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The Effects of "Stress Inoculation" on Anxiety-related Behaviors in a Rodent Model

Rebecca Ruth Clouse
Andrews University, clouse@andrews.edu

ABSTRACT

THE EFFECTS OF "STRESS INOCULATION" ON ANXIETY-RELATED BEHAVIORS IN A RODENT MODEL

by

Rebecca Ruth Clouse

Chair: Pamela S. Coburn-Litvak

ABSTRACT OF GRAUATE STUDENT RESEARCH

Thesis

Andrews University

College of Arts and Sciences

Title: THE EFFECTS OF "STRESS INOCULATION" ON ANXIETY-RELATED BEHAVIORS IN A RODENT MODEL

Name of researcher: Rebecca Ruth Clouse

Name and degree of faculty chair: H. Thomas Goodwin, Ph.D.

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Problem

Humans may face psychological stressors in their everyday life due to their ability to contemplate future events. While long-term stress exposure may be detrimental to health, this study examines the possibility that exposure to unpredictable/controllable, moderate stress may cause resilience against future stressors. This is referred to as the "stress inoculation hypothesis." The effects of unpredictable/controllable stress can be illustrated as a rightward shift in an inverted U-shaped curve, where optimal performance (the top of the curve) can be maintained at higher stress levels.

Method

Thirty-three male Sprague-Dawley rats were tested on the elevated-plus maze (EPM) for trait anxiety. Rats were then placed in housing platforms; 15 rats were exposed

to unpredictable/controllable stress (UST) in the housing platform, and 18 rats were used as a control group (CT). After 21 days in the UST or CT housing, spatial memory and anxiety-related behaviors were tested under aversive conditions on the Barnes maze.

Results

Spatial memory: UST rats took less time to reach the goal box on the Barnes maze ($p<0.05$), and made fewer errors ($p<0.05$). **General locomotor activity:** No significant effect was seen for mobility, which is a measure of general locomotor behavior, on the Barnes maze ($p=0.100$). The UST rats took less time to approach the first hole on the Barnes maze ($p<0.05$). There was no significant effect for the number of holes jumped over in the Barnes maze ($p=0.599$). There was no significant effect for the number of times the rats looked over the edge ($p=0.431$). No correlations were seen between Barnes maze data and EPM behaviors (all $p>0.05$).

Conclusion

These data are consistent with the "stress inoculation hypothesis." As humans, exposure to stress is unavoidable and unpredictable. The results of this study, however, indicate that stress does not always lead to negative consequences, but can be helpful. By causing a rightward shift in the inverted U-shaped curve, stress can better prepare us to face stressful situations in the future and reduce our anxiety about facing those stressors.

Andrews University

College of Arts and Sciences

THE EFFECTS OF "STRESS INOCULATION" ON ANXIETY-RELATED BEHAVIORS IN A RODENT MODEL

A Thesis

Presented in Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

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APPROVAL BY THE COMMITTEE:

_____
Pamela S. Coburn-Litvak, Ph.D., Adviser

_____
H. Thomas Goodwin, Ph.D.

_____
Benjamin Navia, Ph.D.

_____ _____
Brenda Anderson, Ph.D. Date approved

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CHAPTER 1

BACKGROUND

What Is Stress?

In 1936, Hans Selye described a syndrome that occurs when organisms are acutely exposed to non-specific, nocuous agents. Symptoms of the syndrome included adrenal hypertrophy, gastrointestinal ulcers, and suppressed immune function. He described the syndrome as a reaction to the new conditions the organisms were experiencing (Selye, 1936). Selye later used the word "stressor" to describe any stimulus that resulted in this response (Selye, 1976). Currently, "stress" is defined as a response to situations that threaten homeostasis, which is the regulation of the body's internal environment (Johnson, Kamilaris, Chrousos, & Gold, 1992). Humans, like animals, face physical stressors once in a while such as natural disasters, war, and physical assault. Physical threats require physical responses, but the body returns to homeostasis once the threat is gone. However, humans can also experience and even anticipate psychological stress in their everyday lives while trying to meet demands, whether it is by trying to gain approval at school or work, trying to finish assignments before deadlines, or worrying about future life events or disasters (McEwen, 2007). Because humans have the ability to contemplate future events, we are especially prone to psychological or emotional stress (Herman, Prewitt, & Cullinan, 1996; Sawchenko, Li, & Ericsson, 2000).

Stress Activates the HPA Axis

When individuals are exposed to stressors, whether it is physical or psychological, the Hypothalamus-Pituitary-Adrenal (HPA)-axis (Figure 1) is activated. Activation of this endocrine axis results in the synthesis/release of adrenal steroid hormones into the blood stream, predominantly cortisol in humans and corticosterone in rodents (Kalman & Grahn, 2004). In addition, catecholamines such as epinephrine and norepinephrine are released from the adrenal medulla (McEwen, 2007).

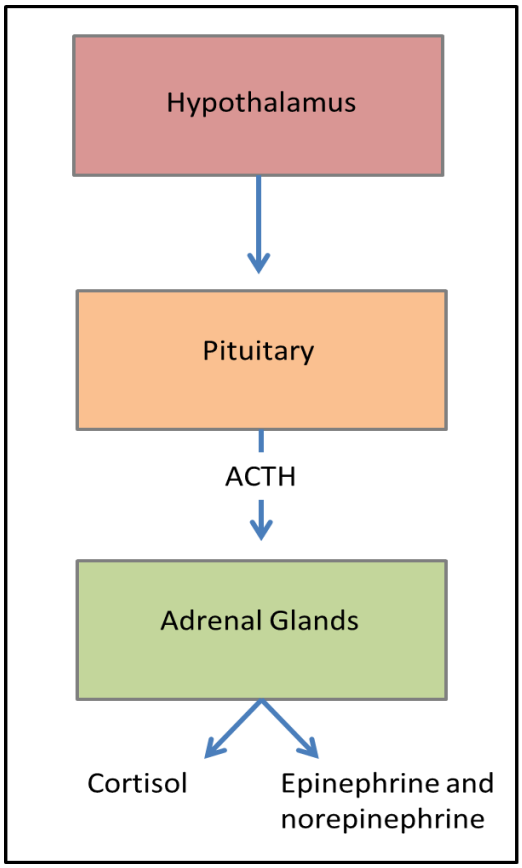

Figure 1. The HPA axis is activated in response to stress.
Exposure to stressors results in the release of hormones from the hypothalamus, pituitary gland, and adrenal glands, ultimately leading to the release of cortisol, epinephrine, and norepinephrine into the blood stream.

Stress Is Described With an Inverted U-Shaped Function

In the short term, elevated stress hormone levels are beneficial because they prepare the body for emergency situations by raising heart rate, blood pressure, and blood glucose levels while at the same time suppressing non-emergency functions such as digestion and urine formation. However, chronic elevations of stress hormones can have detrimental effects (McEwen, 2007). In addition to the cardiac problems associated with chronically elevated heart rate and blood pressure, adrenal steroids cause changes in structure and function of important brain regions that lead to impairment in emotion, cognition, memory processing, and behavior (McEwen, 2007, 2012; McEwen & Gianaros, 2010; Stein-Behrens, Mattson, Chang, Yeh, & Sapolsky, 1994).

Because of possible beneficial and detrimental outcomes, the effects of stress can be illustrated using the inverted U-shaped curve (Figure 2) where optimal performance occurs when stress levels are not too high, or too low.

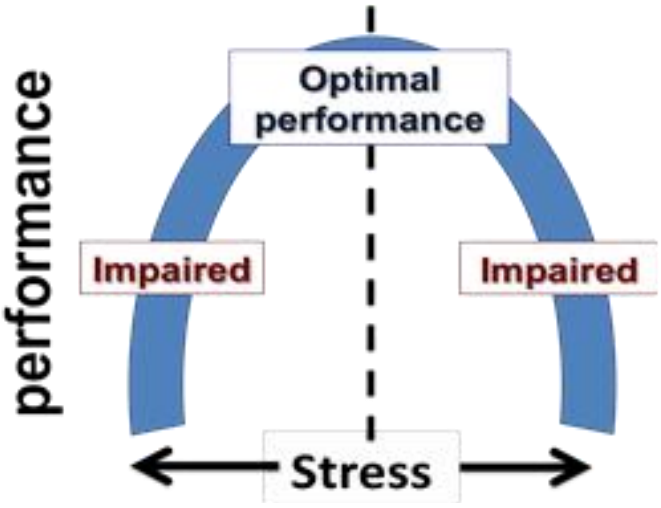

Figure 2. The inverted U-shaped curve illustrates the effects of stress.
The effects of stress can be illustrated using an inverted U-shaped curve, where optimal performance occurs when stress levels are not too high or too low.

Research in this field has focused on finding factors that may "delay the tipping point" from optimal performance to impaired performance due to high stress. One possible factor is examined in this thesis: the "stress inoculation hypothesis." This hypothesis suggests that after an organism is exposed to moderate and controllable stress, it can learn how to cope and be better prepared for future stress (Meichenbaum, 2007). Research presented here suggests that stress inoculation may cause a shift in the stress curve to the right (Figure 3). If the entire stress curve is shifted to the right, then optimal performance can be maintained at a higher stress level. The ability to avoid the detrimental effects of chronic stress on brain physiology behavior is called "stress resilience" (Russo, Murrough, Han, Charney, & Nestler, 2012).

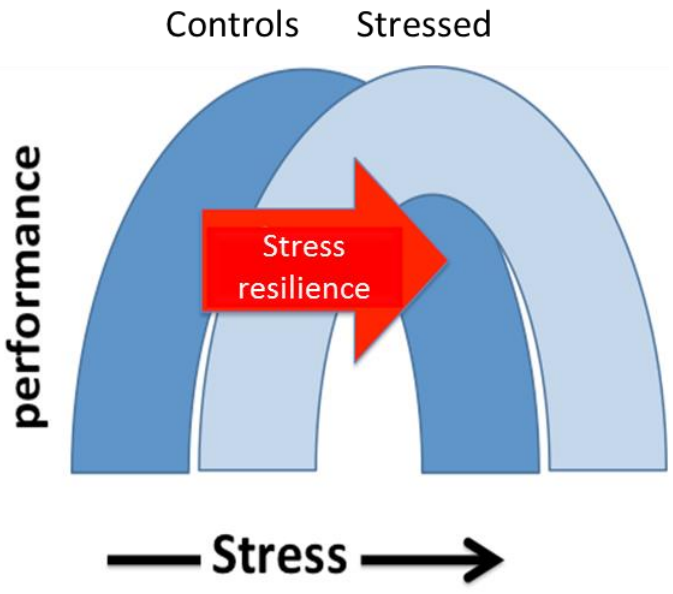

Figure 3. Stress resilience is illustrated as a rightward shift in the curve. Exposure to moderate, controllable stress results in stress resilience. Stress resilience is the ability to avoid the detrimental effects of chronic stress.

The goal of this thesis was to investigate the possible effects of stress inoculation on a specific stress-related mental condition: anxiety. Severe and/or chronic stressors have been shown to worsen anxiety, but recent research also suggests that stress inoculation with moderate stressors may reduce anxiety and anxiety-induced behavior (Jacobson & Anderson, 2013).

What Is Anxiety?

Anxiety occurs when an organism feels or anticipates a threat to its homeostasis. Anxiety is an emotional state (mood) often characterized by apprehension, uneasiness, and dreadness. Physical symptoms of anxiety include palpitations, breathlessness, a choking sensation, tightness in the chest, trembling, and flushing (Miller-Keane & O'Toole, 2005).

Anxiety can be felt in response to real dangers that threaten homeostasis such as war, or changes in the economy that threaten daily living. Anxiety can therefore be a part of the normal stress response to threatening circumstances. Anxiety can also be felt in response to everyday situations that may be mentally or emotionally stressful, such as speaking in front of others or trying to do well on an examination. In contrast, anxiety disorders are characterized by an irrational fear and dread of everyday situations that pose minimal or no real threat. Some people may experience chronic anxiety (e.g., generalized anxiety disorder, panic disorder) or anxiety in response to certain triggers (e.g., social anxiety disorder, post-traumatic stress disorder) (Miller-Keane & O'Toole, 2005).

Brain Regions Involved in Anxiety

Individuals with anxiety disorders display a disproportionally large amount of fear to only mildly threatening sensory stimuli. For example, the sight of a bumble bee or the sound of a dog barking may cause excessive fear and an intense desire to escape. Researchers have made use of this large fear response to study anxiety in animal models (e.g., rodents and primates) in order to discover what brain regions are involved. Pavlovian fear conditioning experiments are performed in which a conditioned stimulus (CS) is paired with an aversive unconditioned stimulus (US). Over time the animals develop a conditioned fear response (CR) to the CS that can be measured behaviorally. For example, fear in rats is measured by freezing and fleeing, both species-specific defense behaviors that may protect them from nearby predators (Bolles, 1970; Fanselow & DeOca, 1998). If the CS is repeatedly presented without the US, then the conditioned fear response will decrease over time or stop altogether. This is termed "fear extinction" (Bishop, 2007).

Two brain regions that may underlie anxiety have been identified using fear conditioning experiments:

Amygdala

The amygdala, an almond-shaped structure located in the temporal lobe, has been found to be important for the development, storage, and expression of fear memory. Information about the current environment enters the basolateral complex of the amygdala, where it is associated with memories about similar situations and stimuli. These associations can then evoke fear responses through connections with the central nucleus of the amygdala and the bed nuclei of the stria terminalis (BNST). The central

nucleus of the amygdala is believed to evoke many of the fear responses including risk assessment behaviors such as freezing and fleeing. The central nucleus is also believed to activate the HPA axis by decreasing inhibitory signals in the hypothalamus (LeDoux, 2000).

Ventromedial Prefrontal Cortex (vmPFC)

The ventromedial prefrontal cortex region helps to distinguish between relevant stimuli and irrelevant stimuli, such as one person's voice in a loud room. The vmPFC is connected to the amygdala, as well as other brain regions, and is thought to guide behavior and emotion based on important stimuli it has identified (Barbas & Zikopoulos, 2007; Price, 2005). The vmPFC activates GABAergic neurons in the basolateral amygdala, which inhibits the central output of the amygdala (Bishop, 2007). This region is also thought to be involved in the regulation of the HPA-axis (Kern et al., 2008) and fear extinction (Vertes, 2004).

Human brain imaging studies confirm involvement of these brain regions in anxiety. The amygdala is active during acquisition of the conditioned fear response, and the vmPFC is active during fear extinction (LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998). Another study reported that the PFC down-regulated amygdala activity during fear extinction (Delgado, Nearing, LeDoux, & Phelps, 2008).

Severe Stress May Cause the Imbalances That Lead to Anxiety

Exposure to severely stressful events such as physical/sexual assault, death of a family member, or natural disasters can predispose a person to developing mental illness. For instance, patients who have experienced assaultive violence during their childhood

are more likely to develop post-traumatic stress disorder (PTSD) after experiencing trauma in adulthood (Breslau, Chilcoat, Kessler, & Davis, 1999). Experiencing many stressful events early in one's life can also increase the chance of developing psychiatric disorders such as depression and substance abuse/dependence (Turner & Lloyd, 1995).

Similar findings are seen in animal studies. Animals that experience certain types of stress show negative changes in the neurocircuitry of anxiety, such as exaggerated fear recall and sensitization of the HPA-axis response (McGuire, Herman, Horn, Sallee, & Sah, 2010; Miracle, Brace, Huyck, Singler, & Wellman, 2006; Vyas, Mitra, Rao, & Chattarji, 2002). One week of chronic restraint stress in rats impairs the recall of fear extinction (Miracle et al., 2006). Rats undergoing chronic variable stress for 1 week had greater fear memory recall and impaired fear extinction (McGuire et al., 2010). The elevated plus maze (EPM) can be used to test for anxiety (Pellow, Chopin, File, & Briley, 1985). Ten days of immobilization stress led to decreased open-arm entries in the EPM, and also increased dendritic length and branching of pyramidal and stellate neurons within the basolateral amygdala (Vyas et al., 2002). These are thought to be excitatory projection neurons to the central nucleus (McDonald, 1982, 1992).

Moderate Stress Does Not Cause Imbalances That Lead to Anxiety

In contrast to the previously mentioned studies, exposures to some stress can lead to resilience against future stress rather than cause mental impairment. Many studies have been done on early life stress since the brain displays great plasticity during this developmental stage. Changes made in the brain during this time can cause lasting changes in behavior (Knudsen, 2004). One model used is maternal separation stress in the squirrel monkey, since mothers often leave their young while foraging for food. During

the initial separations in these studies, young squirrel monkeys made distress calls and had elevated levels of plasma cortisol. Over time, these effects diminished (Hennessy, 1986). When reaching adulthood these squirrel monkeys had lower plasma cortisol levels and were more willing to explore novel environments and novel objects than were monkeys that had been raised in undisturbed social groups (Lyons, Parker, Katz, & Schatzberg, 2009). Neuroimaging studies show that this form of early life stress causes an increase in vmPFC volume and myelination (Lyons, Afarian, Schatzberg, Sawyer-Glover, & Moseley, 2002).

According to these studies, some exposure to stress may help people learn how to mentally and physically deal with future stressors. This phenomenon will be referred to as "stress inoculation" (Meichenbaum, 2007).

Exposure to Unpredictable/Controllable Stress May Alleviate Anxiety: Preliminary Data in a New Animal Model

The studies cited above do not refute the large body of evidence that severe stress results in a hyper-responsive HPA-axis and mental/emotional disturbances (Seery, Holman, & Silver, 2010). Rather they suggest that certain factors may determine whether a stressor causes vulnerability to future stress or resilience to future stress. These factors include stressor type, frequency, duration, intensity, sensory modality, and developmental timing (Parker & Maestripieri, 2011). This thesis will focus on two additional factors: predictability and control.

Predictability is knowing ahead of time when and under what circumstances a stressful event will occur (Miller, 1981). If an organism can predict a stressor, then it can prepare for the encounter. The ability to predict stressors changes the outcome of the

stress. Rats exposed to predictable, chronic mild stress showed fewer depressive and anxiety behaviors and increased neurogenesis in the hippocampus compared to controls (Parihar, Hattiangady, Kuruba, Shuai, & Shetty, 2011).

Control is the ability to effectively escape or avoid harm (Folkman, 1984). Studies in humans (Glass, Reim, & Singer, 1971) and animals (Minor, Jackson, & Maier, 1984) show that those who felt little or no control were cognitively impaired by stress exposure while those who felt control were often not impaired. According to more recent studies, the vmPFC helps prevent the stress response when an animal believes it is in control (Amat, Paul, Zarza, Watkins, & Maier, 2006).

Collaborators in the research behind this thesis at Stony Brook University in Stony Brook, NY, have recently created an animal model of psychological stress that explores these two factors (Figure 4). They have confirmed that this model induces a stress response in the animals (Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2014), illustrated by deceased exploration of the stress environment and increased behaviors such as stretch-attend, rearing, head scanning, and freezing, all species-specific measures of awareness to threat (Blanchard, Griebel, & Blanchard, 2003). The specific type of stress used so far has been unpredictable but controllable stress (UST). This combination seems to facilitate development of coping behaviors toward future stress—following 21 days of this stress condition. UST rats spent more time than controls did burying rags that smelled of ferret dander, a natural predator (Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2014). Defensive burying is a typical rodent behavior that helps them cope with threatening stimuli (De Boer & Koolhaas, 2003). However, it seems that this facilitated coping came at a cost, as UST rats also showed impaired spatial working memory

compared to controls when tested in the Barnes maze under low stress conditions (Kim, Hudson, Molaro, Chorley, & Anderson, 2014).

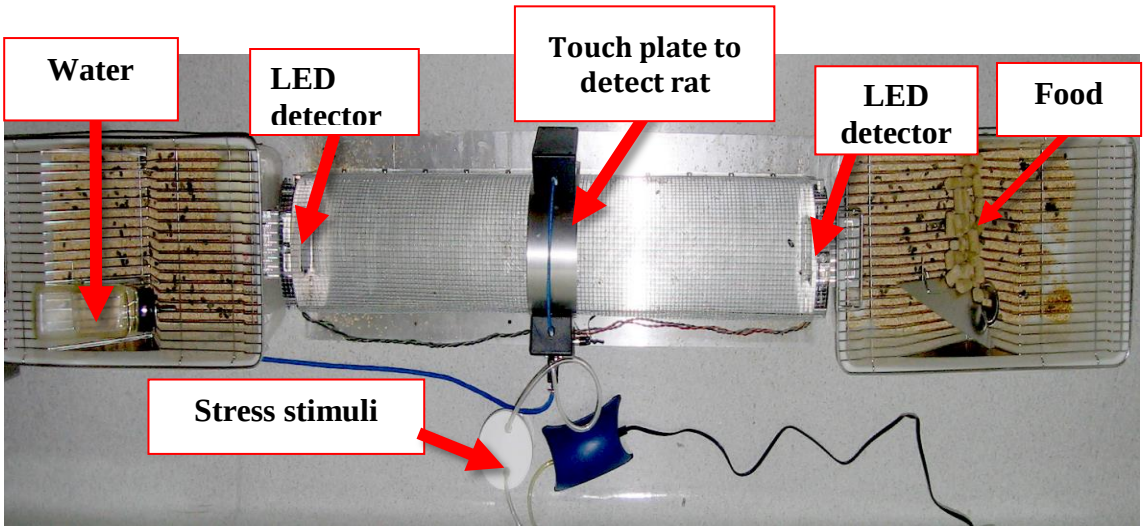

Figure 4. Rats were housed in a psychological stress-inducing platform.
This platform is designed to induce psychological stress in rats by exposing them to unpredictable/controllable stressors.

The **stress inoculation hypothesis** has been proposed to explain these findings (Jacobson & Anderson, 2013). According to this hypothesis, UST rats that experienced unpredictable/controllable stress were "inoculated." This inoculation caused neural and behavioral adaptations that aided the rats in coping with new environmental stressors. These adaptations could represent a rightward shift in the inverted u-shaped curve, helping the rats to maintain effective coping behaviors at higher levels of stress. This could explain why the UST rats exhibited more active coping behavior when exposed to a novel stressor (Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2014). At high stress, the UST rats are at the top of the curve and the controls are on the downward slope. This could also explain why the UST rats showed impaired spatial memory when tested under

low stress conditions (Kim, Hudson, Molaro, Chorley, & Anderson, 2014) (Figure 5). Under low or baseline stress levels, the UST rats are on a downward slope in the curve while the controls are at the top of the curve.

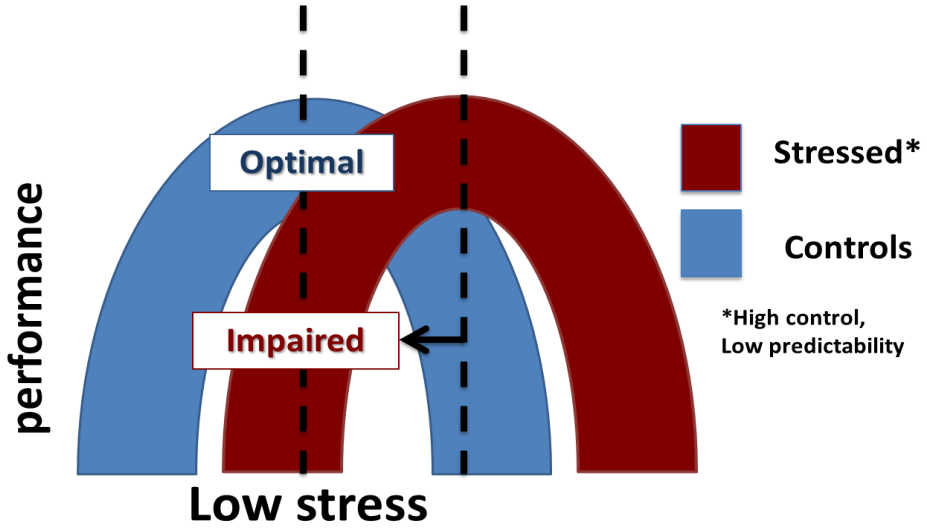

Figure 5. A rightward shift in the curve leads to impairment.
In previous studies, spatial memory was tested under low stress conditions. UST rats exhibited impaired spatial memory compared to controls. Hypothetically, the impairment was due to a rightward shift in the stress curve. However, when tested in the Barnes maze under low stress conditions, the UST rats were on the downward slope of their curve and thus showed impaired spatial memory compared to controls.

Thesis Goals and Hypotheses

The first goal of this thesis was to examine the effects of stress-inoculation on anxiety, a stress-related condition. The second goal was to determine if the stress-inoculation hypothesis explains the previously observed increased resilience, but impaired spatial memory in a low stress environment after exposure to unpredictable/controllable stress (Kim, Hudson, Molaro, Chorley, & Anderson, 2014). In order to address these goals, the animals were housed for the same amount of time (21

days) in the same housing conditions as the previous experiments. As in the previous study, rats were tested on the Barnes maze, but this time under high stress conditions. To address the first thesis goal, anxiety-related behaviors were measured on the maze, such as overall levels of mobility, emotional arousal, and risk assessment. If stress inoculation results in a rightward shift in the stress curve (Figure 6), it was hypothesized that rats previously exposed to 21 days of unpredictable/controllable stress (UST) would show the same or increased mobility compared to controls when tested under high stress conditions. Similarly, it was hypothesized that UST rats would exhibit increased vigilance and risk assessment behaviors, similar to what was seen in previous studies (Kim, Hudson, Molaro, Chorley, St. Louise, et al., 2014).

To address the second thesis goal, spatial memory was measured on the Barnes maze. If stress inoculation results in a rightward shift in the stress curve (Figure 6), it was hypothesized that rats previously exposed to 21 days of unpredictable/controllable stress (UST) would exhibit better spatial memory than the controls when tested under high stress conditions. "Inoculation" with this specific type of stress (unpredictable but also controllable) may help UST rats adapt to higher stress levels in the testing conditions. UST rats would be at optimal performance, but the control rats would be at impaired performance.

It was possible that individual differences in trait anxiety would affect the outcome of this study. Therefore, before housing the animals in the stressful or control environments, anxiety-related behavior was assessed in the elevated-plus maze (EPM) (Pellow et al., 1985). Pre-EPM behavior was analyzed for possible correlations with anxiety-related behavior while in the housing condition (average number of tunnel

traversals, a measure of general locomotor activity). Pre-EPM behavior was also analyzed for possible correlations with anxiety-related behavior (latency to visit first hole) and also spatial memory in the Barnes maze. It was hypothesized that trait anxiety would not affect anxiety-related behaviors or spatial memory, therefore no statistically significant correlations would be found.

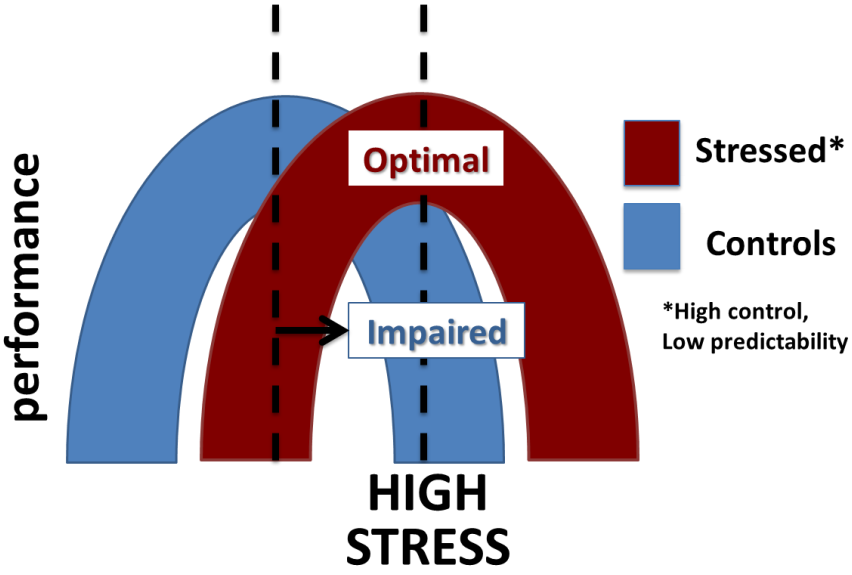

Figure 6. A rightward shift in the curve can lead to adaptive behaviors.
In this study, anxiety-related behaviors and spatial memory were tested under high stress conditions. It was hypothesized that under these higher stress levels, stressed rats would be near or at peak performance for both anxiety and spatial memory on their stress curve, which shifted to the right due to effects of stress inoculation. The control rats would be on the downward slope of their respective stress curve.

CHAPTER 2

METHODS

Subjects and Description of Housing Condition

The housing conditions were identical to those used previously (illustrated in Figure 4) (Kim, Hudson, Molaro, Chorley, & Anderson, 2014). Thirty-three male Sprague-Dawley rats were housed individually for 21 days in a platform (Figure 4), which was made of two cages connected by a 3-foot-long tunnel. Food was supplied in one cage and water in the other, therefore, the rat had to cross back and forth through the tunnel to access both. When traversing the tunnel, the stressed rats (UST, n=15) were exposed to stimuli designed to induce psychological stress (Kim, Hudson, Molaro, Chorley, & Anderson, 2014). The stimuli included a puff of air that smelled of ferret dander (Masini, Sauer, & Campeau, 2005), flashing lights, and a loud noise, presented simultaneously. The stressful stimuli were unpredictable because they occurred only on average in 1 out of 4 tunnel crossings. However, the stress was controllable because the rats always reached the other side safely (there was no real danger), and the rats were always successful at obtaining food and water. The control group (CT, n=18) was housed in the same platform but without the stressful stimuli and in a separate room from the stressed rats. The rats were housed in these conditions for 21 days, which, according to previous studies, is enough time for structural and functional changes to occur in the brain due to stress (Cook & Wellman, 2004; Watanabe, Gould, & McEwen, 1992).

Connected to the tunnel in the platform (Figure 4) was a computer that controlled the delivery of the stressful stimuli. Infrared LED lights at either end of the tunnel were used to track the rats' movement. A break in the LED light at one end and then the other was recorded as a tunnel traversal. After 21 days in the housing conditions, the rats were removed and housed individually in tub cages in a new room.

Spatial Memory and Risk Assessment in the Barnes Maze

Testing on the Barnes maze (Barnes, 1979) began the day after the end of the stress manipulation. The testing took place during the dark cycle for the rats. The procedures and maze were the same as those done previously (Coburn-Litvak, Pothakos, Tata, McCloskey, & Anderson, 2003). The maze (see Figure 7) consisted of a round, white, 1.22m table with 12 holes (10cm in diameter) evenly spaced around the edge. One hole had an escape chamber (which will be referred to as the "goal box") underneath for the rat. Placed around the room were visuo-spatial cues (e.g., a file cabinet, storage cupboard, poster) to help the rat navigate. The previous experiments were done under low or normal stress levels, but aversive conditions were used to test memory and assess anxiety-related behavior in this current study. Aversive conditions for the maze included loud noises, bright light, and a fan placed above and blowing directly on the maze.

Habituation to the maze was done on the first day, and then testing was done over the next 4 days. On the first day, each rat was placed in the goal box for 2 minutes, and then placed back in the cage. The rat was then placed on the maze next to the hole just above the goal box and encouraged to go in where it remained for 2 minutes. Then it was returned to the cage again. Lastly, the rat was placed in the center of the maze and a

walled, rectangular path led to the hole above the goal box. Once the rat entered the goal box, it was allowed to stay for 2 minutes before it was put back in its cage.

Figure 7. The Barnes maze was used to assess spatial memory and anxiety-related behaviors following 21 days of housing in stress or control conditions. Barnes maze testing was done after the 21-day housing in stress or control conditions.

The following 4 days were for memory testing and assessing behavior: the first 2 days with one, randomly chosen, goal box position and the second 2 days with a different goal box position (at least four holes away from the first position). For each trial on the maze, the rats were placed in a start box in the middle of the table in random orientation to the goal box. After 30 seconds, the box was lifted, and the rats had 3 minutes to find the goal box. If the rats did not find the goal box, they were placed next to the hole above the goal box and allowed to crawl in where they remained for 2 minutes before being returned to the cage. Between each rat, the surface of the maze and the inside of the goal box were cleaned with a 30% alcohol solution to prevent the next rat from using odor cues. Two or three investigators, blind to the treatment conditions, were in the room

recording the behavior of the rats. Video recordings were also taken for later analysis of behavior and memory. Four trials per rat were run each day.

The following measurements of spatial memory were recorded: (a) the time the rat takes to find and enter the goal box (latency), (b) the number of wrong holes visited before finding the goal box (errors). Errors were defined as at least one head dip into any hole of the maze except the one above the goal box with the nose dipping below the top edge of the hole. It should be noted that these data were collected and analyzed by me and another student who also used the data in her thesis. The spatial memory data are used as background information for the anxiety data, which is the focus of this thesis.

The following anxiety-related measurements were recorded: (a) mobility (number of holes visited per minute) and latency to approach the first hole (both of which assess overall locomotor activity), (b) emotional arousal (number of fecal boli produced on the maze per trial), and (c) risk assessment behaviors (jumping over holes and looking over the edge of the maze).

Pre-Elevated-Plus Maze Testing

Prior to the 21-day housing of the animals in the stress or control conditions (illustrated in Figure 4), anxiety-related behaviors were measured in the elevated-plus maze (EPM; illustrated in Figure 8). The maze consisted of two open arms and two closed arms (each extending 27 inches from the center) that were elevated 26.25 inches off the floor. Early validation studies indicate that anti-anxiety drugs increase open-arm activity, and anxiogenics such as ACTH and picrotoxin decrease open-arm activity (Handley & Mithani, 1984; Pellow et al., 1985). Therefore, an increase in open-arm activity (number of entries and time spent in arms) indicates low anxiety, while an

increase in closed-arm activity (number of entries and time spent in arms) indicates higher anxiety.

Figure 8. The elevated-plus maze was used to detect trait anxiety.
Rats were tested on the elevated-plus maze prior to 21 days in the stress or control housing.

Rats were habituated to the EPM room for 30 minutes before starting the test. Each rat had one trial on the maze. The rat was placed in the center of the maze facing the open arm opposite the experimenter. It was allowed to explore the EPM for 5 minutes, and then was returned to its cage. The maze was cleaned with a 30% alcohol solution between each rat. This was to prevent the next rat from using odor cues while exploring the maze. Each test was recorded for later scoring by experimenters—a professor and myself—who were blind to the conditions (UST or CT) of the rats.

The following anxiety-related behaviors were measured:

1. Arm entries into open arms (criterion for an arm entry = all 4 paws in the arm)
2. Arm entries into closed arms

3. Total number of arm entries (an index of overall locomotor activity)
4. Time spent in open arms
5. Time spent in closed arms
6. Time spent in center
7. Ratio time: Total time spent in both open arms divided by time spent in any arm of maze (e.g., time in open arms in seconds / total time on maze – time spent in center in seconds)
8. Ratio entry: Total entries into open arms of maze divided by total entries into any arm of maze
9. Composite anxiety score: 1 - (ratio time + ratio entry)/2. (Scores range from 0,which indicates low anxiety, to 1, which indicates high anxiety. This provides a single value for anxiety that can be used in the correlation statistics.)

Statistical Analysis

Comparisons between the control group and the stress group of rats were done using SPSS statistics software (v. 21.0.0, IBM Corporation, New York, NY). Latency and errors from the Barnes maze were analyzed with a repeated measures ANOVA with group as the independent factor and trials as the within-subjects factor. A one-way ANOVA was used to analyze errors and latency on individual trials. Anxiety-related measurements were analyzed with a repeated measures ANOVA with group as the independent factor and trials as the within-subjects factor. A one-way ANOVA was used to analyze anxiety-related measures on individual trials. An alpha level of 0.05 was used. The degrees of freedom were adjusted by the Greenhouse-Geisser estimate of epsilon when the assumption of sphericity was violated. Sphericity occurs when the variances of

the differences between all possible pairs of related groups are equal. The Greenhouse-Geisser correction is used if the assumption of sphericity is violated to ensure the results are still valid (Lund & Lund, 2013). In the results section, the ε value was reported when the sphericity assumption was violated, but the unadjusted degrees of freedom was reported for simplicity.

In order to assess for the effect of trait anxiety, anxiety-related behaviors were measured in the elevated-plus maze (EPM; illustrated in Figure 8) prior to the 21-day housing of the animals in the stress or control conditions (illustrated in Figure 4). One rat was removed from the CT group because the composite anxiety score was felt to be an inaccurate representation of its behavior in the maze: the rat entered an open arm of the maze at the beginning of testing, but then sat in one spot near the center for the remainder of the test. This resulted in a low composite anxiety score, but the rat did not exhibit willingness to explore the maze. One rat was also removed from the UST group because of poor video quality, therefore, the sample size for the EPM for the two groups was CT=17 and UST=14. From the behavioral measures (time spent in open arm and entries in open arms) a composite anxiety score was calculated, with a high score indicating high anxiety and a low score indicating low anxiety. Pearson correlations were then calculated to reveal relationships between this composite anxiety score and behavioral measures in the housing conditions and on the Barnes maze. Pearson correlations are reported after running the statistical analysis on both groups (CT and UST) together and also after running the statistical analysis separately on each group. The analysis was done this way to find out if one or both groups were affecting the results. The tables included in this

thesis report the Pearson *r* values, and *p* values are reported only when the correlations were significant or at least were a statistical trend.

To reveal relationships between trait anxiety and behaviors in the housing condition, Pearson correlations were run between the composite anxiety score in the EPM and average tunnel traversals over the 21 days in the housing conditions.

To reveal relationships between trait anxiety and spatial memory in the Barnes maze, Pearson correlations were run between the composite anxiety score and the number of errors and the latency to the goal box in the Barnes maze. To reveal relationships between trait anxiety and mobility on the Barnes maze, Pearson correlations were run between the composite anxiety score from the EPM and the number of holes visited per minute on the Barnes maze. This statistical analysis was also run at two separate time points in the Barnes maze. Pearson correlations were run on mobility and spatial memory measures from trial 1 on the Barnes maze, since it was the rats' first exposure to the maze under high stress conditions. Pearson correlations were also run against measures from a later trial, chosen after a repeated-measures analysis was run. A trial was chosen where a group difference was seen. The correlation was run to determine if the significance seen was due to trait anxiety. Pearson correlations were run between composite anxiety scores in the EPM and spatial memory measures in trial 7 (which were significantly different between groups). Pearson correlations were run between composite anxiety scores in the EPM and mobility measures in trial 8 (which was significantly different between groups). Both trials 7 and 8 occurred on Day 2; therefore, to avoid inaccurate results based on one trial, Pearson correlations were run between averages for

Day 2 measures on the Barnes maze (trials 5-8) for errors, latency to goal, and mobility and the composite anxiety score in the EPM.

CHAPTER 3

RESULTS

UST Rats Had Better Spatial Memory Than Controls When Tested Under High Stress Conditions

These data will appear in another student's thesis and are reported here as background information for the anxiety data. Memory assessments on the Barnes maze included (a) the time the rat took to find and enter the goal box (latency), and (b) the number of wrong holes visited before finding the goal box (errors). Trial 1 is analyzed separately since it was the rats' first exposure to the maze under high stress conditions, and would not be an accurate measure of spatial memory. Trial 1 was also analyzed separately in order to keep the data analysis consistent with previous studies by collaborators at Stony Brook University. For latency to reach the goal box on the first trial, there was no significant group effect ($F(1,31)=2.004$, $p=0.167$) (Figure 9 A). On the remaining trials, there was a significant group effect with UST rats taking less time overall to enter the goal box ($F(1,31)=5.452$, $p<0.05$). There was also a significant trial effect, with a decrease in latency to the goal box for both groups across trials 2-16 ($F(14,434)=6.065$, $p<0.001$, $\varepsilon=0.414$). There was no interaction ($F(14, 434)= 0.766$, $p=0.593$, $\varepsilon=0.414$). For the number of errors on the first trial, there was no significant group effect ($F(1,31)=1.494$, $p=0.231$) (Figure 9 B). On the remaining trials, there was a significant group effect ($F(1,31)=7.767$, $p<0.05$): UST rats made significantly fewer errors than did CT rats. There was also a significant trial effect, as both groups made

fewer errors over the trials 2-16 ($F(14, 434)=3.806$, $p<0.001$, $\varepsilon=0.501$). There was no interaction ($F(14, 434)=0.358$, $p=0.923$, $\varepsilon=0.501$).

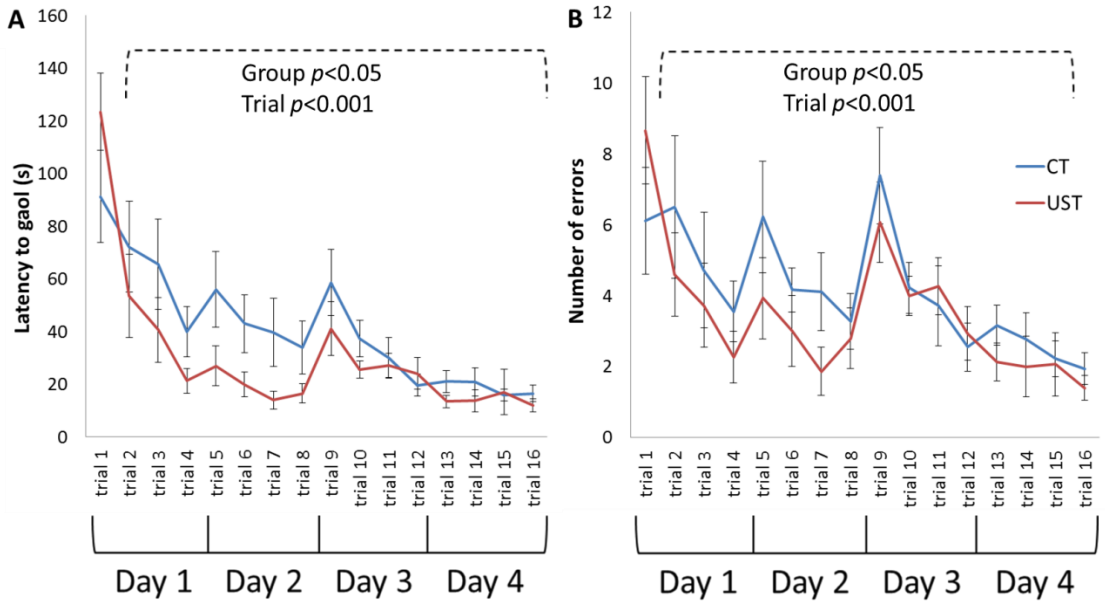

Figure 9. UST rats had better spatial memory than did controls.
(A) UST rats made significantly fewer errors than did CT rats on trials 2-16. (B) UST rats took significantly less time to enter the goal box than did CT rats in trials 2-16.

UST Rats Had Better Mobility Than Controls When Tested Under High Stress Conditions

Two measures of mobility were recorded for each trial on the Barnes maze: latency to visit the first hole, and number of holes visited per minute (mobility). Trial 1 was analyzed separately to assess the rats' behavior on the first exposure to the maze under high stress conditions, and to remain consistent with the data analysis done on spatial memory. On the first trial, mobility was not significantly different between groups ($F(1,31)=0.379$,$p=0.543$) (Figure 10 A). Mobility also was not significantly different between groups on the remaining 15 trials ($F(1,31)=2.753$, $p=0.107$). There was a

significant trial effect, as both groups showed increased mobility over trials 2-16 ($F(14, 434)=7.730$, $p<0.001$, $\varepsilon=0.578$). There was no interaction ($F(14, 434)=1.057$, $p=0.394$, $\varepsilon=0.578$). On the first trial on the Barnes maze, there was no significant difference between groups in the time it took for the rats to approach the first hole ($F(1,20)=2.572$, $p=0.124$) (Figure 10 B). On the remaining 15 trials, there was a significant group effect, as the UST rats took significantly less time than did controls to approach the first hole ($F(1,20)=5.465$, $p<0.05$). There was a significant trial effect, as both groups showed decreased latency over trials 2-16 ($F(14, 280)=4.857$, $p<0.001$). There was no interaction ($F(14, 280)=0.569$, $p=0.888$).

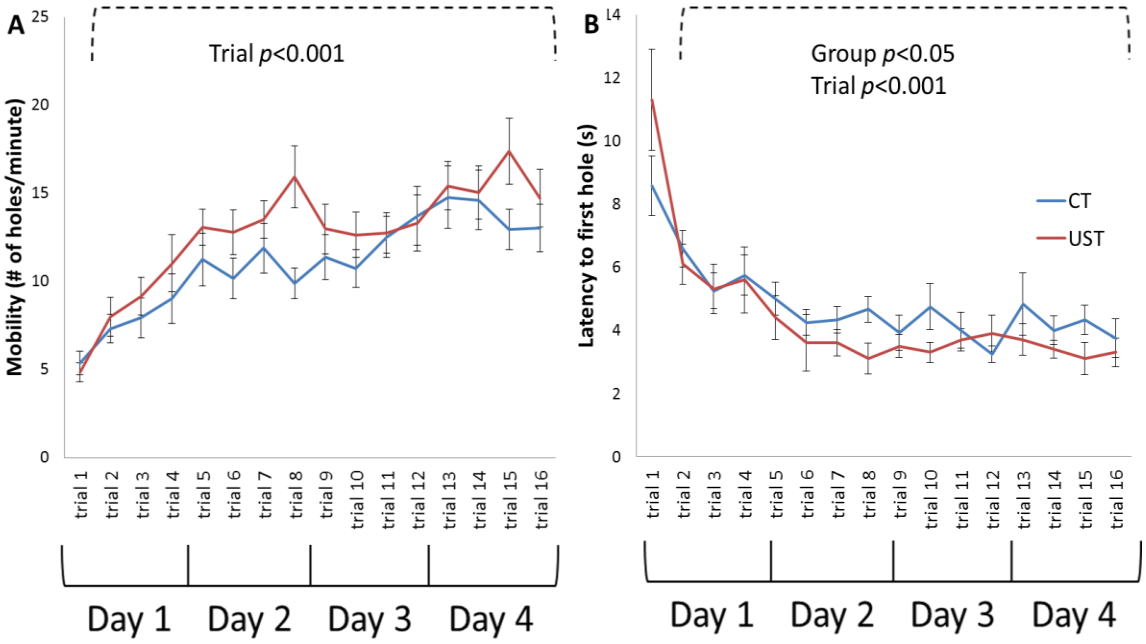

Figure 10. UST rats had shorter latencies to the first hole than did CT rats.
(A) The UST rats did not differ from CT rats in mobility on the Barnes maze. (B) The UST rats took significantly less time to approach the first hole than CT rats did in trials 2-16.

UST Rats Were Not Different From Controls on Arousal and Risk Assessment When Tested Under High Stress Conditions

Two other assessments on the Barnes maze included the number of fecal boli produced, an indirect measure of emotional arousal, and risk assessment behaviors including jumping over holes and looking over the edge of the maze. Trial 1 was analyzed separately to assess the rats' behavior on the first exposure to the maze under high stress conditions, and to remain consistent with the data analysis done on spatial memory. For emotional arousal, there was no significant difference between groups on trial 1 ($F(1,31)=0.159$, $p=0.692$). There was also no significant group effect over trials 2-16 ($F(1,31)=0.001$, $p=0.982$) (Figure 11). There was a significant trial effect across the 15 trials ($F(14,434)=5.012$, $p<0.001$, $\varepsilon=0.450$). Both UST and CT rats exhibit the same pattern of relatively high emotional arousal on the first trial, with a gradual decline by the last trial of the testing day. There was also a decline in emotional arousal overall, so that both UST and CT rats produced fewer fecal boli on testing Day 4 vs. Day 1. There was no interaction ($F(14,434)=0.271$, $p=0.955$, $\varepsilon=0.450$).

Risk assessment behaviors were later measured from videos of the Barnes maze testing, but the first cohort of rats was not video recorded. Therefore, the sample sizes were UST $n=10$ and CT $n=12$. For the number of holes jumped over in trial 1, there was no significant group effect ($F(1,20)=0.783$, $p=0.387$) (Figure 12 A). There also was no significant group effect across trials 2-16 ($F(1,20)=0.928$, $p=0.347$). There was no significant trial effect in the number of holes jumped over across trials 2-16 ($F(14,280)=1.435$ $p=0.227$, $\varepsilon=0.304$). There was no interaction ($F(14,280)=0.599$, $p=0.675$, $\varepsilon=0.304$). The UST rats looked over the edge significantly more times than did the control rats on trial 1 ($F(1,20)=6.076$, $p<0.05$) (Figure 12 B). However, there was no

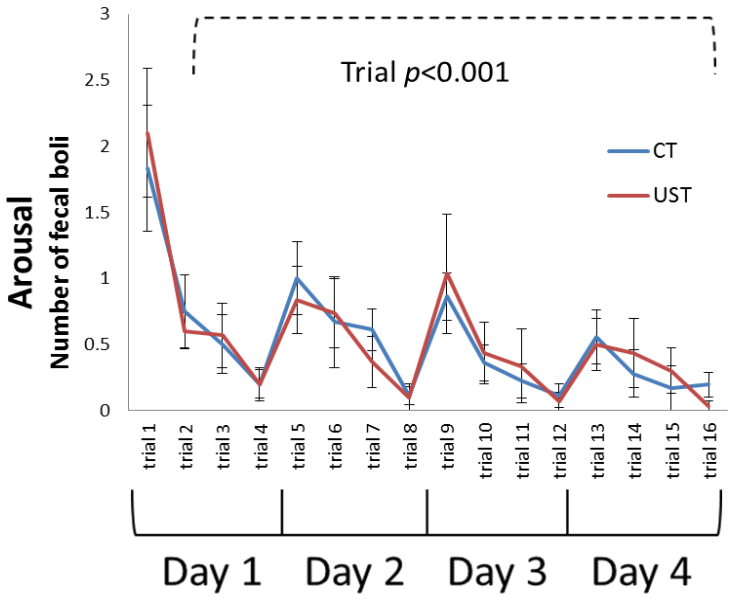

Figure 11. Emotional arousal was not different between UST and CT rats.
Arousal was not different between groups, but it decreased over trials 2-16. Both UST and CT rats produced fewer fecal boli on testing Day 4 vs. Day 1.

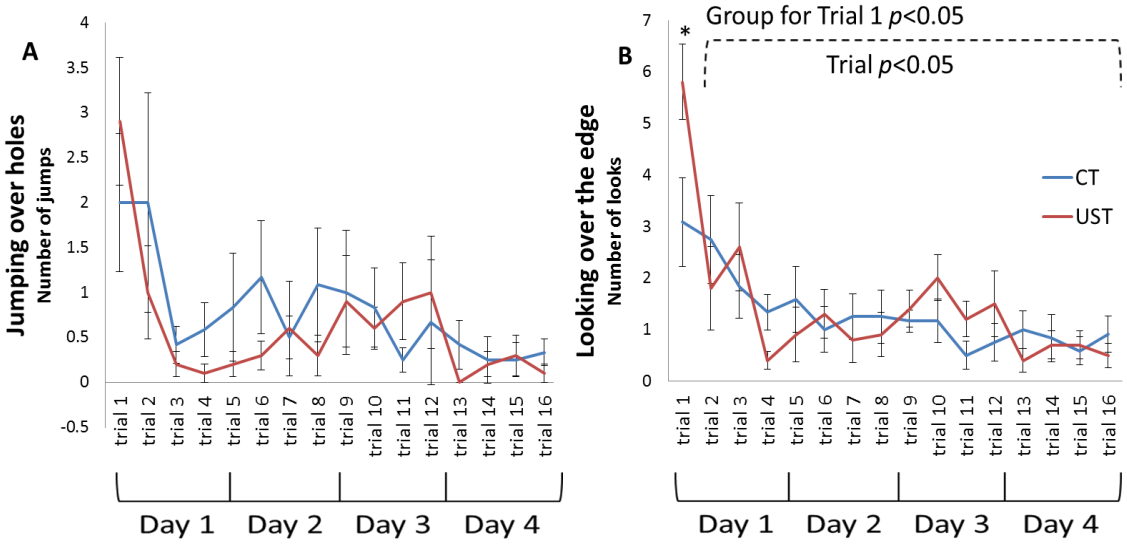

Figure 12. Risk assessment behaviors were similar between UST and CT rats.
(A) Jumping over holes did not differ between groups. (B) UST rats looked over the edge more often than did the CT rats on trial 1, but were similar to CT rats on the remaining trials.

significant group effect between UST and control rats in the number of times the rats looked over the edge across trials 2-16 ($F(1,20)=0.084$, $p=0.775$). There was a significant trial effect—both groups showed a decrease in the number of looks over the edge over trials 2-16 ($F(14,280)=2.566$, $p<0.05$, $\varepsilon=0.383$). There was no interaction ($F(14,280)=0.989$, $p=0.431$, $\varepsilon=0.383$).

No Significant Correlations Were Found Between EPM Anxiety Score and Average Tunnel Traversals in the Housing Condition

No significant correlation was seen between the average number of tunnel traversals made by the UST and CT rats during the 21 days in the housing platform and the EPM composite anxiety score ($r(29)= 0.311$, $p=0.088$) (Figure 13, Table 1), although it is a statistical trend (p between 0.05-0.10). Both groups have composite anxiety scores ranging from 0.2 to 1.0. When correlations were run separately for each group, no correlation was seen for either the CT group ($r(15)=0.400$, $p=0.112$) or UST group ($r(12)=0.346$, $p=0.225$) alone.

Table 1

Pearson Correlations for Composite Anxiety Score and Average Tunnel traversals

	CT[*] and UST[**] animals	**CT alone**	**UST alone**
r	.311 p=.088	.400	.346

[*]CT = 17 (one animal removed due to composite anxiety score of 0).
[**]UST = 14 (one animal removed due to poor video quality).

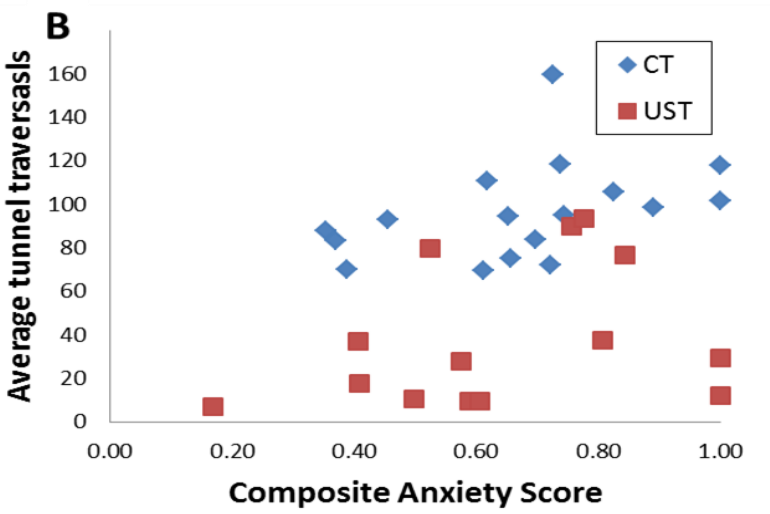

Figure 13. Tunnel traversals do not correlate to composite anxiety scores in the EPM. A statistical trend was seen (p=0.088) between tunnel traversals and the composite anxiety score when the analysis was run for both UST and CT groups together.

No Correlations Were Found Between EPM Anxiety Score and Spatial Memory in the Barnes Maze

No correlation was seen between composite anxiety scores in the EPM and spatial memory by both UST and CT rats, as measured by latency to find the goal, in trial 1 on the Barnes maze (r(29)= -0.006, p=0.973) (Figure 14 A, Table 2), or errors to find the goal box in trial 1 (r(29)=0.013, p=0.944) (Figure 14 B, Table 2). When correlations were run separately for each group, no correlation was seen for either the CT group (r(15)= -0.098, p=0.709) or UST group (r(12)=0.166, p=0.571) alone for latency to find the goal. No correlation was seen for either the CT group (r(15)=-0.119, p=0.649) or UST group (r(12)=0.189, p=0.517) alone for errors.

Table 2

Pearson Correlations for Composite Anxiety Score and Spatial Memory in Barnes Maze on Trial 1

	CT* and UST** animals	CT alone	UST alone
r for Errors	.013	-.119	.189
r for Latency	-.006	-.098	.166

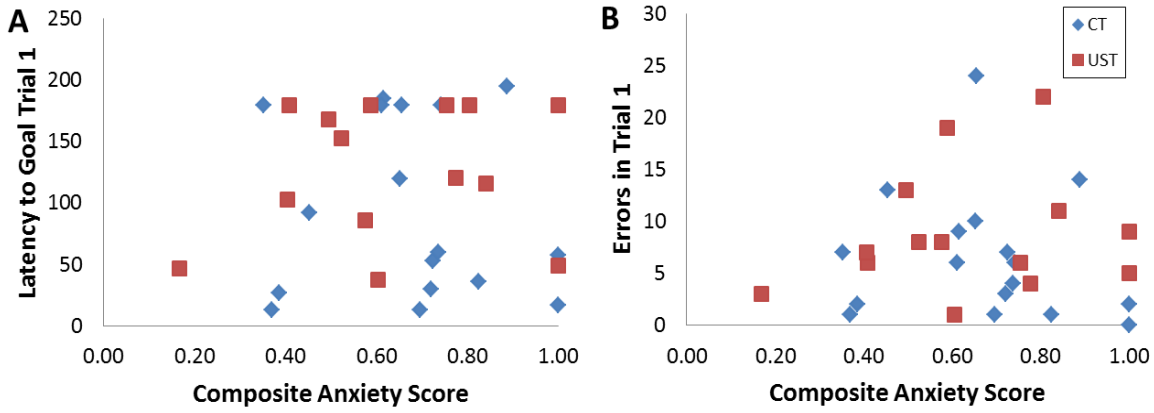

Figure 14. Latency and errors in trial 1 of the Barnes maze were compared to EPM data. (A) No correlation was seen between latency to goal in trial 1 on the Barnes maze and the composite anxiety score from the EPM (p=0.973). (B) No correlation was seen between errors in trial 1 and the composite anxiety score (p=0.944).

No correlation was seen between composite anxiety scores in the EPM and spatial memory for both UST and CT rats, as measured by latency to find the goal, in trial 7 on the Barnes maze ($r(29)$=-0.162 , p=0.384) (Figure 15 A, Table 3), or errors to find the goal box in trial 7 ($r(29)$= -0.288, p=0. 116) (Figure 15 B, Table 3). When correlations were run separately for each group, no correlation was seen for either the CT group ($r(15)$=-0.237, p=0.359) or UST group ($r(12)$=-0.177, p=0.545) alone for latency to find the goal box in trial 7. No correlation was seen for the UST group ($r(12)$=-0.124,

p=0.674) alone for errors on trial 7. However, there was a statistical trend in the CT group between the composite anxiety score and errors in trial 7 (*r*(15)=-0.462, *p*=0.062).

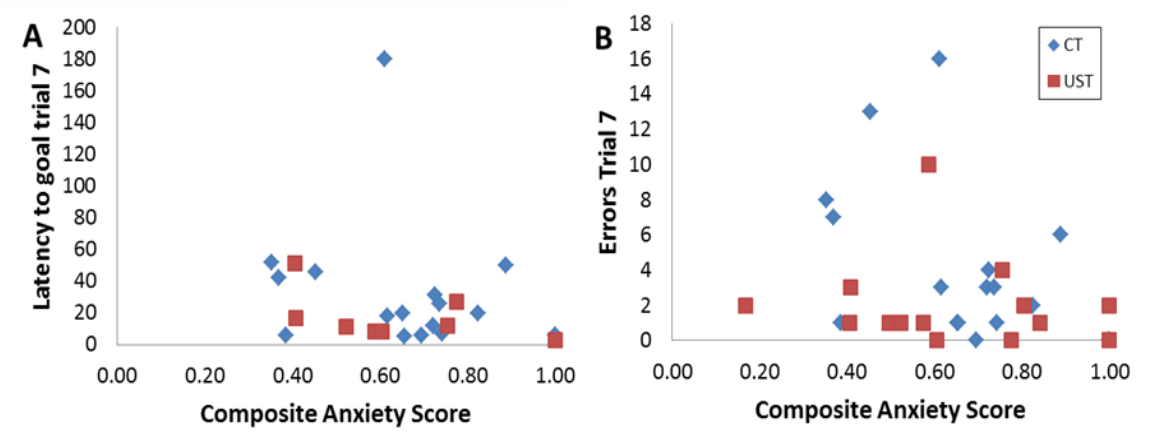

Figure 15. Latency and errors in trial 7 of the Barnes maze were compared to EPM data. (A) No correlation was seen between latency to goal in trial 7 on the Barnes maze and the composite anxiety score from the EPM (*p*=0.384). (B) No correlation was seen between errors in trial 7 and the composite anxiety score (*p*=0.116). A statistical trend was seen for errors in trial 7 for the CT group alone.

Table 3

Pearson Correlations for Composite Anxiety Score and Spatial Memory in Barnes Maze on Trial 7

	CT* and ST animals*	**CT alone**	**ST alone**
r for Errors	-.288	-.462, *p*=.062	-.124
r for Latency	-.162	-.237	-.177

Pearson correlations were also run between the composite anxiety score from the EPM and the average spatial memory measures from all four trials on day 2. No correlation was found for the average number of errors on Day 2 for the UST group and the composite anxiety score (*r*(29)= -0.240, *p*=0. 193) (Table 4). However, a statistical

trend was seen between the average number of errors on Day 2 and the composite anxiety score for the CT group ($r(15)=-0.443$, $p=0.075$), therefore, the same pattern of results is seen when trial 7 is analyzed alone or when all four trials on day 2 are averaged.

Table 4

Pearson Correlations for Composite Anxiety Score and Spatial Memory in Barnes Maze on Day 2 (average of trials 5-8)

	CT* and ST animals*	**CT alone**	**ST alone**
r for Errors	-.250	-.443, *p*=.075	-.147
r for Latency	-.096	-.156	-.127

No Correlations Were Found Between Trait Anxiety in the EPM and Mobility in the Barnes Maze

No correlations were found between composite anxiety scores for both UST and CT rats and mobility in trial 1 ($r(29)=-0.113$, $p=0.544$) (Figure 16 A, Table 5). No correlation was seen for either the CT group ($r(15)=-0.323$, $p=0.206$) or UST group ($r(12)=0.142$, $p=0.628$) alone for trial 1.

Table 5

Pearson Correlations for Composite Anxiety Score and Mobility in Barnes Maze on Trial 1

	CT* and UST animals**	**CT alone**	**UST alone**
r	-.113	-.323	.142

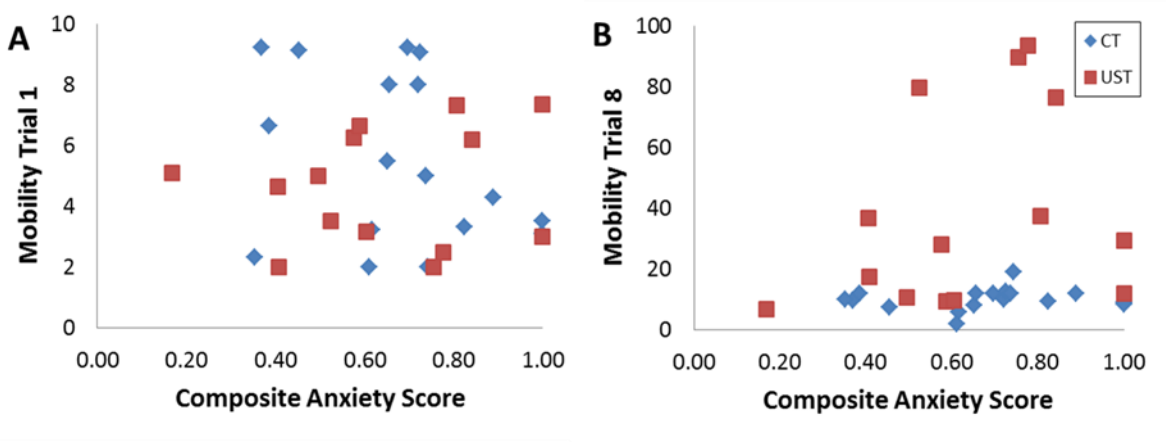

Figure 16. Mobility in trial 1 and trial 7 of the Barnes maze was compared to EPM data. (A) No correlation was seen between composite anxiety scores and mobility in trial 1 (p=0.544). (B) No correlation was seen between composite anxiety scores and mobility in trial 8 (p=0.884).

No correlation was found between composite anxiety scores for both UST and CT rats and mobility in trial 8 ($r(29)$=0.884, p=0.884) (Figure 16 B, Table 6). No correlation was seen for either the CT group ($r(15)$=0.110, p=0.674) or UST group ($r(12)$=-0.037, p=0.901) alone for trial 8.

Table 6

Pearson Correlations for Composite Anxiety Score and Mobility in Barnes Maze on Trial 8

	CT* and UST** animals	CT alone	UST alone
r	-.028	.110	-.037

Pearson correlations were also run between composite anxiety scores from the EPM and the average spatial memory measures from all four trials on Day 2. No correlation was found between composite anxiety scores and average mobility across all

trials on Day 2 ($r(29)=-0.142$, $p=0.446$) (Table 7). No correlation was seen for either the CT group ($r(15)=-0.244$, $p=0.345$) or UST group ($r(12)=0.033$, $p=0.911$) alone for mobility on Day 2. Therefore, the same pattern is seen whether trial 8 is analyzed separately or when the average of all trials on Day 2 is analyzed.

Table 7

Pearson Correlations for Composite Anxiety Score and Mobility in Barnes Maze on Day 2 (average of trials 5-8)

	CT* and UST animals**	**CT alone**	**UST alone**
r	-.125	-.224	.033

CHAPTER 4

DISCUSSION

The Value of Animal Models to Study Stress

An animal rather than human model was chosen for this study for multiple reasons. First, stress environments can be closely controlled and manipulated in a lab setting. Second, the animals can be kept in the stress-inducing environment for an extensive amount of time, which is not practical or ethical in human studies. Third, animals are living creatures that provide a glimpse into the neurocircuitry and neurobiology that underlie stress.

Previous Unpredictable/Controllable Stress Study

A previous study by collaborators at Stony Brook University used the unpredictable/controllable stress platform (illustrated in Figure 4) for 21 days and then assessed spatial memory on the Barnes maze. This previous study was conducted under low stress conditions (i.e., no fan and loud noise were used). Under low stress, UST rats showed impaired spatial memory compared with controls (Kim, Hudson, Molaro, Chorley, & Anderson, 2014). The stress inoculation hypothesis was suggested to explain these results (Jacobson & Anderson, 2013). The impaired spatial memory of the UST rats was thought to be due to a rightward shift in the inverted U-shaped curve (Figure 5). Under low stress, the UST rats were on the downward slope of their curve where

performance is impaired, compared to CT rats who might have been at the peak of the curve and thus were able to perform optimally.

UST Rats Had Better Spatial Memory Than CT Rats Under High Stress Conditions

The goal of this thesis was to confirm the stress inoculation hypothesis and examine the effects of exposure to stress on anxiety. The same methods from the previous study by Kim, Hudson, Molaro, Chorley, St. Louise, et al. (2014) were used, but aversive conditions were added to the Barnes maze.

The rats that were exposed to unpredictable/controllable stress for 21 days took less time to enter the goal box and made fewer errors on the Barnes maze. These results are consistent with the stress inoculation hypothesis. According to the stress inoculation hypothesis, after exposure to unpredictable/controllable stress, the rats experienced a shift in the inverted U-shaped curve (Figure 6). They performed better under stressful conditions on the Barnes maze than the control rats did because this matched the high stress conditions of the previous living conditions.

UST Rats Exhibited No More Anxiety Than CT Rats Under High Stress Conditions

In addition to spatial memory, anxiety-related behaviors were affected by the unpredictable/controllable stress. Mobility was not different between groups, but mobility decreased over the 16 trials. This may indicate that both the UST and CT rats were getting more comfortable with the maze, and were more willing to explore by Day 4. Time to approach the first hole was significantly lower in the UST group. The rats may have been less timid and more willing to explore the maze. According to the stress

inoculation hypothesis, exposure to stress caused a rightward shift in the inverted U-shaped curve (Figure 6). The UST rats then experienced relatively less stress when exposed to the highly arousing testing conditions, when compared with controls. This could explain why the UST rats took less time to approach the first hole.

No clear differences were seen in basic responses to the maze as the stress inoculation hypothesis predicts; although emotional arousal measures varied across trials, they did not vary across groups. Both UST and CT rats appear to have the most emotional arousal on the first trial of each day.

Risk assessment behaviors were similar between UST and CT rats, except for looking over the edge in trial 1. The UST rats looked over the edge more than did CT rats on one trial, but were similar on all other trials. UST rats may have handled the high stress conditions better than CT rats on the first exposure to the Barnes maze, but both groups appear to have experienced decreased anxiety by Day 4, as indicated by the lower number of jumps over holes and looks over the edge.

Trait Anxiety Were Analyzed Between UST and CT Rats in the Elevated-Plus Maze

The EPM was used to test for individual differences in the rats' anxiety-related behavior prior to any stress manipulations. The EPM is often used to test for anxiety-related behaviors after drug application (Cortese & Phan, 2005; Handley & Mithani, 1984; Pellow et al., 1985), but can be used to screen for trait anxiety prior to experimentation (Landgraf & Wigger, 2002).

No Significant Correlations Were Found Between Trait Anxiety in the EPM and Average Tunnel Traversals in the Housing Condition

A statistical trend (*p* between 0.05-0.10) was seen between the EPM composite anxiety score and average tunnel traversals over 21 days in the housing condition (Figure 13; p=0.088), such that higher composite anxiety scores were associated with higher tunnel traversals. This positive pattern was seen when both groups were analyzed separately as well, although it was not close to significant in either group (for UST, *p*=.225; for CT, *p*=.112). Nevertheless, the pattern is somewhat puzzling because higher trait anxiety might have been expected to correlate with lower tunnel traversals if trait anxiety affects willingness to explore. One way to test this would be to run another correlation on the first week of tunnel traversal data to see if the same pattern is seen, or the opposite. There was a general decrease in tunnel traversals over the 21-day period, and there were also group differences in average tunnel traversals as illustrated in Figure 13 A. Both groups displayed a wide range of composite anxiety scores from 0.4 to 1.0 (Figure 13 B), indicating that there was no difference in trait anxiety between these groups prior to putting them in the housing condition.

No Significant Correlations Were Found Between Trait Anxiety in the EPM and Spatial Memory in the Barnes Maze

No significant differences in trait anxiety were seen between UST and CT rats; therefore, the significant differences in spatial memory between the UST and CT groups seen on the Barnes maze are due to the unpredictable/controllable stress the UST rats experienced. However, a statistical trend was seen in the CT group alone in the number of errors in trial 7 (*p*=0.062), and the same statistical trend was seen in the average of Day 2 errors (*p*=0.075). It is puzzling that these statistical trends were seen between trait

anxiety and only one measure of spatial memory (errors) but not the other (latency to goal box). There may be a relationship between trait anxiety and spatial memory on the Barnes maze, but any relationship appears to be eliminated by the 21-day unpredictable/controllable stress, since no correlation was seen between the UST rats' spatial memory and trait anxiety measured in the EPM. Although genetic differences may play a role in anxiety, the environmental conditions that the UST rats were subjected to may override these differences. Wahlsten et al. (2003) tested 8 different mice strains on the EPM in three different labs to compare the effects of genetic differences and environmental differences. Although the difference in strain accounted for some variability in anxiety, the difference in environment had a larger effect on an anxiety measure in the EPM, indicating that environment may play a large role in anxiety-related behaviors.

No Correlations Were Found Between Trait Anxiety in the EPM and Mobility in the Barnes Maze

No differences in trait anxiety were seen between UST and CT rats for mobility; therefore, the significant differences between the UST and CT groups seen on the Barnes maze are due to the unpredictable/controllable stress the UST rats experienced.

Comparison to Other Animal Studies

Other animal studies on exposure to stress have similar results. Rats that experienced predictable chronic mild stress (PCMS) performed better on a water maze than did control rats. The rats that experienced PCMS also exhibited fewer anxiety behaviors in the forced swim test and the EPM than did control rats (Parihar et al., 2011). A similar study reported that adolescent rats exposed to PCMS exhibited fewer anxiety-

related behaviors on the EPM, and responded better to chronic unpredictable stress as adults (Suo, Zhao, Si, Liu, Zhu, et al., 2013). Rats previously exposed to immobilization on wooden boards (IMO) showed decreased ACTH (which leads to the release of corticosterone) levels immediately after a second exposure to IMO. IMO rats also had lower ACTH and corticosterone 90 min after the second exposure to IMO stress, indicating better recovery of hormone levels (Martí, García, Vellès, Harbuz, & Armario, 2001). Young squirrel monkeys experience maternal separation when mothers leave their young to forage for food. The temporary separations cause the young monkeys to make distress calls and experience elevated levels of plasma cortisol. When these monkeys reach adulthood, however, they have lower levels of cortisol and are more likely to explore novel objects than are monkeys raised in undisturbed social groups (Lyons et al., 2009). These animal studies indicate that exposure to certain types of stress may reduce anxiety-related behaviors when exposed to stress in the future.

Implications for Humans

Human studies have had similar results, indicating that some exposure to moderate stress promotes resilience to future stress when compared with those who have experience high stress or no stress (Dienstbier, 1989, 1992; Seery et al., 2010; Seery, Leo, Lupien, Kondrak, & Almonte, 2013). Studies in children also indicate that exposure to moderate stress early in life leads to a decreased cardiovascular response (Boyce & Chesterman, 1990) and lower cortisol activity (Gunnar, Frenn, Wewerka, & Van Ryzin, 2009) in response to stress in a laboratory setting. Similarly, young adults cope better with work-related stress if they have experienced work-related stress as adolescents (Mortimer & Staff, 2004). Adults also display lower levels of anxiety (Edge et al., 2009),

cope better with illness, major accidents, and loss of a spouse if they have had to cope with moderate stress during childhood (Khoshaba & Maddi, 1999).

The anxiety disorder post-traumatic stress disorder (PTSD) can be caused by severe stressors such as sexual assault, war, or natural disaster (American Psychiatric Association, 2013). Stress inoculation has been used to help prevent the development of PTSD in persons who have to work in aversive conditions such as military personnel, police, and firefighters. The stress inoculation process involves exposure to stress-related cues that promote resilience to future stress (Meichenbaum, 2007). Stress inoculation may also be helpful in treating anxiety disorders, panic disorder, and PTSD (Foa & Kozak, 1986).

Conclusion

As humans, exposure to stress is unavoidable and unpredictable. The results of this study, however, indicate that stress does not always lead to negative consequences, but can be helpful. Consistent with the hypothesis, the results indicated that exposure to unpredictable but controllable stress may cause a rightward shift in the inverted U-shaped curve, resulting in stress resilience. It can better prepare us to face stressful situations in the future and reduce our anxiety about facing those stressors.

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