://www.drugabuse.gov/clubalert/clubdrugalert.html

Nikforuk, A. & Popik, P. (2012). Effects of quetiapine and sertindole on subchronic ketamine-induced deficits in attentional set-shifting. *Psychopharmacology*, *220*, 65-74. doi:10.1007/s00213-011-2487-x

Nikiforuk, A., Fikal, K., Potasiewicz, A., Popik, P., & Kos, T. (2013). The 5-hydroxytryptamine (serotonin) receptor 6 agonist EMD 386088 ameliorates ketamine-induced deficits in attentional set shifting and novel object recognition, but not in the prepulse inhibition in rats. *J Psychopharmacol*, *27*(*5*), 469-76. doi.org/10.1177/0269881113480991.

Nurse, S. & Lacaille, J. C. (1999). Late maturation of GABA(B) synaptic transmission in area CA1 of the rat hippocampus. *Neuropharmacology*, 38, 1733-42.

Oliveira, L., Fraga, D. B., De Luca, R. D., Canever, L., Ghedim, F. V., Matos, M. P. P…Zugno, A. I. (2011). Behavioral changes and mitochondrial dysfunction in a rat model of schizophrenia induced by ketamine. *Metab Brain Dis*, *26*, 69-77. doi: 10.1007/s11011-011-9234-1.

Pal, H. R., Berry, N., Kumar, R., Ray, R. (2002). Ketamine dependence. *Anaesth Intensive Care*, *30(3)*, 382-4.

Parada, M., Corral, M., Caamaño,-Isorna, R., Mota, N., Crego, A., Holguín, S. R., & Cadaveira, F. (2011). Binge drinking and declarative memory in university students. *Alcohol Clin Exp Res*, *35*(*8*), 1475-84. doi: 10.1111/j.1530-0277.2011.01484.x.

Park, M. S., Sohn, S., Park, J. E., Kim, S. H., Yu, I. K., & Sohn, J. H. (2011). Brain functions associated with verbal working memory tasks among young males with alcohol use disorders. *Scand J Psychol*, *52*, 1-7. doi: 10.1111/j.1467-9450.2010.00848.x

Parwani, A., Weiler, M. A., Blaxton, T. A., Warfel, D., Hardin, M., Frey, K., & Lahti, A. C. (2005). The effects of a subanesthetic dose of ketamine on verbal memory in normal volunteers. *Psychopharmacol*, *183*, 265-274. doi.org/10.1007/s00213-005-0177.

Paul, M. G., Li, M., Allen, R. R., Lui, F., Zou, X., Hotchkiss, C…Wang, C. (2011). Ketamine anesthesia during the first week of life can cause cognitive deficits in rhesus monkeys. *Neurotoxicol Teratol*, *33*(*2*), 220-30. doi: 10.1016/j.ntt.2011.01.001

Peissig, J. J., Singer, J., Kawasaki, K., & Sheinberg, D. L. (2007). Effects of long-term object familiarity on event-related potentials in the monkey. *Cereb Cortex*, 17, 1323-1334.

Phelps, L. E., Brutsche, N., Moral, J. R., Luckenbaugh, D. A., Manji, H. K., & Zarate Jr., C. A. (2009). Family history of alcohol dependence and initial antidepressant response to an NMDA antagonist. *Biol Psychiatry*, *65*(*2*), 181-184. doi:10.1016/j.biopsych.2008.09.029

Pickles, A., Pickering, K., Simonoff, E., Silberg, J., Meyer, J., & Maes, H. (1988). Genetic clocks and soft events: A twin model for pubertal development and other recalled sequences of developmental milestones, transitions, or ages at onset. *Behavior Genetics*, *28*, 243-53. doi: 0001-8244/98/0700-0243$15.00/0

Pisikas, N. (2007). Effects of scopolamine and L-NAME on rats' performance in the object location test. *Behav. Brain. Res.*, *179*, 294-298.

Pitsikas, N. & Boultadakis, A. (2009). Pre-training administration of anesthetic ketamine differentially affects rats' spatial and non-spatial recognition memory. *Neuropharmacology, 57*, 1-7. doi:10.1016/jneuropharm.2009.03.015

Pitsikas, N., Boultadakis, A. & Sakellaridis, N. (2008). Effects of sub-anesthetic doses of ketamine on rats' spatial and non-spatial recognition memory. *Neuroscience, 154*, 454-460. doi:10.1016/j.neuroscience.2008.04.001

Pope Jr., H. G., Gruber, A. J., Hudson, J. I., Cohane, G., Huestis, M. A. & Yurgelun-Todd, D. 2003. Early-onset cannabis use and cognitive deficits: what is the nature of the association? *Schiophr Bull*, 34, 907-926.

Prabhakar, R. P. & Subbu, V. S. V. (1977). Cardiovascular and other pharmacological actions of ketamine. *Indian J Pharmacol, 9*, 273-7.

Price, R. B., Nock, M. K., Charney, D. S., & Mathew, S. J. (2009). Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. *Biol Psychiatry, 66*(*5*), 522-526. doi:10.1016/j.biopsych.2009.04.029

Rakic, P., Bourgeois, J. P., & Goldman-Rakic, P. S. (1994). Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness. In: van Pelt, J., Corner, M. A., Uylings, H. B. M., & Lopes de Silva, F. H. *Progress in brain research, the self-organizing brain: from growth cones to functional networks*, vol. 102. Amsterdam: Elsevier, 227-43.

Reger, M. L., Hovda, D. A., & Giza, C. C. (2009). Ontogeny of rat recognition memory measured by the novel object recognition task. *J Neurosci Met, 166*, 99-103. doi:10.1002/dev.20402.

Ribeiro, P. P., Rodrigues, P. C., Valentim, A. M., & Antunes, L. M. (2013). A single intraperitoneal injection of ketamine does not affect spatial working, reference memory or neurodegeneration in adult mice: A prospective animal study. *Eur J Anaesthesiol*, [Epub ahead of print].

Richardson, J. T. E. (2011). Review: Eta squared and partial eta squared as measures of effect size in educational research. *Educational Research Review, 6*(2), 135-147. doi: 10.1016/j.edurev.2010.12.001.

Rome, E. S. (2001). It's a rave new world: rave culture and illicit drug use in the young. *Cleve Clin J Med, 68*, 541-50.

Rosat, R., Da-Silva, R. C., Zanatta, M. S., Medina, J. H., & Izquierdo, I. (1992). Memory consolidation of a habituation task: role of N-methyl-D-aspartate, cholinergic muscarinic and GABA-A receptors in different brain regions. *Braz. J. Med. Biol. Res., 25*, 267-273.

Rowland, L. M. (2005). Sub-anesthetic ketamine: how it alters physiology and behavior in humans. *Aviation Space Environ Med, 76*, 52-58.

Rushforth, S. L., Steckler, T., & Shoaib, M. (2011). Nicotine improves working memory span capacity in rats following sub-chronic ketamine exposure. *Neuropsychopharmacology, 36*(*13*), 2774-81. doi:10.1038/npp.2011.224;

Schwartzman, R. J., Alexander, G. M., Grothusen, J. R., Reichenberger, E., & Perreault, M. (2009). Outpatient intravenous ketamine for the treatment of complex regional pain syndrome: A double-blind placebo controlled study. *Pain, 147*, 107-115. doi: 10.1016/j.pain.2009.08.015

Sherril, L. K., Stanis, J. J., & Gulley, J. M. (2013). Age-dependent effects of repeated amphetamine exposure on working memory in rats. *Behavioural Brain Research, 242*, 84-94. doi: 10.1016/j.bbr.2012.12.044

Silvers, J. M. Harrod, S. B., Mactutus, C. F., & Booze, R. M. (2007). Automation of the novel object recognition task for use in adolescent rats. *Inc Dev Psychobiol, 51*, 672-678. doi:10.1016/j.jneumeth.2007.06.032

Silvers, J. M. Harrod, S. B., Mactutus, C. F., & Booze, R. M. (2007). Automation of the novel object recognition task for use in adolescent rats. *Inc Dev Psychobiol*, 51, 672-678. Doi:10.1016/j.jneumeth.2007.06.032.

Sircar, R., Basak, A., Sircar, D., & Wu, L. C. (2010). Effects of gamma-hydroxybutyric acid on spatial learning and memory in adolescent and adult rats. *Pharmacology Biochemistry & Behavior*, *96*(*2*), 187-93. doi:10.1016/j.pbb.2010.04.028.

Smith, K. M., Larive, L. L., & Romanelli, F. (2002). Club drugs: Methylenedioxymethamphetamine, flunitrazepam, ketamine, hydrochloride, and y-hydroxybutrate. *Am J Health-Syst. Pharm*, *59*, 1067-1076.

Snyder, M. A. & Gao, W. J. (2013). NMDA hypofunction as a convergence point for progression and symptoms of schizophrenia. *Frontiers in Cellular Neuroscience, 7(31),* 1-12. doi 10.3389/fncel.2013.00031.

Sorensen, J., Bengtsson, A., Backman, E., Henriksson, K. G., & Bengtsson, M. (1995). Pain analysis in patients with fibromyalgia, effects of intravenous morphine, lidocaine, and ketamine. *Scan J Rheumatol, 24*, 360-365.

Sowell, E. R., Thompson, P. M., Holmes, C. J., Jernigan, T. L., & Toga, A. W. (1999). In vivo evidence for post adolescent brain maturation in frontal and striatal regions. *Nat. Neurosci*, *2*, 859-861. Doi:10.1038/13154

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews*, *24*, 417-463. doi.org/10.1016/S0149-7634(00)00014-2.

Spear, L. P., Shalaby, I. A., & Brick, J. (1980). Chronic administration of haloperidol during development: behavioral and psychopharmacological effects. *Psychopharmacology*, *70*, 47-58. doi.org/10.1007/BF00432369.

Stewart, C. E. (2001). Ketamine as a street drug. *Emerg Med Serv*, *30*(*11*), 30-34.

Taffe, M. A., Davis, S. A., Gutierrez, T., & Gold, L. H. (2002). Ketamine impairs multiple cognitive domains in rhesus monkeys. *Drug Alcohol Depend*, *68*(*2*), 175-87. doi.org/10.1016/S0376-8716(02)00194-1.

Tapert, S. F., Baratta, M. V., Abrantes, A. M., & Brown, S. A. (2002). Attention dysfunction predicts substance involvement in community youths. *J Am Acad Child Adolesc Psychiatry*, *41*, 680-686. doi.org/10.1097/00004583-200206000-00007.

Tapert, S. F., Schweinsburg, A. D., Barlett, V. C., Brown, S. A., Frank, L. R., Brown, G. G., & Meloy, M. J. (2004). Blood oxygen level dependent response and spatial working memory in adolescents with alcohol use disorders. *Alcohol Clin Exp Res*, *28*(*10*), 1577-86. doi.org/10.1097/01.ALC.0000141812.81234.A6.

Thoma, R. J., Monnig, M. A., Lysne, P. A., Ruhl, D. A., Pommy, J. A., Bogenschutz, M…Yeo, R. A. (2011). Adolescent substance abuse: the effects of alcohol and marijuana on neuropsychological performance. *Alcohol Clin Exp Res, 35*(*1*), 39-46. doi.org/10.1111/j.1530-0277.2010.01320.x.

Traverso, L. M., Ruiz, G., Camino, G., De la Case, L. G. (2008). Ketamine blocks the formation of a gustatory memory trace in rats. *Pharmacol. Biochem. Behav., 90*, 305-311.

Tyler, D. B. & van Harreveld, A. (1942). The respiration of the developing brain. *American Journal of Physiology*, 136, 600-3.

Valentim, A. M., Ribeiro, P. O., Olsson, I. A., & Antunes, L. M. (2013). The memory stages of a spatial Y-maze task are not affected by a low dose of ketamine/midazolam. *Eur J Pharmacol, 712*(*1-3*), 39-47. doi.org/10.1016/j.ejphar.2013.04.027.

Venãncio, C. Magalhães, A., Antunes, L., & Summavielle, T. (2011). Impaired spatial memory after ketamine administration in chronic low doses. *Current Neuropharmacology, 9*, 251-255. doi:10.2174/157015911795016912.

Verma, A. & Moghaddam, B. (1996). NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alternation performance in rats: modulation by dopamine. *The Journal of Neuroscience, 16(1)*, 373-379. doi: 0270-6474/95/160373-07$05.00/0

Wagner, D., Becker, B., Koester, P., Gouzoulis-Mayfrank, E., & Daumann, J. (2013). A prospective study of learning, memory, and executive function in new MDMA users. *Addiction, 108*(*1*), 136-45. doi: 10.1111/j.1360-0443.2012.03977.x

Warburton, E. C., Barker, G. R. I., & Brown, M. W. (2013). Investigations into the involvement of NMDA mechanisms in recognition memory. *Neuropharmacology, 1-7.* doi: 10.1016/j.neuropharm.2013.04.013

White, H. R., Marmorstein, N. R., Crews, F. T., Bates, M. E., Mun, E. Y., & Loeber, R. (2011). Associations between heavy drinking and changes in impulsive behavior among adolescent boys. *Alcohol Clin Exp Res*, *35*, 295-303. doi: 10.1111/j.1530-0277.2010.01345.x

Wilcox, R. R. (1987). New designs in analysis of variance. *Ann Rev Psychol*, *38*, 29-60. doi:10.1146/annurev.ps.38020187.000333

Wiltgen, B. J., Sanders, M. J., Anagnostaras, S. G., Sage, J. R., Fanselow, M. S. (2006). Context fear learning in the absence of the hippocampus. *The Journal of Neuroscience*, 26(20), 5484-5491. doi:10.1523/jneurosci.2685-05.2006

Wood, S., Sage, J. R., Shuman, T., & Anagnostaras, S. G. (2013). Psychostimulants and cognition: a continuum of behavioral and cognitive activation. *Pharmacol Rev*, 66(1), 193-221. doi: 10.1124/pr.112.007054.

Wu, L. T., Schlenger, W. E., & Galvin, D. M. (2006). Concurrent use of methamphetamine, MDMA, LSD, ketamine, GHB, and flunutrazepam among American youths. *Drug Alcohol Depend, 84(1),* 102-113.

Yu, H., Li, Q., Wang, D., Shi, L., Lu, G., Sun, L…Yew, D. T. (2012). Mapping the central effects of chronic ketamine administration in adolescent primate model by functional magnetic resonance imaging (fMRI). *Neurotoxicology*, *33*, 70-77. doi:10.1016/j.neuro2011.11.001.

Zarate Jr., C. A., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A.,…Manji, H. K. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry*, *63*(*8*), 856-64. doi.org/10.1001/archpsyc.63.8.856.

Zecevic, N., Bourgeois, J. P, & Rakic, P. (1989). Changes in synaptic density in motor cortex of rhesus monkey during fetal and postnatal life. *Developmental Brain Research, 50,* 11-32. doi.org/10.1016/0165-3806(89)90124-7.