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Effect of Ketamine on Social Avoidance in Socially Defeated Male and Female Syrian Hamsters

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EFFECT OF KETAMINE ON SOCIAL AVOIDANCE IN SOCIALLY DEFEATED MALE AND FEMALE SYRIAN HAMSTERS

by

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Under the Direction of Kim L. Huhman, PhD

ABSTRACT

Social stress can cause or exacerbate neuropsychiatric illnesses such as depression. Unfortunately, currently available treatments for these disorders are slow to take effect and are often ineffective. One promising novel treatment option is the anesthetic ketamine, which may have rapid-acting antidepressant effects in humans and rodents. These rodent studies, however, have primarily used artificial stressors to produce depression-like responses. Our lab uses an ethologically relevant rodent model of social stress in Syrian hamsters. We tested whether a single, intraperitoneal dose of ketamine reduces social avoidance, a common symptom of mental disorders, in male and female Syrian hamsters that have experienced social stress. Social avoidance was tested one day after ketamine injection. Eight days later, subjects were defeated again, and tested for avoidance the following day. Ketamine reduced avoidance one day but not nine days post-injection. These data suggest that ketamine can act rapidly to prevent depressive-like responses to social stress.

INDEX WORDS: ketamine, antidepressant, social stress, conditioned defeat, Syrian hamster

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DEDICATION

I dedicate this thesis to my parents, who fully supported me the entirety of my time as a Master's student, and gave me everything I could ask for. It took a little longer than expected, so I'll never forget the patience that you guys showed me. I also want to dedicate this to the Bio-Bus, who made grad school as fun an experience as you could ask for; I don't think I could ask for a better graduate assistantship.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Huhman, first for accepting me into your lab, and then for all of your help throughout this entire project and paper. You provided me all of the necessary feedback, and believed in me during the times that I couldn't. I know I didn't make it easy in the end, so I'm also very grateful for your patience. Next I want to thank Kate McCann and Alisa Norvelle, who helped show me the ropes around the lab, and for always being optimistic and willing to help whenever I needed it. I'd also like to thank the members of the Huhman lab who helped me in completing this project. Finally, I'd like to thank Dr. Houghton and Dr. Albers for being in my committee, and for their input on this project.

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1 INTRODUCTION

Social stress, which includes bullying and psychological abuse, is arguably the primary stressor that humans face today (Almeida, 2005). Exposure to social stress is thought to induce or exacerbate neuropsychiatric illnesses like depression and anxiety disorders (Bjrokqvist, 2001), which affect approximately one quarter of the population during their lifetime (Mathers and Loncar, 2006). Unfortunately, current treatments for stress-related mental illnesses are inadequate for many of these patients. In the case of depression, the most prevalent mood disorder (Vasconcelos et al., 2015), around 40-50% of affected individuals fail to improve after being given currently available medications, which largely target monoaminergic systems (Browne & Lucki, 2013; Penn, 2012). Even when these treatments do significantly reduce depressive symptoms, they typically take weeks or even months to take effect. This delay often discourages patients from continuing their treatment and can be particularly perilous for patients struggling with suicidality (Hasselmann, 2014). Thus, there is a clear need for novel antidepressants with more rapid and robust efficacy.

Social stress, and in particular social defeat, is considered an ethologically relevant stressor that spans taxa (Blanchard et al., 2001), and similar behavioral responses following exposure to social defeat are observed in organisms ranging from crickets and fish to rodents and primates, including humans (Stevenson & Rillich, 2013; Koolhaas et al., 1997; Huhman, 2006; Krishnan et al., 2008; Earley RL et al., 2013; Fuchs & Flugge, 2003;). Non-human animals subjected to social defeat demonstrate behavioral and hormonal responses that mimic those found in individuals with mood and anxiety disorders (for a comprehensive review, see Bjorkqvist, 2001; Huhman, 2006; Vasconcelos et al., 2015). For example, defeated animals display a variety of anxiety-like symptoms, as well as anhedonia, disruptions in sleep and ingestive behavior,

alterations in hypothalamic-pituitary-adrenal responsivity, and significant social avoidance (Huhman et al., 1991; Huhman et al. 1992; Blanchard et al., PNE 1995; Berton et al, 1998; Solomon et al., 2007; Meerlo et al., 1997).

One treatment that is showing promise as a novel antidepressant is the N-methyl-D-aspartate (NMDA) receptor antagonist ketamine, a commonly used anesthetic. Several studies have suggested that ketamine has fast-acting antidepressant-like effects (ADE) that can last over two weeks when given at subanesthetic doses (Abdallah et al., 2015; Browne &Lucki, 2013; Scheuing et al., 2015), although other studies have failed to demonstrate a therapeutic effect (Bechtholt-Gompf et al., 2011; Browne & Lucki, 2013; Popik et al., 2008). In short, ketamine has been shown to initiate a signaling pathway that results in brain-derived neurotrophic factor (BDNF) exocytosis and the upregulation of synaptic proteins via mTOR (mammalian target of rapamycin) activation, ultimately leading to a reversal of the neuronal atrophy observed in key brain regions of individuals with major depression (Scheuing et al., 2015). Animal studies that have demonstrated an ADE, almost all of which involved rats and mice, primarily used non-social stressors such as electric shocks or genetic models that are more susceptible to depressive- or anxiety-like behavior (Browne & Lucki, 2013). Additionally, behavioral tests used to explore ketamine's potential efficacy in treating these symptoms have included the tail suspension test, forced swim test, sucrose preference test, and elevated plus maze (Browne & Lucki, 2013), all of which are thought to reflect depression- or anxiety-like symptoms. Few, if any, studies have examined other symptoms such as social avoidance, which is a prominent and disabling symptom of a number of neuropsychiatric disorders (Chaouloff, 2013).

Syrian hamsters are an excellent animal model to use in studies examining social stress. Hamsters are territorial animals that readily defend their home cage against intruding

conspecifics even in the laboratory (Nowack & Paradiso, 1983; Potegal et al., 1993). Hamsters that lose an agonistic encounter, however, undergo a drastic behavioral shift such that they abandon all aggression and instead become submissive and defensive, even in the presence of a smaller, non-aggressive intruder. This phenomenon, termed conditioned defeat, is a particularly useful model due to the ease and rapidity with which it can be elicited; agonistic behavior in hamsters requires no provocation or complex social housing, and submission in defeated hamsters can be observed after even a single, brief defeat (Huhman et al., 2003). Furthermore, brief agonistic encounters in hamsters rarely result in any physical injury, unlike in many other social stress models, which allows for an examination of behavioral responses to defeat in the absence of a marked inflammatory response. Finally, this model is particularly valuable in that both males and females display social defeat-induced social avoidance, and so the model allows us to test whether there is a potential sex difference in the potential antidepressant-like effect of ketamine. Thus, the goal of the present study was to establish whether ketamine blocks social defeat-induced social avoidance in male and female hamsters. Specifically, we tested the hypothesis that a single dose of ketamine given after social stress would be sufficient to decrease social avoidance of a conspecific as well as to reduce the response to an additional social stressor one week later.

2 MATERIALS AND METHODS

2.1 Subjects and Housing

Adult male Syrian hamsters were obtained either from our in-house facility or Charles River Laboratories, while adult females were from our in-house facility. Subjects, approximately 8-12 weeks of age and weighing between 88-161 g, were individually housed in polycarbonate cages (24 × 33 × 20 cm) for 8-12 days prior to experimentation, and given unique ID numbers. All cages contained corncob bedding and cotton nesting material. Animals were maintained on a 14:10 h light-dark cycle and had free access to food and water. All subjects were handled daily for eight days prior to the study in order to habituate them to the stress of experimenter handling. Resident aggressors (RA), used for defeat training, are larger hamsters weighing between 140-180 g and over five months of age, and that have previously defeated smaller subjects. All behavioral manipulations were done in a dedicated hamster testing suite within the vivarium. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2 Drug Administration

Ketamine (Ketathesia- 100 mg/ml) was obtained from Henry Schein Animal Health. Sterile physiological saline served as the drug vehicle and control. Dosages were formulated so that animals received a volume of drug proportional to body weight (i.e., 0.1 ml per 100 g), regardless of dose level. Sterile vials and sterile pipette tips were used for preparing solutions. All injections were administered intraperitoneally (IP).

2.3 Behavioral Procedures

The conditioned defeat model has been described extensively (Huhman et al., 2003; McCann & Huhman, 2012). Briefly, animals were taken to the behavioral suite 30 min prior to the start of defeat training to allow for acclimation of the environment. Animals in the defeat-group were placed in the home cage of an RA. This cage contained a plastic mesh box (13.5 × 13.5 × 7 cm) in one corner, which was then used to confine the RA during subsequent avoidance testing. A clear plastic lid was placed on top of the cage to prevent escape. Animals were subjected to a total of seven, 5-min defeats or seven 5-min novel cage exposures over four days (described below; see Figure 1 for an overview of the design). The 5 min interval for the defeated animals' first training session started after the test animal first showed a flight response from the RA, and subsequent defeats were timed beginning the moment the hamster was put into the cage. Each animal was defeated by a novel, randomly assigned RA each day of training to ensure that the effects of social stress were not attributed to a single pairing. Non-defeated animals (No-defeat group), which served as controls for establishing baseline levels of avoidance, were placed in a neutral arena (clean cages with corncob bedding) that contained a small amount of bedding from an RA and a plastic mesh box for seven, 5 min exposures. No-defeat subjects were exposed to bedding from a different RA during each trial. All animals involved in defeat training were monitored closely to prevent injuries, and further checked for injuries after all training sessions ended. Animals were removed if injured in a way that bleeding was present. The short- and long- term effects (20 h and 9 days post-injection, respectively) of ketamine on defeat-induced social avoidance were tested in each experiment as described below.

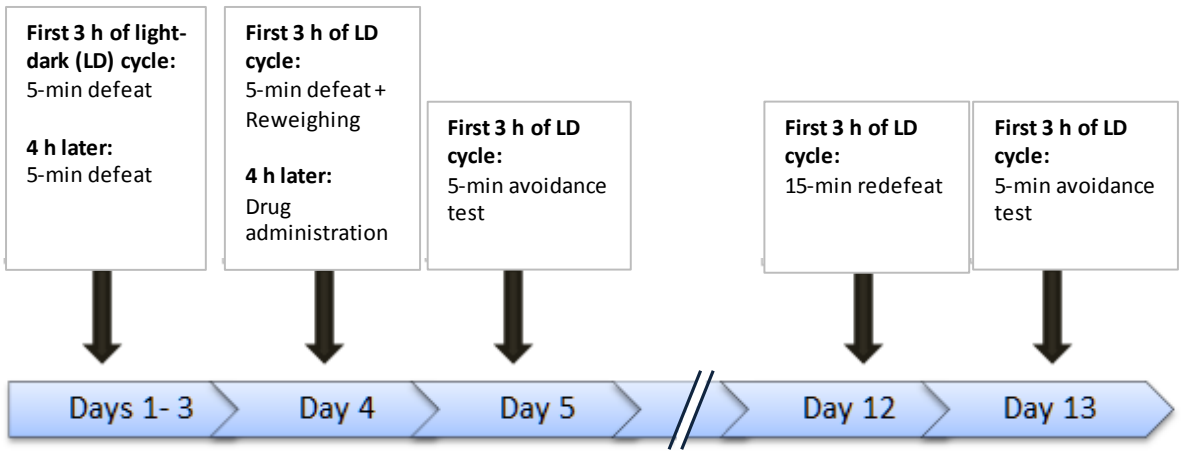

Figure 1. **Overview of design for Experiments 1 and 2.**

2.3.1 Experiment 1

A literature search revealed that no studies had investigated ketamine's antidepressant-like effect in Syrian hamsters. Thus, a purpose of Experiment 1 was to determine a dose-response curve for hamsters. The ketamine dosage range for rats and mice is 0.25-50 kg/mg, with the most effective dose typically being 10 mg/kg (for a review, see Browne & Lucki, 2013). As a general rule, Syrian hamsters metabolize drugs faster or more effectively than do other laboratory rodents (Gad, 2015). Additionally, anesthesiology studies have shown that Syrian hamsters require approximately twice the amount of ketamine (typically 125 mg/kg for hamsters) for proper anesthetization (Fish et al., 2008). Therefore, we tested the following subanesthetic doses of ketamine: 10 mg/kg, 20 mg/kg, and 50 mg/kg.

Male (n= 32) and female (n= 40) hamsters were randomly assigned to one of five weight-matched drug groups the day before experimentation: no-defeat saline, defeat saline, and defeat ketamine (10 mg/kg, 20 mg/kg, or 50 mg/kg). Estrous cycles of females were monitored for at least two cycles and then all females were subjected to defeat training beginning on Diestrus 2 to standardize possible effects of estrous cyclicity (Solomon et al., 2007). A subset of the males was

also included each day to standardize conditions across sex. On Days 1-3 of the experiment, defeats or novel cage exposures occurred during the first three hours of the light-dark cycle and again four hours later. On Day 4, a single 5-min defeat occurred during the first 3h of the light-dark cycle, after which the animals weighed once more to ensure that the most accurate drug volume was injected. Animals were then administered IP ketamine or vehicle 4 h after the last defeat. The 4 h delay was implemented so that the treatment was given after the time that the defeat experience is consolidated in hamsters (Gray et al., 2015). All animals were returned to the housing room immediately after injection. Social avoidance was measured 20 h later (Day 5) as described in 2.3.3 below. To test for possible long-term effects, on Day 12 animals were either re-defeated (defeat groups) or placed into a neutral arena (no-defeat group) for 15 min. In order to test again on Diestrus 2 for females, we chose to test 9 days post-injection, as the review of rodent studies investigating ketamine's antidepressant-like effects by Browne & Lucki (2013) suggested that the majority of the experiments tested for long-term effects between 7 and 10 days post-injection. The next day (Day 13) all animals were again tested for social avoidance as described below.

2.3.2 Experiment 2

The most effective dose of ketamine found in Experiment 1 (20 mg/kg) was further tested on male subjects to determine if the apparent decrease in avoidance was replicable and to include an additional control (no-defeat + drug). Animals (n=24) were assigned to one of four weight-matched groups: no-defeat saline; no-defeat 20mg/kg; defeat saline; defeat 20 mg/kg. Non-defeated animals given ketamine were included to determine if the drug had an antidepressant-like effect independent of previous defeat experience. All training and testing was as described in Experiment 1.

2.3.3 Avoidance Testing

Social avoidance testing occurred 24 hours after the seventh defeat and after the re-defeat and followed the protocol as described by McCann & Huhman (2012). In short, each animal was placed next to the far wall of a neutral arena that contained an unfamiliar, caged RA of the same sex in the opposite corner (see Figure 1). Social avoidance tests were 5 min in duration. Animals were returned to their home cage afterward. A clean neutral arena was used for each animal, and the plastic boxes that held the RAs were cleaned between trials using a 70% alcohol solution. All testing trials were recorded using an infrared CCD camera for future analysis and were later scored by an observer experimentally blind to each animal's drug group. Non-defeated hamsters normally investigate (spend time in contact with the box containing) a conspecifics, thus, we measured the amount of time that the animal spent in the far side of the arena, i.e., the half not containing the caged RA as a measure of social avoidance. An animal was considered to be in the part of the arena where their forepaws and shoulders were, and social avoidance was defined as the amount of time (out of a maximum 300 s) that the subject spent on the half of the arena opposite the caged RA.

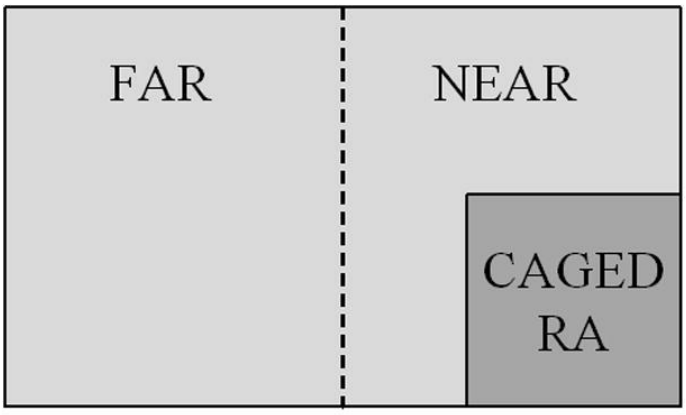

Figure 2. Layout of the arena used for testing and scoring social avoidance (adapted from McCann & Huhman, 2012). Social avoidance was measured by the amount of time subject spent on the "Far" side of arena.

2.4 Statistical Analysis

The data from avoidance testing were analyzed on SPSS (version 23) for Experiment 1 using a three-way between subjects analysis of variance (ANOVA) to determine any sex differences, while a two-way ANOVA was used to determine dosage differences within sexes. A two-way ANOVA was used in Experiment 2 to determine differences between defeat types and doses. Post hoc analysis was conducted using either Tukey's HSD or Fisher's LSD test when appropriate. Non-parametric tests (Kruskal-Wallis, with Mann-Whitney U for post-hoc analysis) were used whenever parametric assumptions were violated, while independent T-tests were performed for comparing 2 groups when appropriate. All significance was set at $p < 0.05$. The data for each group are reported as the mean ± standard error of the mean (S.E.M.)

3 RESULTS

3.1 Experiment 1: Dose response for subanesthetic doses of ketamine on social avoidance in socially-defeated Syrian hamsters.

No animals had to be removed from the experiment due to injury. One outlier from the no-defeat females was found (z-score= 2.00, t= 112 s), and this animal was not included in the statistics. As expected, there was a significant main effect of defeat (U= 77.0, p= 0.000). When split by sexes, there was a significant difference between male groups (Kruskal-Wallis p= 0.039) as well as in females (Kruskal-Wallis p= 0.025). The follow-up Mann-Whitney U test showed a significant difference between the no-defeat control and the saline (vehicle) and 50 mg/kg groups in males, while all female defeat groups (animals defeated by an RA) had a significantly greater mean avoidance 20 h post-injection compared to the no-defeat controls (Figure 3). No significant main effect of sex was observed in the defeat groups, although somewhat surprisingly we found the no-defeat females to have a significantly lower avoidance than the no-defeat males. Additionally, there was no main effect of drug on social avoidance.

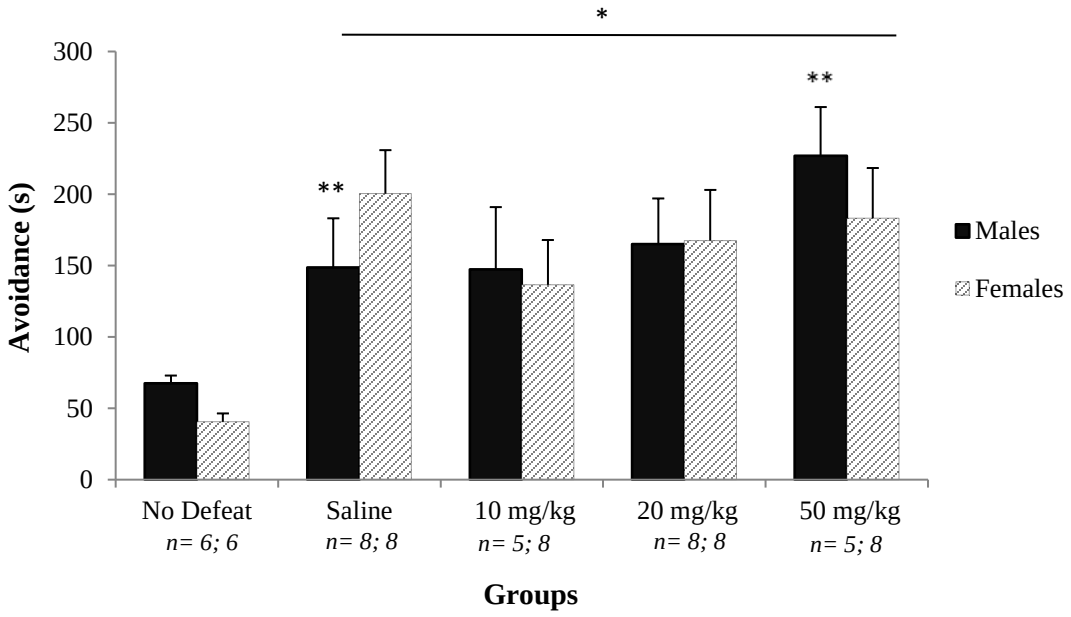

Figure 3. Mean avoidance time ± S.E.M., measured 20 h post-injection, of socially-defeated Syrian hamsters given ketamine. No-defeat and defeat groups administered saline served as controls. Avoidance was measured as the time spent in the far half of the arena from the caged aggressor. There was no significant effect of drug on avoidance during this first avoidance test, but all female defeat groups (Saline, 10 mg/kg, 20 mg/kg, and 50 mg/kg) and the saline and 50 mg/kg male group exhibited significantly greater avoidance than did the no-defeat group (females=* and males =**; $p<0.05$).

Experiment 1 was split into two cohorts. Animals from the first cohort (n=11 males, n=19 females) were redefeated nine days later to test for long-term effects. These additional defeats were only 5 min long, and unfortunately no animals responded with significant avoidance (data not shown). Because of this, we used a more robust 15-min re-defeat for the second cohort of animals (n=21 males, n=20 females). No significant differences were found between sexes, and thus they were collapsed; here we observed a significant different effect of defeat on avoidance after this longer defeat stressor (U= 57.5, p= 0.17) (Figure 4). Further individual group comparisons revealed a difference between the no-defeat and defeat saline groups (U= 13.0, p=

0.27). One outlier was found in the 20 mg/kg group (z= 2.09, t= 296 s), and not included in the statistics.

It is interesting to note that while there was no significant effect of drug on avoidance, there was also no difference between the 10 mg/kg (U= 20.0, p= 0.124) and 20 mg/kg (U= 20.5, p= 0.227) groups and the no-defeat control. This suggested that ketamine at those two doses had a protective effect following a social defeat stressor; among the two drug groups, avoidance was lowest in the 20 mg/kg group (88 ± 20.85 s). Given the fact that males administered 20 mg/kg had the closest mean time to no-defeat controls across both sexes (84.8 ± 22.99 s) and that 4 of the 5 subjects showed avoidance comparable to the no-defeat subjects (Appendix A.1), we further studied this drug dose (20 mg/kg) in additional males for Experiment 2 to determine whether it can lead to a significant decrease.

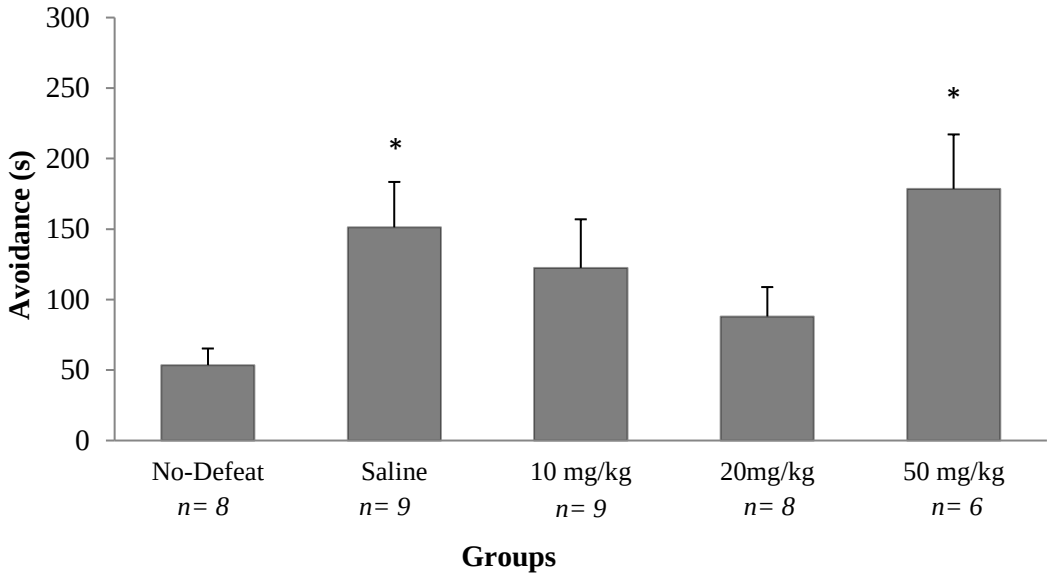

Figure 4. Mean avoidance time (± S.E.M.) in males and females (collapsed across sex) measured nine days post-injection. Animals experienced a single 15 min defeat one day before the re-test. No-defeat and defeat groups administered saline served as controls. Avoidance was measured as the time spent in the far half of the arena from the caged aggressor. Saline and 50 mg/kg defeat groups exhibited significantly greater avoidance compared to the no-defeat group (* p< 0.05).

3.2 Experiment 2: Short- and long-term effects of a 20 mg/kg dose of ketamine on social avoidance in socially-defeated Syrian hamsters

There was no significant difference ($t_{(6)}$= 0.683, p = 0.520) in avoidance between the no-defeat saline (64.1± 12.99 s; n= 4) and no-defeat 20 mg/kg (77.58± 14.87 s; n= 4) groups, indicating that ketamine had no effect on avoidance behavior (Figure 5). Thus, these two groups were collapsed for subsequent analyses. Because these data violated parametric assumptions, we used the Kruskal-Wallis test, which revealed significant differences between groups tested 20 h post-injection (p= 0.000). The defeat saline (U= 0, p= 0.001) and 20 mg/kg groups (U= 2, p= 0.001) displayed significantly higher avoidance than did no-defeat controls, but the 20 mg/kg group was also significantly lower than the defeat saline group (U= 11.5, p= 0.031), indicating that ketamine at least partially reverses the effect of defeat on social avoidance. When animals were tested nine days post-injection, we observed no significant differences between no-defeat, defeat-saline, and defeat-ketamine groups (Kruskal-Wallis p= 0.665). Unfortunately, given the fact that there was not a significant effect of the re-defeat on avoidance behavior, it was impossible to demonstrate a significant drug effect.

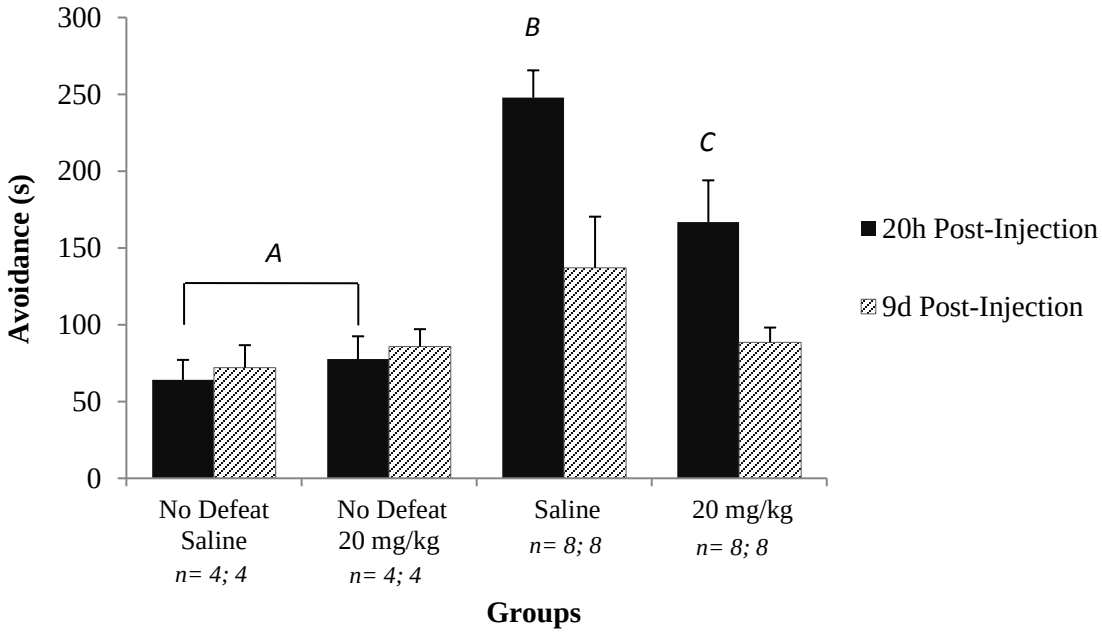

Figure 5. Mean avoidance time ± S.E.M. of socially defeated male Syrian hamsters given a 20 mg/kg dose of ketamine 4 h after the last defeat. Avoidance was first measured 20 h post-injection and then again one day after a re-defeat. Avoidance was measured as the time spent in the far half of the arena from the caged aggressor. No significant difference was found between the two no-defeat groups at either 20 h or 9d post-injection, so these controls were collapsed before analysis. Non-shared letters indicate statistically significant differences ($p < 0.05$). No effect of drug was observed when tested 9 d later in the 20 mg/kg group.

4 DISCUSSION

Neuropsychiatric disorders are multifaceted illnesses that place a heavy burden on society (Eaton et al., 2008). The process of discovering new therapies for treating these disorders frequently makes use of animal models with which researchers can examine the mechanisms whereby stress causes or exacerbates these disorders. Rodent models are by far the most prevalent for studying the effects of stress, and most of these studies use either rats or mice (Shapiro, 1998). While these two species can be extremely useful for testing the effects of stress and the novel treatments for relieving them, they are perhaps not the best models for studying social stress, which is known to be involved in the etiology of numerous neuropsychiatric disorders (Bjorkqvist, 2001). Furthermore, the tests used for measuring stress-induced symptoms, which include tail suspension and forced swimming, may not be readily translatable to humans or be particularly valid measures (Nestler & Hyman, 2010).

What is needed is a more accurate and ethologically relevant way to measure the effects of social stress; thus, we tested the promising novel antidepressant ketamine in Syrian hamsters that were subjected to brief social defeat, an ethologically relevant model of social stress. This study is the first to suggest that ketamine may reduce behavioral responses to social stress in this species. Specifically, we have demonstrated that, in one of two experiments, a single, 20 mg/kg dose of ketamine can reduce defeat-induced social avoidance in males 20 h post-injection. This suggests that ketamine has the potential to promote a fast-acting antidepressant-like action in that it may attenuate the short-term avoidance of a conspecific that is induced by social defeat. The most effective dose of ketamine found in this study (20 mg/kg) is somewhat higher than the dose found to be most effective in other rodent studies (typically 10 mg/kg) (Browne & Lucki, 2013)

but is still well within the range of doses that have been previously suggested to have antidepressant-like effects (0.5- 50 mg/kg).

The low mean avoidance scores of the no-defeat controls and relatively high avoidance in defeat-saline groups are consistent with results previously reported by our lab (McCann & Huhman, 2012), meaning that the avoidance-producing effects of defeat were replicated, as expected. Additionally, in Experiment 2, we found a significant decrease in defeat-induced social avoidance in the defeated males that were given 20mg/kg ketamine and were tested 20 h post-injection. This finding was consistent with other rodent models that have also found that ketamine can reverse other short-term, antidepressant-like effects of stress (Browne & Lucki, 2013); for example, reductions in immobility in the forced swim and tail suspension tests were observed in rodents administered a single dose of ketamine that were subjected to non-social stressors.

Our failure to observe a significant effect of ketamine on Day 13 in both experiments was disappointing. Interestingly, however, all of the animals given ketamine in Experiment 2 displayed avoidance on Day 13 that was nearly identical to that produced by the non-defeated group (Appendix A.2); this failure to observe defeat-induced avoidance could mean that ketamine does, in fact, confer some protection against the effects of later social stress, and we maintain that this possibility warrants further study. The lack of a significant effect in this study could be due to the high variability in the avoidance times of the defeated animals that served as vehicle controls in Experiments 2. In fact, the distribution of the avoidance scores in the control group (i.e., defeated animals given saline) appeared to be bimodal, with 5 of the 8 subjects exhibiting avoidance scores that were exactly like those in non-defeated animals. Such individual

differences in responses to stress have previously been documented in rodents with some animals being more resilient to social defeat than are others (Feder et al., 2009).

It is important to acknowledge that the significant short-term effect of ketamine on social avoidance that was observed in Experiment 2 was completely absent in Experiment 1. There are several possibilities that could explain this inconsistency. The first possibility, of course, is that ketamine is not effective in reducing antidepressant-like behavioral responses in hamsters and that the significant findings in Experiment 2 are an anomaly. Another factor that may be important is that the hamsters in the two experiments were different. In Experiment 1, the subjects were bred in-house and were housed in our animal facility from birth. In Experiment 2, the subjects were purchased from Charles River Laboratories and were shipped from their facility two weeks before experimentation. It is not clear what factors would explain the variation in the drug response between the two groups of animals, but this appeared to be the only obvious difference between the two experiments.

There is unfortunately a lack of research properly studying the sex differences in the efficacy of many psychoactive drugs, which is disturbing given that women are diagnosed with mental disorders at a much higher rate than are men (Bigos et al., 2009). This lack also extends to animal models, including those that have studied ketamine, as a literature search reveals that only a very small proportion have looked at ketamine's effects in females. In addition, none of these studies have used social stressors. Therefore, one of the goals of the present study was to determine whether there is a sex difference in the effect of ketamine on social defeat-induced behavioral responses in hamsters. In fact, our data did not reveal any significant differences in the effect of ketamine on socially stressed males and females. It is perhaps important to note that while there was no significant difference among doses of ketamine in females, the lowest

avoidance on Day 13 was observed at the 10mg/kg dose. Thus, while our study was not consistent with others that have found an effect of ketamine in females, this trend does fit with a study which found that a lower dose (2.5 mg/kg) of ketamine had an antidepressant-like effect in female but not males rats, which required 5-10 mg/kg of ketamine to elicit similar effects (Carrier & Kabbaj, 2013). This finding suggests that the dose response curve for ketamine in females is shifted to the left and that they may require a lower dose than do males to observe significant effects of ketamine. Future studies should examine this possibility.

Additional behavioral studies should also include testing the effects of chronic ketamine administration, as other studies have shown that repeated injections lead to more profound and longer-lasting effects (Browne & Lucki, 2013). Another area of interest is studying susceptibility to ketamine's effects. Appendix A.3 shows another bimodal pattern 20 h post-injection in hamsters given 20 mg/kg of ketamine. These data indicated that, in six of the eight subjects, ketamine reduced avoidance down close to no-defeat levels, while the other two animals showed marked social avoidance at levels similar to the defeated animals given saline. The apparent insensitivity of a subset of the animals to ketamine could arise from genetic variants that dictate sensitivity to ketamine, as has been shown in other studies (Liu et al., 2012). It is also important to understand the molecular and cellular mechanisms by which ketamine administration acts in suppressing social avoidance in Syrian hamsters; one possibility is that brain-derived neurotrophic factor (BDNF) mediates ketamine's effect on defeat-induced behavior, as it has been shown to play an essential role in ketamine's antidepressant-like effects (Scheuing et al., 2015)

As it is one of the root causes of depression and other mental disorders, it is surprising to see how little social stress is taken into account by studies testing ketamine's antidepressant-like

properties. In fact, only a single study had ever investigated how ketamine affects social avoidance behavior using mice that underwent chronic social defeat stress (Donahue et al., 2014). While this study was a step in the right direction, it failed to isolate the psychological aspects of social stress because it used a severe defeat procedure that generally causes tissue damage from bites; pain is known to also cause severe stress, but is confound that is absent in our hamster model. Furthermore, the defeats in mouse studies are usually chronic, meaning that the animal is left in the same cage as its aggressor and is separated only by a clear wall between bouts of social stress. In hamsters, we are able to stimulate social avoidance with only brief exposure to social stress with no tissue damage, and it is this reason that we maintain that this model is a valuable addition to the field. Here, we demonstrated that, at least under some conditions, ketamine confers a rapid antidepressant-like effect in males. While we did not demonstrate a significant effect in females, there was no significant sex difference in ketamine's effects. In conclusion, we maintain that more research is warranted to determine whether ketamine is a potential novel therapeutic for social stress-related symptoms in humans.

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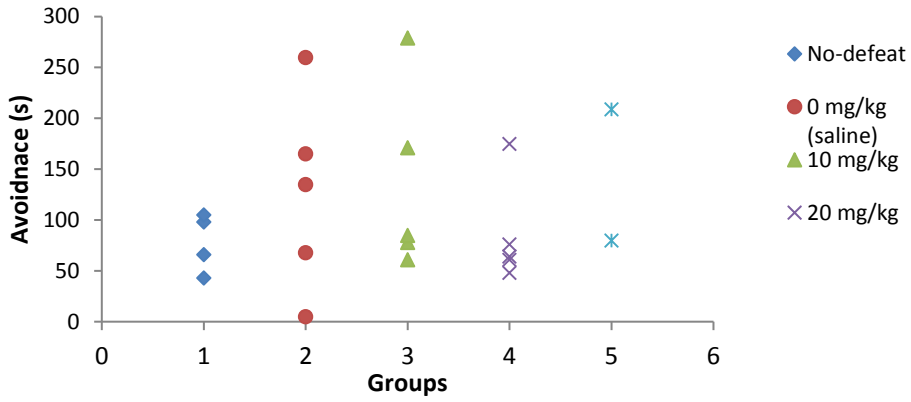
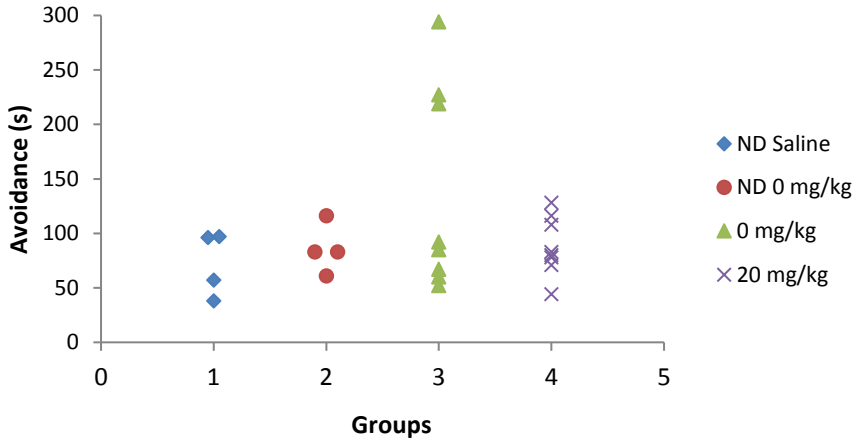
APPENDICES

Appendix A: Raw Data Plots

Appendix A.1: Long-term effects of ketamine in males (Experiment 1)



Appendix A.2: Long-term effect of ketamine (Experiment 2)



Appendix A.3: Short-term effect of ketamine (Experiment 2)