Effects of social defeat stress on the dopaminergic system, behavioral coping, and cocaine self-administration in male mice

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Abstract

Psychiatric disorders, such as depression and other mood disorders, have a high comorbidity with drug use disorders. Research has led to the study of the mesolimbic dopaminergic pathway as a key site for the comorbidity of the two diseases because chronic stress and drug abuse alter dopamine (DA) release in a similar manner. Two different intensities of social defeat stress, brief or moderate, can be used as a model to induce depressive-like symptoms and study the alterations of the DAergic system by examining the behavioral effects, the changes in DA in the nucleus accumbens in response to an amphetamine injection, as well as cocaine taking behavior. Male mice were exposed to ten days of social defeat stress, with behaviors during the defeats analyzed from days 1 and 10, and then either took part in *in vivo* microdialysis or cocaine self-administration. Stressed mice show an increased change in DA in response to a d-amphetamine challenge, with moderately stressed mice showing a longer-lasting effect. Stressed mice also take more cocaine on an FR schedule and briefly stressed mice display defensive behaviors more frequently than moderately stressed mice. The intensities of social defeat stress can produce distinct effects on DA, drug taking, and behavior.

Drug abuse and psychiatric disorders have an alarmingly high comorbidity. According to the National Epidemiological Study, nearly 20% of all respondents report suffering from a mood disorder and 16% report an anxiety disorder within their lifetime (Conway et al., 2006). Additionally, one-fifth of respondents with a mood or anxiety disorder also reported having a drug abuse disorder within their lifetime (Conway et al., 2006). Research has led to the study of the mesocorticolimbic dopaminergic pathway as a key site for the comorbidity of the two diseases because chronic stress and drug abuse alter dopamine (DA) release in a similar manner (Kalivas & Duffy, 1989; Brady & Sinha, 2005). Much of this work has been done in preclinical studies using laboratory animals, often using social defeat stress as a model because of its ethological validity and its ability to increase drug-taking behaviors. This study aims to evaluate the affect that social defeat stress has on extracellular DA in the nucleus accumbens, cocaine self-administration, and the individual differences in coping mechanisms in response to the social defeat stress.

Stress

Nearly two-thirds of Americans report that stress has had a negative impact on their physical or mental health (APA, 2012). Twenty percent of respondents also indicated that their stress levels were very high (APA, 2012). Despite a perceived understanding of the term "stress" from the general public, it is not a clearly defined term. In 1936, Selye first adapted the term stress to describe it in a physiological setting as "the nonspecific response of the body to any demand made on it". Selye also describes a stressor as "an agent that produces stress at any time" (Selye, 1976). Since then, the definition of both a stressor and a stress response has been altered many times, but the stress response is typically defined by the symptoms that an author is studying in response to a stressor (Schuler, 1980). According to the American Psychological

Association (2014) stress response can be defined as an organism's pattern of specific and nonspecific responses to stimulus events that upset its homeostasis and alter the organism's ability to cope. Chronic stress is can be studied in terms of depression, anxiety disorders, or post-traumatic stress disorder (PTSD) in humans. When using preclinical models, however, various types of stressors can be used to mimic some symptoms of these psychological disorders, but there are no complete animal models for any of the disorders.

There are many types of stressors, but they can be categorized into social stressors and other types of stressors. In animals, there are behavioral and physiological responses that differ between social stress and other types of stressors, such as foot shocks, forced swim test or restraint stress. For example, rats exhibit different electrocardiograph responses to social stress compared to restraint or shock-probe stress (Sgoifo et al., 1999) and corticosterone levels differ after exposure to different types of stressors (Koolhaas et al., 1997). In addition, rats tend to groom after encountering most types of stressors, but one study found that the amount of grooming differs based upon the type of stress the Wistar rats were exposed to (van Erp et al., 1994), indicating that different kinds of stressors result in different behavioral reactions. While mammals, including humans, experience both social stress and other types of stress, exposure to social is more common because of frequent interactions with other members of the species, particularly regarding clashes over shared resources. This means that social stress is an ideal model to study because has an ecological validity that is not necessarily present in other stress models.

Social Stress

A variety of preclinical models can be used to induce social stress. Some common methods include the social instability model, maternal separation, the social disruption model,

and social defeat stress. The social instability model is when laboratory animals are paired-housed are frequently pair-housed with a novel partner. This is stressful in rodents, such as rats, and produces short-term effects (Mormede et al., 1990). However, this model produces long-lasting effects in less social animals, such as tree shrews (Raab & Oswald, 1980). Maternal separation is another type of stress that can also produce long-lasting effects. Infants repeatedly separated from their dam for long periods of time have shown to produce depressive-like changes and to effect GABA receptors in adulthood, indicating that developmental stressors can produce life long changes. However, the conditions of the maternal separation, especially the duration and frequency, produce divergent effects on self-administration of alcohol and psychomotor stimulants for unknown reasons (Matthews et al., 1999; Moffett et al., 2006; Plog et al., 2003).

Another model of social stress is group-housing laboratory animals with both male and females. Large, more natural habitats stimulate fighting between the animals, even when they are provided unrestricted access to food and water (Blanchard & Blanchard, 1990; Flannelly et al., 1982). The social disruption model uses the basic group-housing paradigm, but also introduces a dominant, aggressive male into the previously established social group (Pagdett et al., 1998). This alters the dominance hierarchy and the newly introduced, aggressive male defeats the previously dominant male. The social disruption model may be representative of natural behavior, but studies following these methods result in non-linear dominance hierarchies and incomplete dominance or subordination. This makes studying the differences between defeated, victorious, and non-stressed animals difficult because the categories are more fluid.

Social defeat stress is also a common method of social stress that is frequently used in preclinical models. Social defeat stress can consist of a single episode, intermittent episodes of

social defeat or chronic social defeats (Tidey & Miczek, 1997; Yap & Miczek, 2006; Krishnan et al., 2007). Some of the social defeat models involve two naïve rodents that are housed in a single cage, with a clear perforated barrier that allows for visual, auditory and olfactory contact. The barrier is removed for some time period, resulting in interaction and a fight between the two animals. This establishes a dominance hierarchy between the pair. However, as in the current study, a resident-intruder paradigm can also be used. This involved placing an "intruder" rodent into the home cage of a dominant "resident" rodent; the intruder is consequently defeated by the resident (Miczek, 1979). Further, the resident animal is often screened for aggressive behavior prior to the interaction, in order to ensure that one of the pair will be reliably defeated and the other will be victorious (Miczek, 1979; Yap et al., 2006). This resident-intruder paradigm leads to less complicated dominance hierarchies and ensures one of the mice is always subordinate. In this study, the social defeat procedure, specifically as defined by Yap and colleagues (2006) is used because it ensures that the study will be comparing mice that were defeated consistently.

Individual Differences

Individual differences are widespread in human populations. Schuler (1980) suggests that the relationship between a stressor and the stress response differ among individuals because of differences in personality. Differences can also been seen in areas like responsiveness to antidepressants and coping strategies. There are a great number of personality and temperament tests that look for differences in traits among people. Aggression and depressive symptoms, for example, are common traits examined in these questionnaires (Koolhaas, 2008). In addition, it is estimated that only 60 percent of patients respond positively to the first antidepressant they are prescribed and approximately 10 percent of patients do not respond to any category of antidepressant drugs (FDA, 2013). Similarly, about 20 percent of individuals whom experience

traumatic events develop post-traumatic stress disorder (PTSD) (APA, 2014). Antidepressant drug effectiveness, the low subset of the eligible population experiencing PTSD and variances in personality are all examples of individual differences among humans. While individual differences are difficult to study in humans, studying them in animals presents more of a challenge. Preclinical models do not have the luxury of using surveys to evaluate opinions on how a stressor, for example, may impact the individual; other, creative methods must be used to study the differences between how and why individuals react and perceive a stressor in a varying manner. This can be done by evaluating behavioral or neurochemical differences between individuals.

The intensity of and the type of stressor can have varying behavioral and neurochemical effects on individual mice. One way to evaluate the individual differences in how social defeat stress effects the mice is to analyze the behavior of the mice during the social defeat stress procedure so that their coping styles can be determined. A coping style can be defined as the consistent manner in which an animal or group of animals alters behavioral or physiological responses in order to overcome the situation (Koolhaas et al., 1999). Cannon (1915) first described the "fight or flight" response, which can be applied to the social defeat stress behavioral analysis as an "active" behavioral coping response. This includes behaviors such as escaping and locomotor activity (Koolhaas et al., 1999; Gomez-Lazaro et al., 2011). The other type of behavioral response is sometimes called "passive" (Gomez-Lazaro et al., 2011) and was first described as the "conservation-withdrawal" response (Engel & Schmale, 1972). Defensive upright and crouching are two key behaviors that define the passive copying style (Koolhaas et al., 1999; Gomez-Lazaro et al., 2011). The passive and active titles can be used to separate intruder mice into two distinct groups with varying stress responses.

In order to classify intruder mice as maintaining a passive or active coping style, the behavior of both the intruder mouse and the resident mouse during the defeat session should be recorded analyzed. Sideways threats, pursuits, tail rattles, and attack bites are typical aggressive behaviors that are displayed by resident mice (Brain, 1989). On the other hand, defensive upright, crouch and escapes are behaviors typically shown by the intruder mice (Brain, 1989). One study categorized mice as active or passive based upon their behavior during the chronic 21-day social defeat procedure. The passive group was significantly different from controls and the active group in plasma corticosterone levels, hippocampal BDNF, and proliferative capacity of lymphocytes (Gomez-Lazaro et al., 2011). Examining, analyzing and classifying mice as passive or active based upon their behavior during a social defeat has shown to be an effective way of examining individual differences between mice.

Cocaine

According to the NIH, 18 percent of the US population has used cocaine or crack cocaine at least once in their lifetime. Cocaine is a naturally occurring compound that was isolated from the leaves *Erythoxylon coca* plant, which is native to South America. It has both local anesthetic and mood-altering properties, namely inducing euphoria. Although cocaine was once commonly used as an anesthetic, it has a high abuse potential and the toxic dose is low enough to cause lethal overdoses. Over 40,000 fatal overdoses of all drugs were reported in the United States in 2010 (NIH, 2014). Preclinical models have been used to study the abuse potential in an effort to understand the effects that cocaine may have on the brain. One study found that animals with an unlimited access schedule of cocaine (0.2mg/kg) self-administration only survived less than two weeks and an average of five days before dying (Johanson, Balster & Bonese, 1976). Cocaine's abuse potential is due to its psychopharmacological effects, namely its action in blocking

reuptake of dopamine (Ritz et al., 1987; Fischman, 1987). The inhibition of DA reuptake mediates the reinforcing effects of cocaine, in both humans and animals (Ritz et al., 1987; Johanson & Fischman, 1989).

Condition placed preference (CPP) is one method of studying motivational aspects of a drug by using it as an unconditioned stimulus that is repeatedly paired with conditioned, environmental stimuli. If the drug is deemed motivational, the subject will approach this conditioned environment more frequently. CPP is an example of a classical conditioning model that can be used to study rewarding or aversive aspects of drugs. CPP has shown extinction effects when the environment is no longer paired with the drug (Calcagnetti and Schechter, 1993; Hughes et al., 1995; Hinson et al., 1993), which offers validity to its use for studying drug taking behavior. While CPP is a beneficial measure because it can test for both preference and aversion and it is adaptable to multiple animal models, CPP may lack external validity and cannot study dose curves (Bardo & Bevins, 2000). Additionally, chronic mild stress (CMS) has shown to disrupt amphetamine- morphine-, and food-induced CPP (Papp et al., 1991; Papp et al., 1992; Papp et al., 1993), which indicates that stress experience may alter drug-taking behavior.

Intravenous (IV) self-administration studies are another frequently used preclinical model to measure the abuse potential of a drug because self-administration can model the reinforcing effects of the experimental drug (Weeks, 1962; Deneau, Yanagita & Seevers, 1969). Unlike CPP, self-administration studies are based on operant conditioning, can be used in pigeons and primates, in addition to rodents, and have a higher face validity than CPP. IV self-administration is an ideal model because, for example, it bypasses the aversive taste of drugs, which is a hindrance in oral self-administration (George et al. 1990), and it is both fast-acting and the dose is controllable. Previous studies have show that monkeys and rats will work, by lever pressing,

for a cocaine infusion (Weeks, 1962; Deneau et al., 1969). However, mouse studies are inconsistent and mice do not always self-administer cocaine (Deroche et al., 1997; Roberts, Polis & Gold, 1997; Yap & Miczek, 2007). The stages of IV self-administration can be studied in terms acquisition, maintenance using a fixed ratio (FR) schedule, motivational aspects using a progressive ratio (PR) schedule, escalation, binge, and extinction.

Cocaine and Stress

Stress has been shown to increase self-administration of cocaine during acquisition, FR dose-response, and reinstatement (Piazza and Le Moal, 1998). Further, some studies have shown that laboratory animals with a stress history will self-administer more cocaine than non-stressed controls (Haney et al., 1995; Miczek & Mutschler, 1996; Goeders and Guerin, 1994).

Acquisition has also been found to increase in rats receiving non-contingent foot shocks (Goeders and Guerin, 1994). Male and female rats were found to have increased acquisition for cocaine taking after five episodes of social defeats. The stress procedure produced statistically significant differences in the number of cocaine infusions during the two and a half sessions after two days of self-administration access (Haney et al., 1995).

Another study examined rats' cocaine taking behavior after four defeat sessions during the maintenance phase on an fixed ratio (FR-1) schedule for IV self administration. The rats that had been socially defeated acquired, defined as two consecutive sessions of 15 infusions, nearly twice as fast as the non-stressed control rats during the 23-hour session (Tidey & Miczek, 1997). Similar results were seen when rats experienced a single, 60 minute episode of threat, but not actual aggressive confrontation (Miczek & Mutschler, 1996). Foot shocks have also been shown to reinstate cocaine self-administration lever-pressing behaviors in rats that were drug deprived for 4-6 weeks (Erb, Shaham, & Stewart, 1996).While the types of stress and stress intensity vary,

similar conclusions can be made: rats self-administer more cocaine after experiencing stress, including social defeat stress.

Mouse studies, however, are less conclusive. One study found that there was no difference between the number of cocaine self-administration infusions between CFW mice that experienced ten days of social defeat stress and non-stressed controls. The self-administration sessions lasted three hours or a maximum of 50 infusions (0.30, 0.56, 1.00, or 1.78 mg/kg) of cocaine and were on a progressive ratio schedule. However, there was no overall stress effect found (Yap & Miczek, 2007), conflicting with known data from rats. The present study aims to examine different intensities of social defeat stress in mice, with the hypothesis that varying intensities of stress have different effects of cocaine-taking behavior.

Dopamine and Drug Use

It is estimated that 30-40% of patients do not respond to the first antidepressant drug they use and 10% of total patients never respond to antidepressant drugs (FDA, 2013). This indicates the lack of understanding of the way depression and other mood disorders affect the brain. While studies on the rewarding effects of psychostimulants have had a particular focus on dopamine, many studies of depression have focused on serotonin and norepinephrine. However, there is increasing evidence that dopamine, particularly the mesocorticolimbic dopamine system, also plays a key role in mood disorders (Berton et al., 2006; Dunlop & Nemeroff, 2007; Nestler & Carlezon, 2006;). The paradoxical findings that stress and rewarding drug use produce similar effects on the dopaminergic system have been replicated many times, but the reasons behind the effect is not well understood.

In 1969, Deaneau and colleagues first showed that monkeys will freely self-administer morphine, codeine, cocaine, d-amphetamine, pentobarbital and ethanol after a single exposure to

the drug, indicating addictive properties (Deaneau et al., 1969). Since then, many studies have examined neurochemical effects of these drugs these drugs in order to determine what causes their rewarding properties. It has been found that nearly all drugs, including cocaine and amphetamines, increase dopamine levels in the nucleus accumbens (Di Chiara & Imperato, 1988; Wise, 1998). This increase in dopamine is believed to be involved in the drugs' rewarding effects.

Chronic drug administration affects the mesocorticolimbic dopamine pathway (Everitt & Wolf, 2002; Fitzgerald et al., 1996; Ortiz et al., 1996; Saal et al., 2003). The mesocorticolimbic dopaminergic pathway projects from the ventral tegmental area (VTA) to the NAc, causing a release in DA in the NAc. This pathway is believed to be involved in the psychomotor stimulant and reinforcing effects of drugs, including cocaine and amphetamines. The involvement of this pathway has been investigated through experiments that look at lesions, self-administration dopamine and dopamine reuptake inhibitors, and *in vivo* microdialysis. Lesioning the NAc results in a decrease the rewarding effects of cocaine, as evidenced by the amount of lever presses during self-administration (Roberts et al, 1980). Both DA and nomifensine, a DA reuptake inhibitor, also produce rewarding effects when infused into the NAc, more so than other regions of the brain (Carlezon et al., 1995; Dworkin et al., 1986). Microdialysis studies have also shown that DA is increased after drug use (Pettit & Justice, 1995; Vezina, 1993). Overall, the evidence suggests that the mesocorticolimbic pathway, as opposed to the nigrostriatal pathway, is the site involved in reward because the endpoint has been shown in various manners in influence drug taking behaviors.

Sensitization can be defined as an increased responsiveness to a drug or a phenotype related to the drug, typically after intermittent, not continuous access to the drug (Koob & Le Moal, 2001; Robinson & Berridge, 1993). More specifically, sensitization can be behavioral,

such as an increase in locomotion, or neurochemical, such as an increase in dopaminergic response to a drug (Pettit & Justice, 1989; Vezina, 1993). It is an important phenomenon because it alters the mesolimbic dopamine system and the response to a drug, likely increasing addictive properties, cravings and likelihood of later relapse. In rodent studies, three d-amphetamine injections two weeks prior to a challenge injection results in a two-fold sensitized response in DA levels in the NAc (Vezina, 1993). Cocaine also increases DA in the NAc in a dose-dependent manner during repeated exposure (Pettit & Justice, 1989; Kalivas & Duffy, 1995).

Dopamine and Stress

Similar to the effects that psychostimulants have on DA in the nucleus accumbens, stress also increases extracellular DA in the NAc. This means that stress can have a cross sensitization effect. Cross-sensitization is when an animal is exposed to one drug or behavior, and then a different drug is used to show a sensitized response. Previous studies have shown that prior stress exposure can result in sensitization of locomotor activity and extracellular dopamine in response to a d-amphetamine challenge. Microdialysis studies have shown that DA in the accumbens increases during the "threat" phase of a social defeat (Tidey & Miczek, 1996) and while footshocks are being administered (Kalivas & Duffy, 1995). Furthermore, data suggests that stress has a cross-sensitization effect on drug taking. Previously defeated mice acquire cocaine in half the time that non-stressed controls acquire (Tidey & Miczek, 1997), which indicates that social defeat stress likely alters the mesocorticolimbic pathway to make the individual more vulnerable to psychomotor stimulant self-administration. Cross-sensitization of social defeat stress and cocaine in rats also has a great sensitization effect on locomotion and increases cocaine taking during a binge period (Covington & Miczek, 2001).

Most studies conclude that stress and the use of psychostimulant both alter and create long-lasting changes to the mesocorticolimbic pathway. Injection of a drug or exposure to stress increases DA in the NAc and locomotor activity, indicating sensitization or cross-sensitization effects. In addition, exposure to stress, even prenatal stress, can increase the amount of drug taken during self-administration weeks after the stress experience (Koob & Le Moal, 2001). However, there has not been a study that examines the cross-sensitization effect that different stress intensities have on extracellular DA in the NAc after an amphetamine challenge in mice. The present study intends to replicate similar findings in rat studies in order to ensure generalization across species.

Objective

Previous studies have examined and found cross-sensitization effects in dopamine in the nucleus accumbens in response to an amphetamine injection after social defeat stress in rats. However, these studies have not been replicated in mice. It is important for validity for the findings to be found in multiple species. In addition, two levels of stress intensity have not been studied in either the affects that stress on DA in the NAc or cocaine self-administration in mice. Since there is no widely accepted model for depression in rodents, it is important to examine the varying effects that different stress intensities may have on the mice. Finally, individual differences will be examined in DA in the NAc and behavior during the social defeat procedure.

We postulate that the two stress intensities, brief (15 attack bites) and moderate (30 attack bites), will have differing effects on extra-cellular DA levels in the nucleus accumbens, self-administration of cocaine, and behavior during the social defeat stress experiment. The mice are expected to show an increased stress response after the more severe stress experience. While part

of the study mimics rat studies, behavioral analysis in addition to the microdialysis and self-administration add new, differentiating aspects to the study.

Methods

Subjects

Adult male Carworth Webster (CFW) mice (Charles River Laboratory, Kingston, RI, USA) weighed 23-25 grams upon arrival and were assigned to serve as residents, social stress, or non-stress control mice. Social stress and non-stress control mice were group housed upon arrival in a 46 × 24 × 16 cm polycarbonate cage with corncob bedding (Shepherd's Specialty Blend Alpha-dri/Cob Blend, Shepherd's Specialty Papers) and a stainless steel wire lid. After habituating for three to four days, those mice were then singly housed in 28 × 17 × 14 cm clear polycarbonate cages containing pine shavings and topped with stainless steel wire lids. Resident mice were pair-housed with female CFW mice (Charles River Laboratory, Kingston, RI, USA) immediately after arrival. The breeding pairs were also housed in the 28 × 17 × 14 cm polycarbonate cages specified above.

All mice lived under controlled conditions (21 ± 2º C, 20% humidity) under a 12-h reversed light/dark cycle with lights on at 19:00 and off at 07:00. The mice were allowed unrestricted access to water and food (Purina #5001 Rodent Diet, PMI Nutrition International, Brentwood, MO, USA). The procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Tufts University and mice were cared for following the NIH Guide for the Care and Use of Laboratory Animals.

Experimental Design

Experiment 1: Behavioral Analysis and In Vivo *Microdialysis*

Mice (n=20) were randomly assigned to the moderate stress (n=7), brief stress (n=7) or non-stressed controls (n=6) group. The stress groups experienced social defeat stress for ten days and each defeat session was video recorded for later analysis. After the final day of stress, a cannula aimed at the nucleus accumbens was implanted. Five days later, microdialysis was performed (Figure 1).

Experiment 2: Intravenous Cocaine Self-Administration

Mice (n=43) were randomly assigned to the moderate stress (n=11), brief stress (n=18) or non-stressed controls (n=14) group. After the ten day social stress experiment, a catheter was implanted into the jugular vein for intravenous (IV) cocaine self-administration. Six days after the stress period ended, mice were challenged with 1.5 mg/kg d-amphetamine, then began the cocaine self-administration study the following day (Figure 1).

Social Defeat Stress Procedure

A prerequisite for the social defeat procedure was stable, reliable aggressive behavior by resident. To accomplish this, male residents mice were screened daily for their aggression using the resident-intruder confrontation (Miczek, 1979; Miczek, Thompson & Shuster, 1982). This screening process included up to two minutes of instigation, as well as a five-minute defeat session. During the screening, the aggressive confrontation lasted for five minutes. Resident mice that consistently attacked the intruder mice 25 or more times were selected to be used during the social defeat procedure.

After a minimum of six days of habituation to the laboratory, the experimental mice were randomly assigned to either the non-stress control or social stress groups. The control mice were handled daily. The social stress group was exposed to social defeat stress for 10 consecutive days, acting as intruders to an aggressive, resident mouse in its home cage (Miczek et al, 1982).

The social stress mice were subjected to a three-phase social defeat process, as defined by Yap and colleagues (2006). During the social defeat process, the female mouse and any pups were removed from the resident's home cage and placed into a separate, clean cage. The first part was the *instigation* phase, in which the intruder mouse was placed into a small, perforated and protective cage (7 x 7 x 15 cm) within the resident mouse's home cage for five minutes. The second phase was the *defeat* phase in which the intruder mouse was placed into the home cage of the resident mouse without the protective cage and attacked for either five minutes or until the maximum number of bites was received, whichever ended first. The threshold was either set at 15 bites or 30 bites. Brief stress refers to the group limited to 15 attack bites, while moderate stress refers to the group receiving 30 attack bites. The defeat phase was video recorded for further behavioral analysis that will be specified. The final, *threat*, phase mimicked the instigation phase; the intruder was placed back into the protective cage that was inside the resident's home cage for five additional minutes. Residents were rotated daily so that the social stress mice confronted a different resident everyday.

Behavioral Analysis

The defeat phase of the social stress procedure was recorded (JVC camcorder model no. GZ-MG670BU). The videos from days one and ten of the defeat phase were analyzed (Observer XT, Noldus, v. 9.0.436; Wageningen, The Netherlands) to code behavior from both the resident and social stress mice. The objective of the video analysis was to determine differences in coping style between the mice, both based upon the manipulation of number of bites received and to determine if the social stress mice could be classified as having an *active* or *passive* coping style. For the social stress mice, the behaviors analyzed were walk, rear, self-groom, contact, escape, defensive upright and crouch. Submissive posture was not measured because it is rarely seen in

CFW mice. For the residents, sideways threat, tail rattle, pursuit and bites were analyzed. A detailed description of these behaviors is provided in Table 1 (Brain, McAllister & Walmsley, 1989). The frequency and duration of each of the behaviors was recorded for statistical analysis.

The microdialysis sessions were also video recorded during three specific time points and analyzed using the programs specified above. The recordings were all ten minutes in duration and were recorded from five to fifteen minutes after the saline injection, ten to twenty minutes after the d-amphetamine injection and fifty to sixty minutes after the d-amphetamine injection (see Figure 2). Walking, rearing and self-grooming were the three behaviors that were analyzed in order to determine any sensitization effect.

Intracranial Surgery

Mice were anesthetized with a ketamine (100 mg/kg) and xylazine (10 mg/kg) mixture (i.p.) and given carprofen (5 mg/kg, i.p.) as an analgesic preceding surgery. The dorsal skull was shaved and cleaned using alcohol wipes and betadine. After securing the mouse in a stereotaxic frame, a guide cannula (CMA/7, CMA Microdialysis, Chelmsford, MA) was implanted into the nucleus accumbens shell (+1.7 mm anterior posterior, -0.7 mm medial lateral, 4.0mm dorsal ventral), according to a mouse brain atlas (Paxinos & Franklin, 2001). The cannula was secured to the skull using cement. The mice were given 6-8 days of recovery after surgery.

Microdialysis

Following the recovery period, microdialysis was performed. The night before the microdialysis session, the mouse was anesthetized using isoflurane and a microdialysis probe (CMA/7) with a 1 mm active membrane was inserted through the guide cannula. Artificial cerebral spinal fluid (aCSF: 147 mM NaCl, 1.2 mM $CaCl_2$, 0.85 mM $MgCl_2$, 2.7 mM KCl; CMA Microdialysis, North Chelmsford, MA) was perfused overnight at a rate of 0.5 μL/min using an

infusion pump (CMA Microdialysis, North Chelmsford, MA). Sample collection became the next day, after an hour of equilibrium baseline collection at a flow rate of 2.0 μL/min. Samples were collected every 10 minutes into vials that contained 5 μL of a stabilizing agent (20 mM phosphate buffer containing 25 mM EDTA and 0.5 mM ascorbic acid). Five baseline samples were taken before the saline injection (10 ml/kg, i.p.), two samples were collected after the saline injection, and twelve samples were collected after the d-amphetamine challenge (1.5 mg/kg, i.p.). A timeline of the sample collection times and video recording time bins can be found in Figure 2.

The 25 μL samples were analyzed using high performance liquid chromatography (HPLC) to determine tonic and phasic DA concentrations. A manual injector (model 7,125; Rheodyne, Cotati, CA, USA), pump (LC10-AD, Shimadzu, Columbia, MD, USA), and a DECADE II electrochemical detection system (Antec Leyden BV, Zoeterwoude, The Netherlands) were used along with a CAPCELL PAK cation-exchange column (1.5 mm × 250 mm, 5 μm I.D., Shiseido, Tokyo, Japan). The mobile phase contained 150 mM ammonium acetate, 50 mM citric acid, 27 μM EDTA, 10% methanol, and 1% acetonitrile and the pH was adjusted to 4.6. The flow rate was 0.2 mL/min. DA levels were calculated based on the standard curves, which were tested shortly before each microdialysis experiment. Standard curves were run periodically and consistently resulted in a correlation coefficient greater than 0.99. Level of detection was 0.18 pg.

Intravenous Cocaine Self-Administration

Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) mixture (i.p.) and then, using sterile procedures, implanted with jugular catheters. A 7.0-cm section of silastic tubing (Dow Corning; 0.30 mm ID, 0.64 mm OD) was first inserted 1.2 cm into the right jugular vein, then flushed with sterilized saline. It was fixed in place using both silk sutures and

tissue adhesive (VetBond). A 22-guage back-mount cannula connector pedestal (Plastics One, Roanoke, VA) was placed under the skin between the shoulder blades and was connected to the other end of the catheter. Animals were allowed 5 days of recovery periods before starting the training for cocaine self-administration.

After recovery, the mice began the acquisition phase. They nose poked to receive infusions of cocaine on a fixed-ratio 1 (FR-1) schedule of reinforcement throughout the acquisition and testing periods. There was a 28 second time-out period to avoid very rapid infusions that could lead to cocaine overdosing and each self-administration session lasted 2 hours. The acquisition phase lasted three days and the mice were able to administer up to 20 infusions of 1 mg/kg of cocaine. The mice reached stable baselines of responding during this time. After acquisition phase, the testing phase began. There were two test days for each of the doses of cocaine (1, 0.6, and 0.3 mg/kg/inf), as well two test days for saline infusions. There was a 20, 50 or 100 infusion limit for each cocaine dose, respectively, to prevent overdose.

The catheter was flushed daily and at the end of the experiment with heparinized saline (0.02 ml of 30 IU/ml solution) to help maintain patency of the catheter. The patency was evaluated periodically and if drug self-administration behavior seemed to significantly stray from typical behavior. If this happened, the catheter was flushed Brevital (JHP Pharmaceuticals). The mouse was excluded from the experiment if signs of anesthesia did not occur within 10 s of infusion.

Statistical Analysis

SigmaStat 11.0 (Systat Software, San Jose, CA) was used to perform statistical analyses. Two-way, repeated measures analysis of variances (ANOVAs) were used to compare differences between overall effects of time and stress condition, as well as the interaction between time and

stress condition for the percent change of DA in the NAc for the microdialysis experiment. Two-way, repeated measures ANOVAs were also used to compare between and within group effects for the behavioral analysis. The Bonferonni post-hoc test used for all further analysis.

Results

Behaviors During Social Defeat Stress

Several behaviors were analyzed for frequency and duration during days one and ten of the social defeat procedure for both the resident mouse and the stressed mouse. The mean time of all behaviors analyzed during the social defeat can be seen in Figure 3. There was no difference in the frequency per minute of attack bites or pursuits exhibited by the aggressive resident between days or stress group. The residents, however, did show an increased number of sideways threats to the brief stress group, compared to the moderate stress group on day 10 ($t=3.04$, $p=0.01$) (Table 2).

The behaviors of stressed mice were analyzed by percent time, frequency per minute, frequency during the first 40s of defeat and proportion of times that behavior occurred in one second after an attack bite. The first 40s of the defeat was examined because the brief stress social defeat lasted approximately two minutes, while the moderate stress defeats typically lasted around four minutes. Some of the behaviors exhibited by the stressed mice had some marked differences that indicated both a time effect and an effect of stress intensity. Overall, there were more behavior initiations, exhibited by the brief stress group than the moderate stress group on day 10 ($t=2.79$, $p<0.05$), while there were no differences on the first day (Table 3). Mice in the brief stress group spent less time walking on day 10 than day 1 ($t=2.41$, $p<0.05$), although there was no overall difference in activity level (Table 4).

Most of the differences seen were in regards to escapes. When analyzed using frequency per minute, there were more escapes by the brief stress group than the moderate stress group on day 10 ($t=2.77$, $p<0.05$) and an increased number of escapes within the brief stress group on day 10 compared to day 1 ($t=2.56$, $p<0.05$) (Figure 5). In a similar pattern, a higher proportion of attack bites were immediately followed by escapes on day 10 than day 1 for both stress groups (brief: $t=2.43$, $p<0.05$; moderate: $t=3.71$ $p<0.01$), but on day 1, the brief stress group was more likely to escape following a bite than the moderate group ($t=2.41$, $p<0.05$) (Figure 6). Escape behavior during the first 40s of defeats were also examined. There were no differences between escapes for days 1 and 10 in the brief stress group, but the moderate stress group escapes significantly more on day 10 than on day 1, during the first 40s of the defeat ($t=2.82$, $p<0.05$) (Figure 7).

Finally, frequency of defensive upright posture was also found to have some differences over time and between stress conditions. Mice in the brief stress group went into defensive upright more often in the one second following an attack bite on day 10 than day 1 in the brief stress group ($t=2.95$, $p<0.05$), with no difference among the other groups (Figure 8). During the first 40s of the defeat, the brief stress group also displayed defensive upright posture more often on day 10 than day 1 ($t=3.31$, $p<0.01$), while the moderate stress group showed the opposite effect and were more likely go display the defensive posture on day 1 than day 10 ($t=2.21$, $p<0.05$) (Figure 9).

In vivo *Microdialysis*

Five *in vivo* microdialysis DA measurements were taken before the saline injection in order to obtain a stable baseline for each mouse. The measurements were not significantly different between the non-stressed controls and either stress group during baseline. This was

confirmed when analyzed with a two-way repeated measures analysis of variance (ANOVA). The baselines within group varied greatly, so percent change from baseline was used for further analysis. In addition, there was no artifact from the saline injection; baseline DA levels and DA after the saline injection were not different in any group (Figure 10).

After the d-amphetamine injection, there was an increase in extracellular DA in the NAc in all three groups. The peak increase in DA was significantly greater in both stress groups than the non-stressed controls. The DA peak in the non-stressed group and the brief stress group returned to baseline much quicker than the moderate stress group, as the moderate stress condition resulted in a prolonged increase in DA (Figure 10). Two-way repeated measures ANOVA confirmed that there was a main effect of stress intensity [$F(2,306)=6.87$, $p<0.01$], a main effect of time [$F(18,306)=43.37$, $p<0.001$], and an interaction between stress intensity and time [$F(36,306)=3.64$, $p<0.001$] on DA in the NAc. Post-hoc tests showed that overall there was a significant difference in DA concentration between the moderate stress (30 bite) group and the non-stressed control group ($t=3.70$, $p<0.01$), as indicated by the slower decrease in DA levels for the moderate stress group.

Further post-hoc analysis revealed that samples 9, 10, and 12, which correspond to 10, 20, and 40 minutes after the d-amphetamine injections, extracellular DA in the NAc was significantly greater in the brief stress group compared to controls [10 min: $t=5.46$, $p<0.001$; 20 min: $t=4.79$, $p<0.001$; 40 min: $t=2.58$, $p<0.05$]. In addition, the moderate stress group was significantly different from controls at samples 10, 20, 30, 40, 50, 60, 70, 80, 90 and 110 minutes after injection [10 min: $t=3.35$, $p<0.01$; 20 min: $t=5.55$, $p<0.001$; 30 min: $t=4.49$, $p<0.001$; 40 min: $t=4.48$, $p<0.001$; 50 min: $t=3.18$, $p<0.01$; 60 min: $t=2.79$, $p<0.05$; 70 min: $t=2.74$, $p<0.05$; 80 min: $t=2.79$, $p<0.05$; 90 min: $t=2.68$, $p<0.05$; 110 min: $t=2.56$, $p<0.05$]. The moderate stress

group was different than controls for most of the time points after the amphetamine injection, while the brief stress group was only different for 3 time bins shortly after the injection.

Cocaine Self-Administration

After acquisition, the number of IV self-administration infusions mice took was measured using three different unit doses. While the groups were not different in the number of infusions of saline, 0.6 or 1.0 mg/kg of cocaine, both stress groups administered more cocaine at the 0.3 mg/kg dose, based on two day averages (Figure 11). A two-way repeated measures ANOVA indicated there was a main effect of dose [$F(3,114)=44.19$, $p<0.001$] and an interaction between stress group and dose [$F(6,114)=2.58$, $p<0.05$]. Post-hoc tests confirmed that brief and moderate stressed mice took significantly more infusions at 0.3 mg/kg dose than the control mice [brief: $t=3.49$, $p<0.01$; moderate: $t=2.99$, $p<0.05$]. In addition, all groups had more cocaine infusions at the 0.3 mg/kg dose compared to saline [control: $t=2.86$, $p<0.05$; brief: $t=7.65$, $p<0.001$; moderate: $t=6.65$, $p<0.001$]. The two stress groups had more infusions at the 0.6 mg/kg dose compared to saline infusions, as well [brief: $t=4.39$, $p<0.001$; moderate: $t=2.83$, $p<0.05$]. There were no differences between the stress and control groups in the number of infusions taken for saline, or at the 0.6 or 1.0 mg/kg/inf doses.

Discussion

The present set of experiments has shown that mice exposed to 10 days of social defeat stress experience changes that lead to an increase in cocaine self-administration and increased extracellular DA in the NAc in response to an amphetamine injection days after the stress has ended. Further, different stress intensities have distinct effects on DA, with moderate stress (30 attack bites) group having enhanced DA for nearly two hours after the amphetamine injection,

while brief stress (15 attack bites) group was no different than controls after 50 minutes. Behavioral analysis also indicated there may be some differences in coping style between the two groups of varying stress intensities. The two stress intensities result in distinct effects on behavior during the stress experience and long-term effects on DA in the NAc in response to an amphetamine challenge.

Behavioral Analysis During Social Defeat Stress

Brief stress and moderate stress induce different behavioral reactions from the intruder mice during social defeat stress. Experience also alters the behavior of the mice. Mice experiencing brief stress, as characterized by 15 bites in less than 5 minutes, tend to escape and display defensive upright more frequently than moderate stressed mice (30 bites in less than 5 minutes). Few other intruder and resident behaviors showed significant effects of day or treatment group.

Frequency of escapes and defensive upright was higher in the brief stress group than the moderate stress group when examining frequency per minute, frequency during the first 40s of the defeat, and proportion of escapes in one second after a defeat. While all three methods of data analysis did not find significant differences between the brief and moderate stress groups for the same behaviors, overall, it is indicated that mice experiencing brief social defeat escape more often than those experiencing moderate social defeat. This is potentially explained by the moderate stress inducing more depressive-like symptoms than the brief stress. The moderately stressed mice are less likely to avoid the attack bites, by either escaping or displaying defensive upright posture.

Previous studies have shown that passive behaviors, usually including defensive upright, are correlated with other decreased movement, such as non-social exploration (Gomez-Lazaro,

2011). The passive group differed from the active group in BDNF and corticosterone levels (Gomez-Lazaro, 2011). While the passive and active groups experienced the same stress but were categorized based solely upon behavior in the previous study, unlike the present study which mice are categorized by stress intensity, the overall categorizations may still be relevant. Future studies should examine other neurochemical measurements, such as BDNF or corticosterone to determine any differences between stress intensities since corticosterone can be used to measure the stress response and BDNF is related to behavioral changes due to mood disorders (Dunman & Monteggia, 2006). Additionally, a larger sample size would allow for passive and active group distinctions within the two stress intensities.

When analyzing behavioral data to determine frequency of escapes and defensive upright, frequency per minute was often used for analysis to account for the length of defeats lasting between 40s and 5m. Although this accounted for the average frequency of a behavior within a standard time period, there were likely differences in occurrence of the behavior in the beginning and end of the defeat session. In addition, brief stress typically lasted 2 minutes, but moderate often continued for the entire 5 minutes. In order to account for these differences, frequency during the first 40s of the defeat was also examined for escapes and defensive uprights. This time period of 40s was chosen because that was the length of the quickest defeat.

Another way to account for time differences was to examine the behaviors immediately following an attack bite. This was done to account for the differences in timing of the attack bites. One limitation is the shortest duration Observer XT software was able to examine was 1s after an attack bite. This resulted in the proportion of escapes and defensive uprights occurring after an attack bite to be more than 100%, indicating that mice both escaped and displayed defensive

upright behavior within one second after an attack bite, although the behavior that was initiated first is not indicated.

Despite these limitations, all three measures (frequency per minute, frequency during the first 40s of defeat, and proportion of behavior after an attack bite) indicted that mice in both stress groups escaped more frequently on day 10 than day 1. This may reflect that the mice learned that escape behavior was an effect way of avoiding attack bites. The behavior for defensive upright was less conclusive, but trends appeared to show that defensive upright was more common in the brief stress group on day 10 than on day 1 and than the severe stress group on day 10. This is indicated by both significant results and non-significant trends in the three measurements.

There was an overall effect of time, indicating learning, for an increased number of escapes and defensive uprights, both behaviors that assist with avoiding being bit by the resident mouse. In addition, the briefly stressed mice were more likely to show these defensive behaviors, indicating that there was a stress effect in which the moderate stress group was less likely to show defensive behaviors, potentially indicating depressive-like symptoms.

In Vivo *Microdialysis*

An i.p. injection of 1.5 mg/kg d-amphetamine produced distinct effects to the DA in the NAc between the control, brief, and moderate stress groups. While the groups were not different in baseline DA concentration, percent change was analyzed due to the high variability between subjects, which could be due to inconsistent sensitivity of the HPLC.

As predicted, there were significant main effects between stress and time, as well as an interaction between stress and time. Similar to previous studies in rats, all groups showed an increased DA response after a d-amphetamine challenge. In addition, both stressed groups

showed a significantly higher percent change in DA than the non-stressed controls, which indicates cross-sensitization, which is consistent with literature (Robinson & Berridge, 1993; Tidey and Miczek, 1997; Vezina, 1993).

Differences between the moderate and brief stress groups were also seen in DAergic response. The brief stress group's DA response was only significantly higher than controls for 3 time bins, while the moderate stress group remained higher for nearly the entire 110 minutes measured after the d-amphetamine injection. This confirms that there was an overall significant difference between moderate stress group and control group, which was not seen between the brief stress and control. The prolonged effect of elevated DA likely indicates that a more intense stressor alters DAergic response in a different manner than a mild stressor. This could be because of an increased firing rate of DA neurons, an increased amount of DA released, or decreased reuptake activity. The moderate stress, however, cannot be classified as a severe stress because rat studies have shown that chronic social defeat stress results in a blunted DAergic response while intermittent social defeat stress has the heightened response (Shimamoto et al., 2011).

Previous studies have shown that repeated exposure to different types of stress, including social defeat stress, can increase DA in the NAc both during a stress experience (Abercrombie, et al., 1989; Tidey and Miczek, 1996) and days or weeks after the stress experience (Miczek, et al 2008; Krishnan and Nestler, 2010). The long term alteration in DA is important to study as mechanisms for this increase are not well known and could be due to a variety of reasons. Anstrom and colleagues suggest that social defeat stress causes a long-term increase in firing bursts in the ventral tegmentum area (VTA) (2009), which projects onto the NAc. Further studies should examine the mesolimbic dopamine system, particularly dopamine neurons in the VTA to better understand their role in the stress response.

Cocaine Self-Administration

Social defeat stress can increase cocaine self-administration in mice during maintenance while using a fixed ratio (FR) schedule. While the stress and control groups both showed a cocaine reinforcement effect by taking significantly more infusions at the 0.3 mg/kg dose than saline, only the two stress groups took significantly more infusions at the 0.6 mg/kg dose of cocaine compared to saline. The mice self-administered the lowest dose of cocaine (0.3mg/kg/inf) more frequently when they were tested one week after the stress experience ended, compared to non-stressed controls. Previous studies have shown that stress experience can increase self-administration shortly after stress experience (Tidey & Miczek, 1997; Miczek & Mutschler, 1996), and can have an effect more than one week after the stress experience (Haney et al., 1995). Additionally, other studies have found that rats with a stress history self-administer more cocaine during 24-h binges, as opposed to acquisition (Covington & Miczek, 1991). Other mouse experiments have not seen a marked difference in cocaine self-administration as a function of stress (Yap et al, 2006). This may suggest that stressed animals are only more sensitive to cocaine than controls at lower doses.

Logrip and colleagues have suggested that the stress effect produces the largest increase in drug taking behaviors 24 hours after exposure to the stressor, with an immediate drop in drug effect, before an increase over time (Logrip et al., 2012). Future studies could examine the effects of elapsed time between the stress experience and cocaine self-administration to see how long the increased sensitivity lasts and if it increases overtime, as suggested by Logrip and colleagues.

Conclusion

The intensities of social defeat stress can produce distinct effects on DA, drug taking, and behavior. Moderate stress consists of twice as many attack bites than brief stress and these differences are demonstrated in the three phases of the experiment. Since corticotrophin releasing factor (CRF) has been implicated as an important neuropeptide for initiating the stress response (Shekar et al., 2005), future studies should examine the effects of CRF and CRF receptor antagonists to determine if these stress effects can be blocked or attenuated.

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Figure 1. Experimental design timeline for experiments 1 and 2. Timeline of experiments 1 and 2. Both began with ten days of social defeat stress, then either had intracranial (IC) surgery to implant a cannula for the *in* vivo microdialysis experiment or had intravenous (IV) surgery to implant a catheter for cocaine self-administration (self-ad).

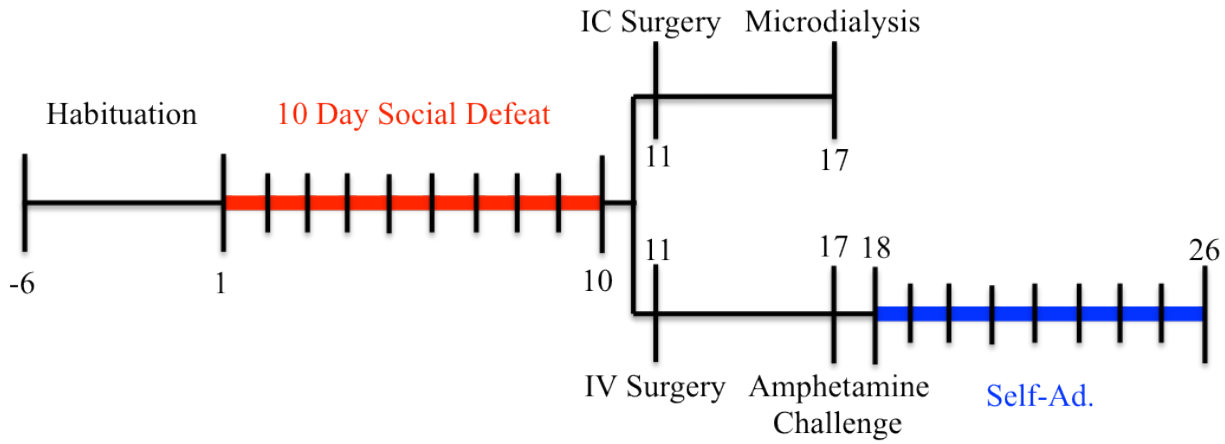


Table 1. Behaviors exhibited by intruder and resident mice during social defeat stress. The behaviors adapted from Brain and colleagues (1989) that were coded and analyzed for intruder and resident mice during social defeat stress.

Animal	Behavior	Brief Operational Definition
Intruder Behaviors	Walk	Walking around the cage
	Rear	Forepaws and front half of the body lifted off the ground in a bipedal manner; activity must be unrelated to the resident's presence
	Self-groom	Licking flanks or abdomen; licking forepaws then stroking the head, specifically the ears and snout
	Contact	Sniffing the head and nose or anogenital region of the resident
	Escape	Rapid movement to move away from the resident animal
	Defensive Upright	Forepaws and front half of the body lifted off the ground in a bipedal manner; directed towards the resident mouse; ears are usually pushed back
	Crouch	Immobility of the body
Resident Behaviors	Sideways Threat	Tripedal posture, oriented towards the intruder mouse. Forepaw nearest intruder mouse is raised; eyes are narrowed and ears are back
	Tail Rattle	Rapid movement or thrashing of the tail
	Bite	Biting the intruder mouse
	Pursuit	Rapidly following the intruder mouse around the cage, typically exhibited following an escape.

Figure 2. Timeline of microdialysis samples and video recording. This schematic indicates d-amphetamine injection at time point 0. Samples were taken every ten minutes, indicated by each line, from -70 minutes to 110 minutes. Behavior of the mouse was video recorded for ten minutes at three different time bins, as indicated by the blue rectangles.

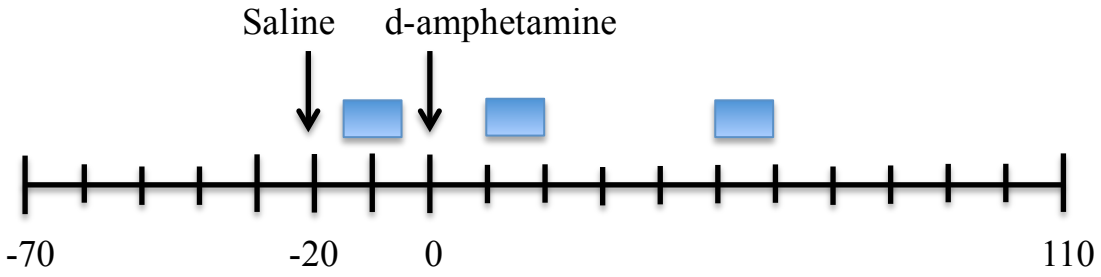
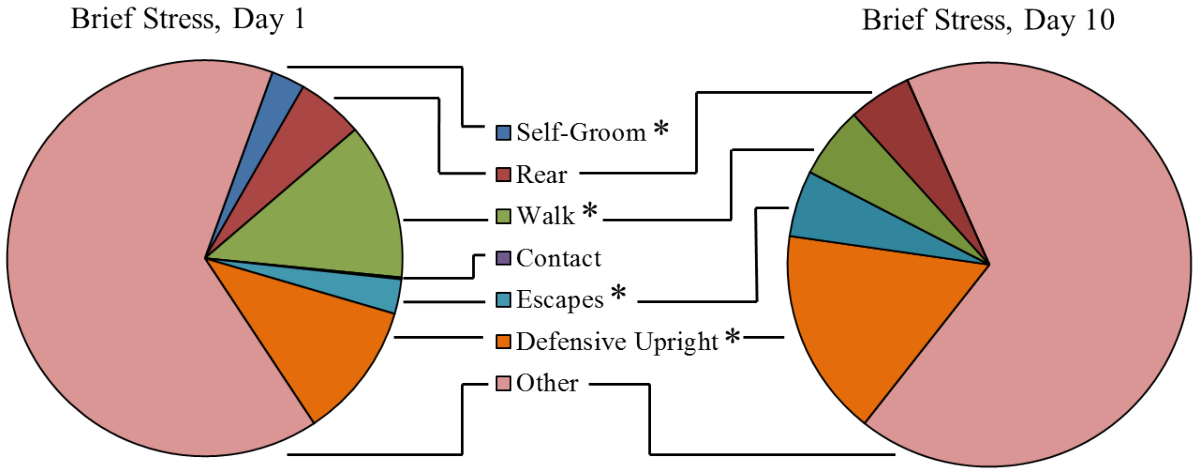


Table 2. Frequency per minute of the residents' behaviors during social defeat stress. Frequency per minute of three behaviors displayed by the residents fighting with a briefly or moderately stressed mouse on days 1 or 10 of the social defeat procedure. Significance ($p<0.05$) between groups, on the same day, is denoted with a pound sign (#).

		Frequency per Minute		
		Bite	Pursuit	Sideways Threat
Brief Stress	Day 1	14.69 ± 3.52	1.22 ± 0.92	11.21 ± 1.93
	Day 10	12.41 ± 2.63	2.24 ± 0.76	15.01 ± 2.17 #
Moderate Stress	Day 1	11.33 ± 3.55	0.876 ± 0.84	12.26 ± 1.88
	Day 10	7.07 ± 1.29	2.63 ± 0.75	7.88 ± 0.87

Figure 3. Behaviors exhibited by stressed mice during social defeat stress. Behaviors analyzed during social defeat stress for the A) brief stressed mice and B) moderate stressed mice on days 1 and 10 of the social defeat procedure.

A



B

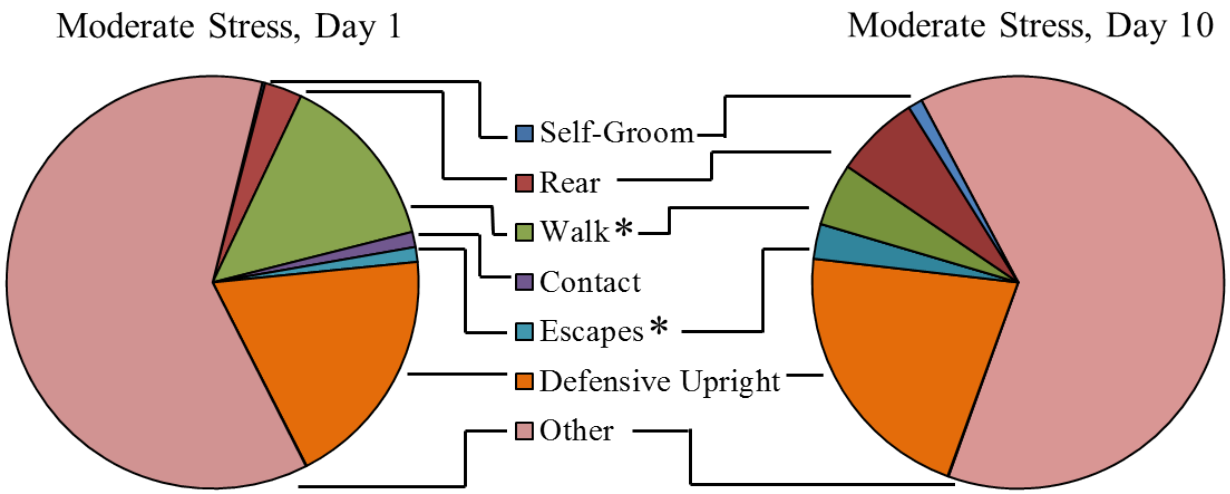

Moderate Stress, Day 1
Moderate Stress, Day 10
Self-Groom
Rear
Walk*
Contact
Escapes*
Defensive Upright
Other

Table 3. Frequency per minute of intruders' behaviors during social defeat stress. The mean and standard error of the mean of the frequency per minute of escapes, defensive upright and behavioral initiations on days 1 and 10 of the social defeat procedure. Behavior initiations are a combined value of all coded behaviors. Asterisks (*) denote significant differences between days 1 and 10 within stress group ($p<0.05$), while pound signs (#) denote significance between groups on the same day ($p<0.05$).

		Frequency per Minute		
		Escapes	Defensive Upright	Behavior Initiations
Brief Stress	Day 1	12.25 ± 4.51	5.32 ± 1.42	34.52 ± 4.44
	Day 10	23.41 ± 5.97 *#	9.50 ± 1.65	39.86 ± 7.71 #
Moderate Stress	Day 1	3.72 ± 2.16	9.07 ± 1.92	23.92 ± 3.37
	Day 10	7.90 ± 1.51	5.86 ± 1.43	20.54 ± 2.29

Table 4. Percent time of intruders' behaviors during social defeat stress. The mean and standard error of the mean of the percent of the social defeat that the intruder spent displaying defensive upright posture, walking, a combined time of non social behaviors (grooming, rearing and walking), and all analyzed behaviors on days 1 and 10 of the social defeat procedure. Behavior initiations is a combined value of all coded behaviors. Asterisks (*) denote significant differences between days 1 and 10 within stress group ($p<0.05$).

		Percent Time			
		Defensive Upright	Walking	Non-Social Behaviors	All Behaviors
Brief Stress	Day 1	10.64 ± 3.36	11.87 ± 2.82	18.65 ± 2.69	32.62 ± 2.28
	Day 10	19.21 ± 4.88	3.73 ± 1.29*	10.90 ± 2.21	38.96 ± 4.77
Moderate Stress	Day 1	19.19 ± 3.03	12.19 ± 2.38	15.33 ± 2.82	36.56 ± 3.36
	Day 10	24.53 ± 5.55	5.37 ± 1.99	12.25 ± 2.76	38.85 ± 5.61

Figure 4. The percent of the time spent walking by brief and moderate stressed mice on days 1 and 10 of defeats. The effect of time and stress condition on the time spent walking that during the social defeat stress on both the first and last days of social defeat stress. Error bars indicate the standard error of the mean. Asterisks (*) denote significant differences between days 1 and 10 within stress group ($p<0.05$).

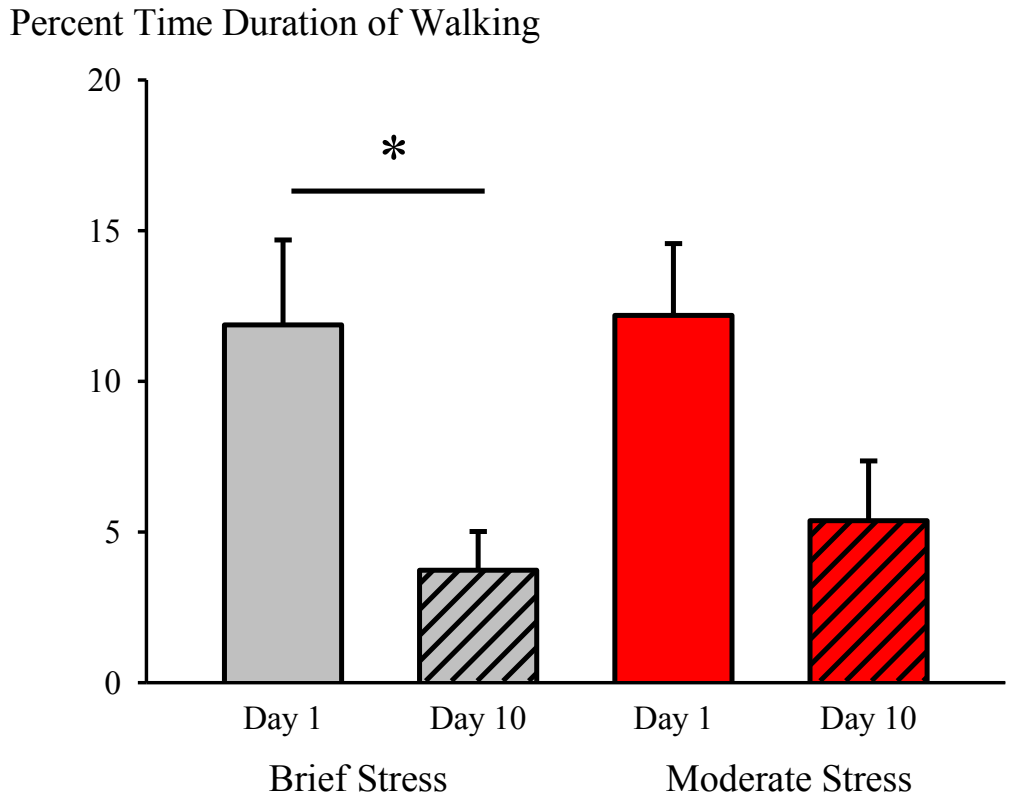


Figure 5. The frequency of escapes per minute by brief and moderate stressed mice on days 1 and 10 of defeats. The effect of time and stress condition on the frequency of escapes that the mice make during the social defeat stress on both the first and last days of social defeat stress. Error bars indicate the standard error of the mean. Asterisks (*) denote significant differences between days 1 and 10 within stress group ($p<0.05$), while pound signs (#) denote significance between groups on the same day ($p<0.05$).

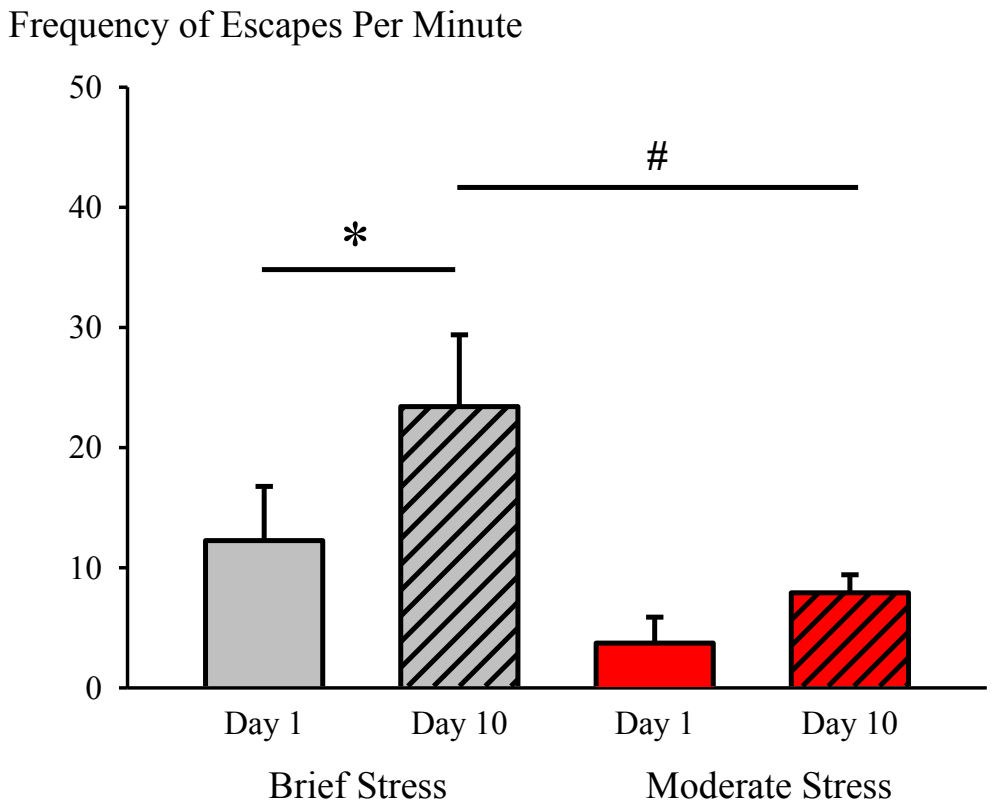


Figure 6. The proportion of times the mice escaped immediately after attack bite by brief and moderate stressed mice on days 1 and 10 of defeats. The effect of time and stress condition on the proportion of times that the mice escapes in the one second immediately following an attack bite during the social defeat stress on both the first and last days of social defeat stress. Error bars indicate the standard error of the mean. Asterisks (*) denote significant differences between days 1 and 10 within stress group ($p<0.05$), while pound signs (#) denote significance between groups on the same day ($p<0.05$).

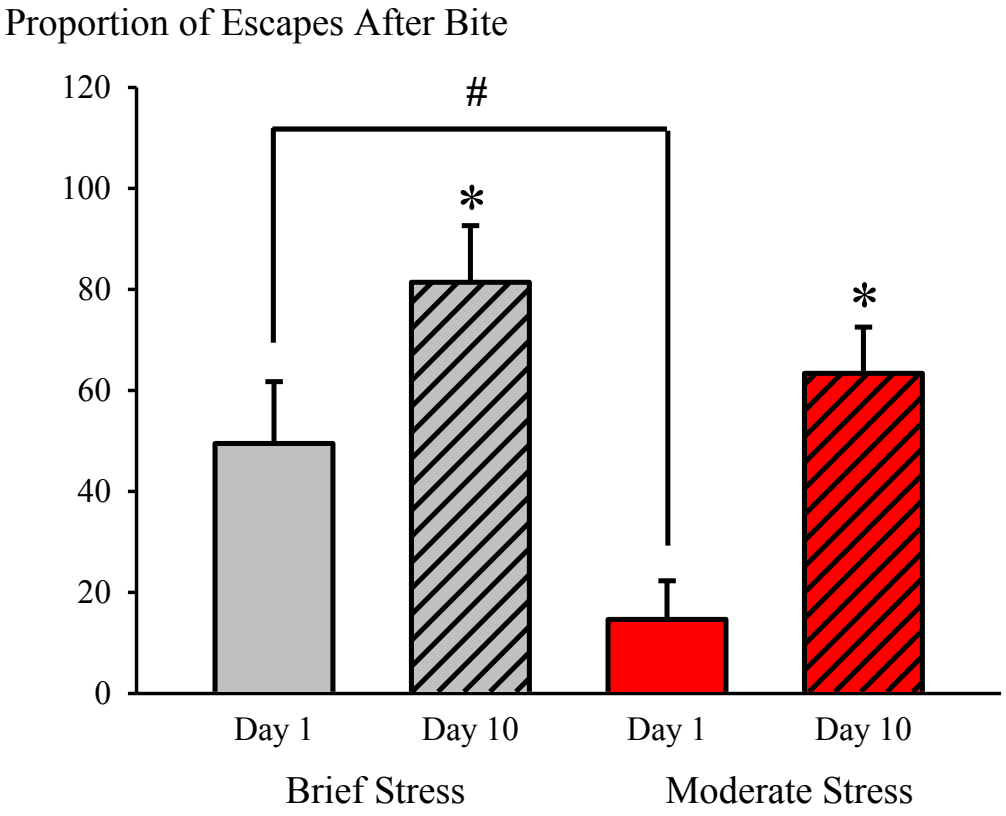


Figure 7. The mean frequency of escapes during the first 40s of defeats by brief and moderate stressed mice. The effect of time and stress condition on the frequency of escapes during the first 40 seconds of social defeat stress on both the first and last days of social defeat stress. Error bars indicate the standard error of the mean. Asterisks (*) denote significant differences between days 1 and 10 within stress group ($p<0.05$).

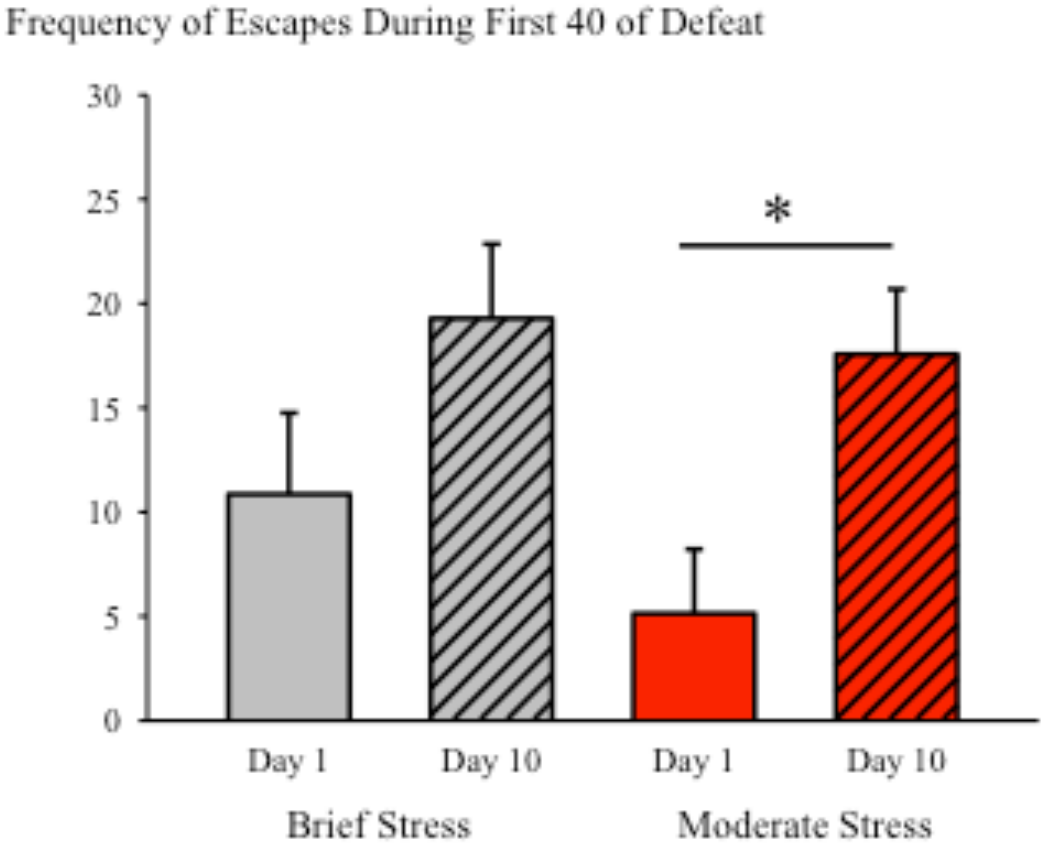


Figure 8. The proportion of times the mice displayed defensive upright posture immediately after attack bite by brief and moderate stressed mice on days 1 and 10 of defeats. The effect of time and stress condition on the proportion of times that the mice displayed defensive upright posture in the one second immediately following an attack bite during the social defeat stress on both the first and last days of social defeat stress. Error bars indicate the standard error of the mean. Asterisks (*) denote significant differences between days 1 and 10 within stress group ($p<0.05$), while pound signs (#) denote significance between groups on the same day ($p<0.05$).

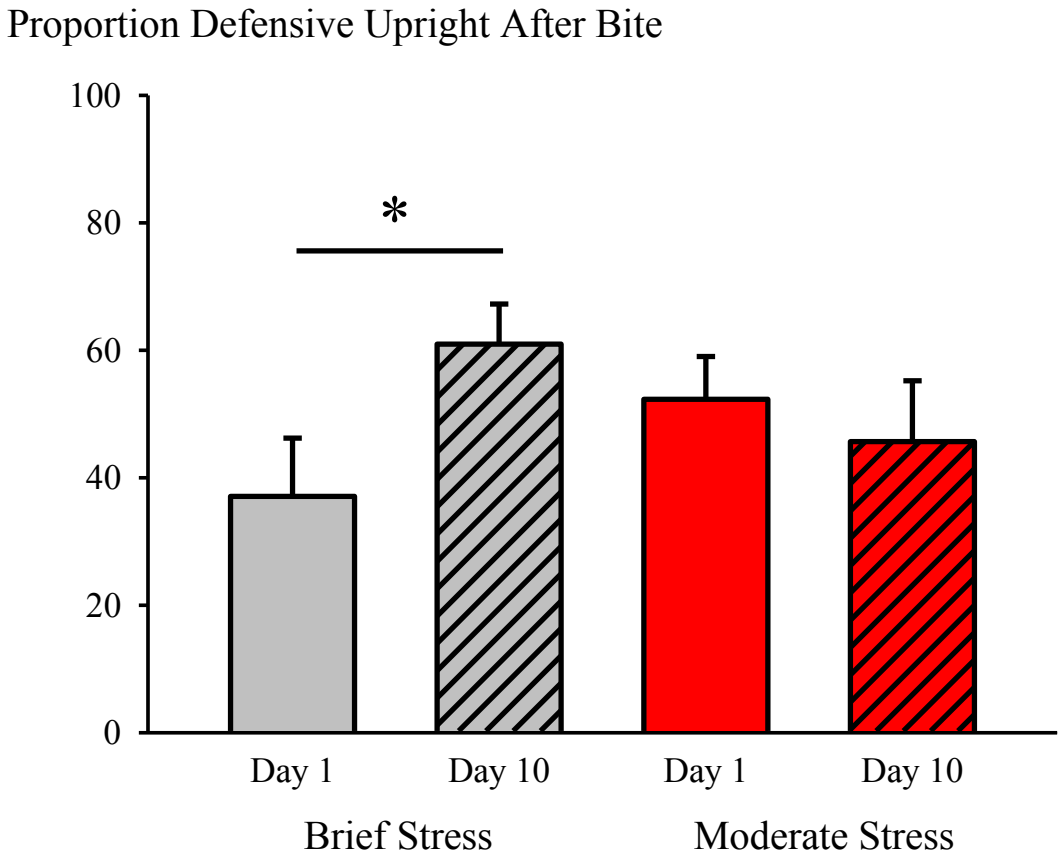


Figure 9. The frequency of defensive upright during the first 40s of social defeat in brief and moderate stressed mice. The effect of time and stress condition on the frequency of defensive upright posture during the first 40 seconds of social defeat stress on both the first and last days of social defeat stress. Error bars indicate the standard error of the mean. Asterisks (*) denote significant differences between days 1 and 10 within stress group ($p<0.05$), while pound signs (#) denote significance between groups on the same day ($p<0.05$).

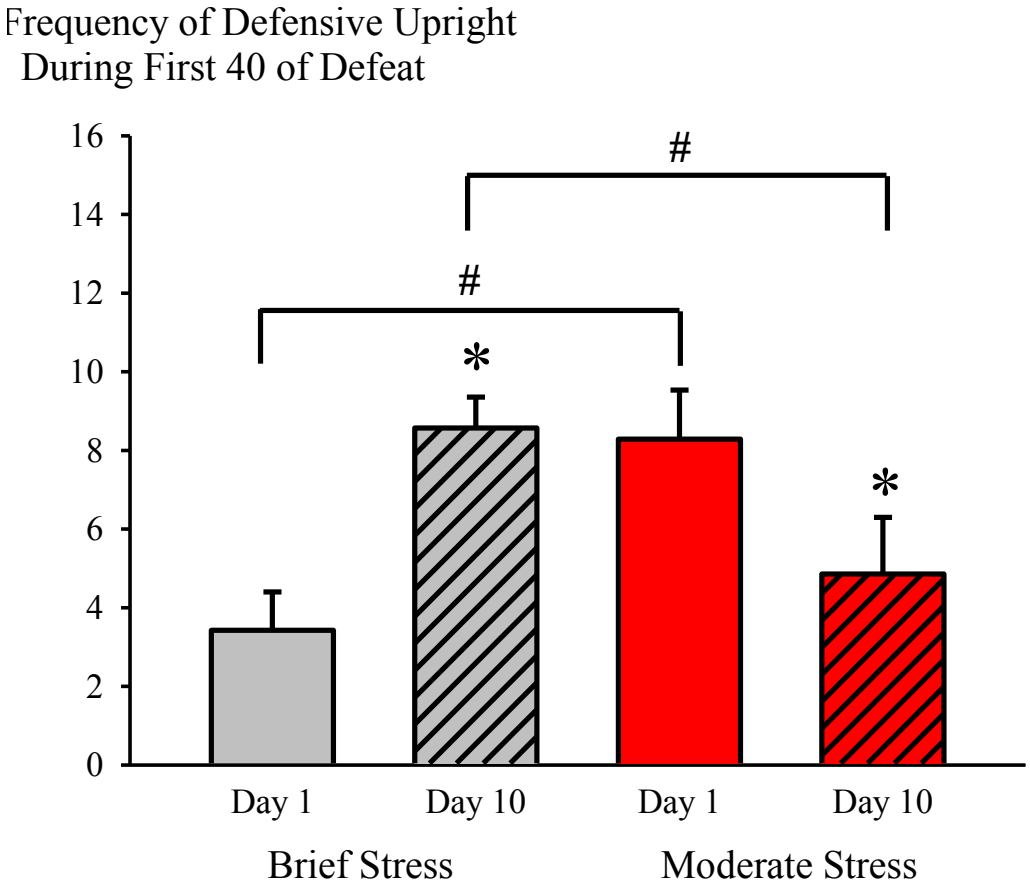


Figure 10. Percent change of dopamine the in nucleus accumbens after an amphetamine challenge. *In vivo* microdialysis results showing baseline, post-saline, and post-d-amphetamine injection DA levels as percent change from baseline for moderately stressed, briefly stressed and non-stressed control mice. Asterisks (*) denote significant differences compared to the non-stressed controls ($p<0.05$).

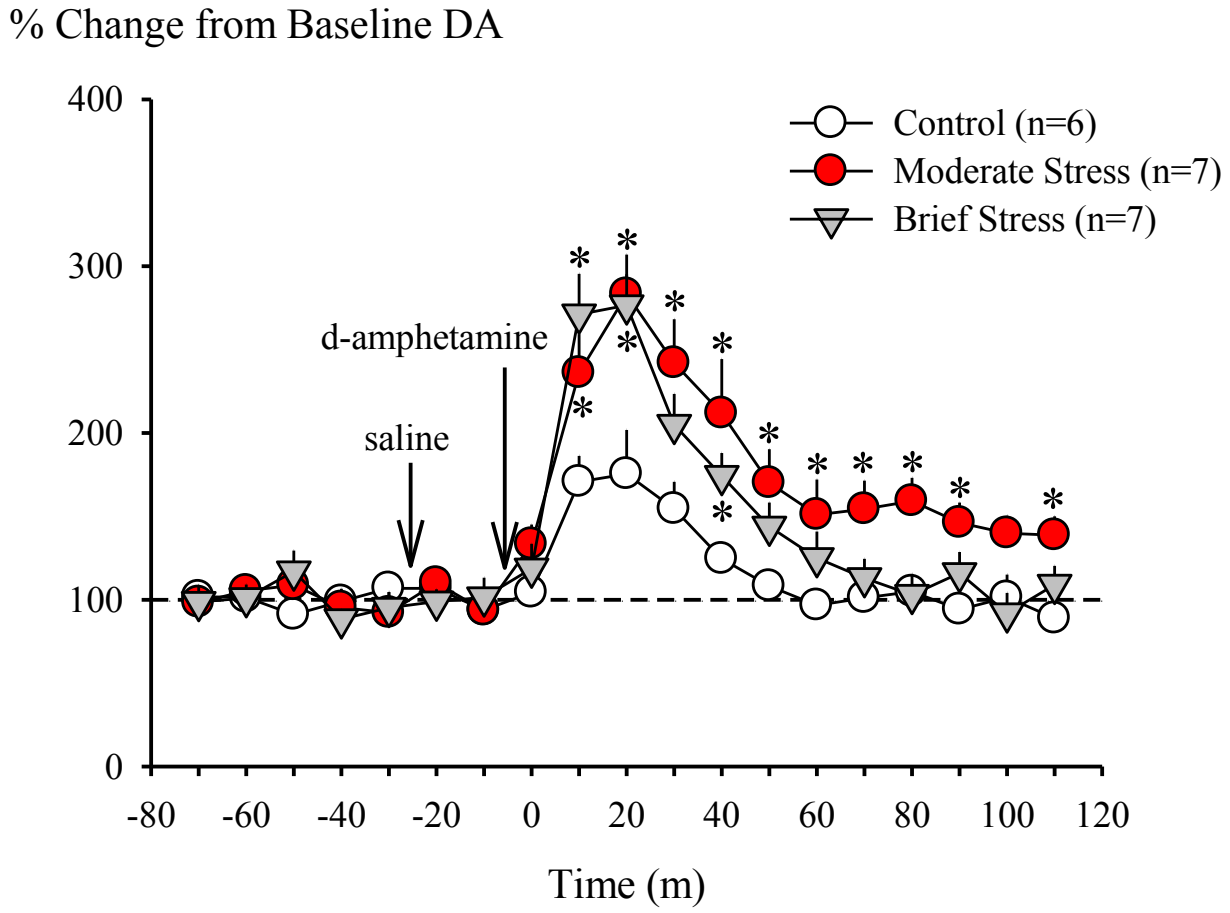


Figure 11. Dose effect curve of cocaine self-administration. Cocaine self-administration data showing the mean and standard error number of responses for both stress conditions and non-stressed controls over the three cocaine doses and saline. Asterisks (*) denote significant differences compared to the non-stressed controls ($p<0.05$) and pound sign (#) indicates within group significance over vehicle.