Spring 5-9-2020

Effects of repeated intermittent episodes of social stress on the acquisition and extinction of a reward-seeking task

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EFFECTS OF REPEATED INTERMITTENT EPISODES OF SOCIAL STRESS ON THE ACQUISITION AND EXTINCTION OF A REWARD-SEEKING TASK

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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford
December 2019

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ACKNOWLEDGMENTS

This study has been supported by the Sally McDonnell Barksdale Honors College, the Neuroscience Minor, the University of Mississippi, and NIGMS/NIH P30GM122733. I am deeply grateful to the people who have helped me throughout this process. First, I would like to thank Dr. Del Arco for being the best advisor I could have asked for. Under his patient and understanding leadership, I have experienced the research process firsthand and have further explored and developed my love for neuroscience. I would also like to thank my readers, Dr. Leary and Dr. Nordstrom, who have both challenged and encouraged me to grow academically and personally both inside and outside the classroom. A very special thank you goes out to Hannah Shaffer, who worked in the lab with me last year, for all of her help and encouragement throughout this process. I am incredibly grateful to the faculty and staff of the Sally McDonnell Barksdale Honors College for the ways the Honors College experience has shaped me and my time as an undergraduate student. Finally, I would like to thank my family and friends for their support throughout this process.

ABSTRACT

Repeated exposure to stress is known to have a myriad of effects on the brain, contributing to the development of psychiatric disorders, such as anxiety, depression, and drug addiction. For example, rats undergoing repeated social stress develop increased cocaine self-administration. These effects of stress are not well-understood and are related to changes in the brain reward system. This study investigated the effects of repeated social stress on reward-seeking behavior via the acquisition and extinction of a discriminative stimulus (DS) task and on anxiety-like behavior in the elevated plus maze (EPM). Male rats underwent intermittent social defeat (4 sessions in 10 days) using the resident-intruder paradigm. Animals were tested in the DS task in between stress sessions for Experiment 1 and one month after the last session for Experiment 2. The EPM was conducted 3 days after the last stress session. In the DS task, stress did not change the acquisition of reward-seeking behavior in the days in between stress sessions, and Stress and Control groups responded similarly to reward-seeking cues. However, stress did affect reward-seeking behavior in the long term, as the Stress group averaged less responses during the first extinction trial one month after stress, indicating a faster extinction response. In addition, the Stress group spent more time in the open arms of the EPM than the Control group, exhibiting a higher tendency towards risk-taking behavior. These results suggest that social stress does not produce effects in the reward system in the short term, but does produce changes in the long term.

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INTRODUCTION

The Stress Response

The stress response is an evolutionary mechanism by which organisms respond to a variety of stimuli that can potentially disrupt homeostasis (Charmandari et al 2005). Organisms respond to stress with behavioral and physiological adaptations to defend against this threat to homeostasis. The first, fast-acting part of the stress response is known as the "fight-or-flight" response, which involves the release of epinephrine (adrenaline) from the adrenal medulla and norepinephrine from the sympathetic nervous system. These compounds cause an increase in heart rate, blood pressure, and blood glucose levels, potentially to help the organism escape from or fight the stressor. The delayed response involves the hypothalamic-pituitary-adrenal (HPA) axis. The hypothalamus is stimulated to produce corticotropin releasing hormone (CRH), which stimulates the production of adrenocorticotropic hormone (ACTH) by the pituitary gland, which stimulates the production of glucocorticoids by the adrenal cortex. Glucocorticoids, in contrast to the catecholamines epinephrine and norepinephrine, are lipophilic molecules and can thus cross the blood-brain barrier and cause long-term modulation of behavior in response to stress (Nelson and Kriegsfeld 2017). A summary of this mechanism is shown in Figure 1.

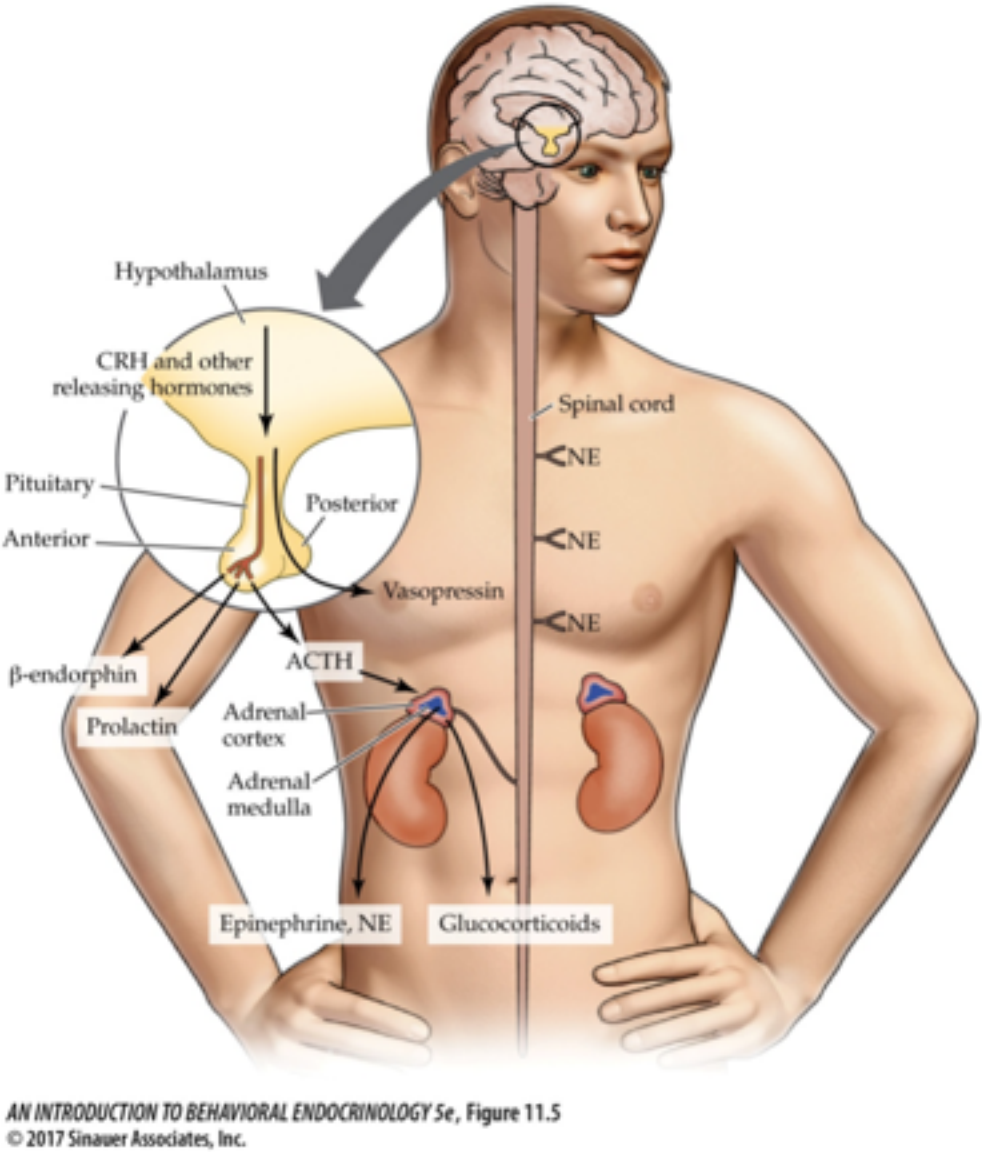

Figure 1. The hormonal stress response mediated by the Hypothalamic-Pituitary-Adrenal (HPA) axis. Stress induces the hypothalamus to produce corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary gland to secrete adrenocorticotropin hormone (ACTH), which stimulates the adrenal gland to produce the "stress hormones" glucocorticoids (such as cortisol in humans). Importantly, glucocorticoids can cross the blood-brain barrier to modulate behavior (not shown in figure).

The Effects of Stress on the Brain

Stress has been shown to induce cellular and behavioral effects in both the short term and the long term that can be detrimental to an individual. Stress can be classified as acute when it occurs one time, or chronic when it occurs repeatedly. These different forms of stress are implemented by researchers to study the effects of stress on brain and behavior. Acute stress protocols usually involve a short-term single exposure to a moderate-severe stressor. This type of stress protocol is particularly useful in studying the reactivity of the HPA axis and modeling post-traumatic stress disorder (PTSD). Acute stress is shown to increase excitatory neural transmission via the effects of corticosterone (James et al 2016). In addition, severe acute stress induces PTSD-like behaviors, such as social avoidance, sleep disturbance, and fear generalization (Flandreau and Toth 2017).

Chronic stress is shown to induce effects in the short-term and the long-term that are distinct from those of acute stress. Chronic stress has been shown to induce dendritic atrophy in the prefrontal cortex (PFC) in rats (Cook and Wellman 2003). These cellular effects in the PFC are thought to explain the deficits in working memory, fear extinction, and cognitive flexibility that are associated with chronic stress (Holmes and Wellman 2009). Chronic stress also induces depression-like symptoms, such as anhedonia and learned helplessness (Willner et al 1992; Song et al 2006). These effects of stress indicate that cellular modifications in the brain cause changes in behavior that leave individuals more susceptible to developing psychiatric disorders, such as PTSD, depression, and drug addiction.

Stress and The Reward System

The ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC) are three of the major brain regions involved in the processing of reward. Over half of the neurons in the VTA release dopamine, and these neurons project into the NAc and the PFC. The dopaminergic connection between the VTA and the NAc has been extensively studied, as an increase in the activity of this pathway has been shown to be the main mechanism of action of drugs of abuse. Neurons within the NAc play a key role in mediating the discrimination of rewarding vs aversive stimuli. The PFC also modulates the NAc through glutamate transmission and is associated with executive function, such as goal-directed behaviors related to reward (Cooper et al 2017). This modulation of the limbic system by the PFC can be altered by pharmacological compounds and environmental stimuli (Del Arco and Mora 2009). This system is depicted in Figure 2.

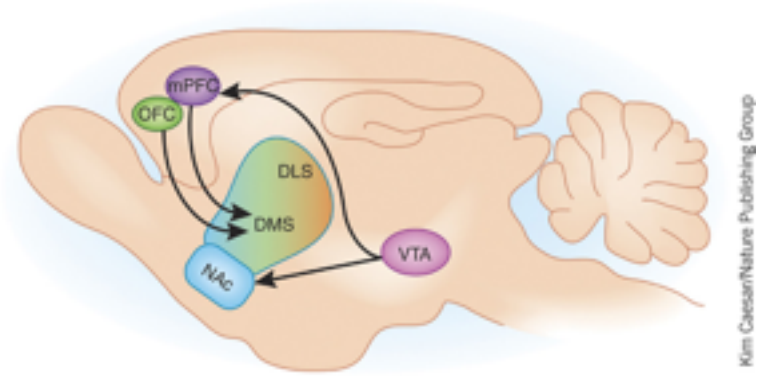

Figure 2. The brain's reward system. Depicted in this figure are the locations of key brain regions in the dopaminergic response to rewarding stimuli, including the medial prefrontal cortex (mPFC), the nucleus accumbens (NAc), the ventral tegmental area (VTA), the orbitofrontal cortex (OFC), the dorsomedial striatum (DMS), and the dorsolateral striatum (DLS).

Stress has been shown to affect the neuronal circuitry associated with the processing of rewards. Chronic stress can induce alterations in mesolimbic dopamine transmission involved in reward processing that can last for weeks or months (Fitzpatrick et al 2018, Sinha 2009). In fact, social defeat stress has been shown to alter signaling between the VTA and NAc (Ch'ng et al 2018). Social defeat stress is also associated with hyperexcitabililty of mesolimbic dopaminergic neurons (Hollon et al 2015). These neuronal modifications to the brain's reward system induce changes in behavior. In one sense, stress depresses behavior, leading to motivational deficits and anhedonia. For example, rats that have undergone social defeat stress show a loss of motivation to escape in the forced swim test (Hollis et al 2010) and a decreased preference for sweet sucrose solution, indicating anhedonia (Rygula et al 2005). On the other hand, some behaviors are enhanced by stress, such as those related to drug and reward-seeking. For example, rodents that have undergone chronic social stress protocols have been shown to self-administer cocaine at higher rates and to relapse more quickly into drug-seeking after extinction of the behavior, indicating a role for stress in susceptibility to drug addiction (Covington and Miczek 2001, Peters et al 2009).

Social Stress as an Animal Model of Susceptibility to Drug Abuse

Social stress has been shown to play a role in the development of certain psychiatric disorders in humans, including anxiety and depression. The term "social defeat" refers to an individual's encounter with a conspecific in which they end up as the "loser". Several animal models of social defeat have been developed using the natural

tendency towards aggression and territoriality in certain animals. A well-developed model is the resident-intruder paradigm in rodents, in which a resident male will attack and defeat a male intruder. After undergoing this defeat stress, rodents have been shown to show symptoms of anxiety, depression, and susceptibility to drug addiction. The most commonly-used timelines of social defeat stress include acute stress, in which the intruder is exposed to a single episode of stress, and chronic stress, in which the intruder is exposed to an episode of stress every day for a certain number of days (Hammels et al 2015). Another variation used is intermittent stress, which is used in this study (Covington and Miczek 2001, Ferrer-Pérez et al 2019, Fanous et al 2010). Intermittent stress involves repeated exposures to the stress separated by days in which the intruder is not exposed to the stress.

Interestingly, chronic (continuous; every day) and intermittent (episodic) exposure to social stress produce different effects on behavior. Specifically, these two stress protocols produce opposite effects on drug-seeking behavior. Rats that have undergone chronic social defeat stress self-administer cocaine at lower rates (Miczek et al 2011). On the other hand, intermittent stress produces increased self-administration of cocaine (Covington and Miczek 2001, Miczek et al 2011). These studies highlight the importance of considering the schedule of stress episodes when studying the effects of stress.

AIM OF STUDY

Exploring the effects of stress on reward processing in rats will further our understanding of the mechanisms by which stress increases vulnerability to develop drug addiction. In this study, we aim to investigate the effects of repeated intermittent social defeat stress on reward-seeking behavior in both the short term and the long term using a discriminative stimulus (DS) task. We hypothesize that social stress produces changes in reward-seeking behavior. Additionally, as repeated exposure to stress has been associated with increased anxiety, we will evaluate anxiety-like behavior using the Elevated Plus Maze (EPM). Our hypothesis is that animals that have undergone social stress will exhibit higher anxiety-like behavior in the EPM.

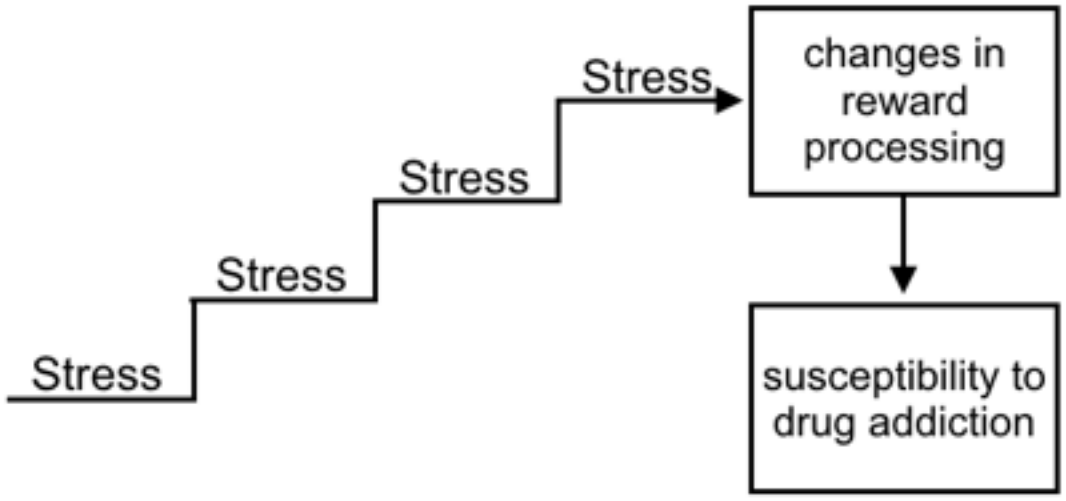

Figure 3. A depiction of the present study's hypothesis that repeated stress episodes will alter reward processing, producing susceptibility to drug addiction.

METHODS

Animals

Thirty-two male Long-Evans rats from 3-4 months of age were randomly split into two groups: Stress (n=16) and Control (n=16). Upon arrival, rats were double-housed on a reverse light/dark cycle, with the lights being off from 9 am- 9 pm. Rats were allowed one week of habituation before handling and training began. One week before the beginning of the social defeat protocol, animals were housed individually. Rats were food restricted to 15 grams of food per animal per day before training in the reward-seeking task in order for them to be motivated to perform for the food pellet reward. This protocol followed the rules of the Institutional Animal Care and Use Committee and was approved by the Institutional Review Board at the University of Mississippi (19-020).

Intermittent Social Defeat Stress

The rats assigned to the Stress group underwent four sessions of social defeat stress (SDS), once every 3 days for 10 days. The SDS protocol was set up according to the Resident-Intruder paradigm (Tornatzky and Miczek 1993), as depicted in Figures 4 and 5.

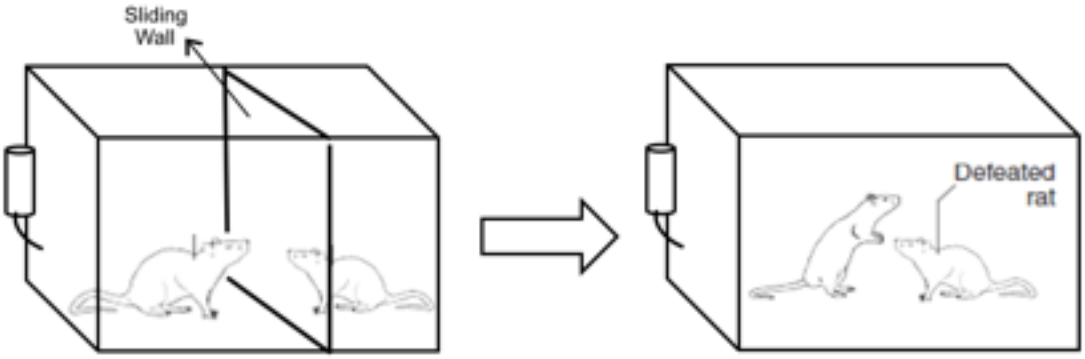

Figure 4. Illustration of the resident-intruder social defeat set-up.

A clear plastic chamber (H x L x W: 45×61×61 cm) was used to house the resident rats. The residents were larger than the intruders. They were housed with a female, and the cage bedding was not changed in order to help induce territoriality in the resident. The female was removed from the cage 15 min before each social defeat session. Then, a sliding clear plastic dividing wall was placed in the middle of the chamber. The intruder was then placed on the opposite side from the resident for 10 min, allowing for stressful sensory exposure, but no physical interaction. Then, the dividing wall was removed, and the resident and intruder were allowed to interact until a bite was witnessed, 6 attacks were witnessed, the intruder was in the supine position for 5 s, or 5 min had elapsed. At this point, the dividing wall was reinserted, and the intruder rat remained in the chamber for an additional 10 min. During the 5 min of physical interaction, latency to first attack, number of bites, and amount of time the intruder spent in supine position were recorded. During this time, the Control rats were moved to another room and handled for 5 min. The timeline for the social defeat sessions in reference to the discriminative stimulus task and EPM is shown in Figure 5.

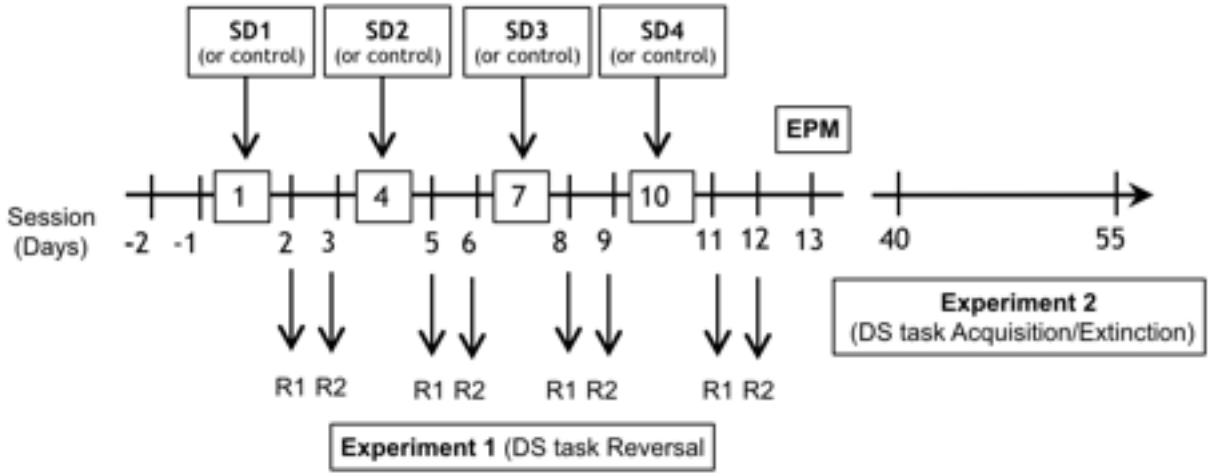

Figure 5. Timeline for social defeat sessions. Prior to the first social defeat session (SD1), Experiment 1 animals were trained in the DS task. Four total social defeat sessions were conducted (SD1-SD4). On the days in between social defeat, Experiment 1 animals were tested in the DS task, reversing reward contingencies between the original protocol (R1) and a new protocol (R2) each day. The animals were tested in the Elevated Plus Maze 3 days after the last social defeat session (SD4). Experiment 2 animals were trained in the DS task beginning 30 days after SD4.

Discriminative Stimulus Reward-Seeking Task

Experiment 1 (n=8, Stress; n=8, Control).

The protocol for Experiment 1 is shown in Figure 6.

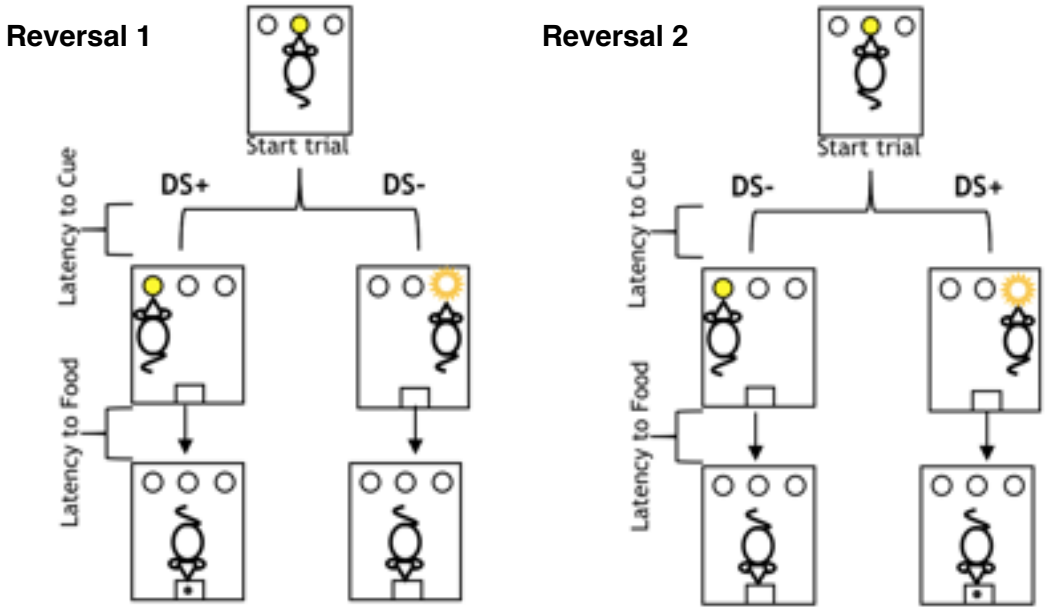

Figure 6. DS task protocol for Experiment 1. The rats had to poke in the lit center cue hole to start the trial, then either a fixed or flashing light appeared. The rats were first trained in Reversal 1, in which a nose poke in the fixed light (DS+) would result in a food pellet reward dropping into the food magazine, while poking in the flashing light (DS-) did not yield a reward. The rat would have to poke in the food magazine to end the trial. When the reward contingencies were switched to Reversal 2, a nose poke in the flashing light resulted in a food pellet dropping into the food magazine. A nose poke in the fixed light no longer yielded a reward. On each day, the animals completed a maximum of 100 trials.

Rats were trained in the discriminative stimulus (DS) reward-seeking task in an operant conditioning chamber with three cue holes and a food magazine. They were trained in the first reversal, in which the fixed light was the DS+, until they poked <35%

of the time in the flashing light. They were then tested in this DS task on the days in between SDS, with reward contingencies being reversed each time. When the task was switched to Reversal 2, the fixed light was no longer rewarding (DS-), and the flashing light became the rewarding stimulus (DS+). For all of these trials, the number of nose pokes in the DS+ and the DS- were recorded. The amount of time it took for the rat to poke in the stimulus light (latency to cue) and the amount of time it took for the rat to retrieve the food pellet from the food magazine (latency to food) were also recorded.

Experiment 2 (n=8, Stress; n=8, Control).

30 days after the last social defeat session, rats were trained in the DS task for an 8-day acquisition period. They were placed in an operant chamber with two cue holes. A fixed or flashing light would appear in one of the cue holes. A nose poke in the fixed light (DS+) would result in a food pellet reward dropping into the food magazine, while poking in the flashing light (DS-) did not yield a reward. Next, the rats underwent a 5-day extinction period. During these sessions, pokes in neither fixed nor flashing lights were rewarded. For acquisition and extinction, the number of nose pokes in the DS+ and the DS- were recorded. This protocol is depicted in Figure 7.

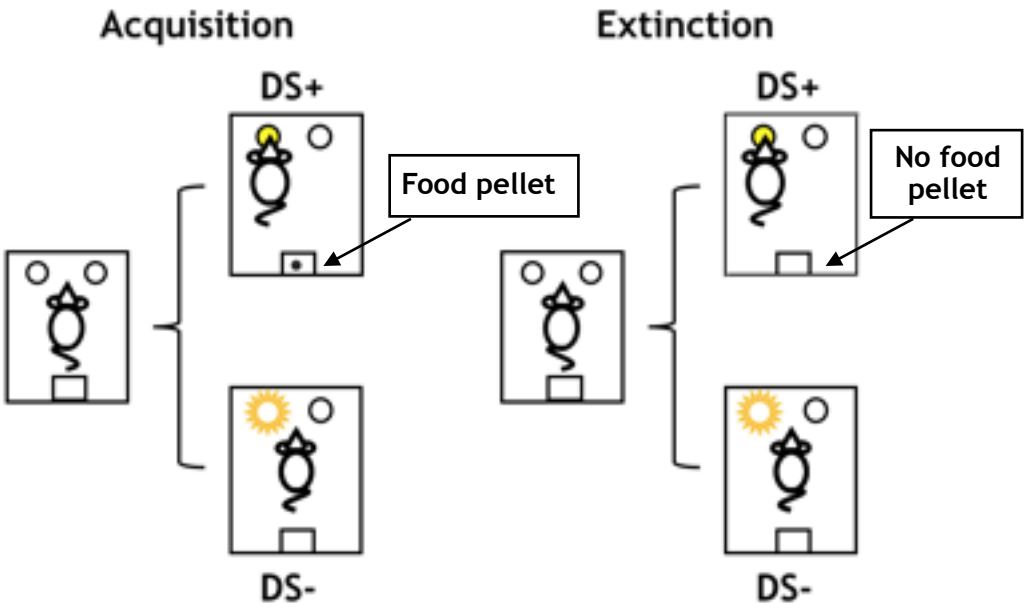

Figure 7. DS task protocol for Experiment 2. During the acquisition period, rats would poke in the fixed light to obtain a food pellet. Nose pokes in the flashing light did not yield a reward. During the extinction period, the previously rewarding fixed light stimulus no longer produced a reward.

Elevated Plus Maze

4 days after the last social defeat session, rats were evaluated on the Elevated Plus Maze (EPM) apparatus, as depicted in Figure 8.

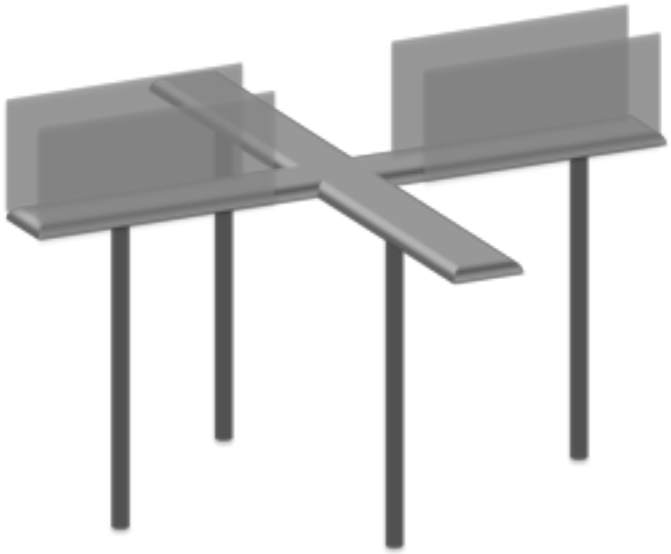

Figure 8. A depiction of The Elevated Plus Maze apparatus. The EPM is a plus-shaped platform raised 76 cm off the ground. It contains two open arms that have no walls, along with two walled closed arms. The rats were placed in the center of the EPM facing the open arm and were able to move freely for 5 minutes. The amount of time spent in both the open and closed arms, along with the number of crosses from one arm to another, were recorded. The animal was counted as entering an arm when all four paws crossed the threshold into the arm.

Data Analysis

Two-way ANOVAs with repeated measures were performed to analyze performance in the DS task. One-way ANOVAs and an independent Student t test were used to analyze EPM results.

RESULTS

Discriminative Stimulus Reward-Seeking Task

Experiment 1.

Reversal 1

Nose Pokes

The number of nose pokes in the DS+ and DS- were recorded as a percent of the total trials and are shown in Figure 9. The number of nose pokes in the DS+ did not differ significantly between the Control and Stress groups [DS+, Group, $F(1,13)=0.09$, $p=0.76$]. The number of nose pokes in the DS- also did not differ significantly between the Control and Stress groups [DS-, Group, $F(1,13)=1.50$, $p=0.242$]. There were significant differences across sessions [DS+, Time, $F(6,78)=7.05$, $p<0.001$; DS-, Time, $F(6,78)=1.40$, $p=0.026$], which shows that both groups of animals learned to discriminate between stimuli after reversals.

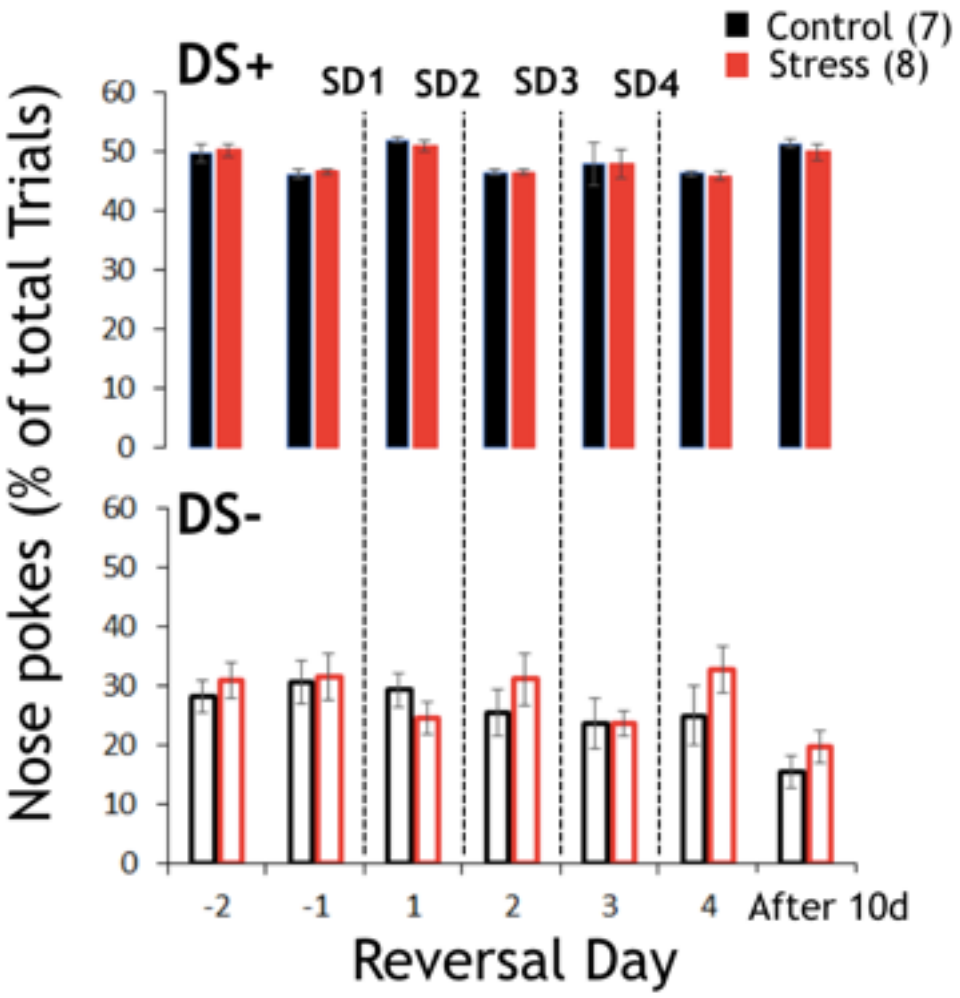

Figure 9. Social stress did not change animals' response to rewarding (DS+) and non-rewarding (DS-) cues on the days in between stress episodes (SD1-SD4) or up to 10 days later. Bars represent the mean ± SEM. Number of nose pokes in response to DS+ (top) and DS- (bottom) (50 trials each) when reward contingencies were reversed to the original protocol (Reversal 1) .

Latency to Cue

The latency to the cue, shown in Figure 10, did not differ between the Control and Stress groups for the DS+ or the DS- [Latency DS+, Group, $F(1,13)= 0.62$, $p= 0.44$; Latency DS-, Group, $F(1,13)= 0.57$, $p= 0.46$]. Differences in latencies to respond to the DS+ and DS- indicate that animals learned to discriminate between stimuli.

Latency to Food

The latency to food, shown in Figure 10 did not differ significantly between Control and Stress groups [Latency food, Group, $F(1,13)= 0.15$, $p= 0.70$], showing no change in motivation in the stress group to retrieve the food pellet reward.

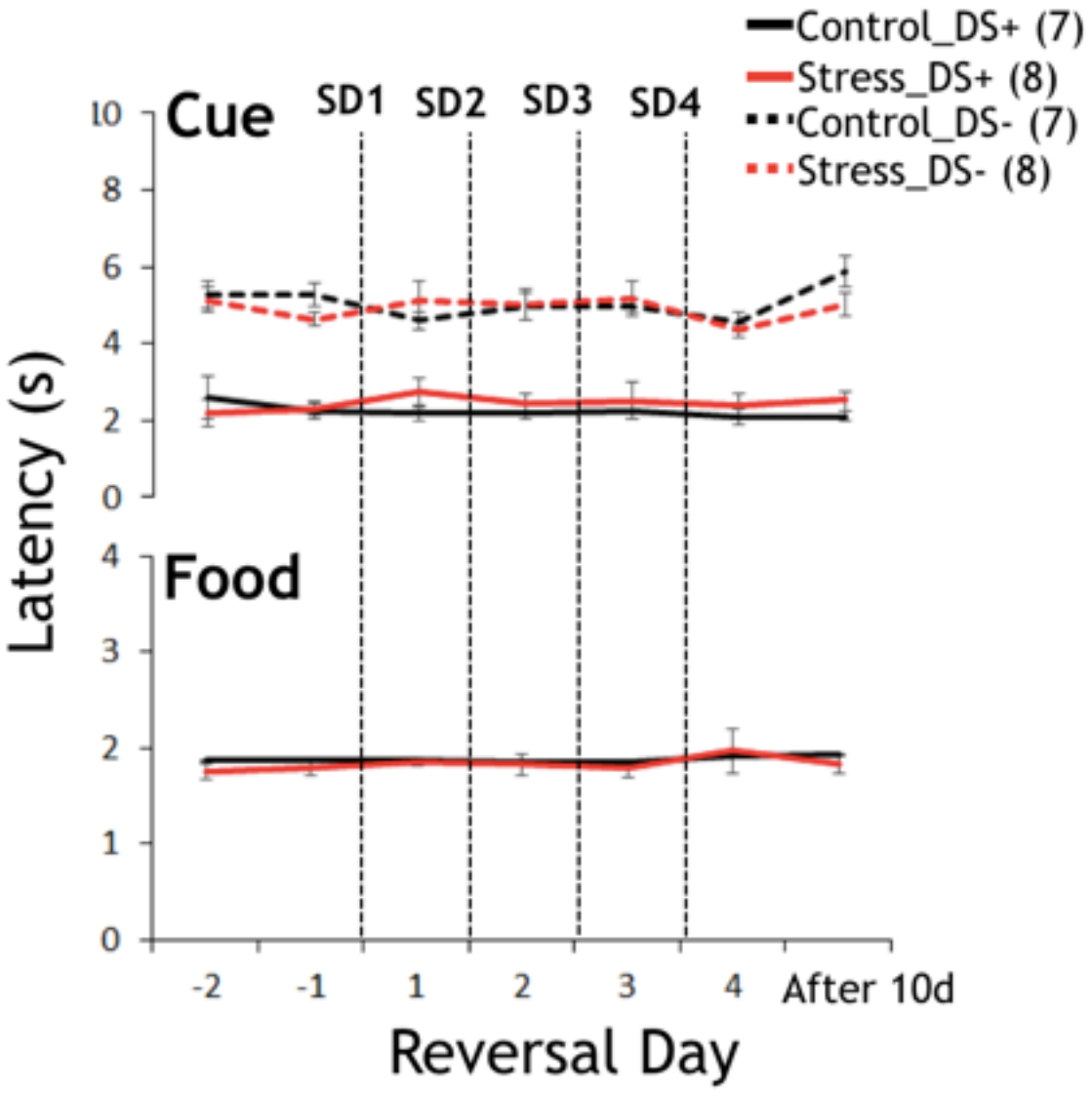

Figure 10. Social stress did not change animals' response time to rewarding (DS+) or non-rewarding (DS-) cues or to food reward on the days in between stress episodes (SD1-SD4) or up to 10 days later. Data represent the mean ± SEM. Latency to respond to DS+ and DS- (top) and to the food trough (bottom) when reward contingencies were reversed to the original protocol (Reversal 1).

Reversal 2

Nose Pokes

The number of nose pokes in the DS+ and DS- were recorded as a percent of the total trials and are shown in Figure 11. The number of nose pokes in the DS+ did not differ significantly between the Control and Stress groups [DS+, Group, $F(1,13)=0.96$, $p=0.34$]. The number of nose pokes in the DS- also did not differ significantly between the Control and Stress groups [DS-, Group, $F(1,13)=0.01$, $p=0.98$]. There were significant differences across sessions [DS+, Time, $F(6,78)=3.65$, $p=0.003$; DS-, Time, $F(6,78)=7.54$, $p<0.001$], which shows that both groups of animals learned to discriminate between stimuli after reversals.

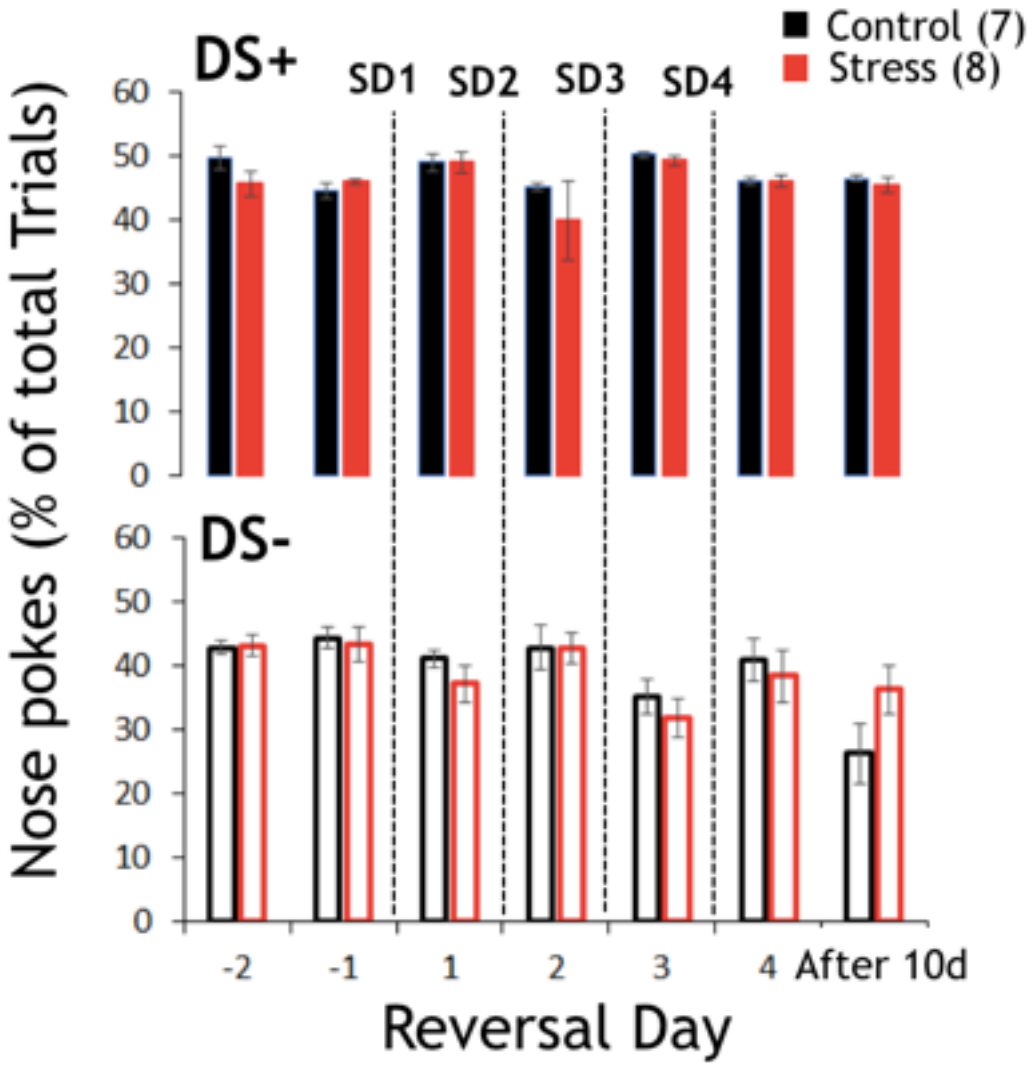

Figure 11. Social stress did not change animals' response to rewarding (DS+) and non-rewarding (DS-) cues on the days in between stress episodes (SD1-SD4) or up to 10 days later. Bars represent the mean ± SEM. Number of nose pokes in response to DS+ (top) and DS– (bottom) (50 trials each) when reward contingencies were reversed to a new protocol (Reversal 2).

Latency to Cue

The latency to the cue, shown in Figure 12, did not differ between the Control and Stress groups for the DS+ or the DS- [Latency DS+, Group, $F(1,13)= 0.83$, $p= 0.38$; Latency DS-, Group, $F(1,13)= 0.06$, $p= 0.80$].

Latency to Food

The latency to retrieve the food pellet, shown in Figure 12, did not differ significantly between Control and Stress groups [Latency food, Group, $F (1,13)= 1.54$, $p= 0.23$].

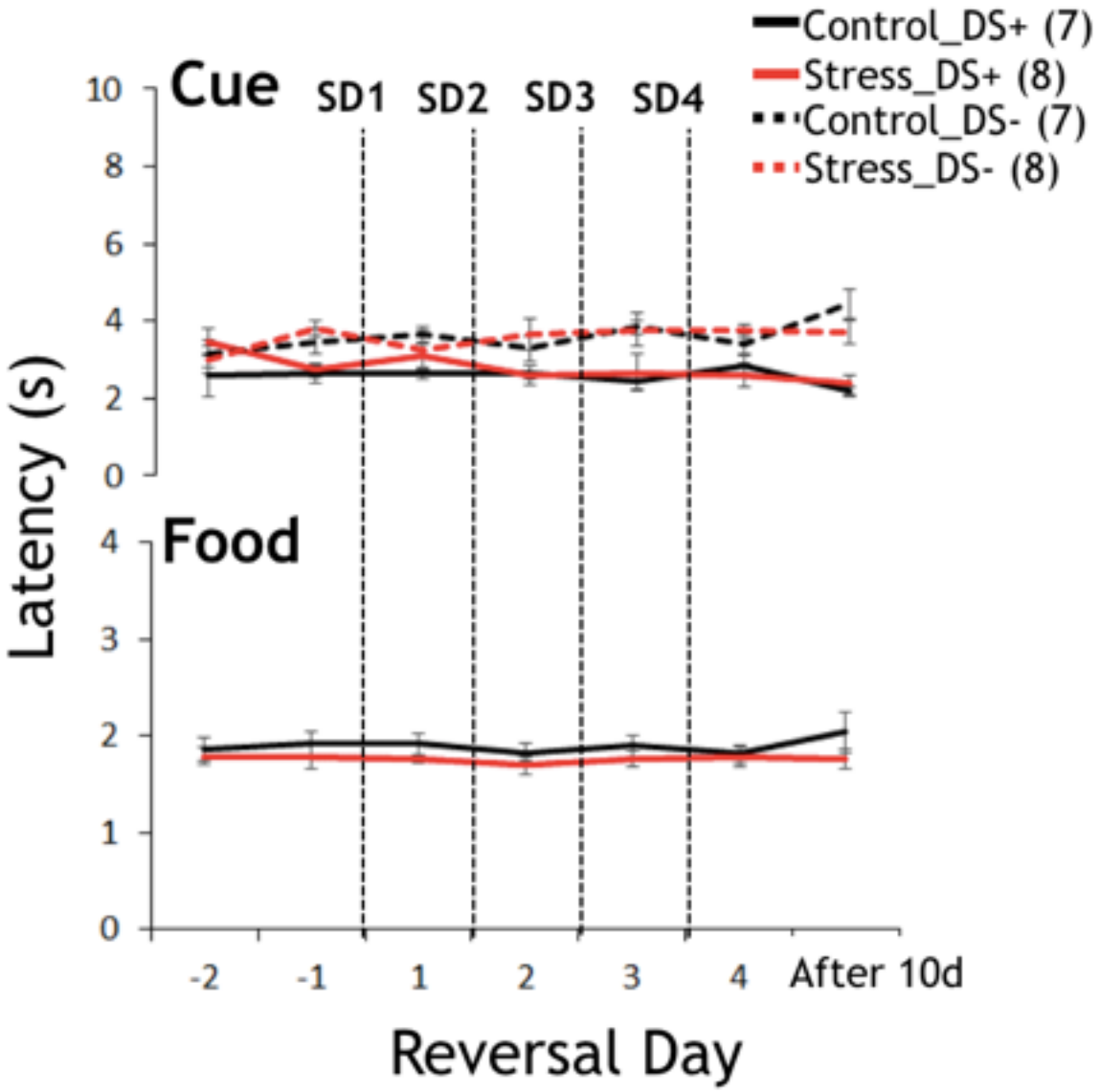

Figure 12. Social stress did not change animals' response time to rewarding (DS+) or non-rewarding (DS-) cues or to food reward on the days in between stress episodes (SD1-SD4) or up to 10 days later. Data represent the mean ± SEM. Latency to respond to DS+ and DS- (top) and to the food trough (bottom) when reward contingencies were reversed to a new protocol (Reversal 2).

Experiment 2.

Nose Pokes

The total number of nose pokes in the DS+ and the DS- for Stress and Control groups were recorded during acquisition and extinction 30 days after stress and are shown in Figure 13. During acquisition, the number of nose pokes in the DS+ and DS- did not differ significantly between groups, but there was a trend towards a decreased response to the DS- by the Stress group [DS-, Group, $F(1,13)= 4.44$, $p= 0.055$]. There were differences in both groups across sessions, as the animals were learning the task. Pokes in the DS- for both groups decreased across sessions [DS-, Time, $F(7,91)= 34.34$, $p< 0.001$]. On the first day of extinction, the Stress group poked significantly less times in the previously rewarding DS+ than the Control group [E1, $F(1,13)= 7.16$, $p= 0.019$].

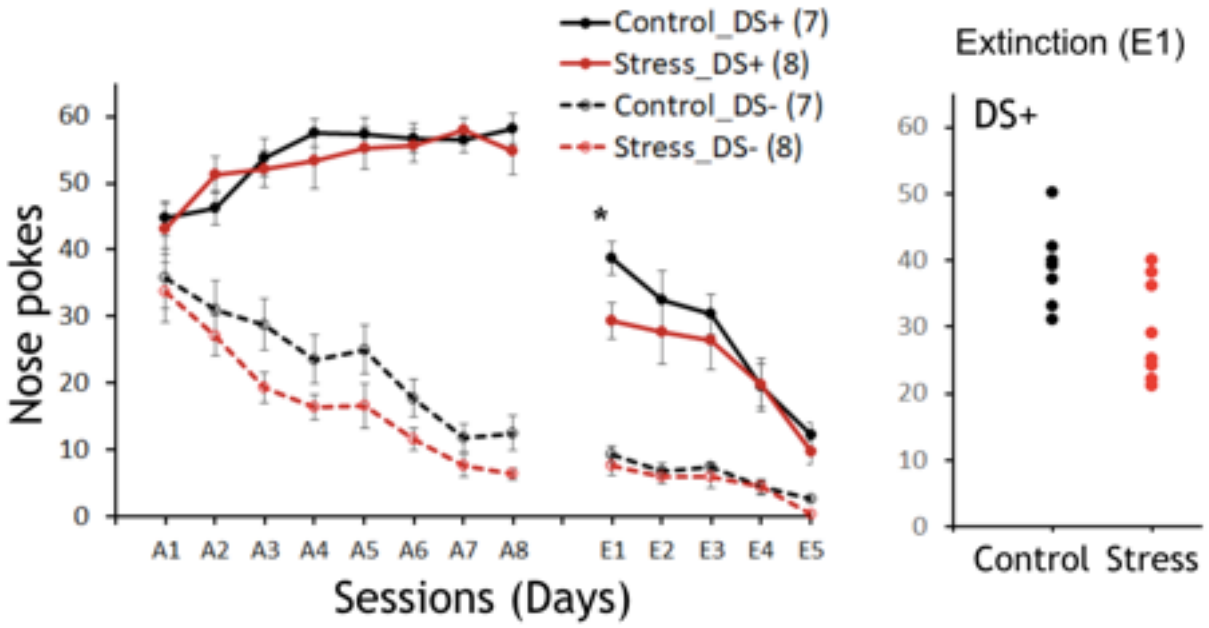

Figure 13. Social stress facilitates the extinction of reward-seeking in the long term during the acquisition (A1-A8) and extinction (E1-E5) of the DS task one month after the end of social stress. **Left**. Number of nose pokes in response to DS+ and DS-. Data represent the mean ± SEM. **Right**. DS+ responses during the first extinction session (E1) (each dot represents one animal). * p=0.019 compared to Control.

Elevated Plus Maze

The Elevated Plus Maze is traditionally used as a measure of anxiety-like behavior, with more time spent in the closed arms interpreted as higher anxiety behavior. The results from the Elevated Plus Maze are shown in Figure 14. The Stress group spent significantly more time in the open arm [$t(30)=3.31$, $p=0.002$] and less time in the closed arm [$t(30)=3.21$, $p=0.003$] than Controls. There was no significant difference between groups in motor activity, as measured by the number of crosses between arms [$t(30)=0.65$, $p=0.520$]. These results seem to indicate an anxiolytic effect of social defeat stress. A more likely explanation, however, is that the social defeat stress increased risk-taking behavior.

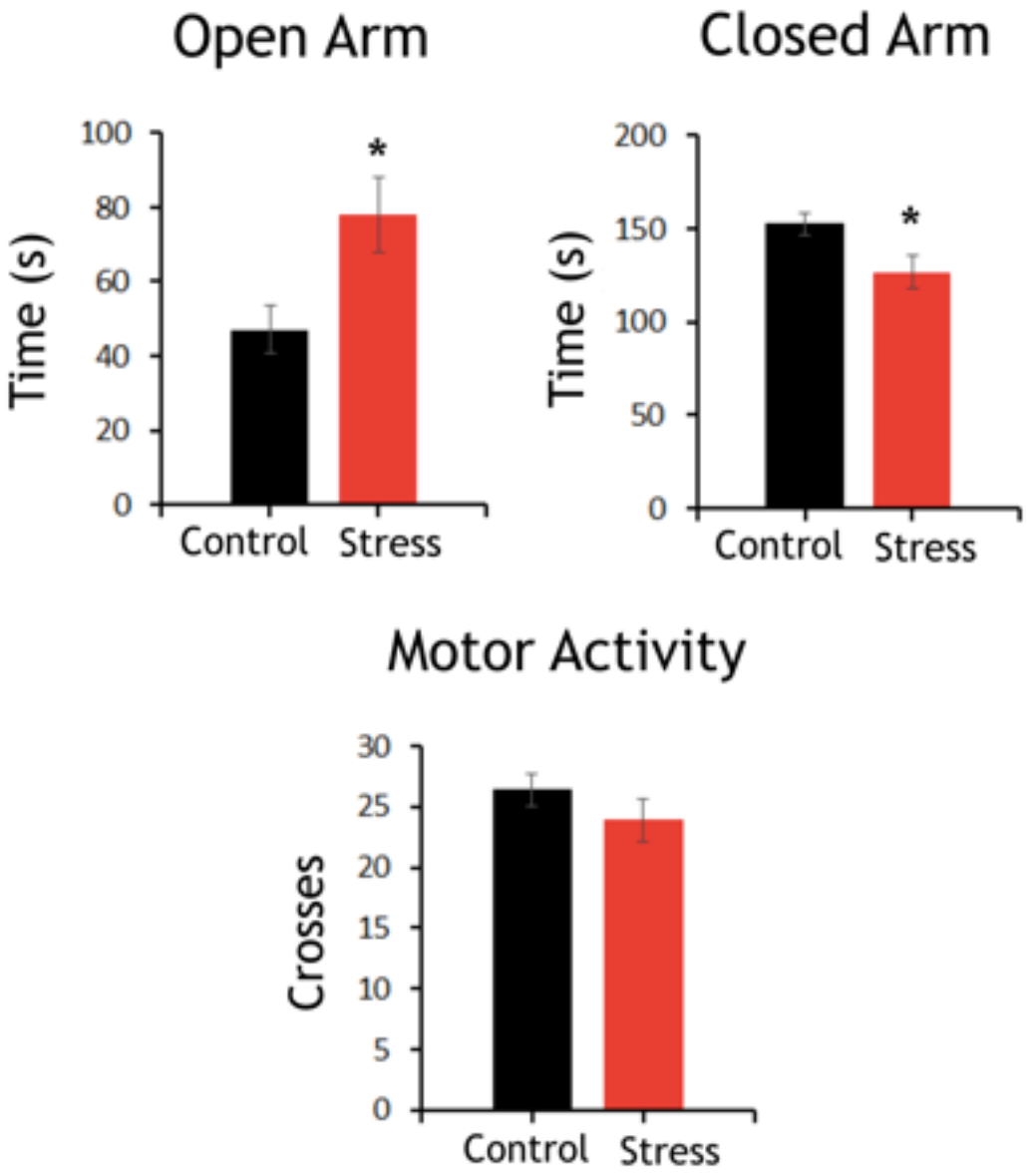

Figure 14. Social stress changes performance in the EPM. **Top.** Mean time (s) spent in the open arm and closed arm. **Bottom**. Mean motor activity based on number of crosses between arms. Bars represent the mean ± SEM. * $p<0.01$ compared to Control.

DISCUSSION

The present study shows that the intermittent exposure to social stress does not alter reward-seeking behavior in the short term, but does produce changes in the long term. Specifically, during the discriminative stimulus task in between stress sessions, Stress animals responded to the rewarding cue similarly to Control animals. However, one month after the last stress session, Stress animals responded less times than Control animals to the previously rewarding cue during the first day of extinction learning. In addition, Stress animals exhibited higher risk-taking behavior in the Elevated Plus Maze. Together, these results suggest that the intermittent exposure to social stress alters the function of the motivation and reward system in the long term. These changes may precede vulnerability to stress-related disorders, such as drug addiction.

As shown, both groups learned to discriminate between rewarding and non-rewarding stimuli. During Experiment 1, rats from both groups responded more times to the DS+ and less times to the DS- across sessions. In addition, rats from both groups responded faster to the DS+ than to the DS-, indicating a learned increased salience for the DS+. Similarly, in Experiment 2, rats from both groups during acquisition sessions responded more times to the DS+ and less times to the DS- across sessions. During extinction learning, both groups responded less times to the previously rewarding stimulus. These results indicate that animals stop responding to a devalued cue. Overall, these results show that animals learned the reward-seeking task.

On the days in between social stress, no differences were observed between Stress and Control groups. There was no change in the Stress group's number of responses for the DS+ or the DS-, along with no change in the latency to cue or to food, indicating no observable change in the reward-seeking behavior in the short term. When the reward contingencies were reversed, the Stress group showed no change in number of responses in DS+ or DS- from controls. These results show that Stress animals are able to discriminate and are flexible to change their behavior when the reward contingency is changed. These results are in agreement with prior literature. In our previous study, we used the same stress protocol and a set-shifting task to evaluate cognitive flexibility. In that study, we found that stress did not impair cognitive flexibility, but did produce long-term changes in salience attribution of rewarding cues (Sullivan et al 2019). The results of the present study indicate that stress does not alter reward processing in the short term.

Of note, social stress has been previously shown to produce short-term changes in the activity of areas of the brain in the dopamine pathway that are involved in attribution of salience. Specifically, one study found increased corticotropin releasing factor (CRF), indicating higher stress levels, and increased dopamine transmission in the ventral tegmental area (VTA) (Nikulina et al 1999). Unlike these studies, our study did not find short-term behavioral effects during social stress. Two considerations can be made in this regard. First, while these effects take place at the cellular level, they simply may not be expressed in behavior at this stage. Alternatively, there may be changes in behavior that are too subtle to be detected by the behavioral task used in this study. For future study, a more powerful behavioral test may be necessary to reveal these changes.

In the long term, 30 days after the end of social defeat stress, stress changed the extinction of the reward-seeking task. Stress animals responded less times to the previously rewarding DS+ during the first extinction trial, indicating that social stress has a facilitative effect on the extinction of the DS task. This finding that social stress has long-term effects on brain reward function is consistent with previous work showing long-term changes in vulnerability to drug addiction. However, we hypothesized a slower extinction response based on previous work showing that stress animals respond more times to a devaluated DS+, which is associated with slower extinction learning (Sinha 2001).

One possible explanation for the unexpected faster extinction learning shown by the Stress group is that stress increases sensitivity to negative outcomes. Under this framework, stress may change brain reward function in the long term by modifying reward circuitry such that the experience of responding and not receiving a reward is more aversive to the animals that have undergone social stress (Koob 2013). An alternative explanation for the faster extinction response is that the Stress group stops responding due to learned helplessness. Learned helplessness is a sign of depression-like behavior in animal models. For example, in the forced swim test, an animal is placed in an inescapable container of water, and the latency to immobility is used as a measure of learned helplessness, with a shorter latency indicating increased learned helplessness (Hollis et al 2010). The correlation between depression and vulnerability to drug abuse is well-established (Deykin et al 1987). Thus, interpreting the decreased DS+ response in the Stress group as learned helplessness is also consistent with previous literature that

shows long-term stress-induced changes in vulnerability to drug addiction. Future study involving behavioral tests designed to measure aversive states and learned helplessness would be helpful to determine the mechanisms of action by which stress causes these long-term effects in the brain reward system.

Interestingly, during the first day of extinction learning, there are individual variations in the magnitude of the effects of stress, as seen in Figure 13. In fact, some individuals appear unaffected by stress (same responses as Controls). Previous work investigating the effects of social defeat stress on brain reward function shows the emergence of two distinct groups: one affected by stress, called the "susceptible" group, and one unaffected by stress, called the "resilient" group (Der-Avakian et al 2014). It is possible that the Stress animals shown in our study could also be separated into these two groups. Further studies investigating these individual differences, along with the neural correlates to susceptibility and resilience, will lead to a better understanding of the effects of stress on the brain.

We ran the Elevated Plus Maze to evaluate anxiety-like behavior and found that the Stress group spent significantly more time in the open arm than the Control group. This behavior of the Stress group did not support our initial hypothesis that social defeat stress would yield an anxiogenic effect on the EPM. Prior studies involving social defeat stress typically report decreased time spent in the open arms (Caldwell and Riccio 2010). However, these studies mostly use a chronic rather than intermittent schedule of stress, which causes different effects. An alternative interpretation to the traditional view of the

EPM, which involves associating increased time spent in the open arms with increased risk-taking behavior, has been used in many studies (Laviola et al 2003, Tillman and Wegener 2019, Toledo-Rodriguez and Sandi 2011, Zhou et al 2015). Therefore, the fact that the Stress group spent more time in the open arms can be explained as a stress-induced increase in risk-taking behavior. Further study involving a task specifically developed to assess risk-taking behavior, such as the predator-odor risk-taking task, would be beneficial to support this explanation (Dent et al 2014).

In conclusion, social stress did not change reward-seeking behavior in the short term, but effects were observed one month after stress. The Stress group responded less times compared to the Control group to the previously rewarding cue during the first day of extinction learning, which suggests changes in the brain's processing of negative outcomes. In addition, stress seemed to produce an increase in risk-taking behavior. Altogether, these results indicate a long-lasting change in the brain's motivational and reward circuitry induced by social stress. Even though the results are statistically significant, the low number of subjects might be a limitation of this study.

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