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Ketamine Pre-Exposure Does Not Influence Later-Life Responses To Reward-Related Stimuli In Female C57bl/6 Mice

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KETAMINE PRE-EXPOSURE DOES NOT INFLUENCE LATER-LIFE RESPONSES TO REWARD-RELATED STIMULI IN FEMALE C57BL/6 MICE

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Dedication

To those who were influential in my education.

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by

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Abstract

Preclinical work indicates that exposure to traditional antidepressant medications, in adolescent and adult female subjects, alters reward-related behavior later in life. In recent years, the anesthetic ketamine (KET), now used as a fast-acting antidepressant, has shown promising therapeutic efficacy for the management of depression. However, the potential long-term behavioral consequences of KET exposure across development have not been thoroughly assessed. Thus, to address this issue, we examined if KET exposure, during adolescence or early adulthood, results in enduring alterations in responsivity to the rewarding properties of sucrose and cocaine later in life. Specifically, female C57BL/6 mice were randomly assigned to receive repeated intraperitoneal injections of KET (0 [vehicle; VEH] or 20 mg/kg) for 15 consecutive days during the adolescent (postnatal day [PD] 35-49), or early adult (PD70-84) stage of development. Twenty-one days after KET or VEH exposure, female mice (PD70+ or PD105+, respectively) were assessed on their reactivity to a sucrose solution (1%) adopting a two-bottle choice procedure, or cocaine (0, 5, or 10 mg/kg) using the conditioned place preference test, two well-established measures of reward-seeking behavior. We found that 21-days post KET exposure, female mice spent significantly higher time in the cocaine-paired chamber ($p<0.05$). However, KET pre-exposure, either during adolescence (PD35-49) or early adulthood (PD70-84), did not influence the preference magnitude for sucrose or cocaine 21-days later (PD70+ and PD105+, respectively). Collectively, our data suggest that exposure to KET does not induce long-term changes to reward-related stimuli, in female C57BL/6 mice.

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Chapter 1: Introduction

Major Depressive Disorder (**MDD**) is a prevalent and debilitating illness that leads to persistent changes in mood, behavior, sleep, and appetite (American Psychiatric Association, 2013). These hallmark syndromes must occur for two weeks or longer, having a direct negative impact on an individual's daily activities. According to the World Health Organization (**WHO**), MDD afflicts close to 300 million people across the globe, with 16 million Americans suffering from this disorder (2017). To make matters worse, MDD also has high comorbidity with other psychiatric illnesses such as anxiety (Kaufman & Charney, 2000) and substance use disorder (Brady & Verduin, 2005); thus, emphasizing the need for the development of better and more effective treatments.

Clinical and preclinical studies indicate that exposure to stress is a risk factor for the development of MDD (Nestler et al., 2002). Nevertheless, most of the research aimed to uncover the underpinnings of MDD has primarily incorporated adult male subjects. This is of concern, since neither stress exposure nor mood-related disorders are limited to adult males in human populations. Indeed, clinical reports indicate that MDD is frequently diagnosed in the adolescent population (Ghandour et al., 2019). According to the 2016 National Survey on Drug Use and Health (**NSDUH**), 3.2 million juveniles (ages 12-17) have experienced an episode of depression (2017). Interestingly, this report also found that adolescent females are more susceptible to MDD relative to their male counterparts (2017). Collectively, this suggests that females and youngsters are more vulnerable and susceptible to developing MDD in relation to adults and/or males.

A challenge for the field of psychiatry has been the lack of an effective treatment for MDD. The first line of management is the pharmaceutical prescription of antidepressant medications, such as selective serotonin reuptake inhibitors (**SSRIs**). SSRIs, like fluoxetine (i.e., Prozac)

increase the amount of serotonin in the brain by blocking the serotonin reuptake transporter at the presynaptic cleft (Leonard, 1993). Unfortunately, SSRIs take several weeks to induce their therapeutic effect (C. Zarate et al., 2013), and up to one-third of MDD patients fail to respond (Gaynes et al., 2009). These subsets of MDD patients are thus diagnosed with treatment-resistant depression, and as such, are in dire need of novel and/or alternative therapeutic approaches to manage their depressive symptoms – particularly because suicide is a constant risk (Sullivan et al., 2015).

Unlike traditional antidepressant medications, recent clinical work indicates that sub-anesthetic doses of ketamine (**KET**), a N-Methyl-D-aspartate receptor antagonist, mediates rapid and long-lasting antidepressant effects (Berman et al., 2000; Diazgranados et al., 2010; Murrough et al., 2013). These KET-induced fast-acting and persistent antidepressant properties are due to KET's ability to increase overall serotonin (Fukumoto, Iijima, & Chaki, 2016; Pham et al., 2017; Sial, Parise, Parise, Gnecco, & Bolanos-Guzman, 2020) and glutamate tone across several brain regions (Duman, 2018). Glutamate is the most abundant excitatory neurotransmitter found in the brain, playing a significant role in learning and memory processes, especially during the adolescent period (Sircar, Wu, Reddy, Sircar, & Basak, 2011). While there has been a significant amount of research examining KET's acute antidepressant effects (Duman, 2018; Murrough et al., 2013; C. Zarate et al., 2013; C. A. Zarate, Jr. et al., 2012), very little research has investigated whether KET history mediates long-lasting side effects (Bates & Trujillo, 2019; Featherstone, Nagy, Hahn, & Siegel, 2014; Parise et al., 2013). In male rats, adolescent and early adult exposure to KET results in long-term changes in reactivity to stress-inducing situations (Parise et al., 2013). Likewise, in female mice, adolescent and early adult exposure to the antidepressant fluoxetine decreases preference for drugs of abuse (Flores-Ramirez et al., 2018). Collectively, this growing body of

literature suggests that early-life psychotropic drug exposure – whether it is conventional (SSRI) or novel (KET) antidepressant medications – result in long-term side effects in later life. Thus, the goal of this thesis is to evaluate for potential long-term changes in reactivity to reward-related stimuli (sucrose and cocaine) in female C57BL/6 mice, as a result of KET pre-exposure during adolescence (PD35-49) or early adulthood (PD70-84).

Specific Aims of Master's Thesis:

Aim 1: Examine the enduring effects of ***adolescent*** ketamine exposure on behavioral responsivity to the rewarding properties of cocaine and sucrose in female C57BL/6 mice.

Aim 2: Examine the enduring effects of ***early adult*** ketamine exposure on behavioral responsivity to the rewarding properties of cocaine and sucrose in female C57BL/6 mice.

Hypotheses: Previous work in female C57BL/6 mice has shown that exposure to the traditional antidepressant fluoxetine, during adolescence or early adulthood, mediates a long-term decrease in preference for sucrose and cocaine (Flores-Ramirez et al., 2018). Thus, the aim of this Master's Thesis is to examine for potential long-lasting changes to reward-related stimuli as a function of exposure to the novel fast-acting antidepressant KET. Specifically, it is hypothesized that independent of the age (adolescence or early adulthood) of KET pre-exposure, female C57BL/6 mice will display an attenuated response to the rewarding effects of cocaine and sucrose in later life.

Chapter 2: Methods and Materials

2.1 Animals

Subjects used in this study were female C57BL/6 mice purchased from Charles River (Hollister, CA). Once the animals arrived to the laboratory, they were group-housed (3-4 per cage) in standard polypropylene cages containing wood shaving bedding (Sani-chip Teklad 7090; Madison, WI). Mice were maintained on a 12-hour light/dark cycle in a humidity and temperature-controlled room (21-23C^{o}) with access to food (Harlan Teklad 7912; Madison, WI) and water *ad libitum*. All experimental procedures were carried out in accordance with the Institutional Animal Care and Use Committee (Protocol A-201609-1) at the University of Texas at El Paso, and the guidelines set forth by the National Institutes of Health.

2.2 Drugs

Ketamine (KET)- and cocaine-hydrochloride were each diluted in 0.9% sterile saline (**SAL**) and administered via intraperitoneal (**IP**) injections at a volume of 2 ml/kg. KET was purchased from Spectrum Chemicals (Gardena, CA), and cocaine was purchased from Sigma Aldrich (St. Louis, MO).

2.3 Experimental design

To examine whether ketamine exposure results in enduring alterations in responsivity to reward-related stimuli, we conducted two experiments (see Fig. 1). In Experiment 1 (Aim 1 in Fig. 1A), mice were randomly assigned to receive IP injections of KET (0 [vehicle; **VEH**] or 20 mg/kg) for 15 consecutive days during adolescence (postnatal day [**PD**] 35-49). After KET or VEH exposure, mice were left undisturbed in their home-cage for a 21-day washout period until mice

reached adulthood (PD70). At this point, KET or VEH pre-exposed mice were assessed on their behavioral responsivity to a sucrose solution (1%) adopting a two-bottle choice procedure, or cocaine (0, 5, or 10 mg/kg) using the conditioned place preference (CPP) approach (see section 2.4 for details). Our rationale for exposing mice to KET from PD35-49 is because this time-frame approximately aligns with adolescence in humans (Spear, 2000). Comparably, the sub-anesthetic KET dose (20 mg/kg/day) was selected because it yields significant effects on depression-related behaviors in both adolescent (Parise et al., 2013) and adult (Iñiguez et al., 2018) rodents. Furthermore, to investigate whether KET induces long-lasting changes in reward-related stimuli, as a function of early adult KET exposure, we conducted a separate, yet similar, experiment using adult (PD70) mice. Specifically, Experiment 2 (Aim 2 in Fig. 1B) investigated if KET exposure during early adulthood (PD70-84) resulted in altered sucrose or cocaine reactivity 21-days after KET exposure (PD105+). Separate groups of animals were used across all experiments in order to avoid potential testing carryover effects (see Table 1 for list of experimental groups). A video tracking system (EthovisionXT; Noldus, Leesburg, VA) was used to record the conditioned place preference behavioral responses.

2.4 Conditioned place preference (CPP)

Conditioned Place Preference is a common behavioral procedure used in basic research to evaluate the motivational and aversive properties of drugs of abuse (Tzschentke, 2007). This well-established paradigm involves repeated pairings between a context (i.e. side of CPP compartment) and drug stimulus (i.e. cocaine), in turn, this learning association results in preference or aversion for a context previously paired with a drug (Bardo & Bevins, 2000). If an animal displays preference for the drug-paired chamber instead of the VEH-paired chamber, then conditioning has

occurred (Iniguez et al., 2008; Iñiguez, Warren, Neve, et al., 2010). Quite the reverse, if an animal spends more time in the VEH-paired chamber over the drug-paired chamber, then aversion has taken place (Tzschentke, 2007). Notably, CPP entails two different behavioral designs (biased or unbiased). In the biased design, the animal's natural preference to either compartment is assessed prior to conditioning sessions. As such, the compartment least preferred is paired with a drug stimulus during conditioning. Conversely, the unbiased method involves the researcher's decision to select and assign the compartment where an animal will receive VEH-parings and drug-pairings (Tzschentke, 2007).

In the present study, CPP was carried out using the biased method and a three-compartment apparatus (see Fig. 2 for a schematic of the testing chamber) – in which each compartment differs in both visual (wall pattern) and tactile cues (floor texture). The CPP procedure is comprised of three separate phases (preconditioning, conditioning, and test day), across six days. We adhered to the protocol that has been previously published by our laboratory (Iniguez et al., 2008). Specifically, on the preconditioning phase (day 1), mice were granted access to freely explore the entire apparatus for 25 minutes – in order to obtain a baseline preference to any of the three separate compartments (side compartments: 23 × 16 × 36 cm; middle compartment: 9 × 16 × 36 cm, L × W × H). During the conditioning phase, mice received an IP SAL injection (2 ml/kg) and placed in the compartment where the animal spent more than 50% of the time during the preconditioning phase (i.e., preferred compartment) for 25 min. Three hours later, mice received an injection of cocaine (0, 5, or 10 mg/kg, IP), however, in this case, they were placed and confined on the opposite (non-preferred) compartment for 25 min. This procedure was repeated across four consecutive days (days 2-5). Lastly, on test day (day 6), mice were allowed to freely explore the entire apparatus for 25 minutes, and we recorded the amount of time (sec) the animal spent in the

cocaine-paired compartment. The dependent variable was a preference score, in which, we subtracted the amount of time spent in the cocaine-paired compartment during test day (day 6) from the amount of time spent on the same compartment during preconditioning day (day 1). As such, a positive score displays preference for the compartment previously paired with cocaine whereas as a negative score indicates avoidance to the compartment previously paired with cocaine (Flores-Ramirez et al., 2018).

2.5 Sucrose preference test (SPT)

The sucrose preference test is used to assess hedonic responses to natural rewards in rodents (Iñiguez et al., 2014; Wallace et al., 2008; Willner, Towell, Sampson, Sophokleous, & Muscat, 1987). This test is sensitive and can produce reliable results by taking advantage of rodents' innate preference of consuming sweetened solutions over water (Liu et al., 2018). The sucrose preference test is also commonly used to assess stress-induced anhedonia, a core symptom of depression in humans, which is defined as the inability (or reduction) of experiencing pleasurable stimuli (Der-Avakian & Markou, 2012). Noteworthy, rodents exposed to chronic stress-based models results in reduced sucrose preference whereas repeated treatment of antidepressant medications (i.e. fluoxetine) during chronic stress exposure ameliorates this stress-induced consequence (Liu et al., 2018).

Here, the SPT consisted of a 2-bottle procedure where mice were given the choice between consuming water or a 1% sucrose solution on a single-day procedure (Iñiguez et al., 2014). Specifically, 21-days after KET pretreatment, mice were habituated to drink water from two separate bottles for five days in their home-cage (see schematic in Fig. 3). Twenty-four hours later, one of the bottles was changed with a 1% sucrose solution while the other bottle contained water.

The placement of the water bottles was counterbalanced (right vs. left) across cages to avoid bottle choice/side preference bias. The dependent variable was liquid intake, where preference for sucrose over water (sucrose/[sucrose + water]) was used as a measure for changes in reward responsiveness (Flores-Ramirez et al., 2018; Iñiguez, Warren, & Bolaños-Guzmán, 2010).

2.6 Statistics

Analysis of Variance (ANOVA) techniques were adopted to analyze the data, with ketamine (between measure), cocaine (between measure), and day of antidepressant exposure (repeated measure for body weight) as sources of variance. Bonferroni post-hoc tests were used to examine all pairwise comparisons. Two-tailed Student's t-test's were carried out to analyze the data from the sucrose preference test. Data is expressed as the mean ± standard error of the mean (SEM), and statistical significance was defined as $p<0.05$.

Chapter 3: Results

3.1 Adolescent KET exposure does not influence cocaine preference in adult mice

Figure 4A displays the effects of juvenile KET exposure on cocaine CPP in adult female mice (PD70+, N=60). Here, a 2-way ANOVA (with KET and cocaine as sources of variance) indicated that preference scores varied as a function of cocaine in adulthood (post-treatment main effect: $F_{2,54}$=59.59, p<0.05), but not as a function of juvenile KET history (pretreatment main effect: p>0.05). Specifically, VEH (n=10) or KET (n=10) pretreatment did not influence preference for any of the compartments when mice were conditioned to SAL (p>0.05). However, VEH-pretreated mice conditioned to 5 (n=10) or 10 mg/kg (n=10) cocaine displayed reliable preference scores to the drug-paired compartment, when compared to VEH-pretreated/SAL-conditioned mice ($^{\beta}p$<0.05). Likewise, KET-pretreated mice conditioned to 5 (n=10) or 10 (n=10) mg/kg cocaine exhibited consistent preference scores to the drug-paired compartment, when compared to KET-pretreated/SAL-conditioned mice (p<0.05, respectively). However, planned comparisons indicted that no differences in preference scores were evident between VEH- and KET- pretreated mice receiving the same doses of cocaine (p>0.05, respectively). No differences in distance traveled (cm) as a function of adolescent KET pretreatment were noted between the groups (Fig. 4B) during the preconditioning phase (p>0.05).

3.2 Adolescent KET exposure does not alter sucrose preference in adult mice

Figure 4C indicates adolescent KET pretreatment does not alter preference for a 1% sucrose solution in adult female mice (p>0.05; n=10 per group). Similarly, no differences in total liquid intake (water + sucrose) between VEH- and KET- pretreated mice were noted (p>0.05; Fig. 4D).

3.3 Adult KET pre-exposure does not alter cocaine preference in later life.

Figure 5A displays the enduring effects of adult KET pretreatment (PD70-84) on cocaine CPP in female mice (PD105+). Here, a 2-way ANOVA (with KET and cocaine as sources of variance) revealed preference scores were influenced by cocaine (post-treatment main effect: $F_{2,54}$=44.44, p<0.05). Specifically, KET (n=10) or VEH (n=10) pretreatment did not influence preference for any of the compartments conditioned to SAL *(*p>0.05). Yet, VEH-pretreated mice conditioned to 5 (n=10) or 10 mg/kg (n=10) cocaine displayed reliable preference scores when compared to VEH-pretreated/SAL-conditioned mice ($^{\omega}p$<0.05). Similarly, KET-pretreated female mice displayed preference for environments paired to 5 (n=10) or 10 mg/kg cocaine (n=10), when compared to KET-pretreated/SAL-conditioned mice (p<0.05, respectively). Further planned comparisons indicated no differences in preference scores were evident between VEH- and KET-pretreated mice receiving the same doses of cocaine (p>0.05, respectively). No differences in distance traveled (cm) during the preconditioning phase were observed between groups, as a function of KET pretreatment (p>0.05; Fig. 5B).

3.4 Adult KET pre-exposure does not alter sucrose preference in later life.

Figure 5C demonstrates adult KET pretreatment (PD70-84) does not alter preference for 1% sucrose solution (p>0.05; n=10 per group). Similarly, no differences in total liquid intake (water+sucrose) between VEH- and KET- pretreated female mice were noted (p>0.05; Fig. 5D).

3.5 Acute and long-term effects of adolescent or adult KET exposure on body weight

Figure 6A exhibits the acute (PD35-49) and long-term (PD70) effects of KET treatment during adolescence on body weight (g). A mixed-design repeated measures ANOVA uncovered that body weight was influenced by KET treatment (between measure: $F_{(1,78)=}$ 20.53, p<0.05), day of antidepressant exposure (repeated measure: $F_{(14,1092)}$= 252.0, p<0.05), as well as their interaction ($F_{(14,1092)}$= 6.02, p<0.05). Post-hoc analyses uncovered that when compared to VEH controls (n=40), KET (n=40) lowered body weight as of the second day of treatment, which remained throughout KET exposure (PD36-PD49; p<0.5, respectively). Twenty-one days later, PD70 mice pre-exposed with KET during adolescence still displayed a reduction in body weight, when compared to controls (p<0.5).

Figure 6B demonstrates the effects of ketamine exposure during early adulthood (PD70-84) on body weight (g). A mixed-design repeated measures ANOVA uncovered that body weight was influenced by day of antidepressant treatment (repeated measure: $F_{(14,1092)}$= 13.72, p<0.05), but not KET exposure (between measure main effect: $F_{(1,78)=}$ 1.72, p>0.05), or their interaction (KET × day of KET exposure; $F_{(14,1092)}$= 1.35 p>0.05). Twenty-one days after KET pretreatment (PD105), no differences in body weight were noted between the groups (p>0.5).

Chapter 4: Discussion

4.1 Summary

The goal of this thesis was to evaluate whether long-term decreases in reward-related behavior would be evident in female C57BL/6 mice as a function of exposure to the fast-acting antidepressant KET. We adopted this approach because previous work in female mice has demonstrated that exposure to the SSRI antidepressant fluoxetine, during adolescence or early adulthood, mediates a long-lasting decrease in preference for sucrose and cocaine (Flores-Ramirez et al., 2018). While KET's primary mechanism of action is to block NMDA receptors (Duman, Aghajanian, Sanacora, & Krystal, 2016; Krystal, Sanacora, & Duman, 2013), like fluoxetine, it also indirectly increases serotonin tone (Fukumoto et al., 2016; Pham et al., 2017; Sial et al., 2020). Therefore, it is possible that similar persistent behavioral outcomes, on reward-related behavior, may be observed as a function of KET pre-exposure. Specifically, we hypothesized that independent of age (adolescence or early adulthood) of KET pretreatment, female mice would display attenuated preference for rewards on the SPT and cocaine CPP paradigms, 21-days after exposure (i.e., a rightward shift on KET's dose response curve).

4.2 Enduring Effects of KET Pre-exposure in female mice

The CPP findings revealed that independent of juvenile KET pretreatment (PD35-49), adult (PD70+) female mice exhibited a preference for the compartment previously paired with cocaine (Fig. 4A), without alterations in general locomotor activity (Fig. 4B). However, the magnitude of CPP scores between the KET and VEH pretreated groups did not differ when animals received the same dose of cocaine (Fig. 4A), indicating that KET pre-exposure did not alter the incentive reward valence for cocaine differently than controls in adulthood. Similarly, no

differences in sucrose preference (Fig. 4C) or total liquid intake were observed between the groups, as a function of KET pretreatment (Fig. 4D). Together, the results from Aim 1 indicate that adolescent exposure to KET does not alter preferences for sucrose or cocaine in adult female mice. These null results were surprising because previous investigations, in female rodents specifically, have shown that early-life exposure to antidepressants (fluoxetine or KET) result in a persistent decrease to the rewarding properties of sucrose, as well as to drugs of abuse like cocaine or ethanol (Flores-Ramirez et al., 2018; Franco et al., 2020). The inconsistent results between the studies could be attributed to differences in the type/class of antidepressant drug evaluated (SSRI vs. fast-acting). For instance, Flores-Ramirez and colleagues exposed female mice to fluoxetine during adolescence [PD35-49; (Flores-Ramirez et al., 2018)], whereas in the present investigation female mice were exposed to the novel fast acting antidepressant KET during the same window of development (PD35-49). Thus, the long-lived fluoxetine-induced decreases on reward preference that Flores-Ramirez and his colleagues reported may be the result of the primary mechanism of action of the antidepressant medication used (i.e., fluoxetine is a SSRI, while KET is a NMDA open channel receptor antagonist). Additional reasons for the discrepancy between the studies may be due to (1) the age window of antidepressant exposure, (2) the drug used to assess changes in reward valence, and/or (3) the animal species used. For example, the laboratory of Dr. Arturo Zavala recently reported that female rats exposed to KET during the prepubertal stage of development (PD21-30) displayed decreases for the rewarding effects of ethanol when these rats reached adolescence (PD31+; Franco et al., 2020). On the other hand, here, we exposed adolescent female mice to KET between PD35-49 (adolescence) and assessed for changes to the rewarding properties of cocaine on PD70+ (adulthood). Also, it should be noted that these previous studies, as well as the work in this thesis, assessed for potential long-term effects of antidepressant

exposure to reward-related stimuli at a single age time point (i.e., PD31+ vs. PD70+, respectively); therefore, it is possible that potential enduring neurobehavioral effects, as result of repeated KET exposure during adolescence, may occur before or after the selected age of behavioral testing in our investigation (i.e., PD70). Because alterations in reward reactivity may be dependent on the age of KET pre-exposure (prepubertal, juvenile, or adulthood), we further evaluated whether KET exposure in early adulthood (PD70-84) would induce change in responses to cocaine and sucrose 21-days post treatment (PD105+; see Fig. 1B).

In Aim 2, adult mice were exposed to KET for 15 consecutive days (PD70-84) and were evaluated on reactivity to cocaine or sucrose 21-days later (PD105+). Similar to the results of Aim 1, we found that PD105+ mice, independent of KET pre-treatment, displayed reliable cocaine CPP (Fig. 5A), without alterations in general locomotor activity (Fig. 5B). However, the preference scores between the KET and VEH pretreated groups did not differ within the different doses of cocaine used, suggesting that KET history does not influence the incentive motivation valence of cocaine in later adulthood. Similarly, no differences in sucrose preference (Fig. 5C) or liquid intake (Fig. 5D) were observed as a function of KET pre-exposure. In general, the data from Aim 2 suggest that KET does not alter responsivity to drug (cocaine) or non-drug (sucrose) rewards 21-days after its exposure in adult female mice (PD105+). These null findings were also unexpected, given that previous published work has demonstrated that KET exposure, in adult rats, results in a decrease in sensitivity to inescapable stressors two months later. Specifically, Parise and his co-workers found that PD149 male rats pre-exposed to KET during adulthood (PD75-89), displayed decreases in immobility on the forced swim test (Parise et al., 2013), a traditional behavioral test used to assess pharmacological antidepressant efficacy in rodents (Castagne, Moser, Roux, & Porsolt, 2010; Iñiguez et al., 2019; Iñiguez, Warren, & Bolaños-Guzmán, 2010; Porsolt, Le Pichon,

& Jalfre, 1977). Decreases in immobility on the forced swim test are indicative of an antidepressant-like behavioral response, thus, suggesting that KET mediates long-lasting prophylactic effects that last up to two-months after treatment. Given the positive relationship between stress and drug-seeking behavior (Cleck & Blendy, 2008; Miczek, Covington, Nikulina, & Hammer, 2004; Piazza & Le Moal, 1998; Sinha, 2008), as well recent clinical work suggesting that KET may potentially be used as a treatment for addiction (Krupitsky & Grinenko, 1997; Witkin et al., 2020), we expected decreases in cocaine and/or sucrose preference 21-days post KET exposure – however, no such effect was observed in this study. Importantly, it should be noted that Parise et al., (2013) conducted their experiments in male rats, and thus, the present null findings in female mice could suggest that males are at higher risk of displaying persistent KET-induced side effects, when compared to females. This is plausible given that a recent meta-analysis found that adult males are more responsive to the acute antidepressant effects of KET, when compared to females (Coyle & Laws, 2015), and thus highlighting that males, but not females, are potentially more likely to display persistent side effects.

4.3 Sex-specific Effects of KET Pre-exposure

The null results in female mice from Aim 1 and Aim 2 indicate that KET history (during adolescence or early adulthood) does not mediate long-term changes for reward-related stimuli. Notably, recent data from our laboratory uncovered that adolescent KET-pretreatment (PD35-49) enhances preference for environments paired with cocaine in adult (PD70+) male mice (Garcia-Carachure et al., 2020). This male-specific effect may be attributed to the brain's monoamine systems, since acute KET administration (i.e., 10-50 mg/kg) increases dopamine levels in the prefrontal cortex, nucleus accumbens, and striatum, while increasing the firing of dopaminergic

neurons within the ventral tegmental area (Kokkinou, Ashok, & Howes, 2018) – brain regions that modulate responses to drug and non-drug rewards. Thus, in male mice, repeated exposure to KET during adolescence may result in persistent and sex-specific changes within the dopamine system, altering reward-seeking behavior in adulthood (Cooper, Francis, Al-Naser, & Barber, 1992; Shinohara, Kamii, Minami, & Kaneda, 2017). Supporting this hypothesis, human recreational KET users display increases in dopamine receptor 1 (DR1) within the medial prefrontal cortex (Narendran et al., 2005), highlighting a potential neurobiological mechanism by which repeated early-life exposure to KET may increase preference for cocaine in later life. Indeed, the expression of cocaine CPP is modulated by DR1 antagonism within this brain region in male rodents (Shinohara et al., 2017), thus it is possible that juvenile KET history may result in altered DR1 expression in a male-specific manner, in a similar fashion as other biological markers across different brain regions. For example, repeated KET exposure enhances hippocampal synapsin levels and serotonin turnover in male, but not female rodents (Thelen, Sens, Mauch, Pandit, & Pitychoutis, 2016). Nevertheless, future work will be needed to evaluate the long-term sex-specific molecular changes induced by KET to uncover the male-specific increases in reward-related behavior.

4.4 Acute and Long-Term Effects of KET on Body Weight

Repeated exposure to KET, during adolescence, significantly lowered body weight when compared to respective VEH-treated controls (Fig. 6A). This KET-induced decrease in body weight was evident as of the second day of KET exposure (days 2-15), and remained 21-day post treatment (PD70), prior to behavioral testing. Because CPP is a behavioral test that is locomotion-dependent, and since repeated exposure to KET resulted in an enduring reduced body weight

effect, it is certainly possible that lowered body weight could have influenced cocaine CPP performance. However, this is unlikely because locomotor activity did not differ between the KET- or VEH-pretreated groups prior to behavioral testing on PD70 (Fig. 4B). Similarly, this KET-induced lowered body weight could have potentially decreased drinking consumption on the SPT. Yet, no differences in total liquid intake between KET- and VEH- pretreated groups were noted on PD70 (Fig. 4D). Conversely, repeated exposure to KET during early adulthood (PD70-84) did not reduce body weight during treatment (Fig. 6B) nor 21-days post KET exposure (PD105). Together, these data suggest that chronic KET exposure, during adolescence, results in reduced body weight, which is in accordance with previous studies (Parise et al., 2013; Venancio, Magalhaes, Antunes, & Summavielle, 2011). The KET-induced reduction in body weight is not well understood, however, in adult humans, chronic KET exposure has been reported to induce nausea, vomiting, and overall decrease appetite (Cvrcek, 2008), which may explain KET's ability to reduce body weight during treatment, specifically during the adolescent stage of development.

4.5 Limitations

A limitation of the present investigation is the utilization of stress-naïve animals – lacking a depression-related phenotype (Iñiguez et al., 2014; Warren, Mazei-Robison, Robison, & Iniguez, 2020). Given that KET is prescribed for the management of various stress-induced illnesses like bipolar depression (C. A. Zarate, Jr. et al., 2012), post-traumatic stress disorder (Krystal et al., 2017), anxiety (Glue et al., 2018), and pain (Bell & Kalso, 2018), future work is needed to assess how female rodents exposed to concomitant KET-and-stress respond to natural and/or drug-rewards 21-days later, thus, increasing the translational implications of this work to the clinical setting.

4.6 Conclusion

Although there are high prescription rates of both traditional (fluoxetine) and novel (KET) antidepressant medications, there is a dearth of research investigating whether long-term side effects are evident in later life – particularly for KET, given that it is now categorized as a novel fast acting antidepressant and has gained popularity in the past decade (Trujillo & Iniguez, 2020). Here, we report that, in female mice, KET pre-exposure does not alter preference for cocaine and/or sucrose 21-days after treatment, unlike the SSRI fluoxetine (Flores-Ramirez et al., 2018). Our results further highlight that female mice display a resilient phenotype, contrasting their male counterparts, to the persistent effects of KET history on reward-related stimuli (Garcia-Carachure et al., 2020). Lastly, our data extend previous work on the safety of antidepressant exposure, wherein KET, unlike fluoxetine (Flores-Ramirez et al., 2018), does not mediate an anhedonia-like behavioral response in later life. Collectively, this preclinical work provides first-line evidence on the safety of repeated KET exposure during adolescence or adulthood, using female C57BL/6 mice as a model system.

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Table 1. Experimental Groups.

Aim	Sex	Age	Treatment	Interval	Preference Test	Dose/n	N	Figure
1	Female	PD35-49	VEH	21 d	Cocaine CPP (PD70-75)	Cocaine **0** (n=10), **5** (n=10), **10** (n=10) mg/kg	60	4(a-b)
	Female	PD35-49	KET	21 d	Cocaine CPP (PD70-75)	Cocaine **0** (n=10), **5** (n=10), **10** (n=10) mg/kg		4(a-b)
	Female	PD35-49	VEH	21 d	SPT (PD70-75)	1% Sucrose (n=10)	20	4(c-d)
	Female	PD35-49	KET	21 d	SPT (PD70-75)	1% Sucrose (n=10)		4(c-d)
2	Female	PD70-84	VEH	21 d	Cocaine (PD105-110)	Cocaine **0** (n=10), **5** (n=10), **10** (n=10) mg/kg	60	5(a-b)
	Female	PD70-84	KET	21 d	Cocaine (PD105-110)	Cocaine **0** (n=10), **5** (n=10), **10** (n=10) mg/kg		5(a-b)
	Female	PD70-84	VEH	21 d	SPT (PD105-110)	1% Sucrose (n=10)	20	5(c-d)
	Female	PD70-84	KET	21 d	SPT (PD105-110)	1% Sucrose (n=10)		5(c-d)

CPP, conditioned place preference; d, day; KET, ketamine; SPT, sucrose preference test; PD, postnatal day; VEH, vehicle.

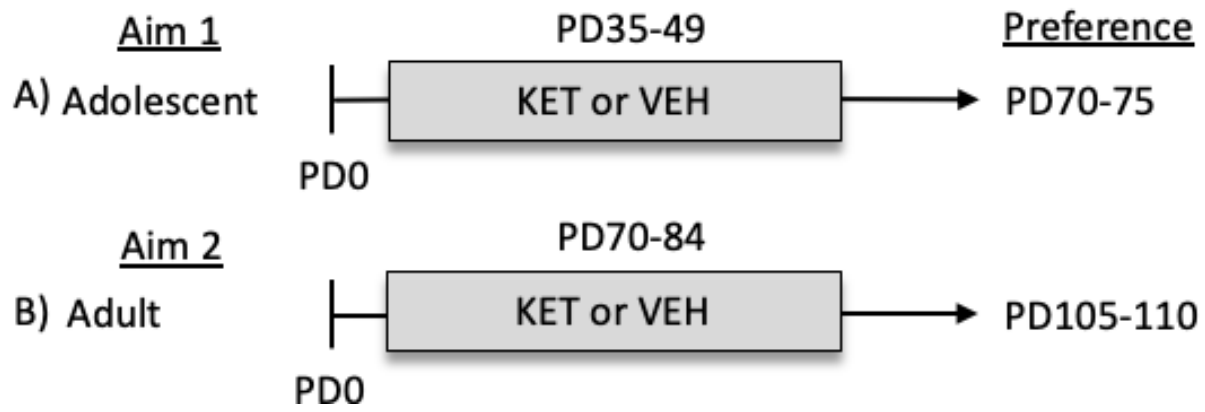

Figure 1. Experimental Timeline. (**A**) To evaluate if juvenile ketamine (**KET**) exposure mediates enduring alterations to reward-related stimuli in adulthood, adolescent (postnatal day [**PD**] 35) female C57BL/6 mice received KET (20 mg/kg) or saline (vehicle, **VEH**) for 15 consecutive days (PD35-49). Twenty-one days after the last KET exposure, mice were evaluated on the cocaine or sucrose preference tests (PD70-75). (**B**) To assess if KET pretreatment influenced preference for rewards as a function of age, adult (PD70) female mice were exposed to KET or VEH for 15 days (PD70-84), and after a 21-day washout period (PD105), they were evaluated on the cocaine or sucrose preference tests (PD105-110).

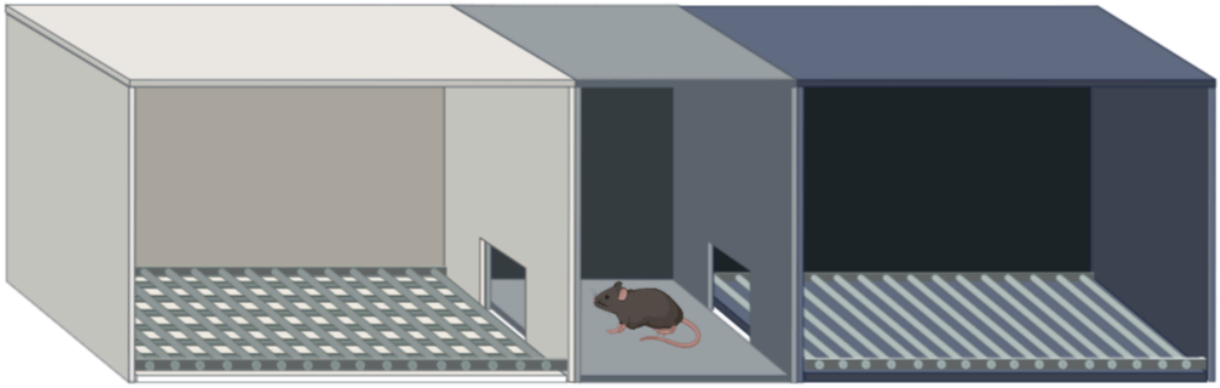

Figure 2. Conditioned Place Preference (CPP) Paradigm. CPP was carried out using a three-compartment apparatus with each compartment differing in visual (wall hue) and tactile cues (floor texture). For specifics, see conditioned place preference section (pg. 5).

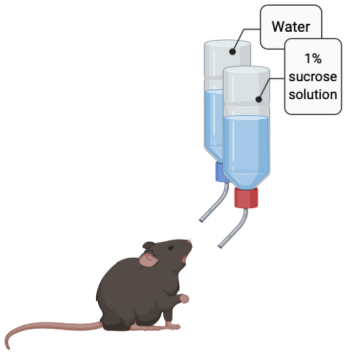

Figure 3. Schematic of the Sucrose Preference Test. The sucrose preference test consisted of a 2-bottle procedure in which mice were given the choice between consuming water or a 1% sucrose solution on a single-day procedure. For details, see sucrose preference section (pg. 7).

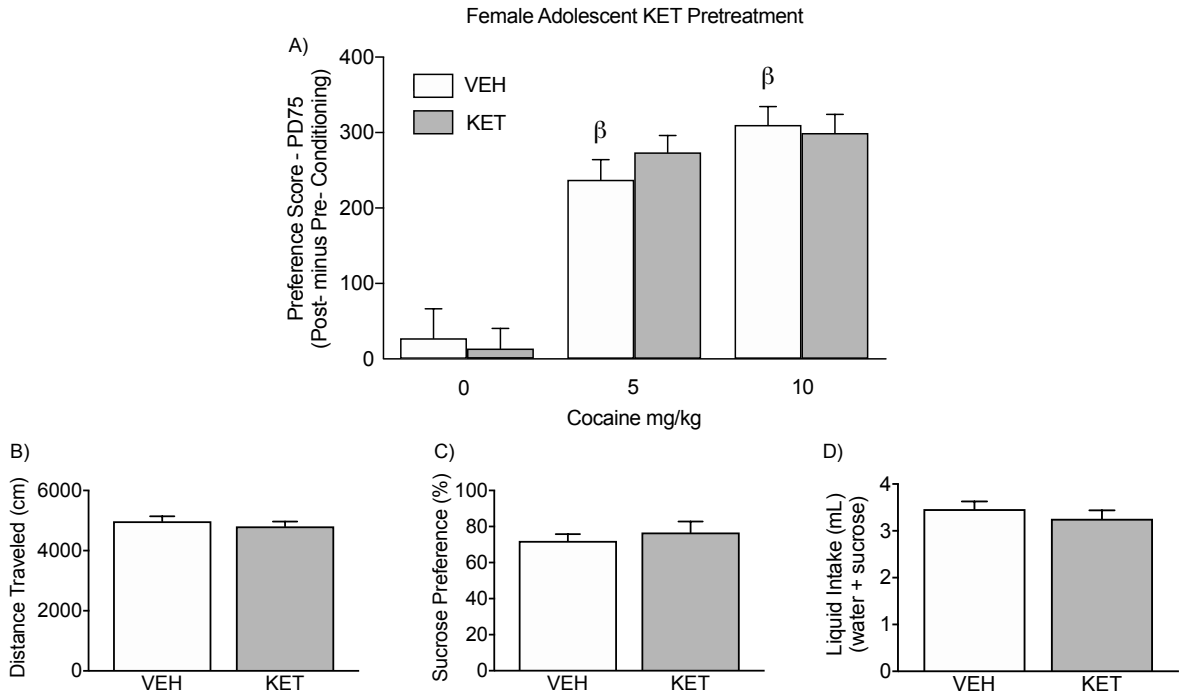

Figure 4. Enduring effects of adolescent ketamine (KET) or saline (VEH) exposure (postnatal day [PD] 35-49) for cocaine and sucrose preference in adult female mice. (**A**) Twenty-one days after adolescent KET exposure (PD 70+), saline (VEH)-pretreated and KET-pretreated mice displayed increased preference for environments paired to 5 (n=10) or 10 mg/kg cocaine (n=10), when compared to VEH-pretreated/SAL-conditioned mice ($^{\beta}p<0.05$). No differences in preference scores were evident between VEH- and KET- pretreated mice receiving the same cocaine doses ($p>0.05$, respectively). (**B**) No differences in distance traveled (cm) were evident during the preconditioning phase, between the experimental groups. (**C**) Adolescent KET pretreatment did not influence preference for a 1% sucrose solution three weeks after KET exposure (n=10 per group; $p>0.05$). (**D**) No difference in total liquid intake were observed between the groups ($p>0.05$).

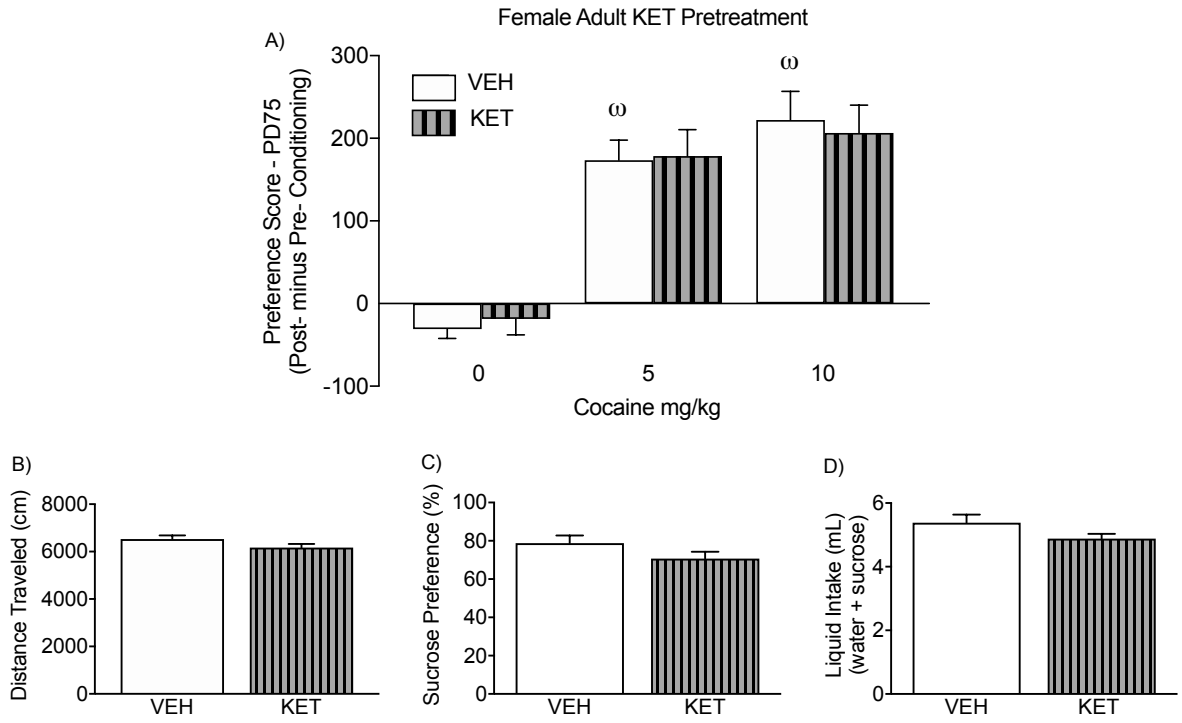

Figure 5. Enduring effects of adult ketamine (KET) or saline (VEH) exposure (postnatal day [PD] 70-84) on cocaine and sucrose preference in female mice. (**A**) Twenty-one days after adult KET exposure (PD 105+), VEH- and KET-pretreated mice conditioned to 5 (n=10) or 10 mg/kg (n=10) cocaine displayed reliable preference scores to the cocaine-paired side, when compared to VEH-pretreated/SAL-conditioned mice ($^{\omega}p<0.05$). No differences in preference scores were evident between VEH- and KET- pretreated mice receiving the same doses of cocaine ($p>0.05$, respectively). (**B**) No differences in distance traveled (cm) were evident during the preconditioning phase, between the experimental groups. (**C**) Adult KET pre-exposure did not influence preference for a 1% sucrose solution three weeks after KET treatment (n=10 per group; $p>0.05$). (**D**) No difference in total liquid intake were observed between the groups ($p>0.05$).

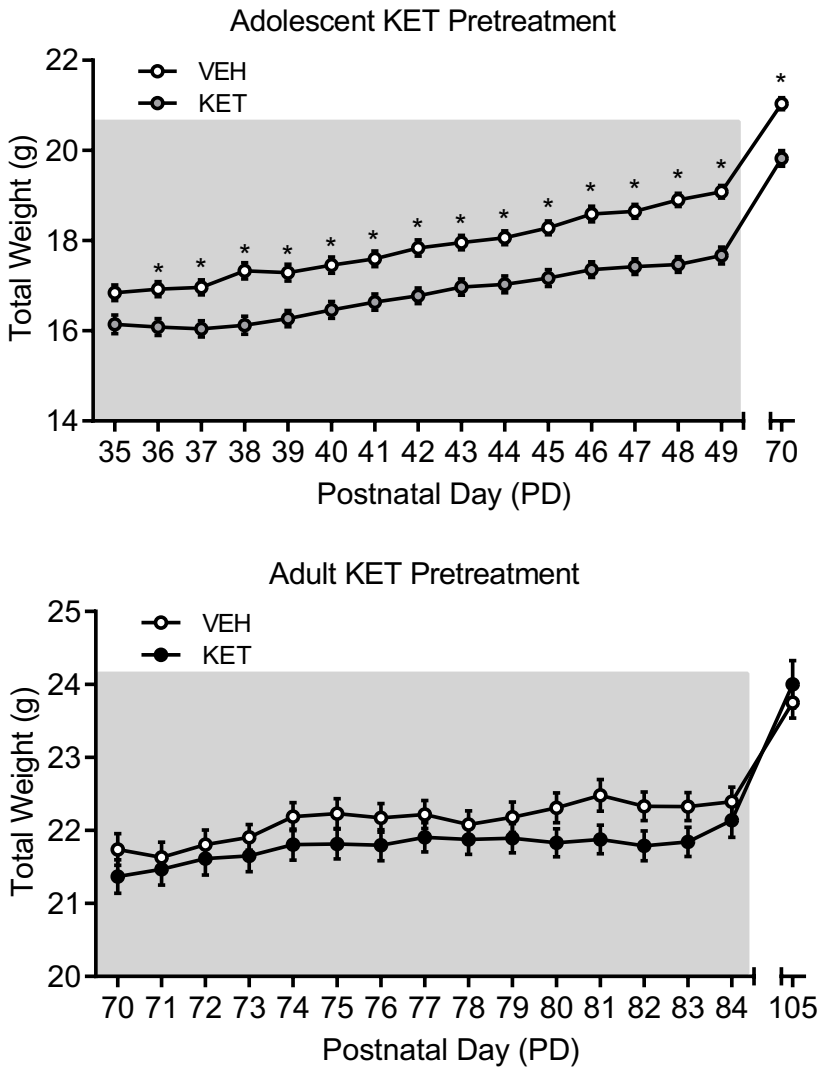

Figure 6. Enduring effects of ketamine (KET) or saline (VEH) exposure on body weight in adolescent and adult female mice. (**A**) KET exposure during adolescence (postnatal day [PD] 35-49; grey area) reduced body weight as of the second day of exposure (PD36-49; *p<0.05, respectively). The KET-induced weight reduction observed during adolescence persisted in adulthood at PD70 (*p<0.05). (**B**) KET exposure during early adulthood (postnatal day [PD] 70-84; grey area) did not reduce body weight in adult female mice (p<0.05) nor twenty-one days after KET exposure (PD105+, p>0.05).

Vita

Israel Garcia-Carachure received his Bachelor's of Arts degree in Psychology in 2017 from California State University, San Bernardino, CA. In 2017, Israel enrolled in the Department of Psychology's *Behavioral Neuroscience* Doctoral program at the University of Texas at El Paso under the mentorship of Dr. Sergio D. Iñiguez. He successfully defended his Master's Thesis August 4, 2020.