ABSTRACT

EARLY EXPOSURE TO KETAMINE DOES NOT AFFECT NICOTINE REWARD DURING ADOLESCENCE IN MALE AND FEMALE RATS

By

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Children are commonly prescribed fluoxetine to manage their depressive symptoms, although evidence suggests many fail to respond to this treatment. Recently, low doses of ketamine were shown to work as a fast-acting and long-lasting antidepressant, however, it is unclear what the long-term effects are of using ketamine in pediatric populations. Thus, this thesis examined whether early-life exposure to ketamine influences the rewarding effects of nicotine in male and female adolescent Sprague-Dawley rats using conditioned place preference. Rats were pretreated with ketamine (0.0 or 20.0 mg/kg) from postnatal day (PD) 21-30 and then assessed for nicotine (0.0, 0.03, 0.1, 0.3, or 0.6 mg/kg) preference during adolescence (PD 32-42). Results indicate that female adolescent rats find nicotine to be more rewarding than male rats, however ketamine pretreatment did not affect nicotine's effects. These findings suggest that ketamine as an antidepressant in children and adolescents may not produce adverse increases in nicotine reward.

EARLY EXPOSURE TO KETAMINE DOES NOT AFFECT NICOTINE REWARD DURING ADOLESCENCE IN MALE AND FEMALE RATS

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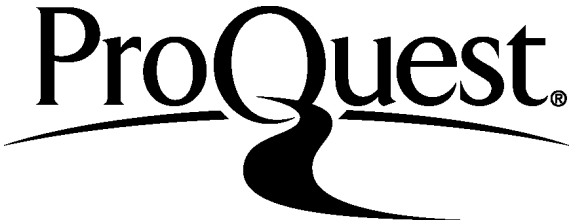

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ANOVA	analysis of variance
BDNF	brain-derived neurotrophic factor
cm	centimeters
CPP	conditioned place preference
CO_2	carbon dioxide
D_1	dopamine 1
D_2	dopamine 2
db	decibel
ERK	extracellular signal-regulated kinase
FST	forced swim test
g	grams
GABA	*gamma*-aminobutyric acid
IP	intraperitoneally
mm	millimeters
mg/kg	milligrams/kilograms
ml/kg	milliliters/kilograms
mTOR	mammalian target of rapamycin
NaOH	sodium hydroxide
NMDA	N-methyl-D-aspartate

PD	postnatal day
PFC	prefrontal cortex
SC	subcutaneously
SEM	standard error of the mean
SSRI	selective serotonin reuptake inhibitor
TRD	treatment resistant depression
VTA	ventral tegmental area
W	watt

CHAPTER 1

INTRODUCTION

Depression is a major psychiatric condition that affects children and adolescents at an alarming rate. Of particular concern is treatment resistant depression (TRD), which can result in suicidality among the pediatric population. According to the Centers for Disease Control and Prevention, suicide is the third leading cause of death for individuals between 10-24 years of age. The high rate of TRD in children and adolescents may in part elucidate why there is a high rate of suicidality in this population (Maalouf, Atwi, & Brent, 2011). TRD is a form of major depressive disorder that cannot be effectively treated with conventional antidepressant medications, such as selective serotonin reuptake inhibitors (SSRIs), which can take up to 6 weeks to induce a therapeutic effect (Mathew et al., 2012). Recently, ketamine, a glutamate noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, has been shown to rapidly reduce suicidal ideation in adult patients with TRD with a single exposure (Murrough et al., 2013), and symptoms of bipolar disorder in a pediatric population that was also unresponsive to traditional antidepressant treatment (Papolos, Teicher, Faedda, Murphy, & Mattis, 2013). These findings suggest that ketamine may be useful in ameliorating TRD. Nonetheless, ketamine is not yet approved by the Food and Drug Administration as an antidepressant, as preclinical and clinical studies are still being conducted. Two concerns about using ketamine in a pediatric population is that the mechanism by which ketamine produces its

antidepressant effect is not well characterized and ketamine is an abused drug (Trujillo et al., 2011). Similar to what is seen with other abused drugs, if given chronically ketamine may have long lasting effects that can influence brain reward systems such as the mesolimbic dopamine pathway (Koob & Volkow, 2010). In particular ketamine exposure may increase vulnerability to subsequently abuse drugs if the outcome is an increase in the rewarding properties and/or a decrease in the withdrawal effects of other drugs of abuse. To examine this possibility, the current thesis proposal will investigate whether chronic treatment with ketamine, early in development, results in an increase in the rewarding effects of nicotine during adolescence, using the conditioned place preference (CPP) paradigm, a validated animal model of reward (Bardo & Bevins, 2000).

Prevalence Rates and Symptomology of Depression

Depression is a psychiatric condition that affects individuals of all ages and is associated with devastating symptoms. Approximately 2% of children and 6% of adolescents are diagnosed with major depressive disorder (Cheung, Emslie, & Mayes, 2005). Major depressive disorder is characterized by experiencing at least five of the following symptoms for at least 2 weeks: feelings of sadness for most of the day, anhedonia, significant weight gain or loss, sleeping too much or not sleeping at all, psychomotor retardation or agitation, decrease in energy, feelings of helplessness and hopelessness, inability to concentrate and/or make decisions, and suicidal ideation (American Psychiatric Association, 2013). Children and adolescents have a more insidious onset of symptoms and experience irritability more than sadness (Hazell, 2009). Before adolescence, males and females are equally affected; however from adolescence and into adulthood, females have a higher rate of depression than males (Hazell, 2009).

Antidepressant Medication Use in Children and Adolescents

The prescription rate of antidepressant medications continues to increase in individuals 6 years old and older. Approximately 2.5% of individuals under 18 years of age are prescribed an antidepressant medication (Olfson & Marcus, 2009). To date, there are studies that show the successful use of SSRIs such as fluoxetine (Emslie et al., 1997; Emslie et al., 2002) and paroxetine (Keller et al., 2001) to alleviate depression in children and adolescents. However, there are also studies that show that paroxetine (Emslie et al., 2006) as well as escitalopram (Wagner, Jonas, Findling, Ventura, & Saikali, 2006) are not more effective than placebo in children and adolescents. One explanation for the differences in efficacy outcomes in a pediatric population is the possibility that individuals experience different effects between types of SSRIs. However, since there are efficacy outcome differences between two studies that examined paroxetine, there could be other contributing factors such as site selection, study population, study design, and outcome measures (Cheung et al., 2005). There is a need for more extensive research into the efficacy as well as safety of prescribing antidepressant medications to children and adolescents.

Research into the efficacy of antidepressants in children and adolescents has identified severe adverse reactions, such as a worsening of depression and an increase in suicidality (Cheung et al., 2005; Hazell, 2009). As such, the Food and Drug Administration issued a warning label to be put onto all antidepressant medications prescribed to children and adolescents. This cautioning label is known as the "black box" warning and it informs the public of the adverse effects. Other side effects from antidepressant medications that have been seen in a pediatric population include anxiety,

panic attacks, agitation, irritability, hostility, impulsivity, severe restlessness, mania, and insomnia (Cheung et al., 2005). The differences in the effects of antidepressants between adults and those in the pediatric population may in part be reflective of the continued maturation of the brain during development.

Brain Development

During childhood and adolescence, there is an abundance of changes in connectivity occurring in the brain. Preclinical data for example suggests that during childhood synapses, or connections between neural cells, are produced at an increased rate (Brenhouse & Andersen, 2011; Penzes, Buonanno, Passafarro, Sala, & Sweet, 2013). However, most of these synapses are redundant and thus are pruned or refined during adolescence (Brenhouse & Andersen, 2011; Low & Cheng, 2006; Spear, 2013). The function of this refinement is to increase the efficacy of neurotransmission and cognitive processing by eliminating unnecessary connections in the brain. Also, as seen in post-mortem studies, there is an increase in myelination during adolescence (Brenhouse & Andersen, 2011; Spear, 2013). Myelin, a fat-enriched substance formed by glial cells, wraps around the axons and increases the speed of neural transmission. This increase in synapses, refinement, and reorganization is also seen in the prefrontal cortex (PFC) during childhood and adolescence (Cousins & Goodyer, 2015). This brain area is of particular importance because it is involved in executive functions such as decision-making and impulsivity.

Changes in the monoamergic systems are also evident across development. Dopamine, which is involved in reward (Nestler & Carlezon, 2006), undergoes considerable changes during adolescence. Dopamine levels in the PFC peak during

adolescence before lessening in adulthood. Also, there is a significant reduction in dopamine receptors following adolescence, specifically D_1 and D_2 receptors in the ventral striatum which is implicated in drug reward (Spear, 2000). Interestingly, male rats experienced an increase in dopamine receptor pruning compared to female rats (Brenhouse & Andersen, 2011; Tarazi & Baldessarini, 2000). Because of this peak in dopamine receptors during adolescence, there is a greater activation of the nucleus accumbens (brain region implicated in reward), which can lead to an increase in sensitivity to drugs of abuse (S. L. Andersen & Teicher, 2009). Moreover, serotonin neurotransmission, which is involved in mood and regulation of dopamine signaling (Cousins & Goodyer, 2015), peaks during childhood and is reduced in adolescence. In contrast, the serotonin receptor is more widely expressed during adolescence, especially in brain areas that are considered to be non-serotonergic (Daws & Gould, 2011). Interestingly, the imbalance between serotonin and dopamine could potentially be what drives the risky behavior of adolescents (Cousins & Goodyer, 2015).

The Use of Animal Models to Examine Drug Effects

Animal models are useful in examining the effects of drugs and the potential long-term consequences of early use of drugs. First, rodents are a useful model because they have a shorter life span (S. L. Andersen, 2003) and have similar brain maturation compared to humans (Spear, 2000). According to S. L. Andersen (2003), childhood in humans is equivalent to postnatal day (PD) 20-30 in rats and adolescence in humans corresponds to PD 30-50 in rats. The rat ages are based on behavioral and brain maturation patterns that resemble human developmental periods. For instance, rats experience a similar pattern of synaptic pruning and reorganization in the prefrontal

cortex, as do humans, before birth and during adolescence (S. L. Andersen, 2003). Interestingly, the dopamine inputs to this area also undergo considerable overproduction and pruning during adolescence in rats as it does in humans (Spear, 2000). Rodents also exhibit similar behavioral patterns that resemble different developmental periods in humans. For example, during adolescence rodents have an increase in social and novelty-seeking behavior (Spear, 2000). In sum, the use of animal models proves to be a useful method of assessing the effects of drugs across different developmental periods and to assess the functional consequences of early use of drugs.

Animal Models of Depression

Animal models of depression have been useful in determining the efficacy of antidepressant medications. Among the animal models that are frequently employed are social defeat stress and forced swim test. These models only mimic specific depressive-like behaviors because a basic understanding of the underlying disease process in depression is lacking (Abelaira, Reus, & Quevedo, 2013).

Social Defeat Stress

Chronic stress can be a predictor of depression in humans and animal models that employ chronic stress procedures result in depression-like behaviors in rodents (Yan, Cao, Das, Zhu, & Gao, 2010). Social defeat stress is one of the most common methods of inducing a stressful experience that leads to depressive-like behaviors in rodents (Abelaira et al., 2013; Czeh, Fuchs, Wiborg, & Simon, 2015; Yan et al., 2010). In a typical experiment, social defeat stress involves subjecting an animal to a threatening situation such as when an animal is introduced to the territory of an aggressive male. The aggressive male characteristically attacks the intruder instantaneously. After 5-10

minutes, the aggressive male and intruder male are separated by a clear plastic divider with holes that allow for visual, olfactory, and auditory contact for the next 24 hours. This paradigm takes place over several days with the intruder male being exposed to different aggressive males on each day. The depressive-like behaviors that are seen in the intruder male following the social defeat stress include decrease social interaction anxiety and anhedonic-like behaviors measured by sucrose consumption (Czeh et al., 2015; Yan et al., 2010).

The effectiveness of social defeat to induce depressive-like symptoms in rodents is also evident across development. For example, adolescent mice exposed to social defeat stress also exhibited depressive-like behaviors (Iñiguez et al., 2014). Moreover, Warren and colleagues (2013) found that it is not necessary for the animal to undergo physical stress, as in the typical social defeat stress model, in order to develop behavioral deficits. Having a mouse witness another mouse undergoing social defeat by a larger, more aggressive mouse invokes the same stress response and behavioral deficits as the mouse that physically underwent social defeat (Warren et al., 2013).

Forced Swim Test

Forced swim test (FST) is used to examine behavioral despair, or learned helplessness. It is also sensitive to a range of antidepressant medications and therefore makes it the most commonly used measure to examine the effectiveness of antidepressant medications (Yankelevitch-Yahav, Franko, Huly, & Doron, 2015). In this test, the rodent is placed into a cylinder of water with the depth of the water being deep enough so the animal's tail cannot touch the bottom. Initially the animal will try to escape, however following these unsuccessful attempts, the animal will eventually "give up" and become

immobile which is taken to be evidence for a depressive-like state. For the experiment, the animal is placed into the cylinder of water for 15 minutes on day one. Twenty-four hours later, the animal is again placed into the cylinder of water, however this time for only 5 minutes. During the second session, the rat will be assessed for swimming, climbing, and immobility behaviors (Abelaira et al., 2013; Yan et al., 2010). These behaviors, specifically immobility, represent behavioral despair (Yankelevitch-Yahav et al., 2015).

Monoamines and Depression

The FST is able to detect behavioral differences between the different classes of antidepressant medications. For example, rats treated with reboxetine (a selective norepinephrine reuptake inhibitor) show an increase in climbing behaviors while the rats treated with fluoxetine (a SSRI) show an increase in swimming behaviors, whereas moclobemide (a monoamine oxidase inhibitor) increased both climbing and swimming behaviors (Cryan, Page, & Lucki, 2005). Similarly, the FST is also sensitive to non-monoamergic antidepressant medication such as ketamine (a glutamate NMDA receptor antagonist), in which you get a reduction in immobility (Maeng et al., 2008). This may be useful to help differentiate the neurochemical mechanisms underlying this behavior (Yankelevitch-Yahav et al., 2015). Regardless of the behavioral phenotype, all of these monoamergic agonist and non-monoamergic antagonist drugs exhibited antidepressant effects by producing a reduction in immobility.

Glutamate and Depression

The discovery of novel pharmacotherapies for the treatment of depression stemmed from the high incidence of TRD with traditional SSRIs. Recent evidence

suggests that over-activation of glutamate neurotransmission is important in the etiology of depression and that drugs preventing the action of glutamate are the most promising as the next generation of antidepressant medications. Glutamate is a major excitatory neurotransmitter, found in more than 80% of neurons in the brain, and is important in neuroplasticity, learning, and memory (Kugaya & Sanacora, 2005). Overstimulation of glutamate, such as during chronic exposure to stress, decreases dendritic branching in hippocampal cells and leads to a reduction in the volume of the hippocampus which is implicated in depression (Kugaya & Sanacora, 2005; Mathews, Henter, & Zarate, 2012).

Indeed, pharmacological attenuation with glutamate NMDA receptor antagonists rapidly reduces symptoms of depression. Specifically, Berman and colleagues (2000) were the first to demonstrate in patients with depression that intravenous doses of ketamine, a noncompetitive NMDA receptor antagonist, resulted in fast-acting and long-lasting relief of depressive symptoms (Berman et al., 2000). Since then, many clinical (Diazgranados et al., 2010; Machado-Vieira et al., 2009; Price, Nock, Charney, & Mathew, 2009; Zarate et al., 2006) and preclinical (Autry et al., 2011; Brachman et al., 2015; Garcia et al., 2008; Garcia et al., 2009; N. Li et al., 2011; S.-X. Li, Zhang, Wu, & Hashimoto, 2014) studies have replicated the antidepressant effects of NMDA receptor antagonists. Moreover, the therapeutic efficacy of ketamine, as a novel antidepressant medication, extends to children and adolescents. Intranasal administration to juveniles suffering from treatment-resistant bipolar disorder improved all symptomology rapidly and with minimal side effects (Papolos et al., 2013). A preclinical study has also shown the efficacy of ketamine during adolescence. Parise and colleagues (2013) examined long-term effects of adolescent exposure to ketamine in rats. Specifically, adolescent

exposure to ketamine reversed the depressive-like behaviors from chronic unpredictable stress. These effects also persisted two months after treatment (Parise et al., 2013). Therefore it is hypothesized that ketamine is causing changes in the connectivity in the brain in order to perpetuate the antidepressant effect for at least two months following treatment. In sum, although the precise mechanisms of ketamine are unknown, the utility of this drug in treating TRD is novel and exciting and has kindled an increase in understanding the role of ketamine and glutamate systems in depression.

Ketamine Mechanisms of Action

The antidepressant effects of ketamine are thought to result from intracellular changes and receptor changes in key brain regions. For example, the mammalian target of rapamycin (mTOR) is a protein kinase that modulates cell growth, proliferation, and survival, which has an effect on synaptogenesis and has been shown to be important in the intracellular mechanisms of depression (Yang, Hu, Zhou, Zhang, & Yang, 2012). Similarly, brain-derived neurotrophic factor (BDNF), has been shown to be implicated in depression with low levels seen in patients with major depressive disorder (Hashimoto, 2010). Ketamine administration increases the levels of BDNF and mTOR in the rodent hippocampus (Akinfiresoye & Tizabi, 2013; Yang et al., 2012) and PFC (N. Li et al., 2010). Interestingly, ketamine also influences *gamma*-aminobutyric acid (GABA) interneurons in the PFC. Specifically, administration of ketamine decreases the firing rate of GABA interneurons, which in turn increases the delayed firing rate of pyramidal cells. The disinhibition of pyramidal cells leads to an increase in glutamate release (Martinowich, Jimenez, Zarate, & Manji, 2013), followed by an increase in synaptogenesis that is thought to restore the dendritic branching lost due to depression.

In sum, ketamine's mechanism of action may produce the antidepressant effects by mediating increases in BDNF and mTOR, which in turn may be facilitating an increase in synaptogenesis.

Potential Long-Term Consequences of Antidepressant Exposure During Development

Little is known about the long-term neurobiological adaptations and behavioral changes ensuing from antidepressant treatments in pediatric populations. This is alarming, given the high rate of antidepressant treatments in children and adolescents (Birmaher, Ryan, Williamson, Brent, & Kaufman, 1996; Pratt, Brody, & Gu, 2011). Preclinical studies, however, suggest that early use of antidepressants, such as SSRIs, have lifelong behavioral consequences. For example, treatment with fluoxetine during adolescence increases anxiety-like behaviors (Iñiguez, Warren, & Bolaños-Guzmán, 2010; Iñiguez et al., 2014a; Warren et al., 2011), aggressive-like behaviors (Ricci & Melloni, 2012), sensitivity to stressors (Karpova, Lindholm, Pruunsild, Timmusk, & Castren, 2009; Warren et al., 2011), sensitivity to natural rewards (Iñiguez et al., 2010), and the rewarding effects of cocaine (Warren et al., 2011) in adulthood. Similarly, treatment with fluoxetine during adolescence decreases learning and memory abilities (Sass & Wortein, 2012) in adulthood. These changes in behavior after exposure to fluoxetine during adolescence could be mediated by a decrease in extracellular signal-regulated kinase (ERK) 1 and 2 within the ventral tegmental area (VTA; brain area implicated in mood regulation), which mediates an intercellular change that has been implicated in stress (Iñiguez et al., 2014b).

The neurobiological and neurobehavioral adverse effects of antidepressant doses of ketamine are not known. Similar to fluoxetine (Warren et al., 2011), ketamine may

alter the reward system such that there would be an enhanced vulnerability to abuse drugs particularly because ketamine causes changes in connections within the hippocampus (Akinfiresoye & Tizabi, 2013; Yang et al., 2012) and PFC (N. Li et al., 2010) and is already an abused drug (Maxwell, 2005; Trujillo et al., 2011). Therefore it is important to investigate whether exposure to ketamine during childhood increases the susceptibility to abuse drugs during adolescence by looking at its effects on reward.

Conditioned Place Preference as an Animal Model of Reward

Conditioned place preference (CPP) is the most common animal model of drug reward (Bardo & Bevins, 2000; Prus, James, & Rosecrans, 2009; Tzschentke, 1998). CPP is performed using a two- or three-compartment apparatus. A two-compartment apparatus has two compartments of equal size that are separated by a wall with a sliding door and distinguished by floor texture and wall color or pattern. The three-compartment apparatus has a neutral start chamber either adjacent to or between the two distinct compartments (Tzschentke, 1998). During conditioning, animals receive drug paired with one of the two distinct compartments. An unbiased design is when the drug is randomly assigned to one of the compartments and a biased design is when the drug is paired with the initially non-preferred compartment. Saline is always paired with the opposite compartment. On the test day animals receive free access to both compartments in a drug free state and their preference for the drug- versus saline-paired compartment is assessed. CPP is evident as an increase in preference for the drug-paired compartment and conditioned place aversion is evident as a decrease in preference for the drug-paired compartment (Tzschentke, 1998). This happens through Pavlovian conditioning.

There are many advantages to using the CPP paradigm to study drugs of abuse. First, there are some drugs that will produce CPP after a single pairing in the conditioning compartment. Another advantage of CPP is that the animals are tested in a drug-free state therefore their behavior is unimpaired by drug side effects. The CPP paradigm relies on the animals associating one compartment with the rewarding effects of the drugs and therefore seeking out those effects on the test day. CPP is also a relatively low stress procedure for the animal since it does not require any surgery. Even with these advantages for using the model of animal reward, there are also disadvantages. One of the biggest issues is novelty-seeking behavior. When an animal is first exposed to a new environment, it is novel and therefore they tend to explore more. This could be a confounding factor in a three-compartment CPP apparatus with the middle compartment as the start compartment. Since the animal will be conditioned in the two conditioning compartments, the animal will not have access to the start compartment. Therefore, during the test day, the middle start compartment will be novel and the animal may exhibit novelty-seeking behavior instead of drug-seeking behavior. Next, the animals could prefer one compartment over the other for no obvious reason. If an animal spends more time in one compartment compared to the other during the preconditioning assessment of the CPP paradigm, that animal is typically not used in the experiment. Lastly, this model has not been validated in human and non-human primates. However, humans do exhibit conditioned drug effects similar to what is seen with CPP (Bardo & Bevins, 2000; Tzschentke, 1998). Although not without limitations, the CPP paradigm is a validated model of drug reward suitable for examining the rewarding effects of nicotine after pretreatment with ketamine.

Nicotine

Prevalence

According to the Centers for Disease Control and Prevention, in the United States tobacco use is the leading preventable cause of disease, disability, and death. In 2010, approximately 60% of new smokers were under the age of 18, and 2.6 million Americans 12 – 17 years old reported using a tobacco product within the last month (Johnston, O'Malley, Miech, Bachman, & Schulenberg, 2014). If this rate continues, 5.6 million of today's Americans younger than 18 years old (or 1 in 13 of today's American youth) will die prematurely of a smoking related illness (Center for Disease Control and Prevention, 2010). Individuals who are suffering from a severe mental illness are also 5 times more likely to smoke daily (Substance Abuse and Mental Health Services Administration, 2012). Accordingly, those suffering from major depressive disorder are already at a heightened risk for developing tobacco dependence.

Effects of Nicotine Use During Adolescence

Preclinical studies suggest that the effects of nicotine in adolescents are qualitatively different from the effects seen in adults. For example, depressive-like behaviors were seen in adolescent rats following 1 week of nicotine cessation and this can happen after only a single dose of nicotine (Iñiguez et al., 2009). Also, adolescent rats have shown a greater amount of nicotine intake compared to adult rats in the self-administration paradigm (Natividad, Torres, Friedman, & O'Dell, 2013). This is due to adolescent rats experiencing more rewarding and less aversive effects of high doses of nicotine compared to adult rats (Belluzzi, Lee, Oliff, & Leslie, 2004; Kota, Martin,

Robinson, & Damaj, 2007; Shram, Funk, Li, & Le, 2006; Shram & Le, 2010; Torres, Tejeda, Natividad, & O'Dell, 2008; Vastola, Douglas, Varlinskaya, & Spear, 2002).

The differences in the rewarding and aversive effects between adolescent and adults rats are thought to be mediated through neurochemical mechanisms. Dopamine levels in the nucleus accumbens are lower during withdrawal in adult rats (Carboni, Bortone, Giua, & DiChiara, 2000; Hildebrand, Nomikos, Hertel, Schilstrom, & Svensson, 1998), however this does not appear to be the case in adolescent rats (Natividad, Tejeda, Torres, & O'Dell, 2010). Interestingly, there is an increase in VTA glutamate levels and a decrease in VTA GABA levels during withdrawal in adult rats while there is no change in glutamate and GABA levels in the VTA of adolescent rats during withdrawal (Natividad, Buczynski, Parsons, Torres, & O'Dell, 2012). Together this shows that there are age-related differences in the neurochemistry involved in processing reward and withdrawal making adolescents more susceptible to the rewarding effects of nicotine.

Sex Differences

Sex-dependent effects of nicotine are also evident. Similar to adolescent rats, female adult rats preferred higher nicotine doses that adult male rats find aversive (Torres, Natividad, Tejeda, Van Weelden, & O'Dell, 2009). Interestingly, estrogen is thought to play a role in the increased rewarding effects of nicotine in females compared to males (O'Dell & Torres, 2014; Torres et al., 2009). In both adolescent and adult female rats, estrogen enhances dopamine release in the nucleus accumbens thereby enhancing the rewarding effects of nicotine (O'Dell & Torres, 2014). What is interesting, however, is that adult female rats experience aversive effects of nicotine withdrawal similar to adult male rats while adolescent female rats do not, again similar to adolescent

male rats (O'Dell & Torres, 2014; Torres et al., 2009). Taken together, adolescent females seem to be the most susceptible to the rewarding effects of nicotine because they exhibit an increase in the rewarding effects of nicotine and a decrease in the aversive effects of withdrawal from nicotine.

Thesis

There is a need for a new type of antidepressant medication that is fast-acting and long-lasting since traditional antidepressant medications can take up to six weeks to have a therapeutic effect and must be taken every day (Mathew et al., 2012). Ketamine, a noncompetitive glutamate NMDA receptor antagonist, has been shown to produce fast-acting and long-lasting antidepressant effects in both clinical (Diazgranados et al., 2010; Machado-Vieira et al., 2009; Price et al., 2009; Zarate et al., 2006) and preclinical (Autry et al., 2011; Brachman et al., 2015; Garcia et al., 2008; Garcia et al., 2009; N. Li et al., 2011; S.-X. Li et al., 2014) studies. Unlike typical antidepressant medications, such as SSRIs which exert their effects on the presynaptic neuron by blocking reuptake of serotonin (Artigas, 2013), ketamine exerts its effects on the postsynaptic neuron by blocking the NMDA receptor and causing an increase in BDNF and mTOR (Akinfiresoye & Tizabi, 2013; N. Li et al., 2010; Yang et al., 2012). These changes result in an increase in dendritic branching (Martinowich et al., 2013) and synaptogenesis (Yang et al., 2012).

Approximately 8% of the pediatric population is diagnosed with major depressive disorder (Cheung et al., 2005). SSRIs such as fluoxetine are being prescribed to this population in order to treat their symptoms of depression (Emslie et al., 1997; Emslie et al., 2002). Prescribing any psychotropic medication during this age range may be problematic since there are still a great deal of development changes that are taking place

in the brain during this time. Specifically, there are changes in brain connectivity in the prefrontal cortex (Cousins & Goodyer, 2015) and limbic regions (S. L. Andersen & Teicher, 2009) as well as changes in the connectivity of dopamine neurons to these brain areas (Nestler & Carlezon, 2006). Also consider that ketamine is a drug of abuse and therefore is affecting the dopaminergic system (Maxwell, 2005; Trujillo et al., 2011). Therefore, the functional consequences of giving ketamine to children and adolescents needs to be examined because of the changes in connectivity that are occurring during these age ranges. This thesis examined the possibility of ketamine changing the reward circuits in children and adolescents that make them more susceptible to the rewarding effects of drugs of abuse, particularly nicotine since it is one of the most abused drugs during adolescence.

Hypothesis

The purpose of the current study was to examine whether childhood exposure to ketamine influences the rewarding effects of nicotine in male and female adolescent rats assessed using the conditioned place preference behavioral paradigm. Childhood-aged rats (PD 21-30) were given a daily injection of either 20 mg/kg ketamine or saline for 10 consecutive days. Two days later (PD 32-42), the rats underwent CPP for nicotine (0.0, 0.03, 0.1, 0.3, and 0.6 mg/kg). It is hypothesized that the rewarding effects of nicotine will be enhanced by exposure to ketamine during childhood in male and female rats. That is, rats will spend more time in the compartment associated with nicotine if they had received ketamine prior to training. However, male and female rats will likely show different behavioral responses to nicotine with males showing a preference for nicotine at lower doses and females showing a preference for nicotine at higher doses.

CHAPTER 2

METHOD

Subjects

A total of 93 male and 92 female Sprague-Dawley rats were used for this study. The rats were born and raised at California State University, Long Beach (CSULB) from rats purchased from Charles River Farms (Hollister, CA). Litters were culled to 10 pups on postnatal day (PD) 3 in order to ensure proper care from the dams. On PD 21, litters were weaned with same sex littermates (2-3 per cage) for the rest of the experiment. No more than one rat from each litter was assigned to an experimental group in order to control for litter effects (Zorrilla, 1997). The cages for the rats were standard ventilated polycarbonate cages (48×24×21 cm) with sani-chip bedding (P.J. Murphey San-Chips®, Murville, NJ). The rats were housed on a 12:12 light/dark cycle with lights on at 7:00 a.m. and had access to food and water *ad libitum*. The colony room was temperature controlled and maintained at 21-24°C. The rats were treated in accordance to the *Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research* (National Research Council, 2003) and with a protocol approved by the Institutional Animal Care and Use Committee at CSULB.

Apparatus

The conditioned place preference (CPP) apparatus was constructed of wood and consisted of three compartments (see Figure 1). The first compartment was a small start

area (17×15×45 cm) that was located on the exterior side between the two conditioning compartments forming a truncated T-shape. This compartment had solid gray walls and a smooth floor. The two conditioning compartments were of equal size (each 25×20×45 cm), however they were distinguishable by stripes on their walls and texture on the floor. One of the conditioning chambers had horizontal black and white stripped walls with a metal rod floor painted black (12.5 mm apart). The other conditioning chamber had vertical black and white striped walls with a sheet metal floor that had been perforated with holes (6.4 mm round perforations centered 9.5 mm apart) and painted black. The walls of the apparatus were painted with a primer and sealed with water-based high-glossy paint while the metal floors were painted with an oil-based flat paint. There was a sliding guillotine door between the start chamber and the two conditioning chambers. There was also a removable door between the two conditioning chambers, which allowed the rat access to both chambers as necessary. Beneath both conditioning chambers were trays with sani-chip bedding. The interior of the apparatus was cleaned with deionized water between each rat and was sanitized with a 50% ethanol solution at the end of each day. A 13W fluorescent lamp was located 68 cm above the chamber in order to ensure proper lighting. Also, a High-Definition digital camera was placed 58 cm above the center of the apparatus in order to record the sessions. In order to reduce external room noise, two sound machines (Brookstone Model #46709, Merrimack, NH) producing white noise at 10 db above background noise were used.

Drugs

Ketamine hydrochloride was obtained from Spectrum Chemicals (Gardena, CA) and (-)nicotine hydrogen tartrate salt was obtained from Sigma-Aldrich (St. Louis, MO).

Each drug was dissolved in 0.9% saline and the pH of nicotine was adjusted to 7.4 using NaOH. Ketamine was injected intraperitoneally (IP) and nicotine was injected subcutaneously (SC) at a volume of 1 ml/kg. Also, saline was injected IP at a volume of 1 ml/kg. Ketamine dosing was based on the salt form of the drug and nicotine dosing was based on the base form of the drug.

Procedures

Ketamine Pretreatment

Rats were randomly assigned to receive an injection of either saline or ketamine (20.0 mg/kg) once per day (between 9-11 am) from PD 21-30. The dose of ketamine is based on a previous study showing that 20 mg/kg ketamine is the most effective dose at producing antidepressant-like behaviors in adolescent rats (Parise et al., 2013).

Procedure

A timeline of the CPP procedure is depicted in Figure 2 and a graph showing the overview of the experimental design is represented in Figure 3. The CPP procedure consisted of a preconditioning test (PD 32), followed by 6 conditioning days (PD 35-40), and concluded with a postconditioning test (PD 42). The CPP procedure took place over 10 days with two rest days between the preconditioning test and first conditioning day, and one rest day between the last conditioning day and postconditioning test. Four days prior to beginning the CPP procedure (PD 28-31), rats were handled for two minutes per day to reduce anxiety/stress due to experimenter handling.

Preconditioning test. Each rat was placed into the start box of CPP apparatus before beginning the preconditioning test (PD 32). Next, the sliding guillotine door was opened to allow the rat to enter the conditioning chambers and explore both chambers

through a small doorway. If the rat did not enter the conditioning chambers after one minute of the door being opened, the rat was gently nudged into either conditioning chamber. Once the rat enters the chambers, the sliding guillotine door was closed and a timer was set for 15 minutes. Once the rat had 15 minutes to explore both conditioning chambers, the rat was removed from the CPP apparatus and placed back into its home cage.

Once the preconditioning test was completed, an experimenter blind to treatment conditions scored the video to determine the time spent on each of the two chambers of the CPP apparatus. The side that a rat spends the most time (i.e., greater than 50% of the total time) was designated as the preferred side while the side that the rat spends the least amount of time (i.e., less than 50% or the total) was designated the least preferred side. Rats that spent less than 315 seconds in the least preferred side were excluded from the experiment. A total of 15 rats were excluded from the experiment due to this strong initial preference. The horizontal compartment was the least preferred side for 60 rats [average time(s) ±SEM in vertical 496.17±4.07 and horizontal 403.83±4.07] and the vertical compartment was the least preferred side for 125 rats [average time(s) ±SEM in vertical 396.48±3.36 and horizontal 503.52±3.36]. Overall, rats spent an average of 428.81±4.33 seconds in the vertical compartment and 471.19±4.33 seconds in the horizontal compartment indicating the unbiased nature of the CPP apparatus. During conditioning phase, rats were given nicotine and confined to the least preferred side of the apparatus for 30 minutes (biased design). A biased design was used in order to enhance the sensitivity for detecting a shift in preference (preference for the nicotine-

paired side). In order to monitor locomotor activity, the number of crossovers between compartments was measured.

Conditioning phase. Two days following the preconditioning test, the rats underwent the conditioning phase (PD 35-40). During conditioning phase, rats were given nicotine (0.0, 0.03, 0.1, 0.3, or 0.6 mg/kg) and confined to the least preferred side of the apparatus for 30 min. These doses were chosen based on a previous study examining the effects of nicotine in female adolescent rats since female adolescent rats experience an increase in the rewarding effects of nicotine (Torres, Natividad, Tejeda, Van Weelden, & O'Dell, 2009). The next day, rats were given an injection of saline and confined to the preferred side of the apparatus for 30 minutes. The order of placement in the preferred and least preferred chamber was counterbalanced across rats, such that half were placed in the preferred side first and the other half in the least preferred first. This two-day cycle was then repeated two more times for a total of six conditioning days. The conditioning sessions lasted for 30 minutes because the half-life of nicotine in the brain is 50 minutes therefore the rats should not be experiencing any withdrawal symptoms during the sessions (Sastry, Chance, Singh, Horn, & Janson, 1995).

Postconditioning test. The postconditioning test occurred one day after the last conditioning day (PD 42). The protocol for this test was identical to that of the preconditioning test. Briefly, rats were placed in the start box and allowed 15 minute access to both chambers of the CPP apparatus. Once the behavioral testing was complete, the rats were euthanized by CO_2 followed by decapitation.

Statistical Analysis

Body Weight

To examine whether pretreatment with ketamine causes a change in body weight for male and female rats, body weight (g) was analyzed using a 2 × 10 mixed-factor analysis of variance (ANOVA). Ketamine pretreatment dose (0.0 or 20.0 mg/kg) was the between-subjects factor and pretreatment day (PD 21-30) was the within-subjects factor. Males and females were analyzed separately due to expected sex differences in weight gain (Spear, 2000).

CPP

To test the hypothesis that the rewarding effects of nicotine was enhanced by early exposure to ketamine, a preference score was calculated. The preference score was determined by taking the time spent in the drug-paired compartment during postconditioning minus the time spent in the same compartment during preconditioning. This preference score was then used to compare each nicotine group to the saline group, given that CPP is defined as a significantly higher preference score compared to saline controls (i.e., rats that were conditioned with saline on both sides of the CPP chamber). Specifically, a 2 × 5 ANOVA was conducted on the preference scores with pretreatment group (0.0 or 20.0 mg/kg ketamine) and dose of nicotine (0.0, 0.03, 0.1, 0.3, and 0.6 mg/kg) as the independent variables. Crossover data (the number of times that the rat crosses between each compartment) was analyzed in order to ensure equal amount of exploration across groups. Specifically, separate 2 × 5 ANOVAs were used to analyze crossover data on the pre- and post-conditioning days. Similar to how the CPP data was analyzed, the factors were pretreatment and nicotine group. Male and female rats were analyzed separately due to the expected sex differences in drug sensitivity during

adolescence in rats (O'Dell & Torres, 2014). Significant main effects and interactions were further analyzed using Dunnett's test. All results were assessed as significant when the alpha level was less than 0.05.

CHAPTER 3

RESULTS

Body Weight

Body weight (g) data for male and female rats across pretreatment days is depicted in Figure 4. Pretreatment with ketamine did not significantly alter the body weight of male and female rats compared to those pretreated with saline. However, there was a significant weight gain across the pretreatment period for males [main effect, $F(9,819) = 4754.39$, $p < .001$] and females [main effect, $F(9,810) = 2600.02$, $p < .001$] as expected due to normal maturation.

CPP

Male and female rats had significantly different nicotine preference scores [main effect, $F(1,165) = 3.81$, $p = .05$]. This confirms the hypothesis to analyze male and female data separately.

Males

The nicotine (0.0, 0.03, 0.1, 0.3, and 0.6 mg/kg) preference score during the postconditioning test for male adolescent rats (PD 42) pretreated with ketamine (0.0 and 20.0 mg/kg) during PD 21-30 is shown in Figure 5. Male rats treated with 0.1 and 0.3 mg/kg nicotine had a significantly higher preference score compared to male rats treated with saline [main effect, $F(4,83) = 4.05$, $p = .005$ and post-hoc analysis] confirming that nicotine does result in a conditioned place preference. Ketamine pretreatment, however,

did not affect the preference scores of male rats. Planned comparisons examining the preference score between the two ketamine pretreatment groups at each nicotine dose were also not significant. When considered together, ketamine did not have an effect on the rewarding properties of nicotine in male rats.

Females

The nicotine (0.0, 0.03, 0.1, 0.3, and 0.6 mg/kg) preference score during the postconditioning test for female adolescent rats (PD 42) pretreated with ketamine (0.0 and 20.0 mg/kg) during PD 21-30 is shown in Figure 6. Female rats exhibited an increase in preference score when given 0.1, 0.3, and 0.6 mg/kg nicotine regardless of pretreatment group [main effect, $F(4,82) = 2.956$, $p = .025$, and post-hoc analysis] again confirming that nicotine results in a conditioned place preference. However, ketamine pretreatment did not affect the preference scores of female rats. Planned comparisons examining the preference score between the two ketamine pretreatment groups at each nicotine dose were also not significant. Similar to the male rats, the rewarding effects of nicotine were not enhanced after pretreatment with ketamine in female rats.

Crossovers

The crossover data during the preconditioning day is presented in Table 1, whereas the crossover data during the postconditioning day is presented in Table 2. Crossover data was analyzed to examine the number of times the rats went between the vertical and horizontal side of the CPP apparatus as a measure of overall locomotor activity. For both male and female rats, there were no group differences in crossovers on either the pre- or post-conditioning days.

CHAPTER 4

DISCUSSION

The purpose of this thesis was to examine the effects of early exposure to ketamine on the rewarding effects of nicotine during adolescence in male and female rats. This is important because ketamine is being examined as a potential antidepressant medication in preclinical (Autry et al., 2011; Brachman et al., 2015; Garcia et al., 2008; Garcia et al., 2009; N. Li et al., 2011; S.-X. Li et al., 2014; Parise et al., 2013) and clinical (Diazgranados et al., 2010; Machado-Vieira et al., 2009; Papolos et al., 2013; Price et al., 2009; Zarate et al., 2006) studies at low doses even though ketamine has abuse potential (Maxwell, 2005; Trujillo et al., 2011). We hypothesized that pretreatment with ketamine would enhance the rewarding properties of nicotine during adolescence in both male and female rats. Contrary to our expectations, early exposure to ketamine did not facilitate the rewarding effects of nicotine during adolescence. This finding is surprising given that nicotine reward is mediated via the dopaminergic system (Matta et al., 2006) and that ketamine has an impact on the dopaminergic system (Tan, Lam, Wai, Yu, & Yew, 2012), as well as the evidence demonstrating that the dopaminergic system is still undergoing developmental changes well into adolescence (Nestler & Carlezon, 2006). The present findings, however, do corroborate previous research demonstrating sex differences in response to nicotine reward (O'Dell & Torres, 2014; Torres et al., 2009).

Administration of nicotine in male adolescent rats resulted in robust nicotine-induced CPP that was dose dependent. Specifically, male adolescent rats exhibited significant CPP when administered the 0.1 and 0.3 mg/kg nicotine doses (see Figure 5). This was evident as a significant increase in preference score compared to the male rats that were given saline in both compartments of the CPP apparatus. As expected, the male rats that received the lowest dose (0.03 mg/kg) of nicotine did not have an increase in preference score compared to the saline controls. Male rats treated with the highest dose (0.6 mg/kg) of nicotine did not exhibit a significant change in preference score compared to the saline controls, likely indicative of a shift towards the aversive effects of nicotine (Torres et al., 2009).

Following administration of nicotine, female adolescent rats also exhibited dose dependent nicotine-induced CPP. Specifically, female adolescent rats exhibited significant CPP when administered 0.1, 0.3, and 0.6 mg/kg nicotine doses (see Figure 6). This preference was evident as an increase in preference score compared to the female rats treated with saline in both compartments of the CPP apparatus. Similar to the male adolescent rats, the female adolescent rats did not have an increase in preference score at the lowest dose (0.03 mg/kg) of nicotine compared to the saline controls. Again, this is a very low dose of nicotine that does not produce rewarding effects. Interestingly, the female adolescent rats treated with the highest dose (0.6 mg/kg) of nicotine did exhibit a significant change in preference score whereas the male adolescent rats did not. This is consistent with previous studies showing that female rats find higher doses rewarding and are less likely to experience the aversive effects of higher doses of nicotine compared to male rats (O'Dell & Torres, 2014; Torres et al., 2009).

Pretreatment with ketamine did not influence the rewarding effects of nicotine during adolescence in either males or females. One possible explanation for this finding may be the dose of ketamine that was used. Specifically, ketamine may not have had an effect on the rewarding effects of nicotine because the dose was too low. One study suggests that a dose of 20 mg/kg ketamine may not be high enough to increase dopamine transmission (Tan et al., 2012), therefore a subanesthetic therapeutic dose of ketamine may not cause an increase in dopaminergic transmission and would not lead to an increased susceptibility to drug reward. Other studies have used doses ranging from 5.0 mg/kg to 40 mg/kg in order to get a therapeutic effect therefore the dose that we chose was within the range of therapeutic doses (Akinfiresoye & Tizabi, 2013, Autry et al., 2011; Brachman et al., 2015; Fukumoto, Iijima, & Chaki, 2013; Garcia et al., 2008; Garcia et al., 2009; N. Li et al., 2010; N. Li et al., 2011; Parise et al., 2013; Xu et al., 2013; Yang, Hu, Zhou, Zhang, & Yang, 2012; Zhang et al., 2013). As previously discussed, ketamine is a noncompetitive glutamate NMDA receptor antagonist which exerts it's antidepressant effects by causing changes to BDNF and mTOR (Akinfiresoye & Tizabi, 2013; Garcia et al., 2008; N. Li et al., 2010; Yang et al., 2012). It is possible that therapeutic doses only influence the effects of antidepressant properties and not those related to nicotine reward. To determine if this is the case, additional doses of ketamine need to be examined in order to determine the lack of effect on drug reward after previous ketamine pretreatment. Moreover, previous studies have mostly been conducted in adult rats. It is also possible that higher doses of ketamine may be needed when administering ketamine in juvenile rats, as in the present study. Consistent with this idea, Parise et al. (2013) gave 20 mg/kg ketamine twice per day to adolescent rats in order to

achieve a therapeutic effect (measured using FST), likely due to the fact that adolescents have an increased rate of metabolism compared to adult rats (Spear, 2000).

Analysis of body weight data revealed no changes between preadolescent rats pretreated with ketamine versus saline. Interestingly, the only study to date that has examined ketamine treatment during adolescence did find differences in body weight. The rats pretreated with ketamine had lower body weights and a decrease in food intake during adolescence and adulthood compared to the rats pretreated with saline (Parise et al., 2013). One major difference between the present study and this one is that we gave the rats 20 mg/kg ketamine once per day versus twice per day. Moreover, the age of exposure to ketamine also differed. In the present study, ketamine was administered between PD 21-30, whereas the previous study gave ketamine between PD 35-49 (Parise et al., 2013). Adolescence is the period where rats have the greatest amount of food intake relative to body weight of any age period during the life span as well as an increase in metabolic activity (Spear, 2000). Aside from the differences in the amount of ketamine given, it is also possible that body weight differences were not found in this study due to the younger age at which the rats received ketamine.

Crossover data was analyzed in order to examine any changes in explorative activity (a rough indication of locomotor activity) caused by pretreatment with ketamine or treatment with nicotine. The results indicated there was no difference in exploration during the preconditioning and postcondtioning tests across all groups. Previous studies have shown that ketamine increases locomotor activity in adolescent (Parise et al., 2013) and adult (Trujillo, Zamora, & Warmoth, 2008) rats while under the influence of the

drug, but our present findings suggest that this increase in ketamine-induced activity did not influence locomotor activity in a drug-free state (i.e., during test days).

Study Limitations and Strengths

CPP measures drug reward but not abuse potential. Therefore one limitation of this research design is that a more sensitive measure of vulnerability to abuse and addiction may be needed. One such approach would be to measure self-administration of drugs. Self-administration involves the surgical implantation of an intravenous catheter into the animal for drug delivery. The animal is presented with a lever that allows for drug delivery through the catheter as the animal desires (Gardner, 2000). Thus, it is possible that although we did not see a change in the rewarding effects of nicotine, it is still possible that we may see an effect on self-administration of nicotine after previous administration of ketamine during the juvenile period. Similar effects have been reported previously. For instance, Crawford and colleagues found no enhanced rewarding effects of cocaine using CPP, whereas they did see an increase in the reinforcing effects of cocaine using self-administration when rats were previously pretreated with methylphenidate (Crawford et al., 2011). As such, an examination of nicotine self-administration after juvenile exposure to ketamine is warranted.

CPP measures drug reward by testing rats in a drug-free state (Bardo & Bevins, 2000). Therefore one strength of this research design is that there is nothing, outside of the context, that is influencing the rat. The rat is then able to associate the environmental context with the rat's experience of the drug without the drug being present. This strengthens the association between the environmental context and the drug. Next, CPP is able to test for both preference and aversion. As stated previously, when a rat has an

increase in time spent in the nicotine-paired side of the CPP apparatus during postconditioning compared to preconditioning, the rat exhibits preference for the drug, and on the opposite spectrum, when the rat demonstrates a decrease in time spent on the nicotine-paired side of the CPP apparatus during postconditioning compared to preconditioning, the rat exhibits aversion to the drug. Importantly, drug aversion effects are difficult to study in self-administration paradigms. These changes between reward and aversion are particularly useful when examining nicotine because nicotine has been shown to produce both of these in adolescent rats (O'Dell & Torres, 2014; Torres et al., 2009).

Lastly, the pretreatment with ketamine was conducted over 10 days. Clinical use of antidepressant medications may last a lifetime depending upon the severity of the depression (March et al., 2007). Therefore a more clinically relevant examination of ketamine use would last throughout development and may even take place during the same time as when rats receive nicotine. This approach may also reveal drug-drug interactions, such as a synergistic effect between ketamine and nicotine. Further research to examine this relationship should be conducted.

Study Implications

As previously stated, it takes monoamergic antidepressant medications approximately six weeks to have a therapeutic effect. Research has already shown that ketamine has a therapeutic effect within hours (Cryan & O'Leary, 2010; Diazgranados et al., 2010; Harihar, Dasari, Srinivas, 2013; Zarate et al., 2006) and in patients with treatment-resistant depression (Diazgranados et al., 2010; Mathew et al., 2012; Papolos et al., 2013; Zarate et al., 2006). This study adds to the literature to further the development

of a new generation of antidepressant medications with a faster onset and longer-lasting therapeutic effects because it shows that there is no increase in the vulnerability to nicotine reward later in life. This was the first study to examine the effects of early exposure to ketamine on the rewarding properties of drugs of abuse during adolescence. Similarly, the efficacy and safety of antidepressant medications in children is underrepresented. Most research in this area focuses on adults and adolescents. Since antidepressant medication is being prescribed at younger and younger ages, it is important to know how it is affecting the developing brain. The brain is plastic during childhood (Cousins & Goodyer, 2015) and antidepressant medications could be mediating neuroadaptations thus increasing the susceptibility to drugs of abuse -- including tobacco products -- and possibly lead to an increase in dependence. Even though this study found that early exposure to ketamine does not increase the susceptibility to abuse drugs later in development, it cannot be said that ketamine is safe and effective for use in a pediatric population. More research needs to be conducted using varying doses of ketamine, alternative age ranges, and a broad range of drugs of abuse before ketamine can be deemed as safe for use in children.

Future Directions

Future research should examine early exposure to ketamine on self-administration of nicotine in order to examine whether the abuse potential of nicotine is influenced by pretreatment with ketamine. Crawford et al. (2011) found varying effects across CPP and self-administration on the rewarding effects of cocaine. Cocaine did not produce CPP however the rats did self-administer cocaine showing that different methods of measuring drug reward can produce different results (Crawford et al., 2011). Similarly, it would be

beneficial to examine early exposure to ketamine on the rewarding properties of nicotine across the phases of the addiction cycle (Koob & Volkow, 2010). One study has shown differences in cocaine self-administration dependent upon the length of abstinence from cocaine (Pentkowski et al., 2014).

It would also be advantageous to examine whether the effects of early ketamine exposure persists into adulthood. Since ketamine is acting through intracellular mechanisms (Akinfiresoye & Tizabi, 2013; Garcia et al., 2008; N. Li et al., 2010; Yang et al., 2012) it would be feasible to assume that these changes would be long-lasting. Lastly, since female adolescent rats have an increased rewarding effect of nicotine (Torres et al., 2009), it would be beneficial to test higher doses of nicotine in order to create a more complete dose response curve.

Conclusion

This thesis is the first study to examine the effects of preadolescent (PD 21-30) exposure to ketamine on the rewarding effects of nicotine during adolescence (PD 32-42). The results confirmed that nicotine results in conditioned place preferences in both male and female adolescent rats. Pretreatment with ketamine however did not influence the rewarding effects of nicotine. In addition, sex differences in nicotine reward were evident. Male adolescent rats preferred lower doses of nicotine compared to female adolescent rats. This is consistent with previous literature (O'Dell & Torres, 2014; Torres et al., 2009; Torres et al., 2013). Future studies need to be conducted in order to fully assess the impact that early exposure to ketamine may have on the rewarding effects of drugs of abuse later in life.

APPENDICES

APPENDIX A

TABLE OF PRECONDITIONING CROSSOVER DATA

TABLE 1. Mean and Standard Deviation (SD) for Preconditioning Crossovers

Sex	Ketamine	Nicotine	Mean	SD
Male	0.0	0.0	44.67	13.87
		0.03	49.78	10.86
		0.1	47.90	9.37
		0.3	52.89	14.79
		0.6	48.78	10.84
	20.0	0.0	42.89	12.17
		0.03	41.22	12.81
		0.1	46.40	14.89
		0.3	49.10	14.50
		0.6	48.33	7.52
Female	0.0	0.0	60.22	15.57
		0.03	43.75	14.95
		0.1	54.20	15.42
		0.3	54.30	13.22
		0.6	51.11	13.91
	20.0	0.0	43.38	6.63
		0.03	55.00	13.10
		0.1	48.40	10.21
		0.3	42.30	18.46
		0.6	49.00	14.41

Male ($N = 93$) and female ($N = 92$) rats were pretreated with 0.0, or 20.0 mg/kg of ketamine during preadolescence (PD 21-30) and conditioned with 0.0, 0.03, 0.1, 0.3, or 0.6 mg/kg nicotine during adolescence (PD 32-42). Crossover data examines explorative behavior similar to locomotor activity.

APPENDIX B

TABLE OF POSTCONDITIONING CROSSOVER DATA

TABLE 2. Mean and Standard Deviation (SD) for Postconditioning Crossovers

Sex	Ketamine	Nicotine	Mean	SD
Male	0.0	0.0	43.33	17.13
		0.03	55.44	14.10
		0.1	46.40	22.65
		0.3	46.44	14.77
		0.6	52.78	14.39
	20.0	0.0	39.89	13.37
		0.03	44.67	21.20
		0.1	49.90	18.00
		0.3	54.60	12.81
		0.6	54.33	12.45
Female	0.0	0.0	62.67	11.89
		0.03	52.13	20.43
		0.1	53.20	15.04
		0.3	60.90	18.80
		0.6	53.22	20.15
	20.0	0.0	41.25	13.85
		0.03	48.13	12.51
		0.1	48.90	13.28
		0.3	55.40	18.68
		0.6	52.40	21.49

Male ($N = 93$) and female ($N = 92$) rats were pretreated with 0.0, or 20.0 mg/kg ketamine during preadolescence (PD 21-30) and conditioned with 0.0, 0.03, 0.1, 0.3, or 0.6 mg/kg nicotine during adolescence (PD 32-42). Crossover data examines explorative behavior similar to locomotor activity.

APPENDIX C

PHOTOGRAPH OF CPP APPARATUS

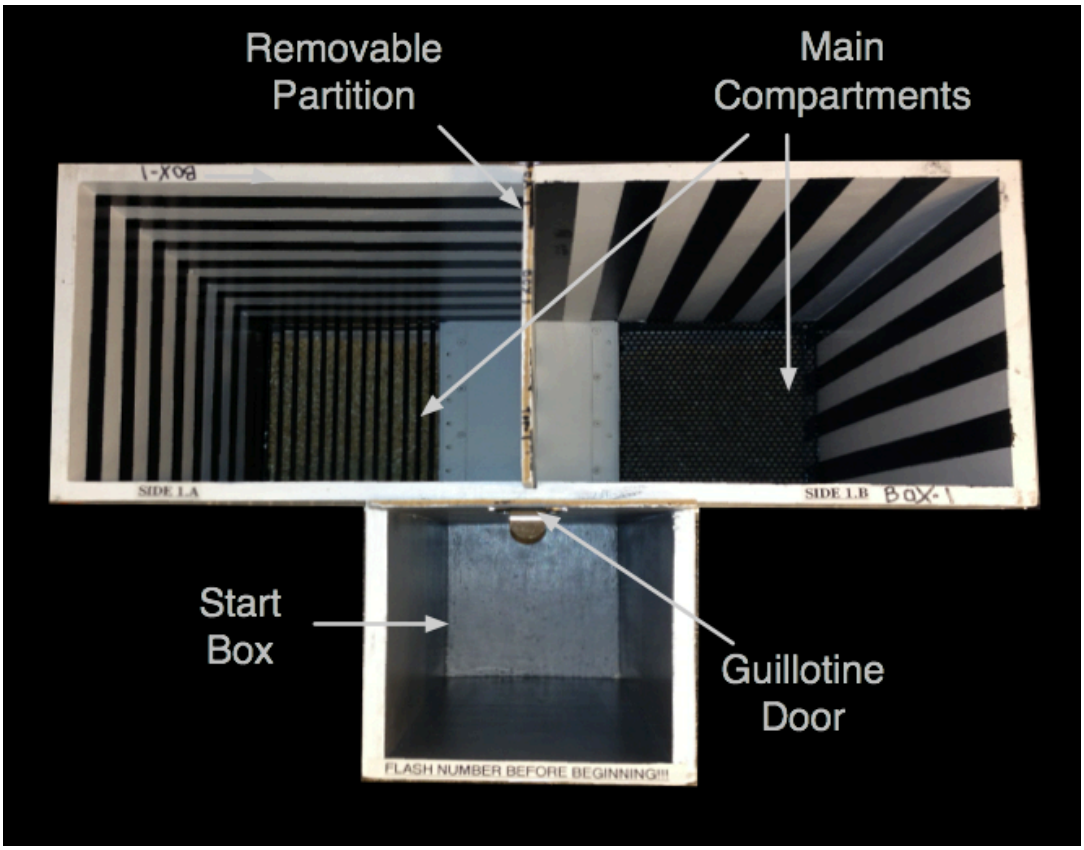

FIGURE 1. Photograph of the CPP apparatus showing the start box and two compartments. The sliding door was raised during pre- and post-conditioning sessions in order to allow the rat access to the two compartments from the start box and close immediately following entrance to one of the two compartments. A removable partition with an opening was inserted during pre- and post-conditioning sessions in order to give the rat access to both compartments. During conditioning sessions, a solid removable partition was inserted in order to confine the rat to only one side.

APPENDIX D

GRAPH OF CPP TIMELINE

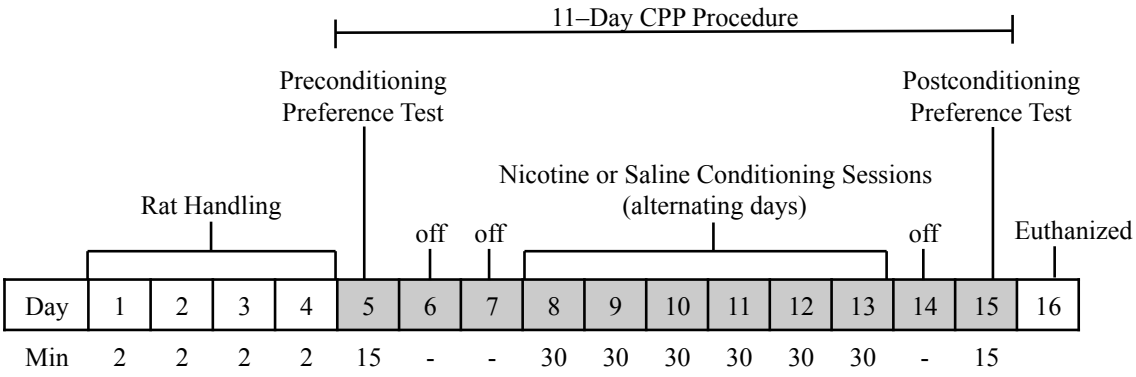

FIGURE 2. This graph represents the timeline and duration of the CPP procedure, including rat handling and euthanasia. Rats underwent handling during PD 28-31. The CPP procedure took place during PD 32-42 and the rats were euthanized on PD 43.

APPENDIX E

GRAPH OF EXPERIMENTAL GROUPS

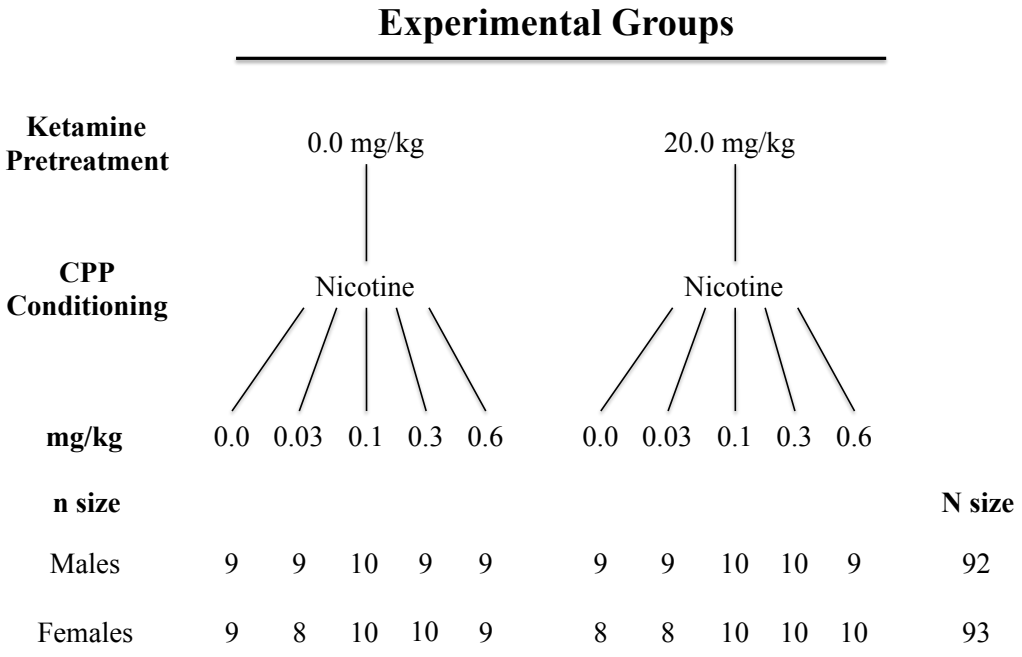

FIGURE 3. Graph representing the different experimental groups and the number of rats per group. The rats were given ketamine pretreatment from PD 21-30 and underwent CPP conditioning from PD 32-42.

APPENDIX F

GRAPH OF WEIGHT DATA FOR MALE AND FEMALE RATS

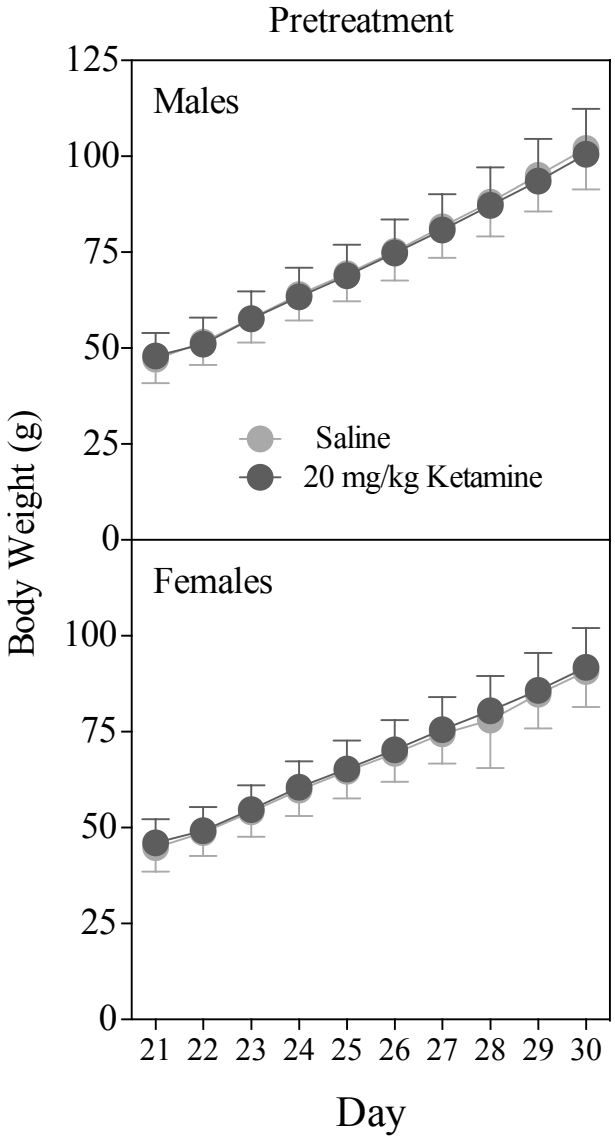

FIGURE 4. Mean weight of male ($N = 93$) and female ($N = 92$) rats pretreated with 0.0 or 20.0 mg/kg ketamine during preadolescence (PD 21-30). Error bars represent standard error of the mean (SEM).

APPENDIX G

GRAPH OF RESULTS FOR MALE RATS

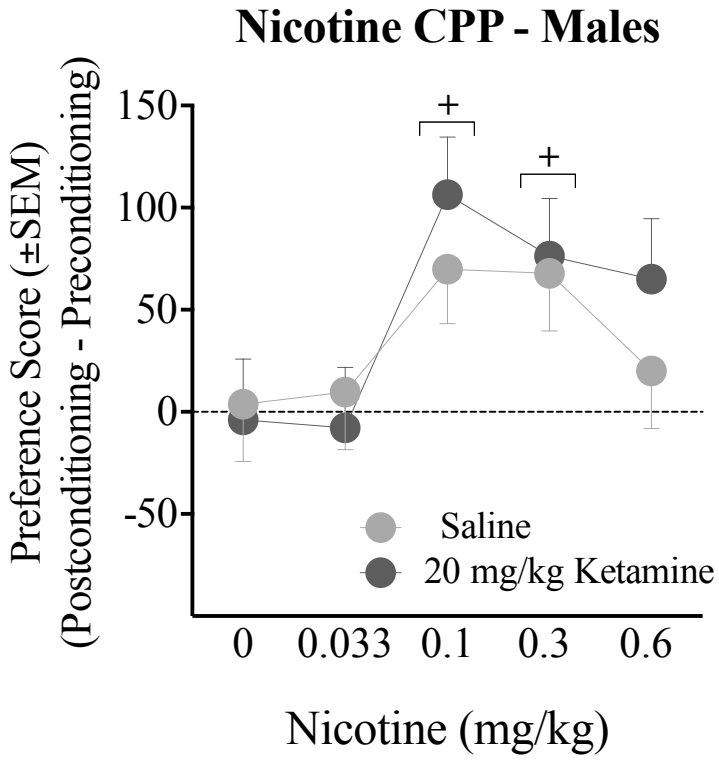

FIGURE 5. The preference score for male rats pretreated with ketamine (20.0 mg/kg) or saline and conditioned with 0.0, 0.03, 0.1, 0.3, or 0.6 mg/kg nicotine. + Represents a significant difference in preference score between the male rats conditioned with 0.1 and 0.3 mg/kg nicotine compared to the male rats conditioned with 0.0 mg/kg nicotine regardless of pretreatment group. Error bars represent SEM.

APPENDIX H

GRAPH OF RESULTS FOR FEMALE RATS

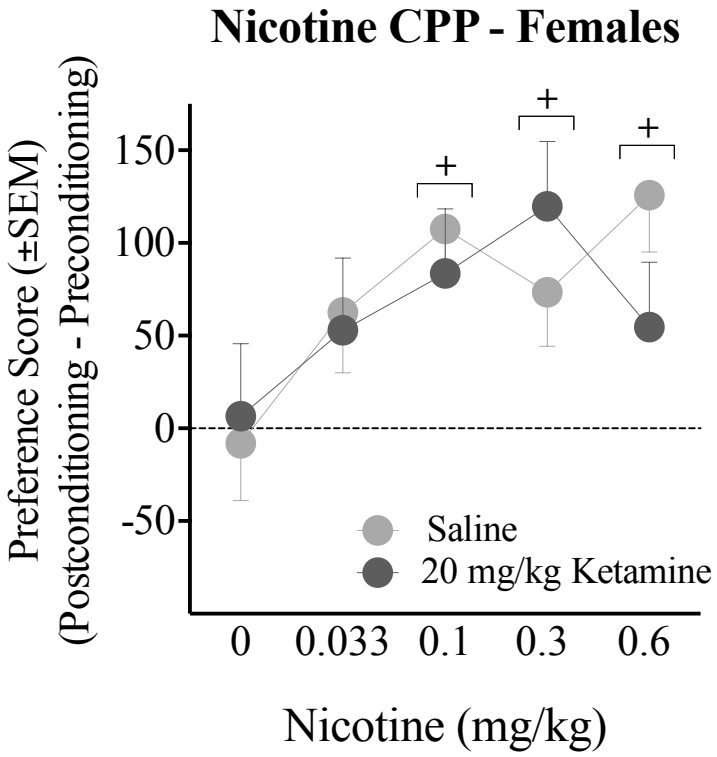

FIGURE 6. The preference score for female rats pretreated with ketamine (20.0 mg/kg) or saline and conditioned with 0.0, 0.03, 0.1, 0.3, or 0.6 mg/kg nicotine. + Represents a significant difference in preference score between the female rats conditioned with 0.1, 0.3, and 0.6 mg/kg nicotine compared to the female rats conditioned with 0.0 mg/kg nicotine regardless of pretreatment group. Error bars represent SEM.

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