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Time Course Effects of Repetitive Social Defeat Stress on a Prefrontal Cortex-Dependent Cognitive Flexibility Task

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TIME COURSE EFFECTS OF REPETITVE INTERMITTENT SOCIAL STRESS ON A PREFRONTAL CORTEX-DEPENDENT COGNITIVE FLEXIBILITY TASK

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ABSTRACT

Stress is known to change the structure and function of the brain in animals and humans as well as their behavior. It has a high correlation with the development of psychiatric disorders as well. We looked to investigate how repetitive intermittent social defeat stress using the resident-intruder paradigm in rats affected a cognitive flexibility task. The task used was a set-shifting protocol known to be associated with the function of the prefrontal cortex. Measurements on the task were taken intermittently between social stress sessions to determine short-term effects and 10 days after the last social stress session to measure long term effects. The rats also underwent testing on an elevated plus maze following the social stress protocol to evaluate anxiety-related behavior. Stressed rats were not impaired in the cognitive flexibility task, but their behavioral performance changed in the short and long term. In the short term, we found a decreased motivation to perform the task. In the long term, we found changes in risk-taking behavior and the processing of salient stimuli. These results suggest that repeated stress alters the neurobiological substrates that regulate the function of the brain reward system.

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INTRODUCTION

Stress and the Brain

Stress is a widely studied topic in both animals and humans and is known to induce anatomical, physiological, and behavioral changes. Stress is a prevalent problem in individuals' lives worldwide. Studies in animals and humans show stress has facilitated the six leading causes of death in the United States: cancer, coronary heart disease, accidental injuries, respiratory disorders, cirrhosis of the liver, and suicide (Rabkin, 1976). The exposure to stress is also a strong determinant for the development of psychiatric disorders. The relationship between stress and mental illness is related to changes in brain function and behavior. However, exactly how stress changes the brain is not well understood. While human studies give us clues into how stress affects the human brain, rat models are easier to manipulate in order to understand the causation of mechanisms behind stress and how it changes our brain.

The stress response is an evolutionary response that is important for survival (Amerman, 2019). The ability to adapt and respond to stressors that pose a threat to survival is crucial for the progression of the human species. Without these physiological changes, we would not be as apt to take on challenges presented to us and endure in the ever-changing, foreboding world. For example, imagine that you are presented with the stressful stimulus of a hungry lion. In order to escape the present danger, the body undergoes multiple physiological adaptations that prepare you to fight the lion or run away to avoid it.

A stress response activates a division of the nervous system called the sympathetic nervous system, often referred to as the "fight or flight" response (Amerman,

2019). Once our sympathetic nervous system is stimulated, noradrenaline is released into the blood stream, and the body makes adjustments in physiological processes that prepare it to deal with the pressing stimulus. Blood is redirected away from digestive and urinary systems, as these are secondary priorities, to the muscular and cardiovascular systems. The enriched blood flow increases heart rate, blood pressure, and muscle tone, all of which help engage in the "fight or flight" behavior the system is named after. The hypothalamus is a key integrator in the response to stress. In particular, the paraventricular nucleus innervates many autonomic centers in the brainstem and spinal cord that induce sympathetic arousal (Ulrich-Lai and Herman, 2009). Stressful stimuli also activate the hypothalamic-pituitary-adrenocortical (HPA) axis, a division of the endocrine system that controls the release of hormones necessary for the physiological and behavioral responses to stress. The activation of the HPA axis involves the release of different hormones such as the corticotropin released factor (CRF) from the hypothalamus which triggers the release of adrenocorticotropin hormone (ACTH) from the pituitary gland. Through this hormone cascade, the adrenal cortex releases cortisol, which plays an important role in modulating brain activity (Lupien *et al*., 2009). A negative feedback loop of the HPA axis allows cortisol release to return to normal levels after the removal of the stressor. A visual representation of this feedback mechanism is shown in Figure 1.

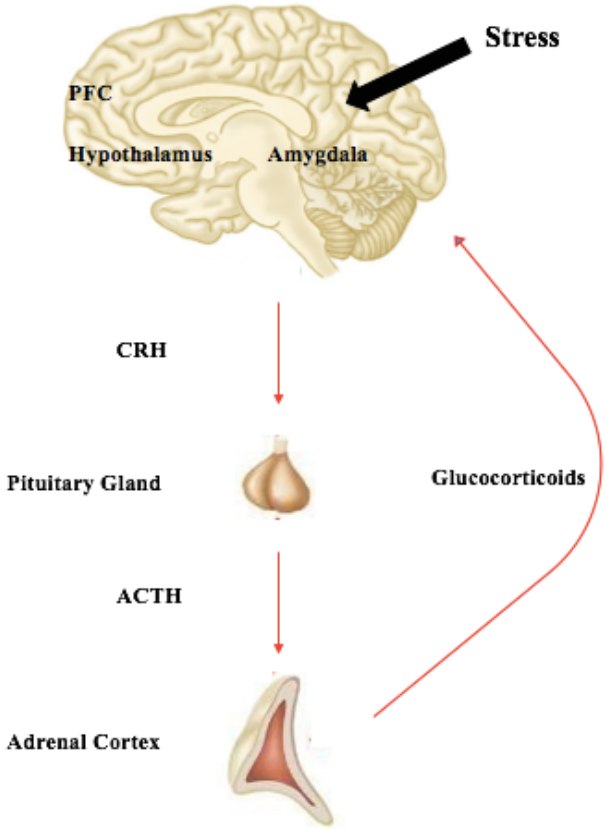

Figure 1. The hormone cascade of the HPA axis. When exposed to stress, the hypothalamus releases CRH which in turn causes the pituitary gland to release ACTH. ACTH stimulates the adrenal cortex to release glucocorticoids. When the stressing stimulus is removed, the levels of gluococorticoids signal the hypothalamus to stop the production of CRH and put a stop to the cascade through a negative feedback loop (Ulrich-Lai and Herman, 2009).

Different areas of the brain are activated during the stress response. The brainstem releases catecholamines (i.e. dopamine, noradrenaline) in the cerebral cortex (Ulrich-Lai and Herman, 2009). The brainstem also activates the HPA axis that begins the hormonal cascade mentioned above. The limbic system plays an important regulatory role as well. In particular, the amygdala, hippocampus, and prefrontal cortex (PFC) are pertinent. The

amygdala is primarily significant in autonomic regulation, while the hippocampus and PFC may be more vital in the cognitive processes involved in decision making in response to the stressor. Different areas of the brain may be more vital in response to specific stressors and may play only a supporting role in response to others (Ulrich-Lai and Herman, 2009). Changes in brain function during stress ensure the behavioral adaptation to stressors. However, stress can produce deleterious effects through maladaptive changes in brain structure and function.

An individual's genetics and previous exposure to stressors influence how capable they are to respond to a stressful event (Zhou *et al*., 2008), but these variables do not stand alone in their influence. Additional factors may also play a role in how stress affects certain individuals, including their age and stage of development. It has been shown that juvenile rats stressed before they reached puberty were more susceptible to both mood and anxiety disorders than their adolescent counterparts. This data indicates that a stress sensitive period may be present in the rat's development that makes the stress they experience more harmful at a certain stage than it is at another. (Tsoory, 2006).

When studying the effects of stress on animal behavior and physiological functioning, two forms of stress are often used: acute and chronic. Acute stress is a short-term, one-time stressor. Acute stress models are useful in evaluating how different conditions affect the activation and functioning of the HPA axis. An acute stressor would be more useful for monitoring some conditions over others. An example of when it is best used is in a study that examined how post traumatic stress disorder may alter the HPA axis by using a polytrauma model in rats. They found an acute stressor especially useful

as it would be applicable to a military situation, as veterans are one of the primary populations suffering from PTSD (Arnaud *et al*., 2018).

Chronic stress involves repeated exposure to a stressor and can induce different short-term and long-term effects than acute stress. Chronic social stress is an animal model often used to investigate the relationship between stress and psychiatric disorders in humans. A resident-intruder model has been used previously to create a chronic stress situation (Koolhaas *et al*., 2013). In one study, the goal was to determine how this paradigm affected motivation and how it related to depressive disorders in humans. It was discovered that the stressed rats had reduced locomotor and exploratory activity which indicates an overall lowered motivation. This lowered motivation was reflected by increased immobility in a forced swim test and decreased consumption of sucrose which suggest anhedonia. Anhedonia, an inability to feel pleasure from things that were previously pleasurable, is a symptom of depression in humans which indicates that chronic social stress in rats may mimic depression in humans (Rygula, 2005).

The behavioral effects of chronic stress are explained by morphological and functional changes in the brain. Following repeated stress, neuronal atrophy in the hippocampus can be observed as well as an increase in dendritic growth of neurons in the amygdala shortly following repeated stress. However, the most susceptible region of the brain to stress is the PFC. After being injected with a small dose of corticosterone, the hormone released in rats in response to stress, there were neuronal alternations in the PFC but not the hippocampus (Holmes and Wellmen, 2009).

Three weeks of daily restraint stress on rats induced changes in their PFC. Compared to the control group, the stressed rats had a significant reduction in apical

dendritic branches in the PFC. A similar reduction of apical dendrites in the same area was shown when rats were given corticosterone injections daily as opposed to undergoing a physical stressor. This study concluded that it is this structural change that may be a cause of the changes in cognition following stress (Cook and Wellman, 2004).

The PFC in the brain deals with decision making as well as the retrieval of memory. Deviations from normal PFC structure have been found in individuals with mood disorders. Changes in the dentate gyrus of the hippocampus have also been demonstrated to play a role in psychiatric disorders. The changes in these brain areas can increase the likelihood of developing depression in humans after chronic stress.

The changes in the PFC in response to stress are also thought to affect different aspects of executive function as measured by behavior. Firstly, acute and chronic corticosterone injections impair performance on working memory tests in rats. Secondly, a number of different stressors including maternal separation and acute foot shock induce impairments in cognitive flexibility. These results are consistent whether the measure of cognitive flexibility is based on performance in a Morris water maze or attentional set shifting. Lastly, chronic stress exposure may impair fear extinction (Holmes and Wellman, 2009).

In this study, we use a repetitive intermittent social stress model that involves the resident-intruder paradigm to investigate PFC-dependent cognitive behavior. We chose this model because it is known to cause changes in behavior and the PFC. A unique aspect of this model is that performance measurements were taken in between social stress sessions instead of only taking interest in the before and after stress measurements.

This allows for the observation of stress-induced effects on behavior and performance over time. The development of these effects has not been studied previously.

Cognitive Flexibility

Cognitive flexibility is the ability to recognize changing stimuli, adapt to the new stimuli, and respond in an appropriate manner (Dajani and Uddin, 2015). Cognitive flexibility is one of the measures often monitored to see how executive functioning is altered under different circumstances. Psychiatric disorders are one of the strongest factors that alter cognitive flexibility. These same disorders also influence other executive functions including fear extinction and working memory. Studies involving human and animal models have directly shown the deteriorating effects of anxiety on cognitive flexibility (Park and Moghaddam, 2017).

There are several models used to measure cognitive flexibility. In humans, the most common test to measure cognitive flexibility is the Wisconsin Card Sorting Test (WCST) (Nyhus and Barceló, 2009). In this task, participants must match a test card with one of three reference cards based on the rule that is currently being applied. The cards have various shapes and colors on them, therefore, the matching rules used are the color rule and the shape rule. One way to evaluate this model is based on the number of total errors made during the task. Using this evaluation, depressed patients with highly suicidal tendencies showed impairment during this task (McGirr *et al*., 2012). More ways to evaluate performance are by looking at the number of trials it takes to complete each rule or the consistency of correctness in response to the rule. These measures show

impairment in patients suffering from eating disorders when compared to controls (Tchanturia *et al*., 2012).

Many studies, including our study, use an attentional set shifting task in rats. This is seen as a variation of the WCST that still involves rule learning. The completion of this task involves the rat being able to switch between two different rules correctly and efficiently. Most often, the rules used are a Light Rule and a Side Rule. Both of these tasks are dependent on the function of the PFC in rats and humans, indicating that they are a consistent measurement between species and a valid measurement of cognitive flexibility (Park and Moghaddam, 2017).

Research has been able to correlate PFC function with the ability to perform a cognitive flexibility task. In order to demonstrate this correlation, researchers train rats in a perceptual attention task. Once rats have shown a capacity to complete the task, the experimental group undergoes a bilateral lesion by injection of acid in the PFC. Following the lesion, performance in the cognitive flexibility task significantly decreases, showing that this area of the brain is involved in cognitive flexibility (Birrell and Brown, 2000).

Drugs have also demonstrated the capability to alter cognitive flexibility performance. The effects of nicotine on the performance of the set shifting task in rats has been researched. The results showed that following acute and chronic nicotine injections, the rats' cognitive flexibility capacity increased, and their performance improved. The process that explains this improvement is thought to be the nicotinic acetylcholine receptors in the PFC. The alterations that nicotine makes in this area of the brain could be responsible for the changes in cognitive flexibility (Allison and Shoabib, 2013).

Stress is another variable that has been shown to alter cognitive flexibility in humans and animals. Humans that underwent a standardized stress-induction protocol showed impaired performance on a behavioral flexibility task that involves the PFC. Salivary cortisol levels were measured and those in the stress group had higher levels than the controls, indicating the activation of the HPA axis. Researchers attributed the impaired performance with the changes in the HPA axis (Plessow, Kiesel, and Kirschbaum, 2012).

In animals, the effects of chronic stress on cognitive flexibility is still unclear. Most studies have shown impairments but others have found improvements (Hurtubise and Howland, 2017). These discrepancies can be attributed to the experimental protocol and the time when the behavior was tested (i.e. immediately or prolonged after stress exposure). Whether the intermittent exposure to social stress alters cognitive flexibility is not yet known and is the purpose of the present study.

AIM OF THE STUDY

A better understanding of the relationship between stress and cognitive flexibility will help to determine how stress facilitates the development of psychiatric disorders. Stress can lead to psychiatric disorders through changes in brain function and behavior, one of which is cognitive flexibility. The purpose of this study is to investigate whether repeated exposure to social defeat stress changes cognitive flexibility performance. Our hypothesis entering this study is that there would be an impairment in cognitive flexibility measured by a poorer performance on the set shifting task. Moreover, we will determine time course effects on performance. We predict the impairing effects will increase as the animal continues to be exposed to stress (Figure 2). The qualifying factors for a decrease in performance would include making more errors and taking additional trials or time to complete the task. In addition, since chronic stress has been shown to increase anxiety, we also evaluate anxiety behavior after the repeated exposure to social stress using an elevated plus maze. We also predict that the rats undergoing social defeat will demonstrate higher anxiety than the control group measured by a plus maze.

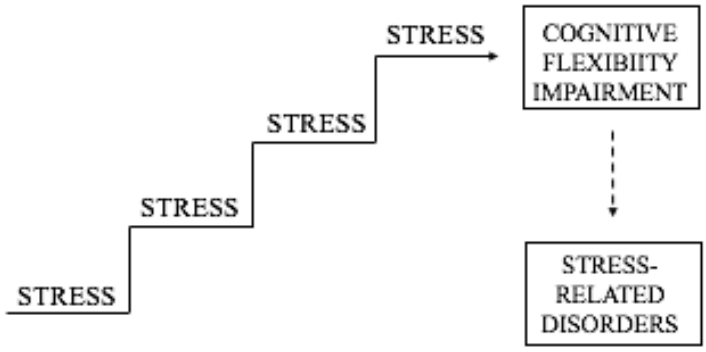

Figure 2. The present hypothesis suggests that the additive effects of stress will cause an impairment in cognitive flexibility which will in turn induce stress-related disorders such as depression and drug addition.

METHODS

Animals

Male Long-Evans rats (n=16) that were 3-6 months old were used in this study and were randomly separated into a control group (n=8) and a stress group (n=8). Rats were initially housed in pairs when they arrived to the animal facility. They were placed on a reverse light cycle that went from 9 a.m. to 9 p.m. (lights on at 9 p.m.). Individual housing began one week before the social stress protocol. The animals' initial weights ranged from 352-385 gr. The animals began a food restriction of 15 gr of food per animal per day two days before behavior training. This caused the weights of the animals to initially drop and then slowly increase. The purpose of the food restriction was to motivate the animals to initially learn and then perform the set shifting task. This animal experiment was approved by the Institutional Review Board at the University of Mississippi and followed the rules of the Institutional Animal Care and Use Committee.

Set-Shifting Task

Rats were taught a cognitive flexibility task in an operant chamber constructed with three nose pokes on one side and a food magazine on the other side (Del Arco *et al*., 2017). This task measured the accuracy and speed that rats were able to complete and alternate between a Light Rule and a Side Rule. The Light Rule required the rats to poke in the nose poke that was lit up. In order for the rule to be successfully completed, the subject was required to poke in the nose poke that displayed a fixed light. The Side Rule depended on the rats being able to poke in either the left, center, or right nose poke only, regardless of where the light was. A model of the set shifting task can be seen below in Figure 3.

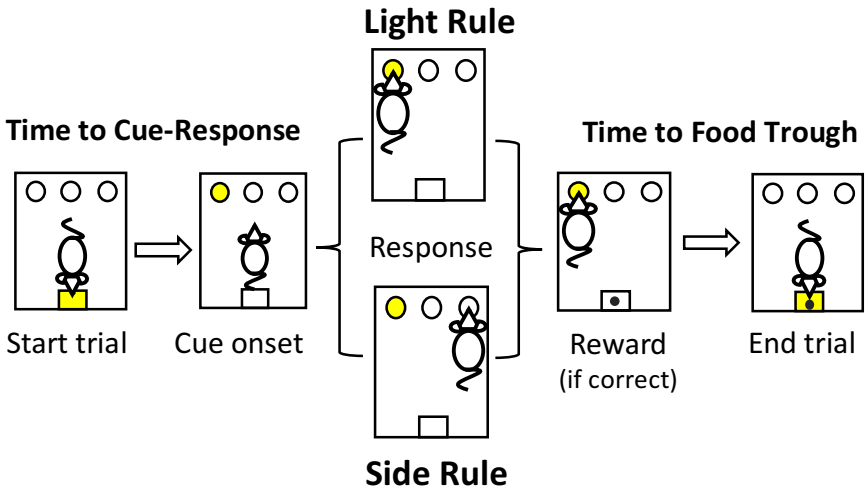

Figure 3. A visual representation of the Light and Side Rules used in the set shifting task to test cognitive flexibility. Initially, the animals would poke in the food trough to start the trial. Then, the cue (the light) would signal that the trial had begun. If the Light Rule was being presented, the rat would need to poke in the nose poke with the light. If the Side Rule was being presented, the rat had to poke in the correct nose poke that corresponded with the rule. After poking in a nose poke, the light in the nose poke would turn off and the light in the food trough would turn on. If the rat poked correctly, a food pellet would also be delivered in the food trough. In order to end the trial, the rat had to poke in the food trough.

The entire task included the completion of two Light Rules and two Side Rules that were presented in an alternating order. In order to move on to the next rule, rats had to get nine out of ten nose pokes correct to show that they learned which rule was being applied and that they were able to successfully complete that rule. There were six protocols of this task that were cyclically presented to the rats in order to ensure that they

were being truly judged on cognitive flexibility as opposed to memory of the task. The different protocols are presented in Table 1. Three of those tasks began with the Light Rule and the other three began with the Side Rule.

	Set 1	Set 2	Set 3	Set 4
Protocol 1	Light Rule	Right Rule	Light Rule	Left Rule
Protocol 2	Right Rule	Light Rule	Left Rule	Light Rule
Protocol 3	Light Rule	Center Rule	Light Rule	Right Rule
Protocol 4	Center Rule	Light Rule	Right Rule	Light Rule
Protocol 5	Light Rule	Left Rule	Light Rule	Center Rule
Protocol 6	Left Rule	Light Rule	Center Rule	Light Rule

Table 1. The order of rules used for the six variations of the attentional set shifting task. These tasks were cyclically repeated in order to prevent memorization and ensure that the task was measuring cognitive flexibility.

Repetitive Intermittent Social Defeat Stress

The resident-intruder paradigm was used to induce social stress (Miczek *et al*., 2011). The chamber for social stress was a large square box made of PVC (0.7 x 0.7 x 0.7 m) that was clear on all sides. There was a removable clear wall that could be placed to divide the chamber in half. This clear wall had holes in it so rats on opposite sides of the cage were still able to see and smell each other. A visual representation of the chamber is shown in Figure 4.

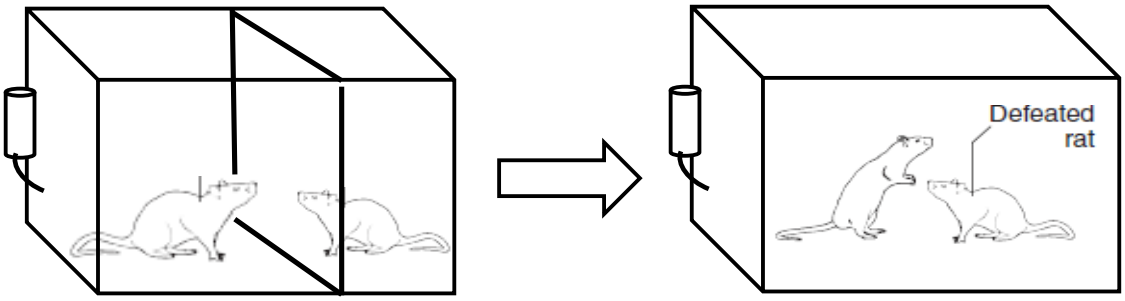

Figure 4. A visual representation of the cage used to carry out the resident-intruder paradigm

The resident rat was the rat that was housed in the social stress cage and would socially defeat the intruder rat, the experimental rats tested in the behavior task. The resident rats were at least 100 gr. heavier than the intruder rats. Each resident was paired with a female in the social stress cage. They were allowed to mate for one week before any social defeat occurred. The placement of the female allowed the resident rats to become territorial of the social stress cage. There was also limited cleaning of the cage in order to maintain the resident's territoriality.

Procedure

Rats in the stress group were individually moved into the stress room to prevent pre-defeat auditory cues. Initially, the female was removed from the social stress cage for at least 30 minutes before any rat entered the social defeat room. The cage was initially separated into two with the removable porous wall. In the first step, the intruder was placed into one side of the cage while the resident was in the other. They remained here for 10 minutes before physical interaction. The social stress starts at this point because the intruder rat was able to see and smell the intimidating resident rat. After the first 10

minutes, the second step began and the wall was lifted. This marked the start of social defeat. During this time the latency of the first attack, the number of attacks, the number of bites, and the supine behavior time were measured. This time of defeat lasted until the intruder showed supine behavior for 5 seconds, the intruder was attacked 6 times, or 5 minutes had elapsed. In order for an interaction to be deemed an attack, the rats must display a clench attack in which they are directly in contact. Whichever of these three conditions came first was when the defeat segment was concluded and the wall was placed again to separate the resident and intruder. The third step lasted for 10 minutes after defeat and allowed the intruder to experience visual and olfactory stimuli from the resident. After post-defeat, the intruder was removed and returned to its home cage. The control rats were removed from the housing room at the same time as the control rats. They were handled for 5 minutes in a room separate from the stress room as not to be influenced by any social stress.

Figure 5 shows the timeline of the social stress protocol. Rats were exposed to social stress every three days and would be tested on the cognitive flexibility task on each day between social stress. The rats underwent a total of four social stress sessions. Following the fourth social stress session, the rats were tested on the cognitive flexibility task once a day for three days. The rats underwent cognitive flexibility testing again ten days after the last social stress scenario once a day for two consecutive days.

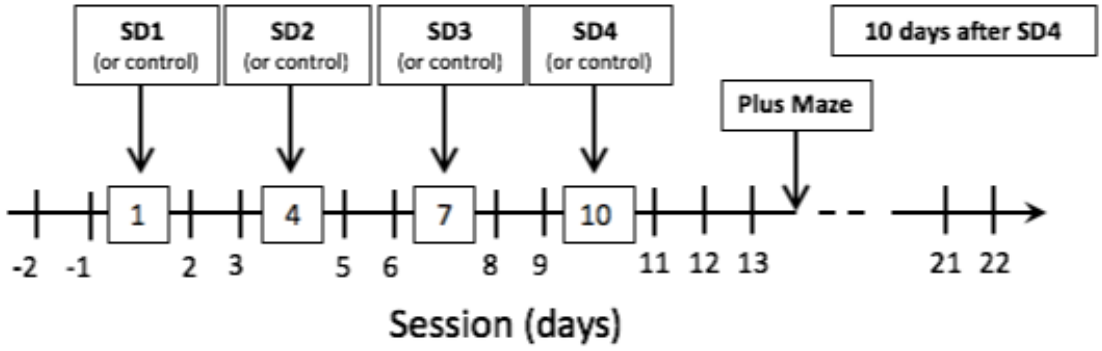

Figure 5. The timeline of the social defeat protocol. Each boxed number represents days when rats underwent social defeat and every other number shows days that they performed the set shifting task.

Plus Maze

The plus maze is a commonly used test to measure anxiety behavior (Tovote *et al*., 2015). It is a plus shaped surface that is raised 76 cm off of the ground with opposite closed and open arms that are 56 cm long. The closed arms have opaque walls that are 15.25 cm tall surrounding the outside so that the rat is enclosed and is not able to see the drop below them. The open arms do not have walls so that the rat is open to the space around them and is able to see the height that they are off of the ground without any barrier. Figure 6 shows an illustration of what the plus maze looks like. The rats were tested in the plus maze on the fourth day after the fourth episode of social defeat. They were first placed in the center of the maze and were free to move about the maze for five minutes. The amount of time spent in the open and closed arms as well as the number of crosses made between arms was measured. To qualify to be in one of the arms, all four of the rat's paws had to be in that arm. Between individual rat measurements in the plus maze, the plus maze was cleaned with the antiseptic Quatricide ®.

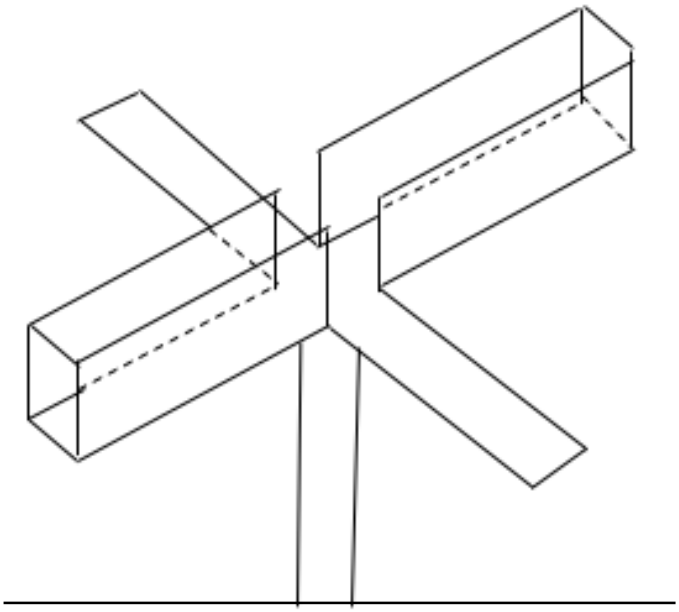

Figure 6. A visual representation of the elevated plus maze used to measure anxiety behavior.

Data Analysis

Two-way ANOVAS with repeated measures were performed to analyze set shift performance. This allowed for a comparison both across sessions within a group as well as between groups (control and stress). An independent t test was used to evaluate changes in plus maze performance and number of free pellets eaten after set shifting sessions.

RESULTS

Set Shifting Performance

Table 2 shows the parameters evaluated during performance and their behavioral significance.

Total Trials and Errors

The number of trials and errors that animals take to adapt their responses to the rule (Light or Side) is used as an index of cognitive flexibility. An impaired flexibility will increase the number of trials and errors. Figure 7 shows the average number of trials and errors per set. This number was calculated for animals in each group that completed at least two sets during the set shifting protocol. Table 3 shows that the average number of sessions completed by stress animals decreased as time went on. For the average number of trials there was a significant difference across sessions [$F(10,140)=2.88$, $p=0.012$] and a significant difference between groups [$F(1,4)=8.05$, $p=0.013$]. For the average number of errors, there was a significant difference across sessions [$F(10,140)=2.96$, $p=0.002$] and a significant difference between groups [$F(1,4)=6.56$, $p=0.023$]. When evaluated 10 days after the end of the stress protocol, there were no significant differences between stress and control groups in the average number of trials and errors. These results show that stress animals require less trials and make fewer errors during the set shifting task in the short term. These effects were not maintained in the long term.

Proportion of Correct Responses for Light and Side Sets

The proportion of correct responses for Light and Side Sets is used as an index of accuracy. An impaired flexibility will lower the proportion of correct responses. Figure 8

shows the proportion of the correct responses for each group for both Light and Side Sets. There was not a significant difference between groups or across sessions in the short term. However, after ten days there was a significant difference between groups in the proportion of correct responses for the Light Set [$F(1,1)=7.36$, $p=0.013$]. These results show that stress animals do not have a higher proportion of correct responses in the short term. However, they are more accurate to respond to light cues that predict rewards in the long term.

Time to Cue Response

The time to cue response refers to the time that occurs between when the light cue comes on, signifying the beginning of the trial, to when the rat pokes in a nose poke. Figure 9 shows the average time that rats in each group took to respond to the cue during the Light Rule. There were significant differences across sessions [$F(10,140)=2.36$, $p=0.013$] and between groups [$F(1,1)=4.95$, $p=0.043$]. These effects were not maintained ten days after the fourth social defeat session. Similar results were found in the Side Rule (data not shown). These results show that stress animals take more time to make a decision and respond to the cue.

Time to Food Trough

The time to food trough represents the time it takes for the rat to go from the nose poke where they made a decision to the food trough to end the trial. Figure 10 shows the time to food trough for stress and control groups following correct (rewarded) and incorrect (unrewarded) responses. Following a correct response, the time to food was not affected across sessions [$F(10,140)=0.42$, $p=0.93$] but it was affected between groups [$F(1,1)=5.98$, $p=0.028$]. Following an incorrect response, there was a significant

difference across sessions [F(10,140)=3.91, p=0.01] and between groups [F(1,1)=10.54, p=0.006]. None of these effects were maintained after ten days. These results show that stress animals took a longer time to go to the food trough after both correct and incorrect responses compared to control animals. They also show that stress animals began to take longer to go to the food trough after making an incorrect response as the stress protocol went on.

To test whether stress and control animals were motivated to eat reward pellets, animals were given 50 pellets following the completion of the set shifting session. All animals ate the total of 50 pellets available. Table 4 shows average time it took for animals in the stress and control groups to eat the 50 pellets. Figure 12 shows the data distribution of the time to eat the 50 pellets for individual animals in each group. There was not a significant difference between groups for the amount of time it took to consume the 50 pellets (p=0.96). These results show that the stress animals ate the same amount of pellets as the control animals and took the same amount of time to do so following the set shifting protocol.

Plus Maze

Less time in the open arm of the elevated plus maze is seen as an index of increased anxiety. Figure 11 shows the average time each group spends in the open arm and closed arm of the plus maze, as well as their motor activity determined by the number of crosses they make between arms. A t-test revealed that the stressed group displayed more time in the open arm (p= 0.0077) and significantly less time in the closed arm (p= 0.0017) compared to the control group. The motor activity between groups was

not significantly different (p=0.3139). These results indicate that stress animals are not more anxious than the control animals.

PARAMETER	BEHAVIORAL SIGNIFICANCE
Trials and Errors	Flexibility to Shift Between Rules
Proportion of Correct Responses	Performance Accuracy
Time to Cue Response	Decision-Making
Time to Food Trough	Motivation to Perform the Task

Table 2. A chart organizing the behavioral significance of each of the parameters evaluated in the results.

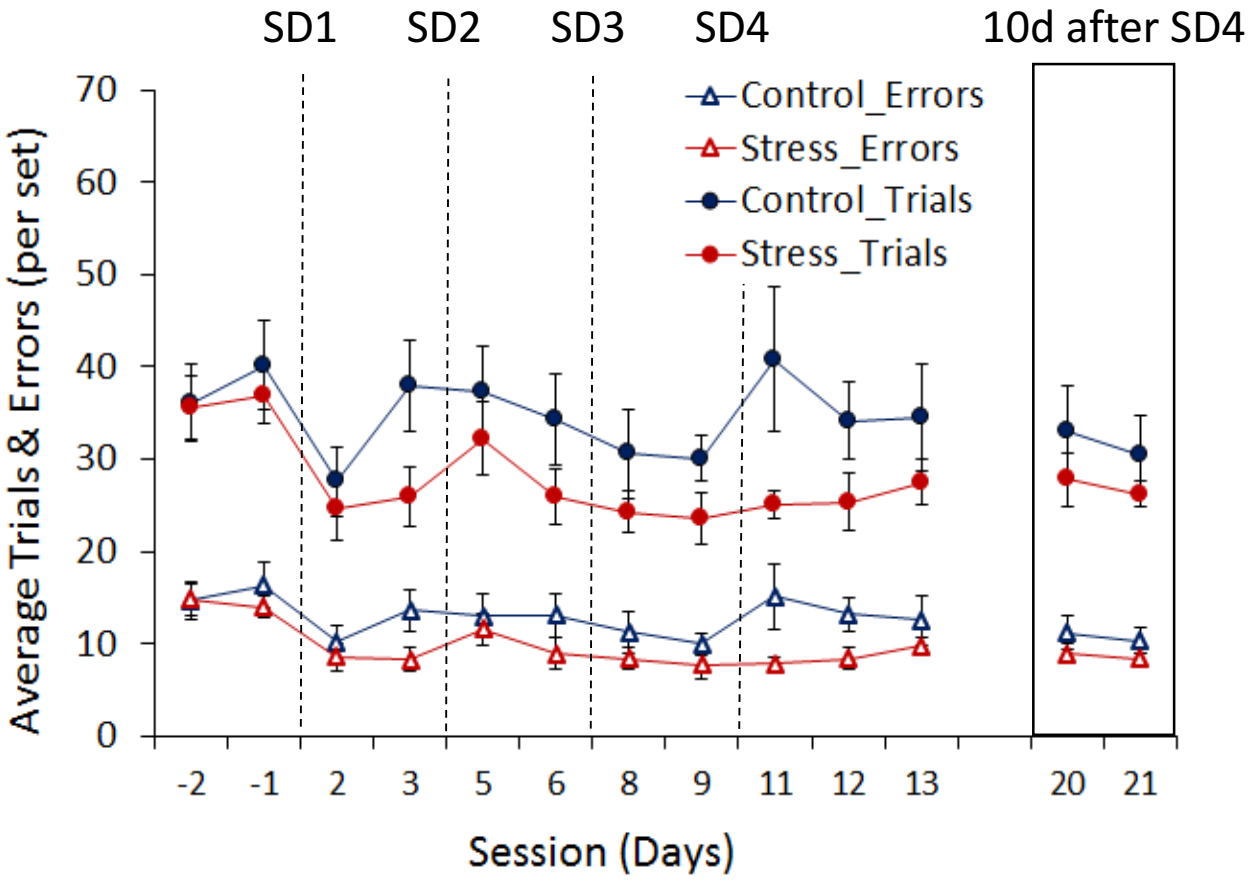

Figure 7. Average number of trials and errors per set for control animals (n=8) and stress animals (n=8). Each data point represents the average number of trials or errors performed ± SEM. Dotted lines represent social stress episodes.

		Control	Stress
	d-2	4.00	3.88 ±0.13
	d-1	3.88 ±0.13	4.00
SD1	**d1**		
	d2	4.00	4.00
	d3	4.00	4.00
SD2	**d4**		
	d5	4.00	3.63 ±0.40
	d6	4.00	3.50 ±0.35
SD3	**d7**		
	d8	4.00	3.13 ±0.43
	d9	4.00	3.25 ±0.39
SD4	**d10**		
	d11	3.50 ±0.40	2.63 ±0.53
	d12	3.88 ±0.13	3.00 ±0.40
	d13	3.75 ±0.27	2.38 ±0.57
	d20	4.00	4.00
	d21	4.00	3.88 ±0.13

Table 3. Number of sets completed every session for control animals (n=8) and stress animals (n=8). Each data point represents mean number of sets complete ± SEM.

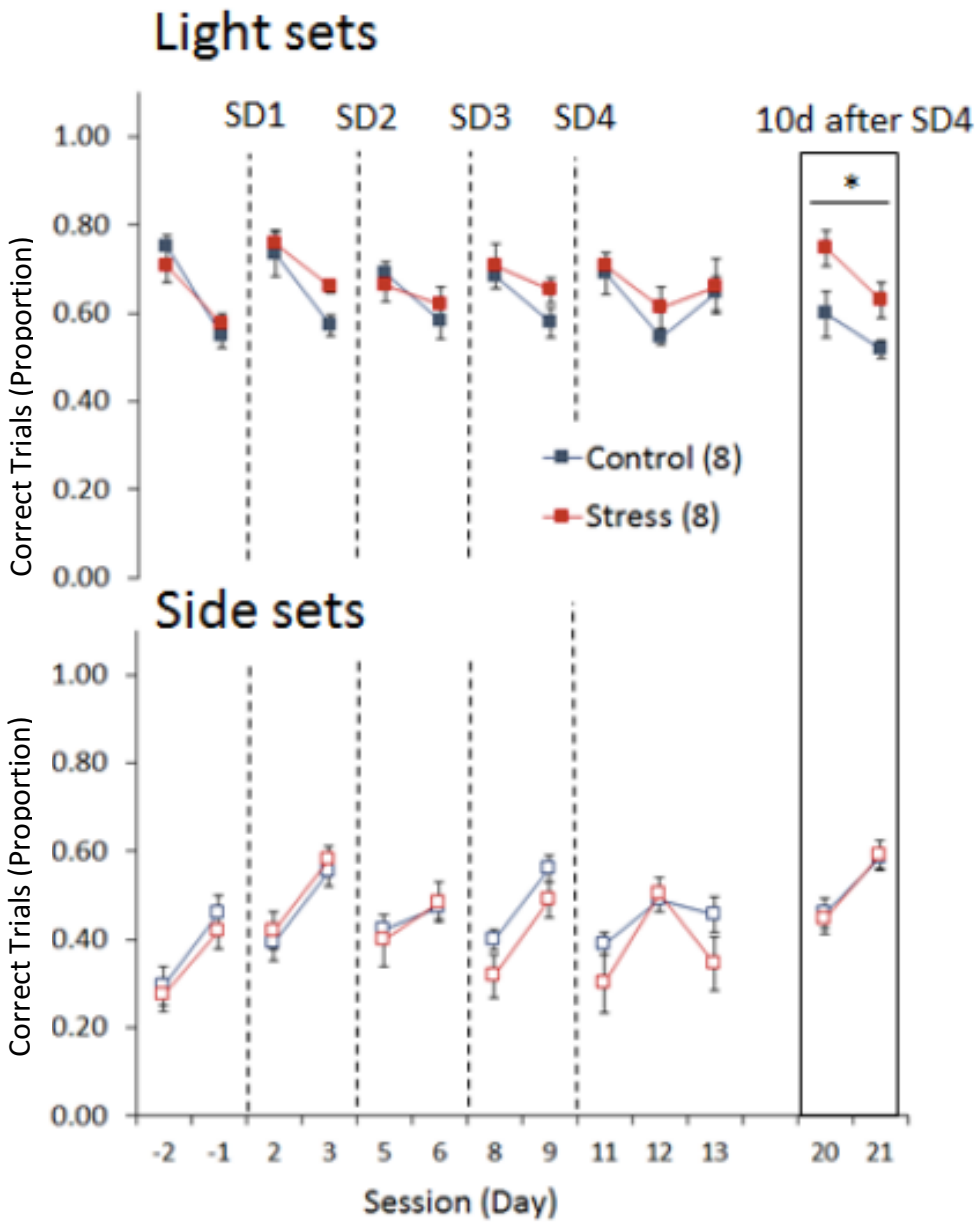

Figure 8. Proportion of correct trials for the Light Rule and Side Rule between control animals (n=8) and stress animals (n=8). Each data point represents percentage of trials correct ± SEM. Dotted lines represent social stress sessions.

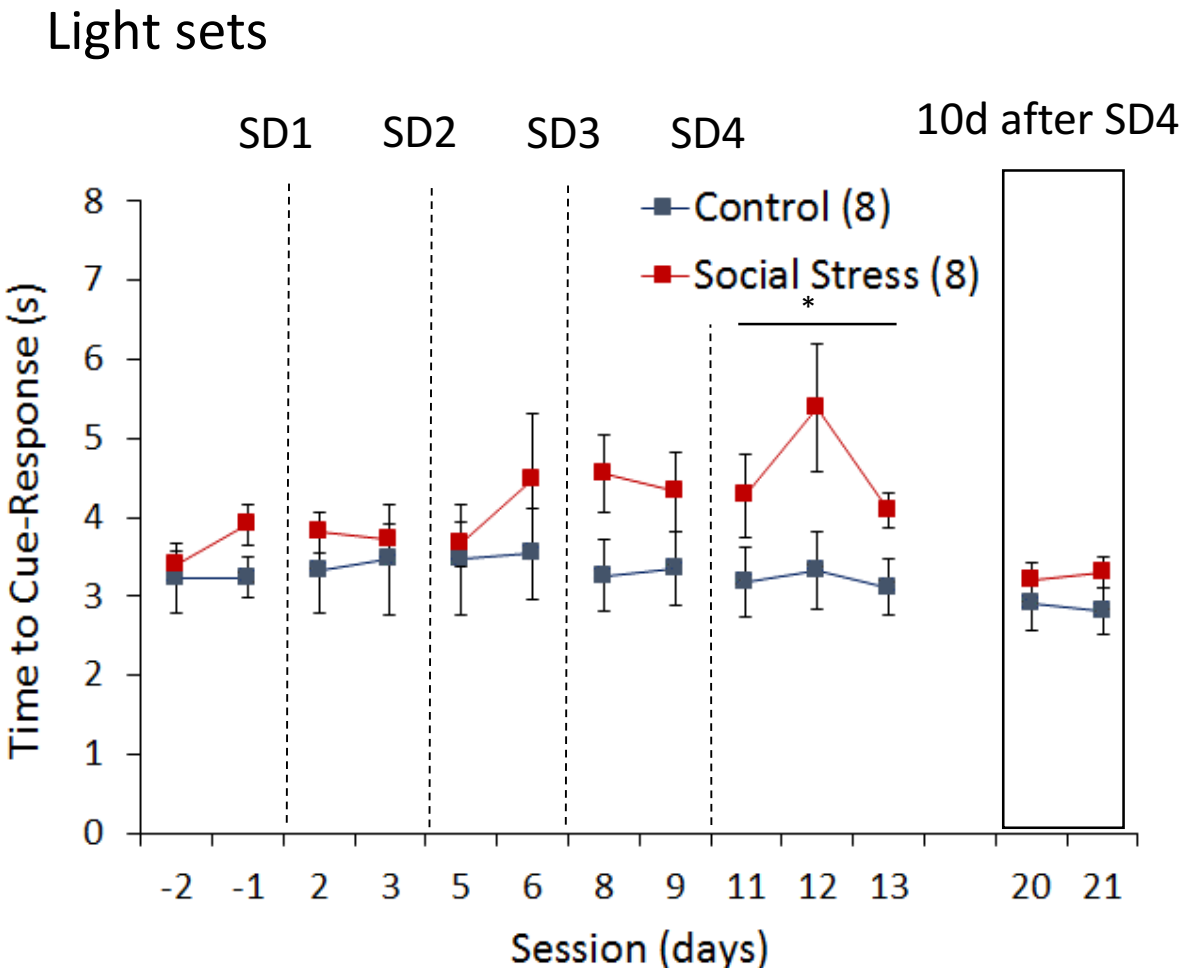

Figure 9. Amount of time to respond to the cue for light sets for control animals (n=8) and stress animals (n=8). Each data point represents the amount of time taken ± SEM. Dotted lines represent social stress sessions.

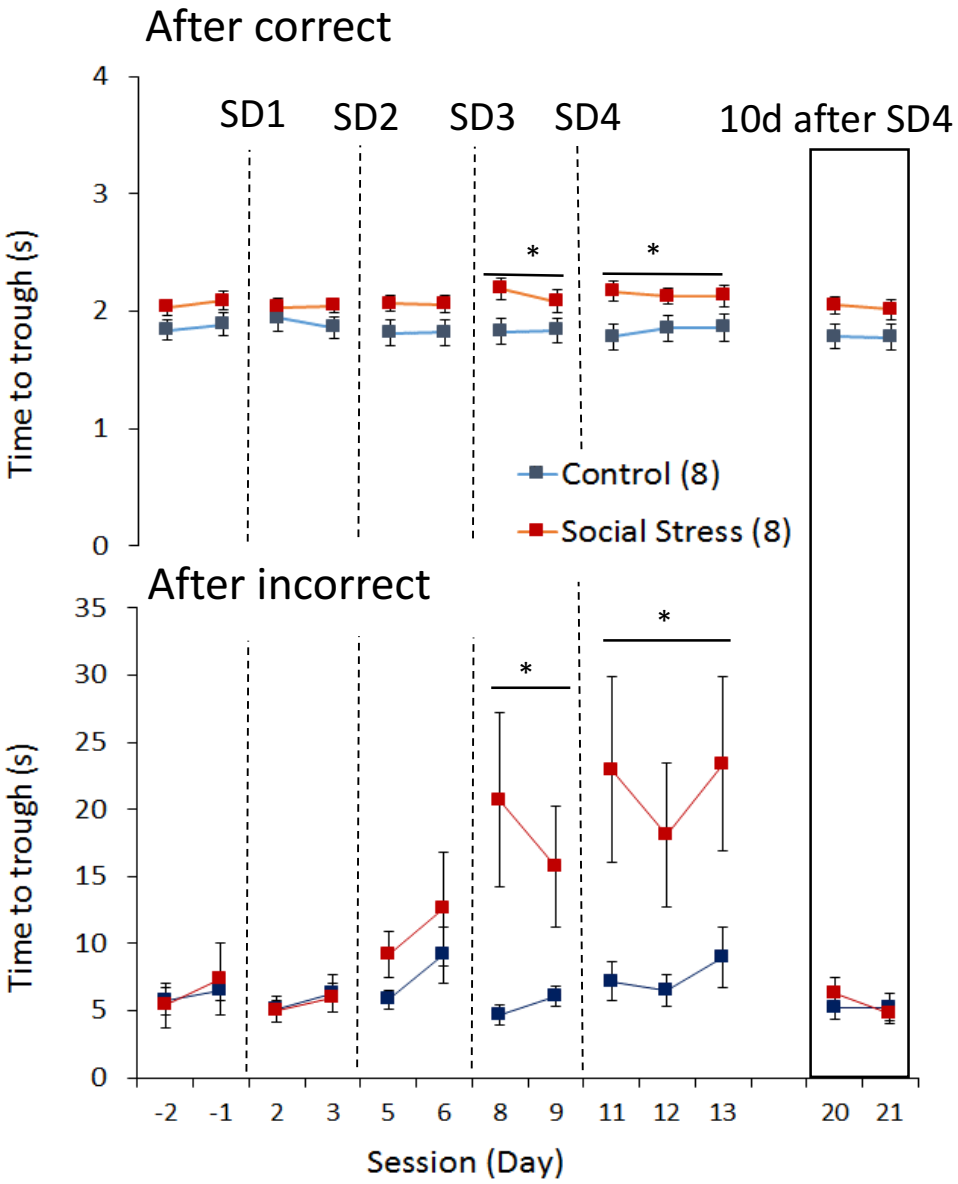

Figure 10. Amount of time to go to the food trough following both correct and incorrect trials for control animals (n=8) and stress animals (n=8). Each data point represents the amount of time taken ± SEM. Dotted lines represent social stress sessions.

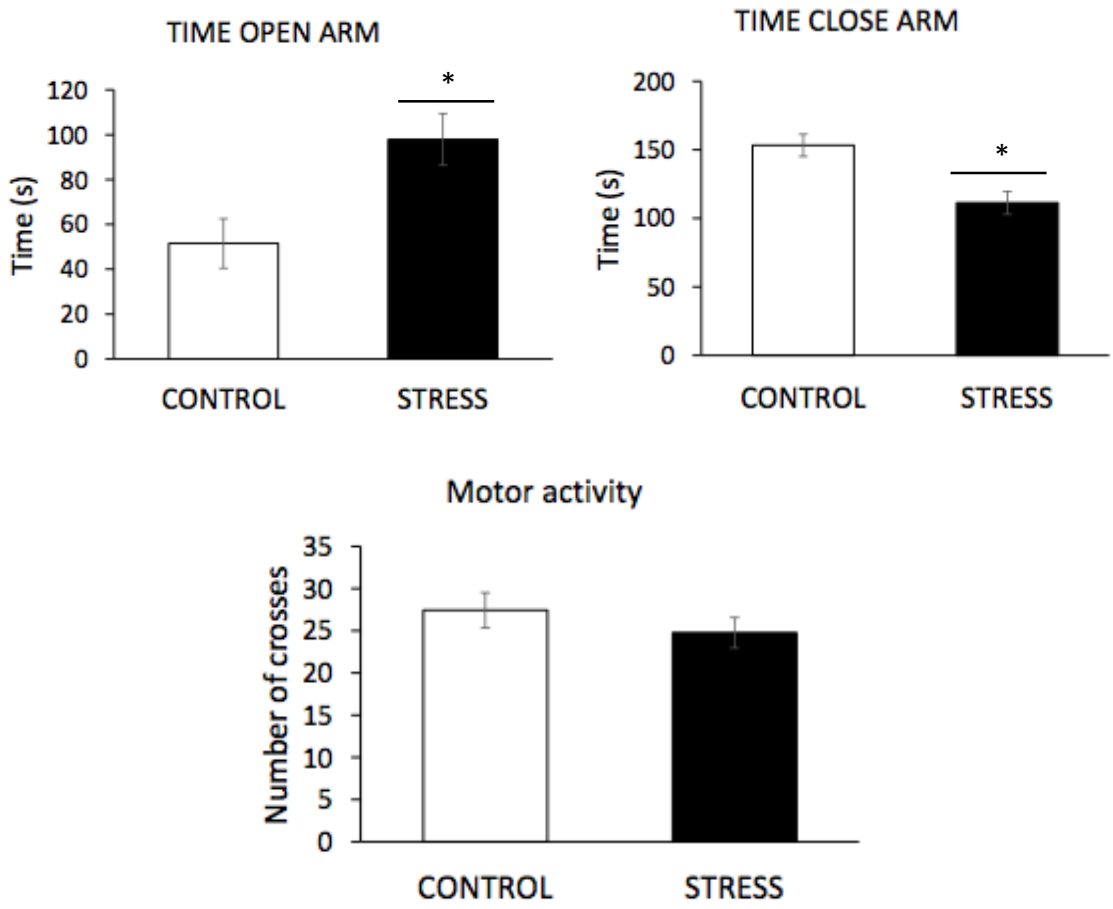

Figure 11. Mean time spent in the open arm and closed arm. Mean motor activity based on the number of crosses made. Bars represent the mean ± SEM. Data was collected on the fourth day after SD4 over a period of five minutes.

Stress	Time (s)	Control	Time (s)
C17	132	**C18**	104
C19	108	**C20**	128
C23	99	**C21**	123
C24	101	**C22**	118
C28	127	**C25**	137
C30	134	**C26**	126
C31	129	**C27**	103
C31	152	**C29**	144
AVERAGE	123	**AVERAGE**	124
SEM	7.03	**SEM**	5.45

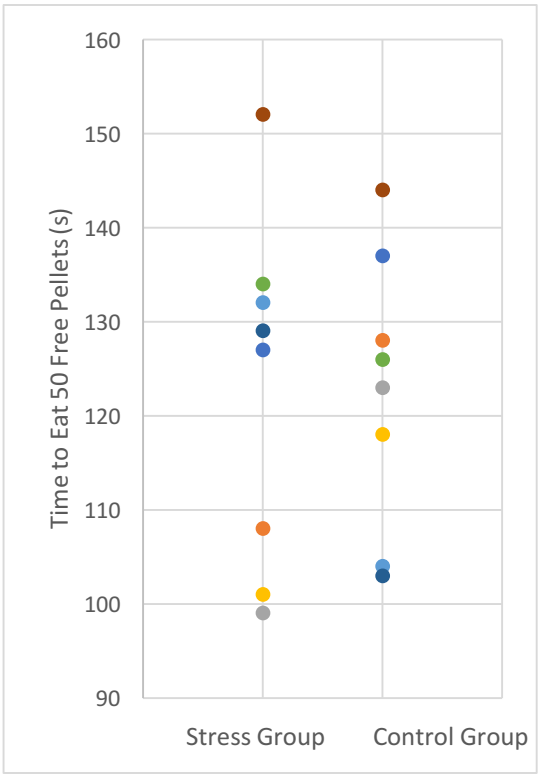

Table 4. Average individual time over all sessions and average group time over all sessions (mean ± SEM) for animals to eat 50 free pellets following set shifting protocol.

Figure 12. Data distribution of the average individual time for rats from each group to eat 50 free pellets at the end of set shifting sessions. Each dot represents one single rat.

DISCUSSION

Our results did not support our hypothesis that repetitive intermittent social stress would impair cognitive flexibility. However, our results showed that changes were present in performance between the stressed and control rats. These changes were present in both the short and long term. In the short term, 1) the stressed rats took fewer trials to complete a set and made fewer errors; and 2) they took longer to respond to a light cue and to end a trial after an incorrect response. In the long term, the stressed animals had a higher proportion of correct responses to the light cue. Finally, the stressed rats spend more time in the open arm of the plus maze than the control group. These results suggest that repeated exposure to stress does not impair cognitive flexibility but alters behavioral responses.

The stressed rats have significantly less trials and errors than the control group, as shown in Figure 7. This interesting finding shows that the stressed group's performance is not impaired in cognitive flexibility. Instead of being impaired, it may appear that the stressed rats are performing better due to the decreased trials and errors. However, there is additional data that contradicts this notion. Figure 9 shows that the stressed rats are taking more time to respond to the cue. By taking more time, in theory, they have more time to make a decision and the chance that they pick the correct nose poke increases. This is called the speed accuracy tradeoff (Heitz and Schall, 2012), in which a competition between those two conditions causes you to have to pick one over the other. Additional support for the fact that the stressed animals are not more flexible is shown in Figure 8 as the percentage of correct trials is not different between the stress and control groups.

The literature has reported mixed findings of the effects of stress on cognitive flexibility (Hurtubise and Howland, 2017). It is thought that impairments may manifest depending on what type of stressor is used. Some studies have shown that acute stress reduced performance in rats (Butts *et al*., 2013). When a repetitive restraint stress was used over the course of ten days, rats took more trials to complete an attentional set shifting task, which was interpreted as a decrease in performance ability (Nikiforuk and Popik, 2014). Other studies are consistent with our findings where an impairment is not found (Thai *et al*., 2013; Chaijale *et al*., 2013). Publications have analyzed how results differ greatly and give some analysis into why the results are so different. They found that it can be attributed to type of stress, sex of rat, and the paradigm used to assess cognition (Hurtubise and Howland, 2017). Our study is the first one using an intermittent exposure to social stress, which is different than chronic (continuous) social stress (everyday exposure) (Miczek *et al*., 2011).

While the performance of the stressed rats is not impaired, it is changed. Table 3 shows that the completed number of sets for the stress group progressively declined as stress continued. These results point to a decreased motivation to work for rewards. However, this is not due to a lack of hunger. An initial assumption that could be made about why the rats stopped performing is that they weren't motivated to eat. However, the stressed rats continued to eat the 50 pellets that were placed in a petri dish in their chamber after the set shifting task had concluded. Table 4 shows that they consistently ate all pellets immediately and quickly, so the motivation to eat the pellets was not the cause for the change in motivation. This assumption is further discredited by Figure 10 that shows that the stressed rats did not change across sessions in the time they took to go

to the food trough after completing a correct trial and eat the earned pellet. The second, more likely possibility for the decrease in performance was that they were no longer motivated to make the effort to pursue rewards. This inference is supported by the increased time that stress animals take to go to the food trough to end the trial following incorrect responses in which no pellet was delivered, as shown in Figure 10. This lack of motivation is a short term effect as it is not maintained after ten days. The number of sets completed and the time it takes to go to the food trough after incorrect trials both return to baseline after the ten-day period.

Other studies have also shown that stress has an effect on motivation. In rodents, it has been shown that the paraventricular thalamic nucleus plays a role in the processing of stress and that this same area is responsible for neurotransmitter regulation in areas that control motivation. Therefore, any alterations or malfunction in the paraventricular thalamic nucleus could induce physiological changes that disrupt motivation. Further support for this possibility is that this area of the brain is altered in psychiatric disorders, in which motivation is also shown to decrease (Hsu, 2014). In humans, it has been shown that after being exposed to an acute stressor, their motivation to learn and learning performance both decreased (Lepine, Lepine, and Jackson, 2004). This is consistent with our findings in that the rats displayed a lower motivation to perform. In addition, current findings show that acute stress causes rats to choose a task that involves lower effort to produce a lower reward task as opposed to a task that requires higher effort for a higher reward (Bryce and Floresco, 2016), which is interpreted as a decrease in this motivation to make the effort. In future studies, a forced swim test could be used to verify the change in motivation. This test involves looking at the animal's immobility when presented with

a situation in which they must swim or float. When the rats become more immobile, they are less motivated to make an effort to perform in the test. A forced swim test is often used to measure the effectiveness of antidepressants, as it is a well respected measure of depression-like behavior in animals (Bogdanova *et al*., 2013).

The long term effect that was present was the increase in proportion of correct responses for the stressed rats in the Light Rule. This change manifested after the ten-day period. This effect could be explained by an increased saliency of the light cue. Increased saliency is associated with an increase in drug-seeking behavior. Also, using this model, it has been shown that stressed rats will become cocaine abusers faster than controls (Miczek *et al*., 2011).

Figure 11 shows that the stress group spent significantly more time in the open arm than the closed arm compared to the control group. Traditionally, more time in the closed arm is interpreted as a measure of anxiety. However, spending more time in the open arm could be translated as an indication of risk-taking behavior (Toledo-Rodiguez and Sandi, 2011). The fact that stressed animals spent more time in the open arm suggests that they were more prone to take risks. This increased risk taking behavior could be further studied in a variety of ways. One method of studying this would be to monitor how social stress affects drug use. This suggestion fits well with the fact that risk-taking animals are more prone to drug abuse (Miczek *et al*., 2011). Previous studies have also found that mild and moderate chronic social stress increased cocaine use in rats (Han, 2015) but no analysis has been done on how this correlated with plus maze performance. The specific neural pathways that cause this change have not been identified either.

In conclusion, the performance of animals that underwent repetitive social stress displayed a change, not an impairment, on the performance of a cognitive flexibility task. In the short term, we found a change in the motivation of the animals. In the long term, we discovered a change in risk taking-behavior and a change in the processing of salient stimuli. These results suggest changes in the neurobiological substrates that regulate the motivation/reward system in the brain. A limitation of this experiment was the low number of subjects. While the significance of the results shows that the number of subjects is sufficient, a repeated study following this model with more animals would be able to demonstrate the results more strongly. The low number of animals make it difficult to address the individual differences between them. One way to better understand what is happening on a neurobiological basis would be to use electrophysiology in stressed and control rats during the set shifting task to see what neural pathways are altered.

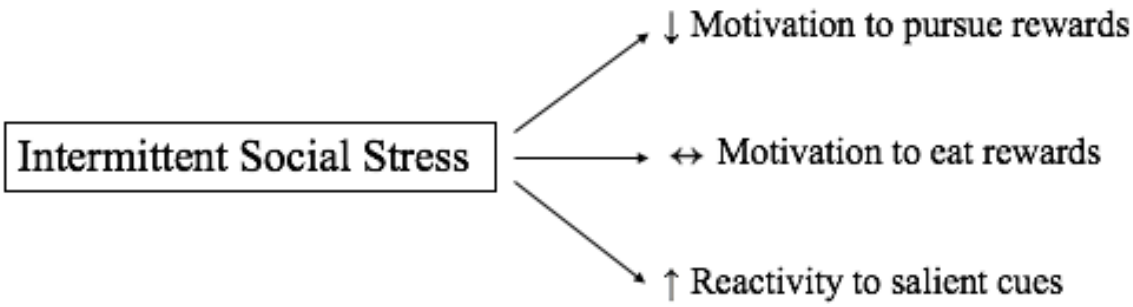

Figure 13. A summary of the three primary findings. Intermittent social stress has been shown to cause a decrease in motivation to pursue rewards, no change in motivation to eat rewards, and an increase in reactivity to salient cutes.

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