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DIFFERENTIAL BEHAVIORAL EFFECTS OF KETAMINE BETWEEN
ADOLESCENT AND ADULT SPRAGUE-DAWLEY RATS

A thesis submitted in partial fulfillment of the requirements for the degree of Master
of Science at Virginia Commonwealth University

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Abstract

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The dissociative anesthetic ketamine has been subject to growing abuse worldwide, particularly in adolescents. This project compared the effects of ketamine in conditioned place preference and intravenous self-administration in adolescent (PND 28-50) and adult (>PND70) Sprague-Dawley rats. Cocaine served as a positive control. In CPP, adolescents demonstrated preferences for ketamine, while adults developed an aversion. In the self-administration procedure, adults acquired the behavior more rapidly, but there was no difference in the percentage of subjects reaching acquisition nor in responding under a progressive ratio schedule for either drug. The CPP results suggest that adolescents have a greater sensitivity to the rewarding and tolerance to the aversive effects of ketamine. The divergent results for ketamine in the adults may reflect differences in the two procedures. However, because cocaine produced only hedonic effects in both age groups, it also suggests unique characteristics of ketamine and differences in its effects based on age.

Differential behavioral effects of ketamine between adolescent and adult Sprague-Dawley rats

Drug use in adolescence

Adolescence is a time period often characterized by novelty and sensation-seeking and impulsive behavior. Studies in human adolescents have shown significantly increased levels of impulsivity peaking between the ages of 12-16, a behavior that on average does not seem to abate until the early 20s (Harden, 2011). Behaviors such as increased novelty-seeking, impulsivity, and risk-taking have been associated with increased potential for drug-use and drug-abuse in adolescents (Wills, 1998; Spear L. , 2000). Adolescent drug use is a common and increasing problem worldwide. According to the *Monitoring the Future* report in 2012, 49% of adolescents in grades 8 through 12 had reported some use of an illicit substance during their lifetime, an increase from statistics reported in 2009 (Johnston, O'Malley, Bachman, & Schulenberg, 2012). There is evidence across many classes of drugs and illicit substances that use during adolescence can lead to permanent neurochemical changes in the reward pathways of the brain, leading these adolescent users to become more susceptible to drug abuse and addiction as adults (Casey, 2008; Wills, 1998). Developmental changes in the brain during adolescence have been reviewed from both psychological and neurobiological viewpoints (Casey, 2008; Wahlstrom, 2010; Sturman & Moghaddam, 2011). Research has consistently shown that physiological development and neurochemical changes in the brain during adolescence can produce highly impulsive and unpredictable behaviors. These notable increases in impulsive and novelty-seeking behaviors can be partially attributed to substantial changes in the neuronal structure of the prefrontal cortex

during adolescence, an area that has been regarded as controlling executive functions such as decision making, impulse control, and inhibition (Miller, 2001).

Adolescent animal models

Using rats as a model to humans requires some interpretation of rodent development and drug-induced behaviors. In rodents and humans, psychostimulants activate dopaminergic neurotransmission and is homologous across species in many respects, although the behaviors exhibited after activation often vary (Wise, 1987). Similar behaviors can be attributed to the dynamic cellular and anatomic changes that take place in the brain during the adolescent period in both species. During human adolescence many different brain structures undergo changes in neuronal myelination, pruning, hyperactivity and hypoactivity, and these changes are mirrored by changes in the adolescent rodent. Changes in the prefrontal cortex, amygdala, and striatum which mediate decision-making and risk-taking behavior are also implicated in analogous rodent behaviors such as exploration and novelty-seeking (Kelley & Schochet, 2004). These aforementioned changes indicate that results from studies using adult subjects should not be readily generalized to the drug-consuming behavior of adolescents (Smith R. , 2003). Additionally, electrophysiology and neuroimaging data have demonstrated that adolescent rats exhibit reduced neuronal coordination and processing efficiency when compared to adult rats. These reductions stem from incomplete myelination and interneuron connectivity, manifesting as lack of impulse control and increased sensitivity to reward (Sturman & Moghaddam, 2011).

Pertinent to this research is the determination of adolescence in the rat model, a topic that has several subtle distinctions depending on the hypothesis in question. In rats the adolescent period is often considered the period after weaning, PND21, up to PND60, which is considered young adulthood. Some studies further subdivide adolescence into early adolescence (PND21-34), which is considered prepubescence, periadolescence (PND34-46), and late adolescence (PND46-59) (Tirelli, 2003). However, PND21 is often too early for many of the hormonal and endocrine changes that heavily influence neurochemistry, and there is evidence that suggests that rats between the ages of PND30-40 tend to have significantly different metabolic and neurochemical profiles than rats older and younger. Because rats between the ages of PND30-40 exhibit increased activity, exploratory behavior, and sensitivities to drugs of abuse, as well as a decreased responsiveness to aversive effects of drugs (Spear & Brake, 1983), this was selected as the age to begin testing of adolescent subjects in the present studies. While controlled studies between adolescents and adult humans may face many logistical and ethical obstacles, there is a considerable amount of literature on developmental differences between the two age groups in spontaneous behavior (Meaney, 1981; Panksepp, 1981), pharmacological profiles (Spear L. P., 1981), and responses to stress (Adriani, 2000; Choi, 1997; Choi, 1996) in the rat.

Ketamine

The focus of this thesis will be on ketamine, an NMDA receptor antagonist that functions as a channel-blocking agent. Ketamine was developed as an anesthetic in the early 1960s at Parke-Davis Laboratories in Detroit, Michigan. It is a

derivative of phencyclidine (PCP), developed for its shorter duration of action in comparison with comparable effectiveness as an anesthetic. Both compounds are classified as dissociative anesthetics due to their cortical dissociation, defined as their similar abilities to induce profound analgesia, sedation, amnesia, and immobilization while maintaining stable respiratory and cardiopulmonary function (Cromhout, 2003). Ketamine has a broad therapeutic index and the risk of fatality from overdose of ketamine is low, with the lethal dose being 3 to 5 times the therapeutic dose in animals (Cohen, Chan, Way, & Trevor, 1973; McCarthy, Chen, Kaump, & Ensor, 1965; McGrath, Lee, & Campbell, 1984). Ketamine was approved by the Food and Drug Administration soon after its synthesis for use as an anesthetic. This expedited approval was to aid soldiers in the Vietnam War who would benefit in the field from an anesthetic with shorter duration of action and a large margin of safety. The clinical use and availability of ketamine also meant a change for dissociative anesthetic drug abusers as PCP was being produced less and more often the source of PCP was through synthesis by illicit drug dealers (Domino, 2010). Ketamine has been subject to misuse and abuse since it was initially synthesized, however a dramatic increase in the prevalence of its illicit use during the 1990s prompted ketamine's reclassification as a Schedule III substance under the United States Controlled Substances Act in August 1999. While the prevalence of abuse nationwide of "club drugs" such as MDMA (Ecstasy), and hallucinogens such as LSD and PCP has declined, the use of ketamine, gammahydroxybutyrate (GBH), and flunitrazepam (Rohypnol) in the United States has remained steady since 2010. Worldwide abuse of ketamine has drastically

increased causing the drug to be rescheduled in the United Kingdom (2006), Canada (2005), and in the city of Hong Kong it has become the most common drug of abuse for users under the age of 21 with increasing rates of abuse since 2005 (Ng, Tse, Lau, & Ng, 2010). The United Kingdom reported over 200,000 users of ketamine in 2011, with at least 50% of users being between the ages of 16-24 (Home Office Statistics, 2012). Ketamine is abused for its purported sensations of euphoria, hallucinogenic properties, its ability to produce a trance-like dissociation. Ketamine has also been labeled as a potential “date rape” drug due to its amnesia-inducing effects. The second volume of the *Monitoring the Future* study that surveys adults ages 19-50 reported ketamine usage among young adults aged 19-20 were three-fold that of all other age groups (Johnston, O'Malley, Bachman, & Schulenberg, 2012). Assessing the prevalence among school-age children, 1.2% and 1.5% of 10th and 12th graders respectively, reported illicit usage of ketamine within the past year (Johnston, O'Malley, Bachman, & Schulenberg, 2012). While these rates of annual abuse are low relative to alcohol, marijuana, and nicotine, they are comparable to heroin, crack cocaine, PCP, steroids, crystal meth, and bath salts in these age groups. In comparison to many other illicit substances such as nicotine and tobacco, abuse has remained relatively low in part due to ketamine’s limited availability as a therapeutic agent, being primarily restricted to use in emergency rooms and veterinary clinics. However, as research grows in favor of ketamine as a possible treatment for depression, (Zarate, et al.; Preskorn, et al.), and chronic pain (Niesters, Martini, & Dahan; Bredlau, et al.), circulation and abuse of ketamine may increase as this drug becomes more widely used. As mentioned above, ketamine

has a large therapeutic index, with the most common physiological side effects being self-resolving tachycardia and hypertensions (Kalsi, Wood, & Dargan, 2011). However, this does not mean the drug is free from acute and chronic toxicities. The primary risk of acute usage of ketamine is physical harm due to drug-induced aggression or agitation, or as a by-product of hallucinations and dissociative effects. This is a dangerous combination as adolescents are characterized by a lack of fully developed impulse control and greater risk-taking behaviors than adults. (Casey, Getz, & Galvan; Spear, 2000). Chronic or frequent usage of ketamine has been linked to various systemic pathologies most commonly gastrointestinal irritation and urological toxicity such as ulcerative cystitis. The correlation between ketamine abuse and ulcerative cystitis is becoming increasingly strong. While cessation of ketamine use and treatments are available to abate symptoms, permanent damage to the bladder tissue and impaired function remain (Shahani, Streutker, Dickson, & Stewart; Tsai, Tsai, & Jang). Chronic abuse of ketamine can also create lasting cognitive impairments. Decreased performance in memory tasks, increased incidences of dissociative and delusional symptoms, and increased depression scores were all recorded after only a 12-month period of ketamine usage (Morgan, 2009). While this sample was primarily composed of adults with a mean age of 25.7 (± 9.32), the significant changes seen in the relatively short amount of time supports further research into how ketamine may affect the behavior of developing adolescents differently from adult abusers. Chronic NMDA receptor blockade using the high-affinity channel blocker (+)- MK-801 during the juvenile period of development in rats has resulted in hyperactivity and decreased anxiety-like

responses when the subjects were tested later in development (Kocahan, Akillioglu, Binokay, Sencar, & Polat). When adolescent non-human primates were administered chronic ketamine, dramatic alterations in neural circuitry occurred such as reduced activation of the ventral tegmental area, substantia nigra in the midbrain, and posterior cingulate cortex. Conversely, hyperfunction was seen in the striatum and entorhinal cortex. These maladaptations in neurotransmission have been implicated in a variety of psychiatric disorders such as depression, schizophrenia, and attention-deficit/hyperactivity-disorder (Yu, 2012).

Animal models of abuse-related behavior

Conditioned place preference and self-administration procedures (described in further detail below) are both thoroughly studied preclinical paradigms used to assess the abuse-related behavioral effects of a drug. In this thesis, both paradigms were used to compare the acute behavioral effects of ketamine between adolescent and adult rats. Since the behavioral effects of ketamine have not been thoroughly characterized in either age group, cocaine was used as a positive control to validate the methods. While the psychostimulant cocaine is from a different pharmacological class of compounds and has direct dopaminergic effects, it has been evaluated in both adult and adolescent rats in the conditioned place preference (Aberg, 2007; Badanich, 2006; Brenhouse, 2008; Tirelli, 2003; Zakharova, 2009), and in the self-administration paradigm (Crawford, 2011; Harvey, 2009; O'Connor, 2011). Results from place conditioning studies using cocaine demonstrated that adolescents developed CPP in fewer conditioning sessions and at lower doses than adults, indicating a greater sensitivity to the drug. In addition, the adolescents exhibited less

locomotor activation, suggesting a greater tolerance to some aspects of cocaine's effects. Results from cocaine self-administration studies indicated differences between age groups, with adolescents exhibiting greater escalation of intake and motivation for psychostimulants (Anker, 2012).

Conditioned Place Preference

Conditioned place preference (CPP), also termed place conditioning, involves the pairing of administration of a drug with a controlled environment to see if the subject will develop a preference for that environment. In some interpretations, this preference has been hypothesized to be a "drug-seeking" response. In this manner, the drug-paired environment serves as a secondary stimulus, or conditioned stimulus (Tzschentke T. , 2007). Typically an experimental apparatus comprised of two to three compartments is used to provide the similar but distinct environments for place conditioning to occur. For those place conditioning chambers with three compartments, the third smaller compartment is situated between the two larger conditioning compartments and serves as a mechanism to address the effect of novelty during post conditioning tests. To summarize the general methodology of CPP, first the subject is habituated to the entire testing chamber, given free exploration of the entire apparatus for one to three sessions. This serves to habituate the animal to the novel environments and to identify any initial bias the animal may have between the conditioning chambers. Unconditioned preference for one compartment plays a larger role if the experimenter is using an unbiased or balanced design, because in this case the researcher must either exclude or counterbalance subjects with an unconditioned preference. After these habituation

trials the subject enters the conditioning phase in which one of the conditioning compartments is designated as drug-paired and the other as the vehicle-paired. Subjects are administered drug or vehicle and placed in the appropriate chamber for a designated time period. Testing will take place after all conditioning sessions have been completed. Post-conditioning testing is generally a single session in which the subject is given free access to the entire chamber and time spent in each compartment is recorded. It is important that both conditioning chambers are distinct from one another but otherwise equivalent. A variety of methods can be used to provide the subjects distinct environmental cues such as colored walls, different textures of flooring, and sometimes scented chambers. The interpretation of whether the drug produces CPP is varies and relies on the initial design of the experiment, specifically if a biased or unbiased design is implemented. In a biased design, subjects are screened during habituation sessions for a preference, and often the drug is paired with the least-preferred chamber. This allows researchers to more readily detect a change in preference, whereas pairing the drug with the compartment that is unconditionally preferred may have less noticeable changes due to a ceiling effect. In some cases, such as expected aversion to the drug dose, the drug may be paired with the biased-chamber. The time spent in the drug-paired chamber before conditioning and after conditioning is calculated as a percentage of the total session time. A drug is considered to have induced a place preference if there was a significant increase in the percentage of time spent in the drug-paired environment following conditioning. In an unbiased design, subjects are randomly assigned a particular chamber as the drug-paired chamber. The development of

CPP in an unbiased procedure is calculated by comparing the time spent in the drug-paired chamber before and after conditioning, as in a biased design, or by comparing the time spent in the drug-paired chamber versus the vehicle-paired chamber after conditioning. Using the latter calculations, a preference is said to be present if the subject spends significantly more time in the drug-paired chamber. Additional information can be obtained regarding drug-induced behavioral effects by implementing extinction and reinstatement procedures in the conditioned place preference paradigm.

Regardless of which methods are chosen, the CPP paradigm offers valuable insight that cannot be replicated by any single assay. One key advantage in comparison to the self-administration paradigm is its ability to test the subject in a drug-free state, so that the motor effects of the drugs such as hyper- or hypoactivity will not interfere with interpretation. While testing for preference following drug-administration has occasionally been performed, data indicate that the results when testing in a drug-state versus a drug-free state are more pronounced and may be due to the behavioral effects of the drug (Bespalov, Tokarz, Bowen, Balster, & Beardsley). Additionally, experiments in which a saline-injection is given on the test day have shown reduced (Dockstader, 2001) or no preference development (Bespalov, Tokarz, Bowen, Balster, & Beardsley) in comparison to an experiment where drug-injections are limited to the conditioning phase and not used in the test session. Another unique ability of place conditioning is its ability to detect both rewarding and aversive effects of drugs. Manipulation of the pretreatment, conditioning session duration, conditioning frequency, and many other factors of the

research design can induce preference or aversion of a single drug dose (Bardo, 2000).

Ketamine has been shown to induce CPP in adult mice at doses of 1, 3, and 10mg/kg i.p. (Suzuki, 1999; Gao, 2003). In rats, ketamine has induced place preference in adults at doses ranging from 5 to 31.6 mg/kg i.p. (van der Kam, 2009; de la Pena, et al., 2012). Other drugs in the same class as ketamine such as dextromethorphan or phencyclidine (PCP) have varying results in the CPP paradigm. Dextromethorphan has induced place preference at doses of 24 mg/kg and 36 mg/kg i.p. (Shin, 2005), but not at a lower dose of 10 mg/kg (Huang, 2003). PCP has been shown to induce place preference in adult rats at doses of 0.45mg/kg (Marglin, Milano, Mattie, & Reid, 1989), 2.5 mg/kg and 5 mg/kg i.p. (Shin, et al., 2005). A dose of 4 mg/kg i.p. has been shown to induce place aversion in adult rats (Kitaichi, Noda, Hasegawa, Furukawa, & Nabeshima, 1996), however a dose of 8 mg/kg s.c. was used to induce both place aversion and place preference in adult mice (Miyamoto, Noda, Komori, Sugihara, Furukawa, & Nabeshima, 2000). Cocaine has consistently been shown to induce place preference in adult rats at doses of 10 to 20 mg/kg (Crawford, 2011; Tzschentke T. , 2007; Brenhouse, 2008). However at a dose of 10 mg/kg cocaine rats in late adolescence (PND 44) did not develop a preference, but adults (PND 60+) did exhibit a preference for the drug-paired chamber (Aberg, 2007). Results demonstrating differential behaviors between adolescent and adult subjects have also been seen in place conditioning experiments using food (Rubinow, 2009), nicotine (Shram & Le, 2010), cocaine and

methamphetamine (Zakharova, 2009). To date, there are no published data available on ketamine-induced place conditioning in adolescents.

Limitations and challenges of conditioned place preference

The conditioned place preference paradigm gives valuable insight into drug-induced behaviors across species and different drug classes. However, like many others, this paradigm is imperfect and subject to criticisms in its methodologies and interpretations. Most frequently discussed is the issue of state-dependency, or the acquisition of a response or knowledge while in a drugged state. According to the theory of state-dependency the subject will not have appropriate recall of information learned in a drugged-state while in a drug-free state (Overton, 1978). Research performed with D₂ knockout mice demonstrated that the mice developed a preference for morphine only when given a priming injection; in a drug-free state no preference was shown. In this same study at the same doses, wild-type mice displayed a significant preference for morphine in a drug-free state as well as when given a priming injection (Dockstader, 2001). This information shows that interpretation of the CPP paradigm must be carefully evaluated for special populations. Additionally, precisely what information is recalled during the test session is often a subject of debate. Traditional classical conditioning requires that the stimulus elicits a response from the subject. Over time the cue to elicit this conditioned response shifts from the unconditioned stimulus to the conditioned stimulus. However, in the CPP paradigm, no response is elicited during testing. Some theorists state that the subjects are emitting an “approach response” during the test session (Aguilar, 2009). However, the distinction between the conditioned

approach response and normal exploratory behavior can be subtle. Consistently and precisely following the methods is necessary to be able to accurately assess conditioned behaviors.

Another challenge presented by CPP is the lack of conventional dose response behavior. This is seen in two forms, across sessions and across doses used within session. It has been shown that the subjects' relative sensitivity to developing a preference can be interpreted by the number of sessions necessary to develop a significant preference (Belluzi, 2004). Belluzi et al. determined that adolescent rats have a greater sensitivity to nicotine and developed a CPP in fewer conditioning sessions than adult rats. This sensitivity to drug-induced conditioning in adolescent subjects was also seen using cocaine (Zakharova, 2009). Additionally, CPP development can be influenced by the duration of the conditioning session, with subjects initially exhibiting insensitivity to the drug developing a preference after longer sessions were implemented. When preference does develop following conditioning, a threshold dose can be determined as the lowest dose producing a significant increase in time spent in the drug-paired environment. While increasing the dose above this threshold can result in a more robust preference, results are highly inconsistent across classes of drugs and highly variable between strains (Cunningham, 1999). For example, an increase or decrease in the dose of drug does not consistently produce a corresponding change in the time spent in the drug-paired chamber, which demonstrates a lack of dose-dependence in CPP. Often the results for CPP are quantal in nature with either a place preference being present or not. Because of this, the amount of time spent in the drug-paired compartment may

not increase across increasing doses which all produce a preference. In addition, in many cases, with drugs known to have rewarding effects, if the drug dose and conditions are not appropriate to induce place preference, they may produce no significant changes at all or in some cases, doses may actually induce conditioned place aversion (CPA). Many factors influence and ultimately determine preference development such as time of conditioning, duration of action of the drug, genotype and strain of the subject, as well as age and sex of the subject. For example, mice that underwent cocaine conditioning during their light phase of their dark/light cycle developed a significantly greater preference than those that were conditioned during the dark portion of their cycle (Kurtuncu, 2004). The pineal gland was found to play an important role in this study; mice with intact pineal glands developed preferences differentially based on the time of day of their conditioning sessions whereas mice that had been pinealectomized displayed no diurnal differences in cocaine-induced preferences. While this presents another facet of conditioning that must be carefully attended to, the conditioning of subjects exposed to different light cycles has not been thoroughly studied. Duration of action of the drug is thought to play a pivotal role as well. Some studies have alluded to the act of administering the drug and the immediate subsequent action of placing the subject into the conditioning chamber as the key to successfully developing CPP (Ikemoto, 2005). This is supported by other studies that provide evidence that onset of drug action is the temporal key to CPP development (Tzschentke, 2004). Onset of drug action also needs to be considered for its euphoric or dysphoric effects, as some drugs may have both effects over an extended length of time (Tzschentke T. , 2007). While methodologies vary widely,

there are many temporal considerations in regards not only to the onset of drug action, but also the rate of drug elimination and to the duration of the conditioning session itself when performing CPP research.

Finally, age of the subject can have profound effects on the development of CPP. Age has been shown to play a role in sensitivity to the conditioning effects and tolerance to the aversive effects of drugs. This is due to a variety of factors such as metabolism and rate of elimination as well as neurochemical changes that can occur throughout adolescent development. It has been repeatedly demonstrated that adolescents and adults often display different responses to the same drug and/or drug dose. MDMA (Ecstasy) used to pretreat adult and adolescents rats for 7 days prior to cocaine-conditioning had significantly different effects between groups. After 3 days of cocaine-conditioning adolescents treated with MDMA exhibited a greater CPP development whereas adults treated with MDMA demonstrated a diminished CPP (Aberg, 2007). Rats tested at ages PND 35, 45, and 60, displayed differential preference development for cocaine with only the youngest rodents developing a preference for the lowest dose tested and a more robust preference at the other doses relative to the other age groups (Badanich, 2006). Nicotine tested at range doses among ages PND 28, 38, and 90 also showed varied tolerance and sensitivities. Younger rats developed a significant preference after fewer sessions and displayed greater tolerance to the motor-suppressing effects of nicotine than the older rats (Belluzi, 2004). When tested in cocaine extinction paradigms, adolescent rats have shown a greater resistance to extinction of CPP than adult rats and a more robust initial preference development (Brenhouse, 2008). While some may attribute

increased preference development to a generalized increase in activity or sensitivity to rewarding substances, CPP performed using food as the reinforcer demonstrated that adolescents did not develop a preference at all whereas the adult rats did develop a preference for the food-paired compartment. Overall the adolescents ingested the least amount of novel food (Rubinow, 2009). While there are many considerations such as growth, available food, and exposure to novelties, Rubinow *et al* implemented many controls such as daily exposure to minimal amounts of the novel food (fruit cereal) and adjusted recorded values for age and weight. Overall these findings suggest that adolescent rats display greater sensitivity to the rewarding effects of drugs of abuse and a greater tolerance to the aversive effects across many different doses and classes of drugs.

In addition to age considerations, there are many methodological considerations regarding the apparatus itself when testing adolescents in CPP. It is suggested that two-chamber apparatuses should always be used when testing adolescents due to increased novelty-seeking and exploratory behavior in adolescents when compared to adults even when no drug is being used (Stansfield & Kirstein, 2006). In light of these findings, a two-chamber apparatus was used to perform all place-conditioning in this experiment.

Self-administration

The self-administration paradigm has long been used to investigate the contingencies of drug-reinforcement and assess the reinforcing properties of drugs. The self-administration paradigm gives researchers the ability to control the contextual environment of the subject and requires the subject to elicit specific

behavioral responses in order to receive the drug. A drug is termed reinforcing when receipt of the drug increases the probability of the response that preceded drug delivery. The route of administration for drug delivery is often intravenous (i.v.), although other routes including oral, insufflation, and inhalation have been used (Panlilo, 2007). A plethora of environmental cues and pharmacological variables impact the self-administration paradigm. This allows the nuances that may affect abuse-related behaviors to be measured and manipulated by the researcher. Environmental cues such as lights and tones within the operant chamber can be paired with the reinforcer to function as conditioned stimuli. Additionally schedules of reinforcement and stressors can inhibit or facilitate the subject's probability of emitting an operant response. Depending on the relative reinforcing efficacy of the compound, subjects will, in some cases, continue to emit responses in the absence of drug delivery solely for the conditioned stimuli (i.e. visual and audio cues) associated with the drug delivery. This phenomenon typically lasts a brief period of time when the cues are presented without any reinforcement before extinction occurs and the subjects' responses diminish to significantly lower rates of responding, or placebo conditions if applicable (Reynolds, 1968). These visible and audible cues have been likened to the environmental cues in place conditioning, in that these cues stimulate drug-seeking behaviors.

The natural world rarely reinforces every response emitted, and operant conditioning can be modified to create a variety of reinforcement schedules. A schedule of reinforcement is the frequency with which the contingent response is reinforced (Reynolds, 1968). Schedules can be divided into ratio schedules which

require a specified number of responses before reinforcement can occur and interval schedules which require a specific amount of time to elapse before reinforcement occurs. Schedules of reinforcement can be highly complex and the impact of schedule manipulations on a drug's effects has been extensively examined. In the current studies both a fixed-ratio-1 (FR1) and a progressive ratio (PR) schedule were used to assess two different aspects of self-administration behavior. An FR1 schedule of reinforcement is useful in tracking rate of drug intake between groups of subjects and between doses (Arnold, 1997). Progressive ratio schedules differ from fixed ratio schedules in that presentation of each subsequent reinforcer requires an increasing number of responses. The subject is required to increase their responses gradually to achieve reinforcement until the cost no longer outweighs the reinforcement and the subject discontinues emitting responses, designated the breaking or break point (Arnold, 1997). The break point under a progressive ratio schedule is used to assess the relative efficacy of the reinforcer. Under PR schedules, it is the persistent efforts of responding rather than solely the rate of responding, that is measured and analyzed. This can be critical in operant conditioning where rate of responding is often altered by drug infusions (Panlilo, 2007). Additionally, progressive ratio schedules allow the use of behavioral economics to measure the unit price of drug consumption. In other words, the number of responses emitted per milligram per kilogram of drug is usually stable, so that although dose may change total consumption does not (Madden, Smethells, Ewan, & Hursh, 2007; Hursh & Silberberg, 2008). Another valuable feature of using different schedules of reinforcement is that the subject's behavior can be

manipulated and analyzed prior to drug delivery, and therefore similar to place conditioning, aspects of behavior can be analyzed in a drug-free state.

The specific focus of this study will be on the differential reinforcing effects of ketamine between adolescent and adult Sprague-Dawley rats. Ketamine has been evaluated in the self-administration paradigm in rats (Rocha, 1996), including the Sprague-Dawley strain (Marquis, 1987; Collins, 1984), non-human primates (Yanagita, 1975; Moreton, 1977; Young, 1981; Lukas, 1984; Carroll, 1983), and clinical studies using human subjects for its subjective effects (Morgan, 2009). While the popularity of ketamine has risen in specific settings such as dance clubs and “raves,” preclinical literature has given credence to the importance of environment in ketamine abuse using the rat self-administration model. De Luca and Badiani demonstrated in their 2010 study that rats housed in their self-administration chambers administered only the highest available dose of ketamine and at levels significantly lower than rats that were transferred to the self-administration chambers for discrete behavioral sessions (De Luca, 2011). While drug-environment interactions will not be explored in this study’s self-administration paradigm, only through the CPP paradigm, it is clear that environment plays a critical role in the acquisition and maintenance of ketamine administration.

The self-administration assay has also been used in adolescent rats and non-human primates across a variety of schedules and drugs to provide valuable insight into differences in acute behavioral effects of drugs of abuse between adolescents and adults. In addition to assessment of reinforcing effects during adolescence, this procedure has been used to examine the effects of adolescent drug self-

administration on subsequent drug administration during adulthood as well as on persistent neurological changes. Adolescents self-administer methylphenidate and cocaine after several training sessions at rates comparable to adult rodents (Burton, 2010). A study performed using heroin showed adolescents administering significantly more drug under an FR1 schedule but exhibiting rates under a PR schedule that were similar to adults (Doherty & Frantz, 2012). There is also evidence to support that adolescent self-administration can have lasting sensitization effects. Two groups of adolescent rats were trained in self-administration procedures, one reinforced with intravenous methamphetamine and the other group was reinforced by saccharin pellets. When comparing both groups of rats as adults, there was no difference in adult self-administration of cocaine. However, increased amphetamine-induced locomotion was seen in the adults that had self-administered methamphetamine as adolescents. In addition to increased sensitization, other characteristics of adolescent drug-use that make them highly susceptible to drug abuse and addiction are their resistance to extinction of drug-seeking behaviors (Andrzejewski, Schochet, Feit, Harris, McKee, & Kelley) and their tolerance to drug-associated aversive effects. Under long-access self-administration conditions (6-hours) for methamphetamine, adolescent rats have been shown to take more infusions and escalate the number of infusions per session over time in comparison to adult subjects in the same study (Anker, 2012). This displays tolerance to high doses of methamphetamine and increased risk for binge-like behavior. Animal models have also shown that basal levels of dopamine in the nucleus accumbens are higher for adolescent rats in comparison to juveniles and adults as well as

increased levels of cocaine-stimulated dopamine in comparison to juveniles (Badanich, 2006). Other drugs have clearly shown a myriad of different behavioral effects between adolescents and other age groups, therefore studying ketamine under the self-administration paradigm could offer valuable insight into age-related differences for this distinct pharmacological class.

Limitations and challenges of self-administration

While the self-administration paradigm offers invaluable information; most importantly results that can often be generalized with both predictive and face validity, the model is not without weaknesses. The most common route of administration for self-administration studies is intravenous (i.v.) - which relies on the maintained patency of the catheter. Complete or partial occlusion can distort results if not addressed in a timely manner. The life of the catheter also determines the length an experiment may run, which is highly unpredictable. To address this possible disruption, experimenters must calculate sufficient numbers of subjects to allow for the loss of catheter patency during the experiment and determine which aspects may need to be analyzed between-groups since there will be insufficient time to perform complete within-subjects analyses. As with many other behavioral paradigms, individual differences play a significant role in both the rates of responding as well as the sensitivity to drug effects. While specific parameters must be determined prior to study initiation, such as criteria for acquisition and extinction, individual responding can vary greatly between subjects. In some instances this variability can be compensated for by analyzing data as a percent change from baseline responding rather than comparing raw data responding between subjects.

Summary

To summarize, findings within the literature provide evidence that adolescent substance abuse, specifically of “club drugs”, is increasing and trending towards higher levels of abuse in the future. Ketamine abuse during adolescence presents risks to the user from both acute effects of the drug as well as the impact of disruption of the NMDA receptors which play an essential role in CNS development. While the role of the NMDA receptor system during CNS development has yet to be fully determined, our understanding of damage to this system by ketamine abuse, particularly in an adolescent population, is also lagging behind more commonly studied drugs such as nicotine and alcohol. The question posed in the current studies is whether or not there is a difference in the acute abuse-related behavioral effects of ketamine between adolescents and adults that makes the former more susceptible to ketamine abuse. The primary objective of this project was to use these two well-established paradigms to further investigate ketamine-induced abuse-related behavior in adolescent rats and contrast it to ketamine-induced abuse-related behavior in adult rats. Currently there are only a limited number of controlled studies with ketamine in the conditioned place preference and self-administration paradigms, and more specifically a complete absence of studies assessing adolescent subjects’ response to ketamine in these assays. In accord with previous findings in adolescent subjects using different drugs from different pharmacological classes, my central hypothesis is that adolescents will display increased sensitivity to the reinforcing effects of ketamine, and increased tolerance for the aversive effects of ketamine. In experiment 1, the development of CPP for ketamine and the

positive control cocaine in adolescents will be compared with those in adults using a conditioned place preference procedure. In experiment 2, the effects of ketamine and cocaine in adolescents will be compared with those in adults for acquisition of self-administration behavior under a FR1 schedule and for reinforcing efficacy under a PR schedule for cocaine and ketamine deliveries.

Research Design & Methods

Experiment 1: Conditioned Place Preference

A place conditioning procedure was used to evaluate differences in the acute behavioral effects of ketamine across two age groups, adolescents and adults, in male Sprague-Dawley rats. Cocaine was used as a positive control in both age groups to validate the methods employed. Data were analyzed by determining the change from baseline in the percent of time spent in the drug-paired chamber after conditioning. The change in time spent was compared for statistically significant differences using a Two-Way ANOVA with age and drug dose as the independent variables and the percent change in time from baseline being the dependent measure.

Experiment 2: Self-administration

Intravenous self-administration was used to assess differences in the reinforcing effects of ketamine between adult and adolescent rats. As a positive control, different doses of cocaine were also tested in both age populations. Subjects that reached stable acquisition criteria were tested under a progressive

ratio schedule of reinforcement to assess the differences, if any, in the reinforcing efficacy of ketamine and cocaine between adolescent and adult rats. The rate of acquisition, the percentage of subjects reaching acquisition criteria, and break point under a progressive ratio schedule were all analyzed independently using Two-Way ANOVAs with age and drug dose as the independent variables. All statistically significant differences were at $p < 0.05$.

Subjects

Rats have historically been used in behavioral experiments for over a century, thus their behavior as well as neuroanatomy and neurochemistry are well-characterized. Sprague-Dawley rats have been used extensively within this laboratory and others in both self-administration and CPP procedures providing validation of their use as an appropriate research species in these behavioral assays. Male, Sprague-Dawley rats (Charles River) were used for all studies. Adult rats were older than post-natal day (PND) 70 at the time of testing. Adolescent rats were delivered on PND 23 for the conditioned place preference assay and allowed to acclimate to the vivarium, the laboratory and handling for 5 days prior to beginning conditioning. Adolescent rats for the self-administration assay were delivered on PND 22-23 and given 7-8 days to acclimate to the vivarium, the laboratory and handling prior to catheter implantation (PND 30). All subjects were housed in clear, plastic microisolator cages in a temperature controlled vivarium (20-22 C°) under a 12/12-hour reversed light-dark cycle (0600-1800 lights off). All subjects were given *ad libitum* food and water regardless of the behavioral assay to which they were assigned. All self-administration subjects were initially pair housed but individually

housed subsequent to catheter implantation. All place-conditioning adolescent subjects were pair-housed throughout the study. All place-conditioning adult subjects were singly-housed throughout the study. All subjects were weighed bi-weekly (Tuesdays and Fridays) and drug solutions were modified to compensate for any changes in body weight.

As previously mentioned due to loss of catheter patency, specifically a greater concern in adolescent subjects as catheter patency is more difficult to maintain with the rapid growth rate, greater numbers of subjects were needed to ensure sufficient statistical power. Previous unpublished research within our laboratory has shown that, despite habituation to handlers and acclimation periods, adolescent rats exhibit a higher degree of variability than adults in these behavioral tests. Therefore, larger group sizes were selected for adolescents in the place conditioning paradigm as well.

Table 1.
Experiment 1: Number of subjects per group for conditioned place preference

Groups		Conditioning Dose (mg/kg)	# of subjects entered testing	# of subjects completed testing	
Adults	Cocaine	3	10	7	
		10	12	7	
		15	12	9	
	Ketamine	1	10	9	
		3	10	9	
		10	11	8	
	Saline	0.9%	10	8	
	Adolescents	Cocaine	3	12	12
			10	12	12
15			13	10	
Ketamine		1	15	12	
		3	15	11	
		10	14	11	
Saline		0.9%	14	12	

Table 2.
Experiment 2: Number of subjects per group for self-administration

Groups		Acquisition Dose (mg/kg/inf)	# of subjects entered testing	# of subjects completed testing
Adults	Cocaine	0.01	4	4
		0.1	6	6
		0.3	6	6
	Ketamine	0.03	8	8
		0.1	7	7
		0.3	7	7
Adolescents	Cocaine	0.01	0	0
		0.1	4	4
		0.3	4	4
	Ketamine	0.03	2	1
		0.1	5	4
		0.3	6	6

Apparatus

Conditioned place reference

Commercially purchased open field arenas with place preference inserts were used (Med Associates, Inc., St. Albans, VT). This apparatus consists of two large compartments, approximately 8.5"x17" each, both with 4 acrylic walls and an acrylic door with ventilation holes to cover the top. The left compartment of each CPP chamber had black walls, a smooth acrylic floor, and an additional opaque cover over the top to create a dim environment. The right side had white walls, grid flooring, and no additional cover over the top. Time spent in either compartment of the chamber was measured by sequential infrared beam breaks. During habituation and test sessions a sliding partition was removed from the chamber so that subjects could freely cross between the two sides. Chambers were deodorized with a 50% ethanol & 50% water solution between each subject.

Self-administration

Eight standard operant conditioning chambers equipped with two retractable levers housed inside individually ventilated, sound-attenuating enclosures (Med Associates, Inc., St. Albans, VT) were used for the self-administration assay. A 5-watt house light was located at the rear wall of each chamber and a white LED light was located above each of the retractable levers. Infusion tubing covered by a stainless steel spring tether (Plastics One, USA) was connected to a back-mounted pedestal implanted in each rat. Infusions were delivered by individual peristaltic pumps located outside of each chamber. Pumps were calibrated to deliver a volume of 0.1ml per infusion over 5 seconds. The self-administration program was

controlled by MED-PC IV software (Med Associates, USA) running on an IBM PC compatible computer.

Procedures

Conditioned place preference

The conditioned place preference paradigm is widely used to assess a drug's ability to produce drug-seeking behavior or drug-induced aversion. However the methodological approaches to this question are highly variable and therefore interpretation of results must vary according to the methods employed. Because a biased procedure was used, efforts were made to control for variability by conditioning drug-states on the overall less preferred side of the chamber. Additionally, a range of doses and a saline control were used in conditioning. Subjects were randomly assigned to receive saline, ketamine, or cocaine conditioning. Ketamine was the primary compound of interest in this study, however there is limited literature available on the results for testing ketamine in this procedure in adult rats and no place-conditioning experiments have been performed with ketamine in adolescent subjects. Cocaine was therefore chosen as a positive control for its well-established behavioral characteristics. All doses of drug were administered via intraperitoneal (i.p.) injection at a volume of 1 ml/kg for adults or 2 ml/kg for adolescents. Throughout the experiment each subject was conditioned and tested in the same chamber each day. Previous testing within our laboratory had shown a significant preference for the side of the chamber with grid-flooring and white walls. Based on this, a 7-day, biased-design CPP procedure was used under the following schedule: Days 1 and 2 – (habituation) a single 20 min session each

day where subjects were permitted access to both sides of the chamber and activity recorded. The purpose of preconditioning is to habituate the subjects to the chambers and to exclude any outliers that demonstrated an extreme preference for either chamber. Any subjects that spent greater than 75% of the habituation session in either chamber were excluded from further conditioning and subsequent testing. Days 3 through 6 (conditioning) each rat was injected with its assigned treatment (dose of ketamine, dose of cocaine or saline) and then placed immediately into the appropriate chamber compartment for 20 minutes. If drug was administered, the subject was placed in the chamber with the smooth-flooring and dark walls; whereas subjects receiving saline were placed in the chamber with the grid-flooring and white walls. The one exception was the saline control group. These subjects received saline injections prior to placement in both the smooth- and grid-floored compartments. The four conditioning days included two sessions each day with a minimum of 4 hours between sessions. Subjects were administered their treatment (ketamine, cocaine or saline) prior to one session and saline prior to the other session. Administration of drug or saline during AM and PM conditioning was counterbalanced across days for each subject to control for any circadian effects. On day 7 (test day) the subjects were placed centrally in the chamber facing the doorway between the two compartments, once again permitted access to the full chamber and activity recorded for 20 minutes. During the test sessions subjects were in a drug-free state and did not receive any injections. Previous research has indicated that administering a vehicle or drug injection prior to a test session can act as a discriminative cue (Bespalov, Tokarz, Bowen, Balster, & Beardsley, 1999),

therefore no injections were used in this procedure during the test session. The table below summarizes an example of a subject's drug administration across the study. The group of subjects in each age group designated as the saline control received saline injections prior to every conditioning session.

Table 3.

Example of a drug-treatment schedule in CPP

This table displays an example of how drug and saline were administered in the conditioned place preference assay. Subjects in the saline control group would receive a saline injection regardless of the compartment into which they were placed. Each subject was exposed to 4 pairings with saline and 4 pairings with drug (or the drug-side paired with saline) with order of presentation counterbalanced across days. Additionally, the order of presentation was counterbalanced between subjects such that half the subjects began on the drug-designated side on Day 3 and half on the saline-designated side.

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Treatment	AM	-	Saline	Drug (left)	Saline (right)	Saline (right)	Drug (right)	No Injection
	PM	-	-	Saline (right)	Drug (left)	Drug (left)	Saline (right)	
Session Type		Habituation	Habituation	Condition	Condition	Condition	Condition	Test

Self-administration

Surgical procedure. Tapered catheters were fabricated using 3.5 French polyurethane tubing (Access Technologies, Skokie, Illinois). The catheters were implanted in each subject's right jugular vein under isoflurane anesthesia following morphine pre-treatment approximately 20 minutes before anesthesia induction. The distal end of the catheter was threaded subcutaneously to a cannula connector pedestal (Plastics One, USA) that was implanted subcutaneously in the subject's midscapular region. Subjects were given a minimum of 5 days recovery prior to beginning the self-administration acquisition phase. Carprofen at 5mg/kg was administered once daily for the first 72 hours following the surgical procedure. Catheter patency was periodically verified with a 15 mg/kg i.v. bolus ketamine infusion, assessed by the immediate onset of sedative effects. Subjects with a failed catheter were excluded from acquisition data as the number of sessions required to meet acquisition criteria was inexact, however these subjects were recatheterized using the left jugular vein to assess their performance under a progressive ratio schedule.

Operant procedure. Subjects were randomly assigned to self-administer either cocaine or ketamine at varying doses (see Drugs section). Two-hour self-administration sessions were run daily (Sun – Sat) throughout the experiment. Catheters were maintained with a daily 0.1 ml i.v. infusion of an ampicillin/sulbactam antibiotic diluted with a solution of 25% heparin, 25% glycerol, and 50% saline. The resultant solution contained 250 IU of heparin per milliliter, and 150 mg/kg ampicillin/sulbactam. Data analyzed included the number of days required to meet

acquisition criteria and the number of subjects as a percentage of total subjects exposed to the drug that met acquisition criteria under a FR1 schedule of reinforcement. Animals were permitted up to 21 days to reach acquisition criteria before being removed from the study. Acquisition criteria included a minimum of 3 consecutive days with > 15 responses on the active lever and responses on the active lever exceeding responses on the inactive lever. Once behavior was acquired, subjects continued in daily behavioral sessions until behavior stabilized. Stability criteria was defined as 3 consecutive days with no upward or downward trend in infusion numbers, and each day's responding had to be within 25% of the mean of those 3 days. These criteria have been used within our laboratory for self-administration assays in other experiments and have proven to be a reliable set of parameters for acquisition and stabilization. Following stabilization of behavior or 7 days under FR1 conditions, subjects were tested under a progressive ratio (PR) schedule of reinforcement for 4 consecutive days to evaluate the reinforcing efficacy of ketamine and cocaine across the two different age groups. Adult rats were assigned to a drug dose, ketamine 0.03, 0.1, or 0.3 mg/kg/inf, or cocaine 0.01, 0.1, or 0.3 mg/kg/inf, and began the acquisition phase of the experiment. Subjects were given free-access to self-administer under an FR1 during a two-hour session. If subjects met acquisition and stability criteria in less than 21 days, they began self-administering the same acquisition dose under a progressive ratio schedule. The progressive ratio schedule used was adapted from Roberts & Richardson (1992). Subjects not meeting acquisition and stability criteria in less than 21 days were excluded from further testing. Once a subject completed 4 days of progressive ratio

testing, the subject was returned to their baseline dose until stability was reached once again and they were subsequently switched to a different self-administration dose of the same drug for a maximum of 7 days followed by 4 days of testing under a PR schedule. This was continued until the subject had completed all test doses of either ketamine or cocaine as well as a saline test point or until their catheter had failed. Due to the short timeframe given for testing during the period of adolescence, the adolescent subjects were tested for acquisition of a single drug dose and 4 days of PR testing for this same assigned dose if they met acquisition and stability criteria within 21 days. The adolescent subject was then removed from testing therefore all dose comparisons within the adolescent group were based on a between subjects design.

Drugs

Ketamine HCl (Phoenix Scientific, Inc., St. Joseph, MO, USA) was diluted in 0.9% physiological saline from 100 mg/ml commercial stock solution to provide the desired test concentrations. Cocaine [National Institute on Drug Abuse (NIDA), Bethesda, MD] was solubilized in 0.9% physiological saline. Drugs and doses tested in CPP were saline, cocaine (1, 3, and 10 mg/kg), and ketamine (1, 3, 10 mg/kg). Drugs and doses tested in the self-administration procedure were cocaine (0.01, 0.1, and 0.3 mg/kg/infusion) and ketamine (0.03, 0.1, and 0.3 mg/kg/infusion).

Overview of Data Analysis

General. All data are presented as the group mean \pm 1S.E.M. All data were analyzed for significance using IBM SPSS 20. $P < 0.05$ was considered to be statistically significant. Significant differences in tests with greater than two groups

were further analyzed using a Tukey post-hoc test.

Conditioned Place Preference. Test session data were analyzed as the difference in time spent in the drug-paired chamber pre- and post-conditioning. This difference is represented as a percentage in the figures. Data were analyzed using a Two-Way ANOVA for main effects of 1)Age, 2)Drug condition, or an interaction of 'Age x Drug.' A Tukey post-hoc analysis was used to detect any significant differences between the drug-conditioned and saline-conditioned subjects. The Turkey post-hoc test was also used to determine if there was any significant preference or aversion development.

Self-administration. A two-way ANOVA was performed to analyze the effect of Age and Drug Dose on the percentage of subjects to acquire. This was measured as the number of subjects to meet acquisition criteria in 21 days or less divided by the total number of subjects assigned to that drug condition. Acquisition criteria was defined as 3 consecutive days of greater than 15 responses and responses on the active lever were greater than responses on the inactive lever. Subjects who failed to maintain a patent catheter during the 21-day acquisition period were excluded from this analysis. The dependent measure for responding under a progressive schedule was assessed by calculating the mean break points. Break point is defined as the last ratio completed within a session (Roberts & Richardson, 1992).

Results

Place Conditioning

Preconditioning Results

Figure 1 presents the mean time spent in each compartment of the conditioning apparatus during the 1200 sec preconditioning session. Overall the rats spent an average of 683 secs (approximately 57% of total time) in the compartment with the grid flooring and an average of 516 sec in the compartment with the smooth flooring. An independent samples t-test comparing differences between the mean time spent in the two chambers revealed significant differences in baseline preferences for both age groups combined, $t(272) = -8.892, p < 0.001$. When evaluating preconditioning behavior for the individual age groups, the data show that adults spent approximately 60% of their preconditioning time in the grid-floored chamber whereas adolescents spent approximately 55% of their preconditioning time in the grid-floored chamber (Figure 2). Individual t-tests were performed for each age group to assess preferences for the grid-flooring in each group. For the adult group the time spent in the smooth-floored compartment ($M = 477.17$) was significantly less than the time spent in the grid-floored compartment ($M = 722.43$), $t(112) = 11.02, p < 0.001$. A less robust preference was seen in the adolescents, however the time spent in the grid-floored compartment ($M = 655.25$) was still significantly greater than the time spent in the smooth-floored compartment ($M = 544.76$), $t(158) = 4.07, p < 0.001$. To compare the adolescent bias with the adult bias, multiple independent samples t-tests were performed to compare the means of both age groups relative to the time spent in each compartment during pre-conditioning.

Assessment of the adolescent group and the adult group showed that adults preferred the grid-floored chamber significantly more than the adolescents, $t(135) = 2.55$, $p = 0.011$, (Figure 2). However both groups independently still showed a significant preference for the grid-floored chamber in comparison to the smooth-floored chamber. This overall inherent preference supports the use of a biased-design approach for the place conditioning procedure with drug administration paired with the smooth-floored compartment and saline with the grid-floored compartment.

Preconditioning

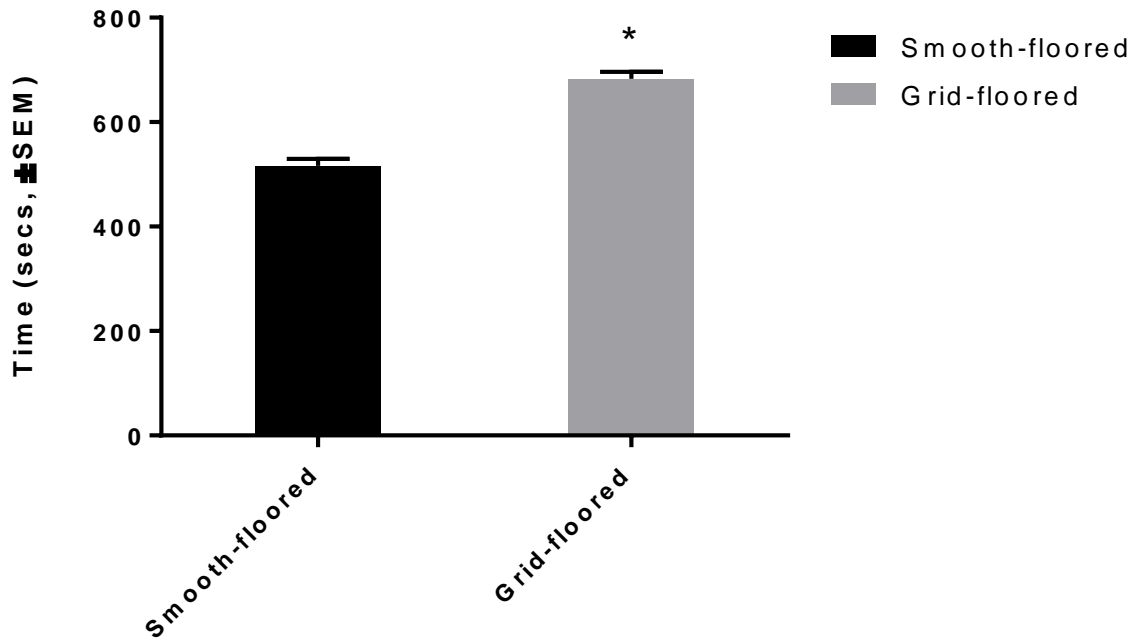


Figure 1. Preconditioning data for both age groups combined
Average time spent (sec, ±SEM) in the smooth-floored and grid-floored compartments for adult and adolescent rats combined during the preconditioning session. Total session time was 1200 seconds. The asterisk denotes statistically different means at $p < 0.001$.

Preconditioning

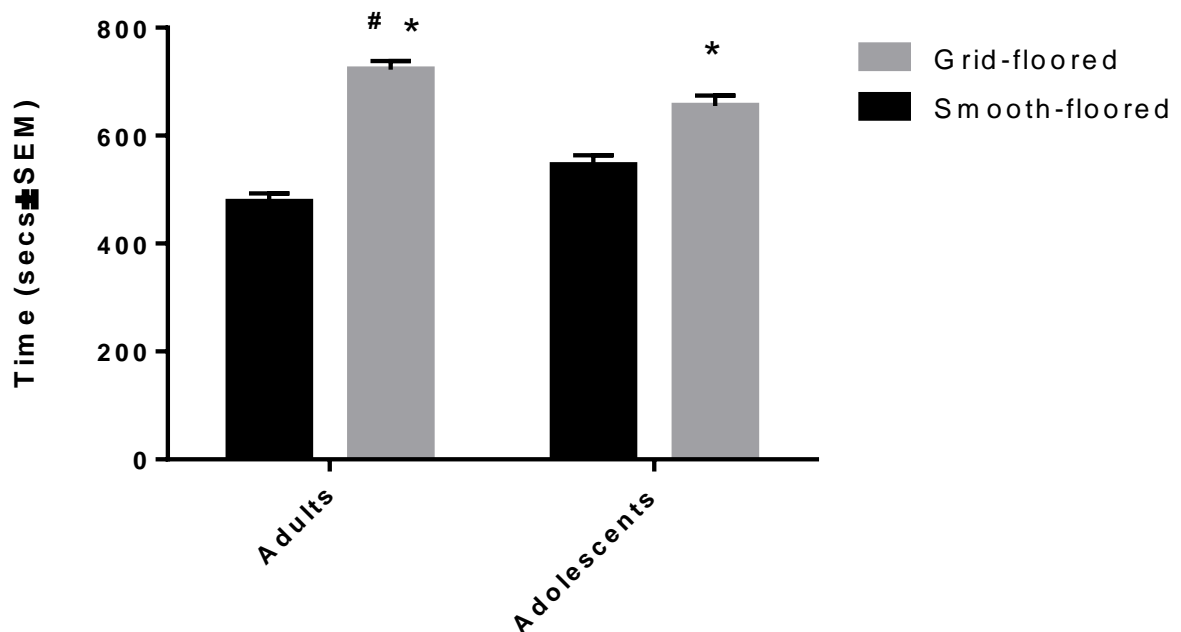


Figure 2. Preconditioning data for all subjects based on age

The average time in seconds (sec, ±SEM) with black bars representing time spent in the smooth-floored compartment for each age group, adolescent and adult. The grey bars represent the amount of time spent in the grid-floored compartment for each age group, adolescent and adult. * denotes significantly different time spent in the grid-floored compartment relative to the smooth-floored compartment within the age group $p < 0.001$. # denotes significantly different time spent in the grid-floored compartment between age groups, $p < 0.001$.

Post-conditioning Results

Adult subjects spent either the same or less time in the drug-paired compartment after conditioning with different doses of ketamine (Figure 3). While repeated pairing of 1 mg/kg ketamine did not alter the time spent in the drug-paired compartment (1mpk: $n=9$, $M = -0.09$) both the 3 and the 10mg/kg ketamine groups showed obvious decreases in time spent in the drug-paired environment however neither of these doses were significantly different from saline, (3mpk: $n=9$, $M = -15.83$; 10mpk: $n=8$, $M = -8.26$). Adolescent subjects demonstrated different effects across the doses of ketamine tested and their behavior in general was highly variable (Figure 3). While the 1 and 3 mg/kg ketamine groups exhibited an increase in the percent time and 10 mg/kg actually decreased the percent time spent in the drug-paired environment post conditioning these changes were not significant at any dose of ketamine (1mpk: $n=12$, $t = 1.881$, $p = 0.087$; 3mpk: $n=11$, $t = 1.813$, $p = 0.099$; 10mpk: $n=11$, $t = 0.996$, $p = 0.343$). Subjects conditioned with ketamine exhibited a significant main effect for Age [$F(1,72) = 7.374$, $p=0.008$], no significant main effect for Drug Dose [$F(3,72) = 1.383$, $p=0.255$], nor a significant interaction for Age x Drug Dose [$F(3,72) = 1.856$, $p=0.145$]. Subjects conditioned under a saline schedule were included in both analyses, ketamine-induced conditioning and cocaine-induced conditioning, and results from a Tukey post-hoc analysis revealed that saline was not found to be significantly different from any dose for either age group. Subjects receiving repeated conditioning with saline in both compartments did not present any significant

change from baseline in either age group (Adults: $n=8$, $M = -2.10$; Adolescents: $n=12$, $M = 2.48$). All doses of cocaine were associated with an increase in time spent in the drug-paired compartment after conditioning in all subjects, regardless of age. Conditioning with cocaine resulted in no significant main effect for Age [$F(1,70) = 0.042$, $p=0.893$]. Additionally, there were no significant differences in the degree of the preferences that developed in adults or adolescents across the doses of cocaine. Due to the high variability in behavior, the time spent in the drug-paired environment by adolescent subjects was highest for the 10mg/kg dose of cocaine, but none of the doses were significantly different from the saline control group (3mpk: $n=13$, $M = 7.28$; 10mpk: $n=12$, $M = 13.43$; 15mpk: $n=10$, $M = 8.58$). Adult subjects exhibited increases in percent time spent in the drug-paired environment relative to their baseline for all doses of cocaine (3mpk: $n=7$, $M = 13.45$; 10mpk: $n=7$, $M = 12.55$; 15mpk: $n=9$, $M = 11.18$). Therefore, there was no main effect for Drug Dose in cocaine-conditioned subjects [$F(3,70) = 1.935$, $p=0.132$]. There was also no significant interaction for Age x Drug Dose in cocaine-conditioned subjects [$F(3,70) = 0.324$, $p=0.808$].

Ketamine Place Conditioning

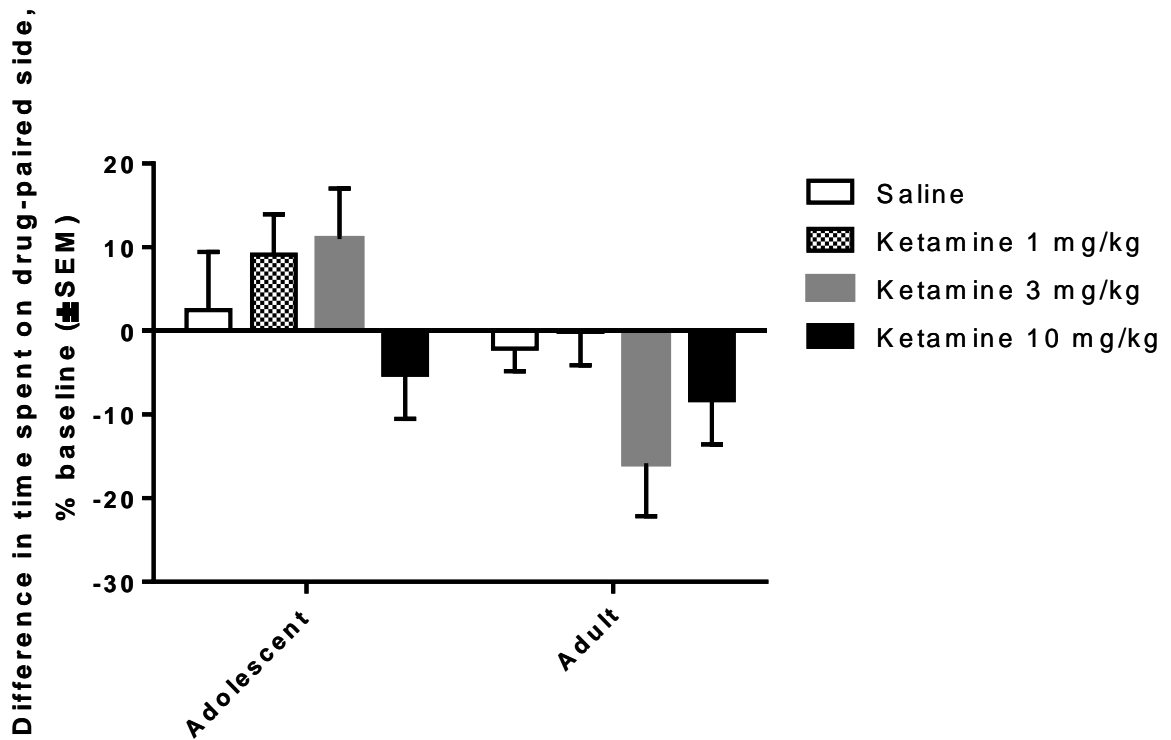


Figure 3. Ketamine postconditioning results

Shown are the differences in time spent in the drug-paired environment relative to baseline for saline and each dose of ketamine tested in place conditioning with results grouped by age. Change is expressed as the difference in the total percentage of time spent in the drug-paired environment pre and post conditioning.

Cocaine Place Conditioning

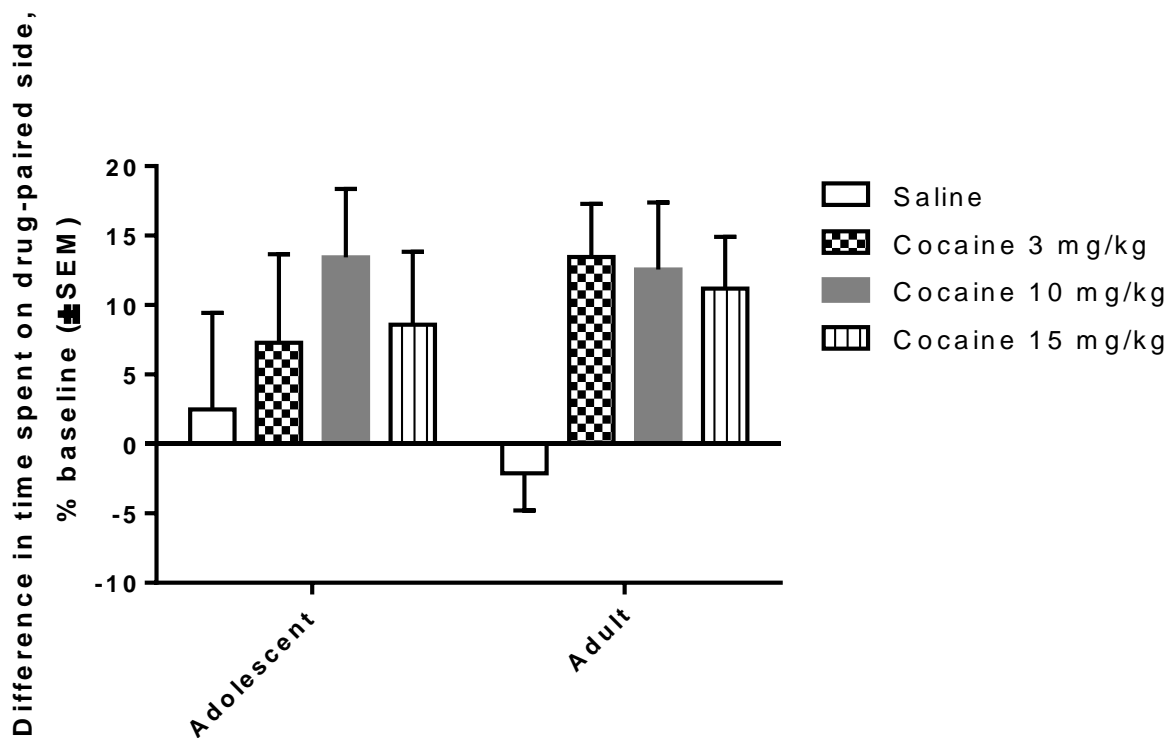


Figure 4. Cocaine place conditioning

Shown are the differences in time spent in the drug-paired environment relative to the baseline for saline and each dose of cocaine tested in place conditioning with results grouped by age. Change is expressed as the difference in the total percentage of time spent in the drug-paired environment pre and post conditioning.

Self-Administration

Percentage Acquisition. Age was not a contributing factor to whether or not a subject met the acquisition criteria. There was no main effect of age [$F(1,4) = 1.768, p=0.254$], however there was a distinct difference in the number of subjects that met acquisition criteria at the lowest dose of ketamine tested. At the dose of 0.03 mg/kg/inf of ketamine, 5 of the 8 adult subjects tested reached acquisition criteria. At this same dose, neither of the 2 adolescent subjects tested met acquisition criteria; however this number of subjects is insufficient to draw a conclusion based on age. This same low dose of ketamine, 0.03 mg/kg/inf had the lowest percentage of adult subjects meeting acquisition criteria out of all the doses tested. There was no significant main effect of drug dose [$F(4,4) = 3.43, p=0.129$]. Insufficient adolescent subjects have been evaluated for acquisition at the lowest dose of ketamine to answer whether adolescents are more, less, or equally sensitive to ketamine's reinforcing effects in comparison to the adults. A characteristic that was regularly observed in the adolescent group was increased inactive lever presses. The responding on the inactive lever frequently exceeded the active lever presses, and inactive lever responding was often observed to be higher than the adult subjects. This factor either prevented subjects from meeting acquisition criteria or for many subjects extended the number of days required to meet acquisition criteria. This effect was not dose-specific, and was seen across different doses of ketamine and cocaine.

Percent Acquisition

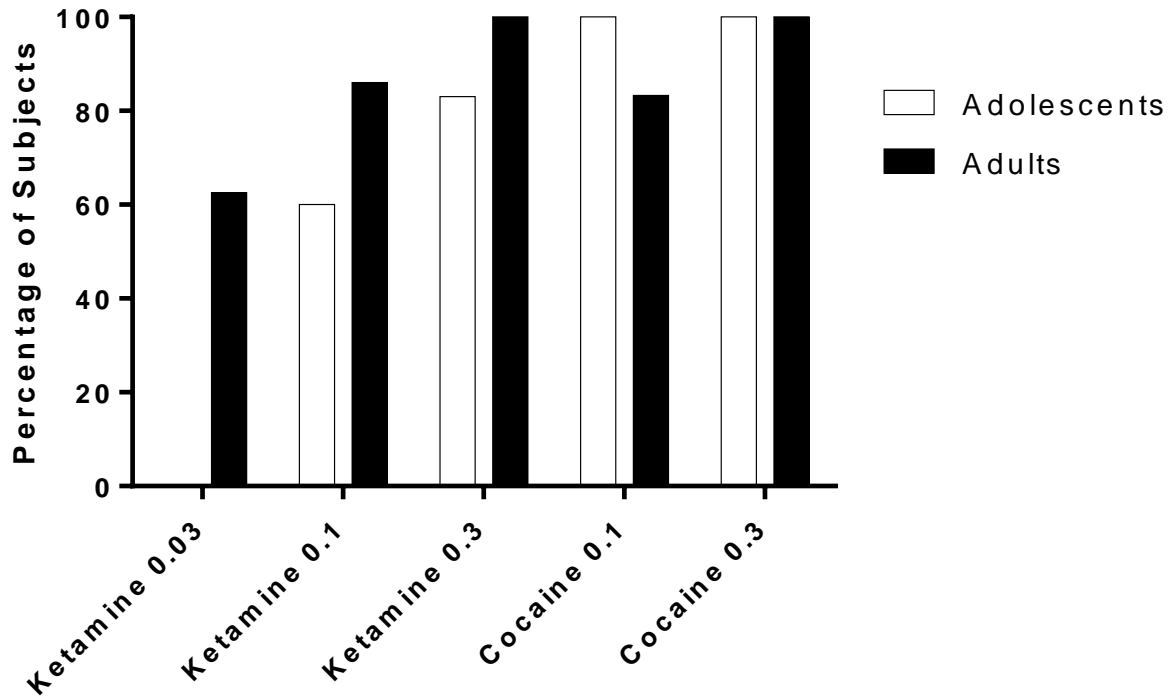


Figure 5. Percentage of subjects meeting acquisition criteria

The graph presents the percentage of adult and adolescent subjects that met acquisition criteria grouped by drug and dose. No adolescent subjects (0%) met criteria for acquisition at the 0.03 mg/kg/inf dose of ketamine; therefore, no bar is presented for this value on the graph.

Rate of acquisition. Rate of acquisition was defined as the mean number of days required to meet acquisition criteria. As shown (Figure 6) the mean number of days required to achieve acquisition was higher in the adolescent subjects than the adults for both doses of ketamine and cocaine. While the main effect of age was significant [$F(1,30) = 5.322, p=0.028$] across all doses the effect was more pronounced in subjects self-administering ketamine. The rates of acquisition for the ketamine and the cocaine doses tested were all very similar for the adult subjects. However, for the adolescent subjects the mean rates of acquisition for the doses of ketamine were greater than the mean rates of acquisition for cocaine. While the differences in the adolescent rates of acquisition between ketamine and cocaine were not significantly different, the trending data may indicate a specific point in development where ketamine's reinforcing potential increases to become more similar to that of cocaine.

There was not a main effect of drug dose [$F(3,30) = 1.492, p=0.237$], however the 0.1 mg/kg/inf ketamine dose and the 0.1 mg/kg/inf cocaine dose had the largest means for the number of days to meet acquisition criteria. These dose-dependent effects for rate of acquisition were seen in both adolescent and adult groups. There was no interaction between age x drug dose [$F(3,30) = 0.7504, p=0.5307$]. The lowest dose of ketamine 0.03 could not be included in this analysis because no adolescent subjects acquired at this dose.

Rate of Acquisition

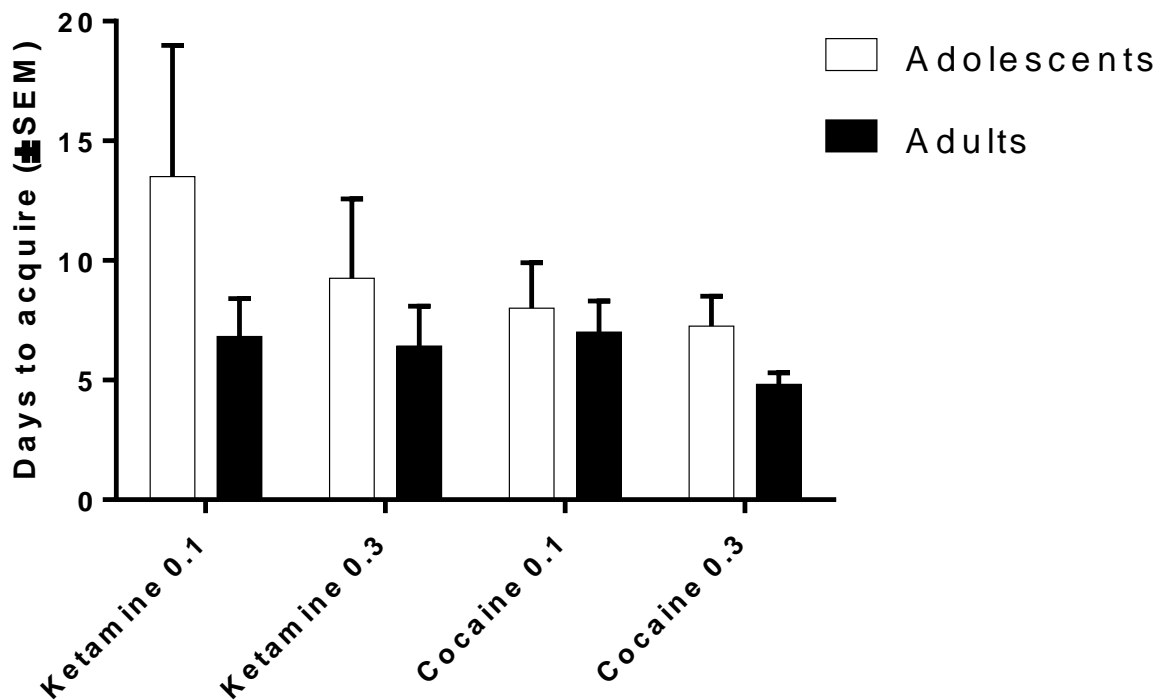


Figure 6. Rate of acquisition

Shown is a comparison of the rates of acquisition of self-administration behavior based on mean number of days to reach acquisition (\pm SEM) for adolescent subjects (white bars) and adult subjects (black bars) across different doses of ketamine and cocaine.

Break points under progressive ratio. Age did not play a significant factor in determining break points reached under a progressive ratio schedule across all doses of [F(1,28) = 0.03421, p=0.8546] and cocaine [F(1,28) = 1.18, p=0.2866]. The dose of 0.03 mg/kg/inf ketamine was not included in the analysis because no adolescent subjects met acquisition criteria and therefore did not qualify for testing under a progressive ratio schedule at this dose. Similarly, saline was not included in the analysis because only adult subjects were tested using saline. There was no significant difference in break points reached under a progressive ratio schedule between the doses of ketamine [F(1,28) = 0.02515, p=0.8751]. This was consistent between adolescent and adult subjects. There was also no significant interaction between age x drug dose for subjects responding for ketamine [F(1,28) = 0.386, p=0.5394]. Conversely both adolescents and adults reached significantly higher break points when self-administering 0.3 mg/kg/inf cocaine in comparison to break points reached for self-administered 0.1 mg/kg/inf cocaine [F(1,28) = 4.28, p=0.048]. There was no significant interaction between age x drug dose for breakpoints of subjects self-administering cocaine [F(1,28) = 0.01393, p=0.906].

Progressive Ratio

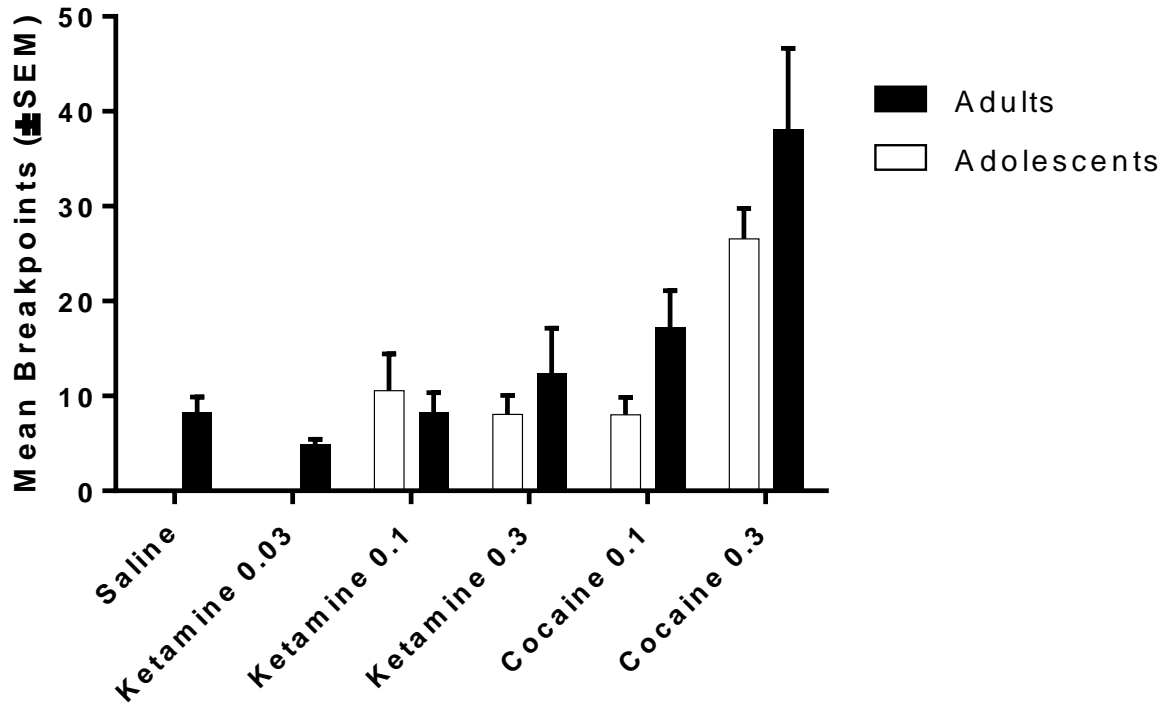


Figure 7. Mean Breakpoints

Mean break points (\pm SEM) achieved during testing with saline, ketamine, and cocaine under a PR schedule of reinforcement are presented grouped by dose.

Adolescent subjects (white bars) were not tested under a PR schedule for saline and the low dose of ketamine. Adult subjects are represented by the black bars.

Discussion

Place Conditioning

The purpose of performing this assay was to determine if there was a difference in the rewarding effects of ketamine based on age. This was accomplished by assessing what, if any, dose of our test drugs would induce place preference in adolescent subjects and contrasting these results with the same assay performed in adults. Adolescents trended towards preferences for the drug-paired chamber at the 1 mg/kg and 3 mg/kg doses of ketamine administered as shown by an increase in time spent in the drug-paired chamber following conditioning (Figure 3). While these values were not significantly different from baseline, this is likely due to the fact that the adolescent rats exhibited a high degree of variability which decreased statistical power. This trend is different from the results for place conditioning in the adult subjects where either no preference or aversion developed across the same ketamine doses. The addition of subjects to both the adolescent and adult groups may reduce variance and strengthen the comparison of the divergent trends between the two age groups. To date, there is no literature available on ketamine, or any similar drugs in its class such as PCP, being evaluated in a place conditioning assay during the adolescent period. Age-related effects have been noted in studies of place conditioning with drugs from other pharmacological classes such as nicotine and alcohol. Nicotine induced place preference in young adolescence (PND27-30), but not in late adolescence (PND38-41), nor in adulthood (PND90-93) in studies conducted in rats. These data suggest that young adolescents may have a higher sensitivity to the rewarding effects of nicotine in comparison to older subjects. In that study no subjects exhibited any

development of aversion at the doses tested (Belluzi, 2004). In contrast, adult subjects developed a preference for alcohol where adolescent subjects (PND28) did not develop a preference at any dose (Song, Wang, Zhao, Wang, Zhai, & Lu, 2007). However, Song's alcohol study used a three-chamber apparatus in Kunming mice. While differences can depend on species and strain, it has been shown that using a two-chamber apparatus can reduce variability in adolescent subjects due their inherent increased novelty-seeking and exploratory behaviors in comparison to adult subjects (Stansfield & Kirstein, 2006). Thus, the novelty of the third chamber may have confounded the results in the adolescent subjects in the Song et al study (2007). In the current study, adolescent subjects also exhibited a slight but not significant aversion to the 10 mg/kg dose of ketamine. However the aversion displayed at the high dose of ketamine in the adolescents was not as robust as the aversion displayed by the adult subjects at the 3 mg/kg dose. Overall the results suggest that adolescents are more sensitive to the rewarding effects of ketamine and more tolerant to its aversive effects in comparison to adult subjects.

Ketamine has been shown to induce place preference in adult rats in the literature; however the experimental design may influence the development of preference. Subjects in this experiment were conditioned twice daily for 4 consecutive days. A dose of 10 mg/kg ketamine has been shown to induce significant preference in adult rats; however, only when administered every 48 hours. This experiment conditioned subjects once per day for 8 consecutive days, alternating drug administration with saline administration (Xu, Mo, Yung, Yang, & Leung, 2006). Certain drugs that have a long action of duration could explain the

lack of place preference, such as buprenorphine. When tested at doses of 3.16 and 10mg/kg, no place preference was induced by the drug until a 1- and 2-day delay was inserted between conditioning sessions. It was hypothesized that the drug effects were carrying over into the vehicle sessions due to the long duration action and slow kinetics of the drug (Tzschentke, 2004). While ketamine is rapidly eliminated, there may be a continuing effect due to altered neurotransmission. A single dose of ketamine in humans can cause significant impairments in memory and cognition days after the drug has been eliminated (Curran & Morgan, 2000). Clinically, ketamine has long been used as a treatment for post-operative pain (Ito & Ichiyonagi, 1974), and more recently as a treatment for chronic pain (Niesters, Martini, & Dahan, 2013; Bredlau, et al., 2013) and depression (Zarate, Singh, Carlson, Brutsche, Ameli, & Luckenbaugh, 2006; Preskorn, Baker, Kolluri, Menniti, Krams, & Landen, 2008; Liebrez, Borgeat, Leisinger, & Stohler, 2007). Patients report lasting effects from a single dose of ketamine, greater than a week. This has also been replicated in animal models with ketamine-induced behavioral phenotypes persisting up to 10 days in the forced-swim and passive avoidance tests (Chatterjee, Ganguly, Srivastava, & Palit, 2011). The lingering behavioral effects of ketamine may offer an explanation of the discrepant results presented in the current study.

Alternatively, looking at drugs with similar durations of action and elimination time courses may help clarify the unique results presented in this study. By manipulating the length of the conditioning session as well as the pretreatment time, cocaine has been shown to induce place preference, place aversion, and no effect at all. A low dose of 1.25 mg/kg given immediately before a 1 hour conditioning

session had no effect at all. However, this same dose given immediately before a 15-minute conditioning session induced place preference. In contrast, the same dose given with a 15-minute delay prior to a 1 hour conditioning session resulted in the development of place aversion. It was hypothesized by the authors that the onset and elimination of cocaine occurring during the 1 hour session prevented any positive or negative associations. The 15-minute session would only be associated with the onset of drug action, usually perceived as an euphoric event. The protocol with the 15-minute delay prior to the 1 hour session would associate the conditioning chamber with the elimination of drug, or an acute withdrawal effect. Cocaine withdrawal symptoms can create a depression-like state and dysphoria in the subject (Pliakas, Carlson, Neve, Konradi, Nestler, & Carlezon Jr., 2001; Ettenberg, Raven, Danluck, & Necessary, 1999). However, in the current study the conditioning sessions were only 20 minutes in duration, and cocaine was also tested in adult subjects who exhibited significant preferences. Therefore, it is unlikely that the session length was the cause for ketamine aversion as cocaine and ketamine have very similar pharmacokinetics.

There is no published literature in laboratory animals that provides evidence of ketamine-induced conditioned aversion. However, conditioned place preference and place aversion have been demonstrated with PCP in adult rats. Indeed it requires specific methodological manipulations in order to avoid production of conditioned place aversion and induction of place preference. Subjects chronically pretreated with PCP for 14 or 28 days developed preferences for PCP in accordance with their length of pretreatment; that is, subjects who were pretreated for 28 days

displayed more robust preferences than subjects who were pretreated for 14 days. In this same study, subjects that were given no pretreatment developed a significant aversion to PCP. Several other studies have shown place avoidance induced by PCP as well. (Kitaichi, Noda, Hasegawa, Furukawa, & Nabeshima, 1996; Miyamoto, Noda, Komori, Sugihara, Furukawa, & Nabeshima, 2000; Acquas, Carboni, Garau, & Di Chiara, 1990). While it can be demonstrated that drugs from the same class do not consistently produce place preference and can often be aversive, it does not explain the disparity between adolescent and adult subjects.

Although a complete discussion of the neurobiology of the adolescent brain is beyond the scope of this thesis, there are hypotheses that offer further explanation of the differential behavioral effects seen in the place conditioning procedure. The NMDA receptor is comprised of four subunit proteins. The vast majority of NMDA receptors in the brain include two NR1 subunits combined with two NR2 subunits (Laube, Kuhse, & Betz, 1998) (Lynch, et al., 2001). Expression levels of the four different types of NR2 subunits (A, B, C and D), particularly NR2A and NR2B, have been shown to go through extensive changes during development (Landwehrmeyer, Standaert, Testa, Penny, & Young, 1995; Monyer, et al., 1992). Therefore it might be possible that changes in the composition of NMDA receptors could explain the difference in the effects between the two age populations. At birth, the NR2B is the predominant subunit, with NR2A expression weak and confined to few areas of the brain. Within a few days after birth NR2C activity emerges and over time NR2A expression begins to outnumber NR2B. While the time course for these neurological changes has not been completely determined, the ratio of NR2A to NR2B receptors

was comparable to adult levels of expression within 21 days after birth (Wenzel, Fritschy, Mohler, & Benke, 1997) (Liu, Murray, & Jones, 2004). While this is considered to be early adolescence in the male rat it is outside the age range of all behavioral data presented in this thesis. Therefore, it would appear unlikely that developmental changes in NMDA receptors could account for the age-related differences.

A final point of discussion that may elucidate a mechanism for the differences between the adults and adolescents is the activity of ketamine at other receptors and binding sites. Ketamine has been shown to bind to sigma-receptors and kappa-opioid receptors (Smith, et al., 1987). Activation of sigma- and kappa-receptors has been shown to play a role in drug-induced aversion in animal subjects (Mori, et al., 2012). Similar to the ketamine data presented in this paper, kappa-agonists administered to adult rats have also been shown to induce place-aversion (Suzuki, Shiozaki, Masukawa, Misawa, & Nagase, 1992). Discrimination studies have shown that, although not from the same drug class, ketamine will dose-dependently substitute for kappa-agonists, but only fully substitute at doses that cause locomotor suppression (Mori, et al., 2006). The authors hypothesized that an interoceptive cue of drugs activating the sigma- and/or kappa-opioid receptors has an aversive component and this aversion plays a role in the discriminative stimulus of these drugs. While the developmental changes of the opioid receptor system during adolescence are not well-characterized, it is possible that physiological differences in the opioid receptor system in the adolescent subjects could contribute to tolerance to the aversive effects of ketamine.

Self-Administration

The purpose of performing this assay was to determine if there was a difference in the reinforcing effects of ketamine based on age. This was accomplished by determining differences, if any, in the rate acquisition of self-administration behavior as well as the percent of subjects reaching acquisition criteria between adolescent and adult rats. Subjects that met stable acquisition criteria were then tested under a progressive ratio schedule of reinforcement to assess the differences in the reinforcing efficacy of ketamine and cocaine between adolescent and adult rats. There was no effect of age in the percentage of subjects reaching self-administration acquisition criteria under either ketamine or cocaine availability. There was however an effect based on the drug dose within each age group for ketamine. The adolescent subjects appeared to only acquire self-administration behavior at higher doses of ketamine than the adults; however, this conclusion is tentative. While the percentage of adult subjects to reach acquisition criteria at the 0.03 mg/kg/inf ketamine dose was the lowest percentage of all the adult groups tested, to date only 2 adolescent subjects have been tested for acquisition under this low dose of ketamine. It is plausible to think that adolescent subjects may ultimately demonstrate an equal or greater sensitivity to the rewarding characteristics of ketamine once a greater number of subjects have been evaluated.

A component of stable acquisition is the subject emitting greater responses on the active lever than the inactive lever. This component may be confounding when testing adolescent subjects. Impulsive action has been recognized as a determinant as well as consequence of drug abuse (de Wit, 2008). Greater

impulsivity and lack of inhibition has been specifically linked with polysubstance abusers who have specifically used 'club drugs' frequently in the past (Verdejo-Garcia, 2010). Impulsivity is often measured in operant assays as the portion of non-contingent responses emitted by the subject. While adolescents are well-characterized as having greater impulsivity in comparison to adult rats, many studies of self-administration demonstrate adolescent subjects responding more on the active lever than the inactive lever (Anker, 2012). The non-contingent responding on the inactive lever has been interpreted by the authors as drug-seeking behavior. Additionally, many experiments studying adolescent self-administration implement lever-training and food restriction prior to self-administration; this promotes greater responding on the active lever as opposed to the inactive lever (Burton, 2010; Harvey, 2009). This higher degree of impulsivity needs to be further investigated as a possible reason for the greater amount of indiscriminate lever pressing that was seen in several of the adolescent subjects.

To analyze the rate of acquisition, the average number of days required for a subject to meet acquisition criteria was compared across age groups and drug doses. There was a significant effect of age for the rate of acquisition across subjects self-administering ketamine and subjects self-administering cocaine (Figure 6). This means that consistently across all doses of drugs tested, ketamine and cocaine, adult subjects reached the acquisition criteria faster than adolescent subjects. Conversely there was no effect across drug doses, meaning that the rate of acquisition was independent of the dose of the drug being self-administered. The finding regarding the effect of age is unique thus far. Other studies comparing rates

of acquisition of self-administration of cocaine (Harvey, 2009), heroin (Doherty & Frantz, 2012), and methamphetamine (Anker, 2012), displayed no significant differences between adolescent and adult subjects in the rate of acquisition across sessions. There are no studies available in the literature evaluating the rates of acquisition of self-administration between adult and adolescent subjects for ketamine or any drugs within its class to provide additional comparisons.

Progressive ratio schedule of reinforcement. A progressive ratio schedule of reinforcement was tested to assess if there would be differences in the relative reinforcing efficacy of ketamine and cocaine based on age. A significant effect was seen for drug dose, with the highest dose of cocaine, 0.3 mg/kg/inf, having a significantly higher break point in compared to the other doses tested. Adult subjects displayed a dose-dependent increasing trend in break points for both ketamine and cocaine, with higher doses eliciting higher break points. Both adult and adolescent subjects exhibited higher break points administering 0.3 mg/kg/inf cocaine in comparison to break points achieved at a dose of 0.1 mg/kg/inf of cocaine. Age did not influence break points reached, as neither age group, adults nor adolescents, significantly differed from one another in their progressive ratio breakpoints at any doses tested of ketamine or cocaine (Figure 7). However, there were not sufficient numbers of adolescent subjects tested under the progressive ratio with other doses of ketamine and cocaine to draw a sound conclusion on their behavioral patterns. Furthermore, a lack of difference in responding based on age is supported by the literature on rats self-administering heroin in that there was no significant difference in the breakpoints reached by adolescent subjects in

comparison to adult subjects self-administering the same dose (Doherty & Frantz, 2012).

Conclusion

The overarching goal of the present study was to investigate age-related differences in the acute abuse-related behavioral effects of ketamine. Differences noted between adolescent and adult subjects in the place-conditioning assay support the hypothesis that adolescents exhibit a greater tolerance to the aversive properties and greater sensitivity to the rewarding effects of abused drugs. Specifically in regards to ketamine, the CPP data suggest that there is an age-dependent factor in the risk for abuse. Results from the self-administration assay also suggested age-related factors in the rate of acquisition of ketamine self-administration behavior, with adults reaching acquisition criteria faster than adolescents. While this might seem protective in terms of vulnerability to abuse, additional testing of adolescent subjects will be required to verify this difference. The prolonged acquisition rates observed with both ketamine and cocaine in the adolescent group suggests that this may be an outcome dependent on learning or mechanical issues between the age groups. Overall, there appear to be disparate results between the two abuse-related behavioral assays supporting important differences in the effects being measured. Adult subjects found ketamine to be reinforcing; whereas, in the context of place conditioning, where the drug is administered in a bolus dose by the experimenter, the adult subjects demonstrated aversion to the drug-paired environment. The mechanisms of self-administration

that allow subjects to titrate their dose may play a more integral role in the reinforcing effects of the drug. It has been noted that self-administered doses of cocaine cause a greater and more persistent increase in dopaminergic transmission than non-contingent administrations of cocaine (Miguéns, et al., 2008; Bardo, 2000). However, the divergent results cannot be credited solely to these procedures, as cocaine was found to have hedonic properties in both age groups in both paradigms. This suggests that ketamine has unique characteristics in comparison to other drugs of abuse and that these characteristics may have age-dependent mechanisms that may induce different behaviors in different age groups.

Future Directions

While the results in the conditioned place preference experiment suggest that adolescent subjects have a greater sensitivity to the rewarding effects and a greater tolerance for the aversive effects of ketamine, the variability between subjects prevented statistical support for this conclusion. Increasing the number of subjects in the place conditioning assay would add power and strengthen the conclusions drawn from the study. The trending data of adolescents' preference development and the adults' development of aversion could be examined further by evaluating differences in anhedonia or dysphoria between adolescent and adult subjects. There is evidence to support that, in addition to greater tolerance to the aversive effects of drugs, adolescents display significantly fewer negative responses to aversive stimuli, such as quinine (Wilmouth & Spear, 2009). While relative aversion to quinine can be dependent on the development of taste receptors and the gustatory system, it would be interesting to use additional models of anhedonic behavior to measure aversion

to ketamine's effects in these two populations.

The data presented for the acquisition of self-administration behavior did not show any significant effects for the percentage of subjects reaching acquisition based on age. Moving forward, there are additional methodological considerations that could be implemented to help maximize the testing potential of adolescents. Priming and lever-training implementation have been shown to improve adolescent acquisition rates in animals responding to cocaine (Lynch W. , 2008; Burton, 2010; Harvey, 2009). No techniques were implemented to promote self-administration behavior in the present study. In fact, many aspects of this study such as no dietary restriction, no priming, and no lever training are all factors that can hinder self-administration behavior. This is beneficial in many adult studies to preserve the reinforcing effects of a drug and to avoid stress-induced behaviors. In an attempt to elucidate differences between adults and adolescents and maximize the data collected during the short time frame of adolescence, instituting methodological changes to facilitate self-administration behavior could be considered. Additionally, analyzing within session patterns of responding could reveal escalation or multi-modal effects of ketamine due to its short duration of action. These data have not been analyzed, but could reveal age-related differences due to adolescents' higher tolerance for aversive effects.

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