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The influence of daily social stimulation in ameliorating PTSD-like behavioral and physiological changes in rats exposed to chronic psychosocial stress

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The Influence of Daily Social Stimulation in Ameliorating PTSD-Like Behavioral and Physiological Changes in Rats Exposed to Chronic Psychosocial Stress

by

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This work is dedicated to my dad, who has never stopped believing in me, and never let me stop believing in myself. I would also like to dedicate this to my wife, Lucky. Without her love, patience, and encouragement, this would not have been possible.

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The Influence of Daily Social Stimulation in Ameliorating PTSD-Like Behavioral and Physiological Changes in rats Exposed to Chronic Psychosocial Stress

Shyam Seetharaman

ABSTRACT

Individuals exposed to life-threatening trauma are at increased risk for developing post-traumatic stress disorder (PTSD). Not all people exposed to trauma, however, go on to develop PTSD. Some evidence suggests that individuals who receive social stimulation, such being involved in supportive social networks, are less likely to develop PTSD compared to those lacking social interactions. Although human research has been effective in demonstrating associations between higher levels of social stimulation and lower incidences of PTSD, there has been a lack of experimental evidence suggesting that social stimulation protects against the onset of the disorder after trauma. Here, we tested the hypothesis that providing animals with daily social stimulation (DSS) would ameliorate psychosocial stress-induced changes in behavior and physiology produced by our previously developed animal model of PTSD which generates responses comparable to patients with the disorder. The major findings of this study revealed that providing animals with DSS initiated shortly after an acute stress experience blocked the

development of PTSD-like responses in adult rats exposed to chronic psychosocial stress, such as heightened anxiety, exaggerated startle, and contextual fear. These results are consistent with human research suggesting that social stimulation may confer resistance of a subset of the traumatized population to develop PTSD. This level of analysis in an animal model of PTSD underlies the importance of continuing clinical research examining social phenomena in identifying risk factors for PTSD, as well as non-pharmacological treatments (e.g. social support systems) for the disorder.

Chapter One

Background

Introduction to Post Traumatic Stress Disorder and Factors which Influence its Etiology

Individuals exposed to horrific, life-threatening trauma are at increased risk for developing post-traumatic stress disorder (PTSD). It is a debilitating disorder which can produce symptoms such as hypervigilance, heightened anxiety, and intrusive memories (Elzinga & Bremner, 2002; Stam, 2007a). Although trauma exposure increases the risk of developing PTSD, not all traumatized individuals go onto develop PTSD. Findings suggest that only about 25% of those exposed to trauma develop PTSD (Yehuda, 2001). Evidence has suggested that environmental factors, such as social support, education and cognitive stimulation are associated with decreased rates of PTSD among victims of trauma. For instance, post-combat social support was associated with decreased rates of PTSD among Vietnam veterans (King, King, Fairbank, Keane & Adams, 1998). Although findings in social and clinical research have been effective in demonstrating relationships between environmental factors, such as social support, and lower rates of PTSD (King et al, 1998), there is a lack of experimental evidence suggesting that environmental factors are effective in protecting against PTSD. Experimentally manipulating environmental factors in an animal model, therefore, may be useful in

facilitating conclusions based on causality. This aim of this study was to manipulate social and environmental factors in an animal model of PTSD previously developed by our group (Zoladz, Fleshner & Diamond, 2008) through the use of daily social stimulation (DSS) to examine its influence on the development of symptoms in rats analogous to those seen in patients with PTSD. In this study, I tested the hypothesis that providing animals with social and environmental enrichment will ameliorate PTSD-like behavioral and physiological changes of rats exposed to chronic psychosocial stress. Findings of this study may provide experimental insight into the influence of social phenomena on the development of PTSD-like symptoms.

Post Traumatic Stress Disorder

Individuals exposed to horrific, life-threatening trauma such as rape, combat, or natural disasters, are at increased risk to develop PTSD. These individuals respond to traumatic experiences with intense feelings of helplessness, panic, fear, anxiety, and horror which can persist for many years after trauma exposure (American Psychiatric Association 1994). PTSD is a debilitating disorder involving symptoms of increased anxiety, exaggerated startle, hyperarousal, and cognitive impairments (Elzinga & Bremner, 2002; Stam, 2007a). These symptoms may be further exacerbated by intrusive flashback memories of the original trauma which are not simply reminders but, rather, are reported as feeling like actual re-experiences of the trauma (Reynolds & Brewin, 1999). As a result, PTSD patients often make great efforts to avoid situations or stimuli which remind them of the traumatic experience.

There are various biological outcomes that are prevalent among those diagnosed with PTSD. Relative to control participants, PTSD patients have significant elevations in cortisol (CORT; cortisol in humans and corticosterone in rodents), a main physiological marker of stress, in response to laboratory stressors (Bremner, Vythilingam, Vermetten, Adil & Khan, 2003), and reminders of traumatic experiences (Elzinga, Schmahl, Vermetten, van Dyck & Bremner, 2003). Although inconsistent, findings have also indicated lower baseline CORT levels among those with PTSD compared to control participants (Yehuda, Golier & Kaufman, 2005). PTSD patients also demonstrate significantly elevated levels of cardiovascular reactivity (i.e. heart rate and blood pressure) when exposed to acute laboratory stressors (Orr, Lasko, Shalev, & Pitman, 1995), and reminders of traumatic experiences (McFall, Murburg, Ko, & Veith, 1990). Research also suggests that PTSD patients exhibit significant increases in baseline heart rate and blood pressure levels compared to controls (Pole, 2007).

Animal Models. Preclinical researchers have utilized different paradigms to model PTSD in rodents. Such paradigms have included the use of electric shock, underwater trauma, predator exposure, or predator-related cues such as odor (Stam, 2007b). Our group has developed an animal model of PTSD that generates PTSD-like responses in rats based on multiple physiological and behavioral tests (Zoladz et al, 2008). It utilizes cat exposure, an ethologically relevant stressor, shown to elicit intense defensive, fear-related behaviors in rats (Blanchard, Blanchard, Rodgers & Weiss, 1990; Blanchard, Yang, I Li, Gervacio & Blanchard, 2001; Blanchard, Canteras, Markham, Pentkowski & Blanchard, 2005). This model, which incorporates two cat exposures in

conjunction with daily social instability, produces robust behavioral changes such as exaggerated startle responses, heightened anxiety, cognitive impairments, as well as physiological changes consistent with symptoms analogous to those exhibited by people with PTSD (Zoladz et al, 2008). Developing an understanding of social factors, which may mitigate PTSD severity and development, will be beneficial in understanding individuals' susceptibility to develop PTSD, and may contribute to the body of clinical research focused on treating the disorder.

Social Stimulation

Accumulating evidence suggests that PTSD risk and recovery are associated with factors such as social stimulation, such as social support, which refers to individuals' social network size and complexity (Charuvastra & Cloitre, 2008). Positive social interactions with spouses, family members, or a community may provide individuals with support in the form of attachment, love, or advice which may promote better health (Cohen & Wills, 1985; Uchino, Cacciopo & Kiecolt-Glaser, 1996). There is evidence indicating that social interactions may be beneficial to those who have experienced intense and, possibly, traumatic events. Specifically, studies have found an association between social support and an attenuation of detrimental effects of stress on psychological or physical well-being in victims of sexual assault (Kimerling & Calhoun, 1994), and childhood abuse (Runtz, 1997). High levels of social support have also been related to lower cardiovascular reactivity to laboratory stressors compared to low-social support controls (Lepore, Allen & Evans, 1993**;** Pruitt & Zoellner, 2008)**.**

Social Stimulation & Stress

Stress can generate general feelings of helplessness (Cohen & Wills, 1985), increasing the likelihood of individuals turning to maladaptive coping strategies such as alcohol abuse (Brady & Sonne, 1999). Social support can increase feelings within individuals that others will provide necessary resources for coping with stressful situations and, in turn, reduce feelings of helplessness and uncontrollability, compared to those who lack support (Cohen & Wills, 1985). In this fashion, social support may, in theory, "buffer" against the onset of pathological disorders by intervening between a stressor and stress reaction by attenuating the perceived threat of the experience and promoting healthier behaviors (Cohen & Wills, 1985). A more recent report, however, suggested that social support was associated with actual decreases in the occurrences of stressors, as opposed to being mobilized during stressful situations in a meta-analysis examining workplace stress (Viswesvaran, Sanchez & Fisher, 1999). These findings imply that social support may simply reduce the likelihood of stressful experiences, rather than act to buffer against the detrimental effects of stress on health. Conflicting correlation-based findings evident in human research reinforces the need for more experimental evidence which may provide insight into the causal effects of social stimulation on responses to stress and its impact on health. Nonetheless, there is evidence indicating that individuals may be able to draw on their own social networks for

support after traumatic stress (Ullman, 1999), and may confer resistance of a subset of the traumatized population to develop PTSD.

Social Stimulation & PTSD

Research has shown that only about 25% of individuals exposed to horrific, life-threatening trauma develop PTSD (Yehuda, 2001). Studies have shown a significant relationship between social support received after trauma exposure and PTSD severity, development, and, in some cases, remission of the disorder. Specifically, analyses of responses from Vietnam veterans revealed a significant correlation between social support received at homecoming and lower rates of PTSD (King et al, 1998). Other evidence indicates higher incidences of PTSD in those not receiving social support among a sample of Jews and Arabs exposed to repeated acts of terrorism in Israel (Hobfall, Canetti-Nisim, Johnson, Palmieri, Varley & Galea, 2008) compared to individuals with support from others. In addition, Vietnam veterans diagnosed with PTSD reported significantly lower levels of social network sizes and positive social interactions in the first 1-3 months following military discharge compared to those combat veterans not diagnosed with PTSD (Keane, Scott, Chavoya, Lamparski & Fairbank, 1985). The association between post-combat social support and lower rates of PTSD was also illustrated in studies examining former prisoners of the Korean War (Sutker, Winstead, Galina, & Allain, 1991), World War II (Engdahl, Dikel, Eberly & Blank, 1997; Gold, Engdahl, Eberly, Blake, Page & Frueh, 2000), and veterans of the

Persian Gulf War (Ozer, Best, Lipsey & Weiss, 2003). In a 14 year longitudinal study, Vietnam veterans diagnosed with PTSD with higher levels of community involvement were significantly more likely to show a remission of symptoms over time compared to patients who withdrew from society (Koenen, Stellman, Stellman & Sommer, 1998, 2003). Interestingly, this relationship between social interaction and symptom remission implies that social support may contribute to PTSD recovery even after clinical diagnosis. Social support received after traumatic events, such as interpersonal violence (Astin, Lawrence & Foy, 1993; Ozer et al, 2003; Perrin, Van Hasselt, Basilio & Hersen**,** 1996), accidents (Perry, Difede, Musgni & Frances, 1992), natural disasters (Ozer et al, 2003), and sexual abuse (Kimerling & Calhoun, 1994; Ozer et al, 2003) were also significantly correlated with lower rates of PTSD. These findings indicate that post-trauma social support may play a critical role in protecting against the development of PTSD.

Although limited, there is some evidence indicating that social support received *during* ongoing trauma may be effective in lowering risk for PTSD development. One such study conducted on Israeli-Lebanon war veterans showed a significantly negative correlation between rates of PTSD diagnosis and cohesiveness within military units during combat (Solomon, Mikulincer & Hobfall, 1987). The authors posited that soldiers who did not form positive social bonds with fellow combatants were more likely to have increased feelings of uncontrollability and helplessness and, in turn, develop PTSD, compared to those experiencing supportive relationships within their military units (Solomon et al, 1987). This finding suggests that social support may protect against the onset of PTSD when it is received by individuals during situations, such as combat, in

which both acute trauma (e.g. witnessing the mutilation of bodies) (King et al, 1998), and chronic social stress (Solomon et al, 1987) may be experienced.

Limitations of Social Stimulation & PTSD Studies. Human studies have indicated a strong relationship between social stimulation and lower rates of PTSD. Although human research has demonstrated correlations between higher levels of social stimulation and lower rates of PTSD, it has lacked experimental evidence suggesting that social factors are effective in protecting against PTSD. In addition, there may also be various sources of error in studies focused on combat veterans stemming from their retrospective nature. In studies examining social phenomena, for instance, combat veterans are asked to recall events, such as levels of social support they received during combat, or after returning home. Veterans who, in some cases, have developed a debilitating disease such as PTSD, may not be able to, for instance, accurately recall the level of social support they received after returning home from combat many years prior to assessment by researchers or clinicians. As a result, their responses during assessment sessions may not reflect actual experiences and, therefore, may lead investigators to flawed conclusions. Nonetheless, there is evidence suggesting that social stimulation may exert a profound influence on the brain and, in turn, influence the impact of stress on the development of PTSD.

Effects of Social Stimulation on the Brain.

There is accumulating research in the social neuroscience field which has attempted to identify the possible neural underpinnings of evidence suggesting that social

stimulation buffers against the detrimental effects of stress, and is associated with lower rates of PTSD. Patients with PTSD, in some cases, are withdrawn in nature and fail to seek out others for support (Norris & Kaniasty, 1996). There is some evidence suggesting that this failure to seek support is the result of emotional numbing, which is related to deficits in brain reward circuits. One study revealed that, relative to normal controls, participants diagnosed with PTSD exhibited significantly smaller activation in areas related to reward seeking, such as the nucleus accumbens in response to social reward stimuli (pictures of attractive or pleasant faces). The investigators, utilizing fMRI, also found that PTSD patients exhibited significant less activation in the prefrontal cortex (PFC) compared to healthy controls performing the task (Elman, Frederick, Ariely, Dunlap & Rodolico, 2005). This study suggests that PTSD may produce deficits in reward circuitry and those who, for instance, are provided with, or seek social stimulation may be less likely to develop such deficits. Additionally, it indicates that PTSD patients may exhibit inhibited executive control based on impaired PFC functioning. Other research has examined specific neurochemical changes which may underlie the possible protective nature of social stimulation against PTSD. Specifically, some investigations have examined the neuropeptides oxytocin (OT) and vasopressin (AVP), which have been identified as essential chemical mediators of social stimulation, pair bonding, mating, (Charuvastra & Cloitre, 2008; DeVries, Glasper & Detillon, 2003) and may relate the effects of social stimulation on PTSD-related circuitry. Animal studies have shown that a large number of AVP and OT receptors are located in the amygdala, which governs emotional responses. Stimulating OT receptors in the amygdala may, in turn, inhibit its

activity under situations which may provoke, for example, fear, or anxiety (LeDoux, 2000). Some work has indicated, in fact, that exogenous administration of OT to female rats decreased anxiety-like behaviors. In one human study, male participants administered OT or placebo intranasally were assessed for amygdala activity utilizing fMRI after viewing pictures of threatening, or not threatening faces. Findings revealed that OT significantly suppressed amygdala activation relative to the placebo in the threatening face condition (Kirsch, Esslinger, Chen, Mier, Lis et al, 2005). Another study, which specifically examined PTSD patients, found that administering OT to Vietnam veterans with the disorder resulted in a significant decrease in their physiological responses to combat imagery relative to placebo (Pitman, Orr & Lasko, 1993). These findings suggest that enhanced social stimulation may elevate neuropeptides, such as OT, thereby suppressing the activation of brain areas governing fear and anxiety-related responses, and, in turn, physiological responses to reminders of the patients' trauma. Social stimulation, therefore, maybe an important non-pharmacological candidate of treating symptoms associated with PTSD.

Environmental Enrichment

The modern concept of EE is based on early anecdotal evidence by Donald Hebb who, in 1947, noted that rats he kept as domestic pets exhibited superior maze-performance compared to cohorts housed in small cages in his laboratory. He speculated that these animals may have had larger brain sizes, as a result of increased opportunities to explore the relatively diverse environment of his home, which contributed to their enhanced performance. Pioneering experimental work demonstrated that EE, involving

exposing groups of animals to complex environments, was indeed effective in significantly increasing neuroanatomical changes in the brain relative to a few animals housed together in smaller, less complex standard cages (Rosenzweig & Bennett, 1984, 1996). Specifically, they found that when rodents were provided with EE, involving placing groups of animals into a large apparatus containing blocks, running wheels, and increased opportunities for social interactions compared to standard laboratory housing conditions, they had significantly larger overall cortical weights (Bennett, Rosenzweig, Diamond, Moromito & Hebert, 1974; Diamond, Krech & Rosenzweig, 1964; Rosenzweig, Love & Bennett, 1968; Diamond, 2001; Welch, Brown, Welch & Lin, 1974), increased dendritic branching **(**Diamond et al, 1964; Diamond, 2001), and increased numbers of synapses (Mollgaard, Diamond, Bennett & Rosenzweig, 1971;West & Greenough, 1972) in the cerebral cortex, suggestive of enhanced cognitive abilities compared to animals housed under standard conditions. More recent studies established that EE significantly enhanced hippocampal synaptic plasticity, (Abel & Nguyen, 2001; Artola, Frijtag, Fermont, Gispen & Schrama, 2006; Duffy, Craddock, Ickes et al, 2000; Faherty, Kerley & Smeyne, 2003; Fernandez-Truel, Gimenez-Llort, Escorihuela, Gil & Aguilar, 2002; Leggio, Mandolesi, Federico, Spirito & Ricci, 2005; Olsson et al, 1994; Segovia et al, 2008; Sharp, McNaughton & Barnes, 1985), neurotrophic factors relating to plasticity (Ickes, Pham, Sanders, Albeck & Mohammed, 2000; Olsson, Mohammed, Donaldson, Henrikkson & Seckl, 1994; Segovia, Arco, de Blas, Garrido & Mora, 2008), neurogenesis (Brown, Cooper-Kuhn, Kempermann, Van Praag, Kempermann, & Gage, 2000; Bruel-Jungerman, Laroche & Rampon, 2005; Kempermann, Kuhn & Gage, 1997;

Kempermann, Gast & Gage, 2002; Nilsson, Perfilieva, Johansson, Orwar & Eriksson, 1999), and reduced hippocampal cell death (Young, Lawlor, Leone, Dragunow & During, 1999) compared to controls, all neurobiological changes related to increases in cognitive performance. Other work indicates that EE improves behavioral performance on cognitive tasks (Lee, Hsu & Ma, 2003; Leggio et al, 2005; Nilsson et al, 1999; Meshi, Drew, Saxe, Ansorge & David, 2006; Schrijver, Bahr, Weiss & Wurbel, 2002), and reduces anxiety-like behaviors (Brenes, Rodriguez & Fornaguera, 2008; Bruel-Jungerman et al, 2005; Chapillon, Manneche, Belzung & Caston, 1999; Fernandez-Truel et al, 1997; Friske & Gammie, 2005; Klein, Lambert, Durr, Schaefer & Waring, 1994; Meshi et al, 2006; Teather, Magnusson, Chow & Wurtman, 2002; Widman & Rosellini, 1990; Zimmerman, Stauffacher, Langhans & Wurbel, 2001) compared to standard housing. Experimental evidence also suggests that the beneficial effects of EE on the brain and behavior may play a role in protecting against the detrimental effects of stress on the brain, behavior, and physiology of organisms.

Environmental Enrichment & Stress

Although limited, findings suggest that EE is effective in reversing the adverse effects of stress on the brain and behavior of rodents. EE, for instance, has been shown to block increases in behavioral measures of fear and anxiety in rodents exposed to shock (Benaroya-Milshtein, Hollander, Apter, Kukulansky, Raz & Will, 2004). In addition, rats exposed to early-post natal maternal separation (Bredy, Humpartzoomian, Cain & Meaney, 2003) and low maternal care (Yang, Hou, Liu, Zhang, Zhou, Xu & Li, 2007)

exhibited memory impairments which were blocked by EE. Exposing animals to EE also protected against stress-induced reductions of synaptic plasticity (Yang et al, 2007), impaired behavioral performance on spatial memory tasks (Larsson, Winblad, & Mohammed, 2002; Wright & Conrad, 2008), and increased anxiety-like behaviors of rats exposed to predator odor (Roy, Belzung, Delarue & Chapillon, 2001).

Utilizing EE to Study PTSD

EE may exert its protective efficacy by mitigating stress-induced increases in physiological markers of stress. This hypothesis has been supported with evidence showing that EE blocked the effect of maternal separation stress on elevated corticosterone (CORT), a main physiological marker of stress, levels when rat pups were subjected to acute restraint stress as adolescents (Francis, Diorio, Plotksy & Meaney, 2002), adults (Morley-Fletcher, Rea, Maccari & Laviola, 2003), and when adult mice were exposed to predator odor (Benaroya-Milshtein et al, 2003; Roy et al, 2001). These findings suggest that EE may render the stress response system of the brain more adaptive and efficient by reducing hormone levels released in response to stress and, in turn, facilitate recovery after exposure to stressful situations (Fox, Merali & Harrison, 2008). As a result, studying the effects of environmental manipulations on the etiology of PTSD-like symptoms may provide insight into identifying mechanisms responsible for the development of PTSD, and reinforcing the importance of social and environmental treatments for the disorder.

Chapter 2: Experiment

Purpose and Hypotheses of Present Study

Although extensive research has revealed EE to produce robust changes in the brain and behavior of organisms, the majority of studies have included running wheels in the EE apparatuses. This can result in possibly confound findings in that exercise alone has been shown to exert profound changes related to the learning and memory of animals, such as neurogenesis, long-term potentiation, neurotrophic growth factors and performance on spatial memory tasks (Neeper, Gomez-Pinilla, Choi & Cotman, 1996; van Praag, Kempermann & Gage 1999; van Praag, Christie, Sejnowski & Gage, 1999; van Praag, 2008). This experiment addressed the possible confounding influence of exercise on findings in the EE literature by eliminating direct sources of exercise (i.e. running wheels) in the enrichment apparatus. Although it is possible that animals in the current apparatus were more active relative to those housed under standard conditions, they were not provided with a source of direct exercise. In doing so, this study focused on addressing the specific influence of daily social stimulation (DSS) on behavioral and physiological responses of rats exposed to the chronic psychosocial stress regimen.

The present study was also designed to build upon the limited literature examining the interaction between chronic stress and enrichment in adult rats. Importantly, in contrast to the majority of studies in this area, this experiment examined

the influence of brief, daily stimulation implemented in an animal model of PTSD which assesses a variety of symptoms associated with the disorder. Additionally, this study addressed the impact of social complexity on stress effects on behavior and physiology by placing groups of animals together, in contrast to some EE studies which utilized smaller group sizes (Marashi et al, 2003; Bennett, McRae, Levy & Frick, 2006). This work investigated the possible profound influence which non-pharmacological experience-related manipulations can exert on the behavior and physiology of organisms, and how observed changes may relate to their brain function. This study tested the hypothesis that 1) rats subjected to our laboratory's previously developed chronic psychosocial stress regimen (Zoladz et al, 2008) would demonstrate robust increases in behavioral and physiological changes analogous to those seen in patients clinically diagnosed with PTSD, and 2) that providing rats with DSS starting the day after an acute stress experience would significantly ameliorate these PTSD-like changes relative to animals kept exclusively under standard housing conditions.

Methods

Animals and Housing Conditions

Adult male Sprague-Dawley rats (225-250g) arrived from Charles River laboratories and were housed in pairs on a 12:12 h light-dark schedule (lights on at 0700h) in Plexiglass cages (46 x 25 x 21 cm) with free access to food (Harlan Teklad Global 18% Protein Rodent Diet; Harlan Laboratories; Indianapolis, IN) and water. Rats

were given one week to acclimate to the animal housing room before any experimental manipulations took place.

Power Analysis: Sample Size Estimation

Given an accepted level of power ($1 - \beta = .80$), a relatively large effect size ($\omega^2 = .60$), and an estimated 60 degrees of freedom for the mean square error, the appropriate sample size per group can be estimated as follows:

$n' = \Phi^2 / \omega^2$ for $1 - \beta = .80$ and 60 df, $\Phi = 1.65$

$n' = (1.65)^2 / (.60)^2$

$n' = 2.7225 / .36$

$n' = 7.5625$

Previous work in our laboratory has shown sample sizes of 8 to be sufficient in reasonably interpreting data by reducing the probability of Type I and II errors (Zoladz et al, 2008). A sample size of 10 per group, therefore, was thought to be more than sufficient for this study.

Design

This study will be designed to examine the effects of chronic psychosocial stress and SES on rat behavior and physiology utilizing a 2x2 factorial design with psychosocial stress (psychosocial stress, no psychosocial stress) and environment (home cage, SES) as the between subjects factors.

Psychosocial Stress Procedure

Animals underwent stress manipulations similar to those employed previously by our group which have produced robust enhancements of fear and anxiety-like behaviors, memory impairments, as well as several physiological changes (Halonen, Zoladz, & Diamond, 2006 ; Zoladz et al, 2008). Rats were randomly assigned to psychosocial stress or no psychosocial stress groups. Rats in the psychosocial stress groups were exposed to two acute stress sessions lasting one hour each. The first session took place during the light cycle (between 0800 and 1500 h) after the one week housing room acclimation period. The second acute stress session occurred 10 days later during the dark cycle (between 1900 and 0200 h) based on research indicating the enhancement of synaptic activity in the amygdala 10 days after restraint stress, indicative of enhanced fear based processing (Mitra, Jadhav, McEwen, Vyas, & Chattarji, 2005; Vyas, Mitra, Shankaranarayana, & Chattarji, 2002). The second stress session, in theory, served to reinforce any stress-induced changes in the brain and behavior initiated during the first session.

During each acute stress session, rats were placed in a box for 3 minutes. The box (25.5 x 30 x 20 cm; Coulbourn Instruments; Allentown, PA) consists of 2 aluminum sides, an aluminum ceiling, and a Plexiglass front and back. The floor of the chamber consists of 18 stainless steel rods, spaced 1.25 cm apart. During the last 30 sec of the exposure, a 30- sec tone was played (74dB @ 2400 Hz)**.** Immediately following box exposure, rats were immobilized in Decapicones (Braintree Scientific; Braintree, MA)

and transported to a different room where they were individually placed in triangular-shaped wedges within a circular Plexiglass "pie" enclosure (20 x 20 x 8 cm; Braintree Scientific; Braintree, MA) located inside of a large metal cage (61 x 53 x 51 cm). An adult female cat was placed on top of the pie enclosure and cat food was smeared on top of the enclosure to direct cat activity towards the rats. The door to the metal cage was then securely closed. Rats were subjected to only non-tactile cues of the cat, as the pie enclosure prevents any physical contact between the two animals.

As with the psychosocial stress groups, rats in the no psychosocial stress groups were brought to the laboratory on two occasions separated by 10 days and placed in the box for 3 minutes, after which they were placed back in their home cages where they remained for one hour. In addition to the two cat exposures, rats in the psychosocial stress groups were subjected to unstable housing conditions (i.e. social instability) where, on each day, rats were pseudo-randomly paired with a different cage mate from the previous day. Social instability manipulations started on the day of the first cat exposure, and continued daily until the initiation of behavioral/physiological testing. Rats in the no psychosocial stress groups were housed with the same cage cohort for the duration of the experiment.

Daily Social Stimulation Exposures

Animals in the psychosocial stress groups were randomly assigned to one of two SES conditions (home cage or DSS). The DSS apparatus (91.44 x 63.50 x 157.48 cm; Ferret Nation; Muncie, IN) consists of three interconnected levels containing plastic

platforms, tunnels, metal ladders, two cloth hammocks, and a climbing rope. Animals in the DSS groups were housed under standard conditions until the day after the first stress session. Twenty-four hours after the first cat exposure, this group of animals (N=10) was transported from the housing room to the laboratory and placed into the DSS apparatus for 2 hours. These daily 2 hour exposures continued until the first day of testing. Animals in the home cage groups were housed under standard conditions throughout the experiment and did not receive any exposures to the DSS apparatus. Between sessions, all objects and platforms were removed from the DSS apparatus and cleaned with soap and tap water. All procedures adhered to the University of South Florida's ethical guidelines on the treatment of animals in research and the University of South Florida IACUC regulations.

Behavioral Testing

Three weeks after the second stress session, animals were subjected to a battery of behavioral tests. Prior to the start of testing, all animals were handled for three consecutive days (2 min each). Body weights were recorded on the day of the first stress session and on the first day of testing. On each behavioral testing day, rats were transported from the housing room into the laboratory and remained in their home cages for 30 minutes in order to acclimate them to the surroundings.

Fear Memory. On the first day of behavioral testing, rats were assessed for their memory of the specific context and cue temporally associated with the two cat exposures. Memory was behaviorally measured by assessing the percentage of time rats spent

immobile (typical fear responses in rats) upon exposure to the context and cue which were paired with the two cat exposures. To assess contextual fear memory, rats were placed in the box previously temporally paired with the two cat exposures, and remained there for 5 minutes. Approximately one hour later, rats were tested for their memory of the cue (tone) presented in the box prior to each cat exposure. During cue testing, rats were placed in a different box (25 x 22.5 x 33 cm; Coulbourn Instruments; Allentown, PA) than the one used during contextual testing. It consists of two aluminum sides, an aluminum ceiling, and a clear Plexiglas front and back with the shuttle door in the closed position. The floor consists of a metal plate (21.5 x 21.5 cm) to eliminate the sensation of stainless steel rods beneath their paws. These conditions serve to reduce any similarities between the training context and the cue testing chamber. The cue testing period commenced with 3 three minutes without an auditory stimulus (tone), followed by 3 minutes with tone (74dB @ 2400 Hz). Tones were presented through a speaker located on one of the sides of the shuttle box, and a house light was turned on inside the chamber. This procedure provided a behavioral measure in a novel context (pre-tone period) and a more direct measure of cue-based memory (tone period). Immobility behaviors are monitored by a 24-cell infrared activity monitor (Coulbourn Instruments; Allentown, PA) mounted on the top of the boxes which uses emitted infrared body heat images (1300 nm) from the animals to detect movement. Immobility was defined as periods of inactivity lasting $\geq$ 5 sec. A Microsoft Excel spreadsheet with a macro designed to analyze immobility behavior calculated the total number of seconds spent immobile by each animal in 30 second epochs. This time divided by the total amount of time in the chamber

yielded a percentage of time immobile for each animal, thus providing behavioral indices of both context and cue-based memories.

Elevated Plus Maze (EPM). The EPM has been extensively used in testing for anxiety in rodents (Korte & De Boer 2003). Twenty-four hours after the context and cue tests, all rats were transported to a room within the laboratory and tested for anxiety-like behaviors on the EPM. The EPM (Hamilton-Kinder; San Diego, CA.) consists of two (10.80 x 51.17 cm) open and two closed (10.80 x 51.17 cm) arms which intersect to form the shape of a "plus" sign. The intersection area is 10.80 by 10.80 cm, and the walls of the closed arms are 40.01 cm in height. Each rat was placed on the EPM apparatus for a period of 5 minutes where 48 infrared photo-beams (located along the perimeter of the open and closed arms) connected to a computer program (Motor Monitor, Hamilton-Kinder, San Diego, CA) scored its behavior. The computer program monitors various types of behaviors for further assessment such as movement, total time spent in each area of the apparatus, and head dips exhibited over the edges of the apparatus. Anxiety-like behaviors were assessed by measuring times spent in certain areas of the EPM. Rats which spend more time in the open arms than the closed arms are considered to be less anxious as this exposes them to open view and, in theory, possible threat. A primary dependent measure of interest, therefore, was the percentage of total time rats spent in the open arms of the EPM. Another measure of exploratory, or risk-taking behavior, the total number of head dips (scored by the computer program each time rats' heads cross photo-beam sensors along the edges and ends of the open arms) was another main focus of

analysis. Fecal boli was removed from the EPM between each ten minute session and cleaned using a 25 % ethanol solution to reduce odor buildup in the apparatus.

Startle Response. Approximately one hour after the EPM assessment, all rats were administered an acoustic startle response test. These startle responses were measured using a large startle monitor cabinet (Hamilton-Kinder; San Diego, CA; 36 x 28 x 50 cm). Each rat was placed inside a small Plexiglas box (19 x 10 x 10 cm) which was located inside the larger cabinet. At the beginning of each trial, rats were placed on a sensory transducer, contained within the small Plexiglas box, which was be connected to a computer program (Startle Monitor; Hamilton-Kinder; San Diego, CA). The program records startle responses by measuring the maximum amount of force (in Newtons) that rats exert on the sensory transducer for a period of 250 ms after the presentation of each auditory stimulus. Differences in body weight were controlled for by adjusting the sensitivity settings on the sensory transducer (a range of 0-7 arbitrary units) prior to each trial. Each startle trial began with a 5 min acclimation period, followed by the presentation of 24 bursts of white noise (50 ms each) consisting of 8 bursts at 3 different auditory intensities (90, 100, 110 dB) presented in sequential order. The time between each noise burst was pseudo- randomly varied between 25 and 55 seconds. After the start of the initial noise burst, the startle apparatus provided an uninterrupted background noise of 57 dB. Each session lasted approximately 20 minutes.

Novel Object Recognition. Twenty-four hours after acoustic startle response testing, rats were transported back in the laboratory where they were placed in an open field. The apparatus consists of a plastic box with black walls and an open top

(Hamilton-Kinder; San Diego, CA; 40 x 47 x 70 cm). The rats spent 5 minutes in the open field to habituate them to this environment prior to training and testing periods. All behaviors were monitored by a video feed to a computer program (Any Maze; Stoelting; Wood Dale, IL). The computer program enables the assessment of behaviors exhibited in the open field such as total distance traveled in each area (center and perimeter), total time spent in each area, and entries into each quadrant of the apparatus, which provide a source of assessment for the general behavior of the rats. Twenty-four hours after habituation to the open field, rats were placed in the same open field containing two identical (plastic/metal) objects for 5 minutes (training phase). The objects were placed in opposite, diagonally oriented corners of the open field and secured to the flooring with tape to prevent rats from manipulating and possibly displacing them. The objects and their locations were counterbalanced across rats to control for place or object preferences. The testing phase commenced three hours later in which rats were placed back in the open field, and one of the objects used during training was replaced by a novel object. A 16 cm^2 zone was specified around each object to assess the time spent with the each object, and was measured by the computer program by tracking the rats' head movements in relation to the location of the object. During testing, greater time spent by rats in proximity to the novel versus familiar object was taken as an indication of intact memory for the familiar object.

Physiological Testing

Blood Sampling and Cardiovascular Measurements. Physiological testing commenced twenty-four hours after the last day of behavioral testing. On this day, blood samples and heart rate (HR)/ blood pressure (BP) measurements were collected and recorded from all rats in order to assess corticosterone (CORT), a main physiological marker of stress, and cardiovascular responses respectively. For baseline and stress-level blood sample collections, petroleum jelly was spread across the saphenous veins of rats to facilitate vein access from which blood was collected within a 2 minute time interval via venipuncture. For baseline blood sampling, rats were transported from the housing room to an adjacent procedure room in which a single blood sample was collected after which they were restrained in plastic Decapicones (Braintree Scientific; Braintree, MA) and transported to the laboratory. Twenty minutes later, rats were removed from the restrainers, and another blood sample was collected to examine their CORT response to restraint stress. For HR/BP measurements, rats were placed in a Plexiglass tube (IITC Life Science; Woodland Hills, CA) within a warming test chamber (approximately 32 degrees Celsius) which serves to facilitate blood flow to the tail. This enables HR and BP measurements to be taken using tail cuffs fitted with photoelectric sensors (IITC Life Science; Woodland Hills, CA). Rats were then returned to their home cages for one hour, after which a sample of trunk blood was collected via rapid conscious decapitation to determine post-stress CORT levels. Following the trunk blood sample, adrenal glands, thymus glands, kidneys, and hearts were harvested and weighed for further analysis. After all blood collections clotted at room temperature, they were centrifuged (3000 rpm

for 8 minutes) after which serum was extracted and stored at -80º C until it was shipped for assay.

Statistical Analyses

Experimental Design and General Analyses. The current study utilized a between-subjects, 2 x 2 factorial design. The independent variables were psychosocial stress (psychosocial stress, no psychosocial stress) and environment (home cage, DSS). The majority of data were analyzed utilizing between-subjects, two-way ANOVAs with psychosocial stress and environment serving as the between subjects factors. Planned comparisons (independent samples *t*-tests) were also conducted between groups that were predicted to differ *a priori* based on previous findings (Seetharaman, Zoladz & Diamond, 2008; Zoladz et al, 2008). For all statistical analyses, alpha was set at .05, and LSD post hoc tests were employed when applicable.

Fear Memory. Contextual and cue-based fear memory tests were analyzed separately. Contextual fear memory, as measured by immobility, was analyzed utilizing two-way, between subjects ANOVAs. The number of fecal boli produced by rats during both context and cue tests were also analyzed separately. For each assessment, psychosocial stress and environment served as the between subjects factors. For analysis of the cue fear test, a three-way mixed model ANOVA was utilized with tone (before tone, during tone) serving as the within subjects factor, and psychosocial stress, and environment serving as the between subjects factors.

Elevated Plus Maze. The amount of time rats spent in the open arms of the EPM was calculated as a percent of the total 5 minute trial time (i.e. percent time spent in the open arms). To further examine anxiety-like behaviors in rats, the percent time that animals spent in specific areas of the open arms on the EPM was examined. The percent time spent in both the near open arms (close to the intersection leading to the closed arms) and far open arms (further away from the intersection leading to the closed arms) were analyzed to further examine rats' anxiety-like behavior on the EPM. Mean head dips, which provide an index of exploratory-like behavior, were also analyzed. The percent time rats spent in the closed arms of the EPM was assessed in order to complete the overall analysis of behaviors relating to anxiety, exhibited by animals on the apparatus**.** Additionally, motor movement was assessed by examining overall ambulations made on the EPM, as well as the velocity of animals in the open and closed arms of the apparatus. All measures were assessed separately utilizing between-subjects, two-way ANOVAs with psychosocial stress and environment serving as the between subjects factors.

Startle Response. Startle responses for each of the three auditory stimulus intensities (90, 100, and 110 dB) were analyzed separately. For each assessment, between-subjects, two-way ANOVAs were employed to analyze the data, with psychosocial stress and environment serving as the between-subjects factors.

Novel Object Recognition. Single ratio time values were calculated for each rat by dividing the time spent with the novel object by the time they spent with the familiar object (ratio time = time spent with novel object / time spent with familiar object) for the

entire 5 minute testing trial, as well as the first minute. These data, obtained from behavior measured in the open field were subjected to between- subjects two-way ANOVAs with psychosocial Stress and environment serving as the between-subjects variables.

Heart Rate and Blood Pressure. Separate between-subjects, two-way ANOVAs were utilized to examine differences in HR and BP, where psychosocial stress and environment served as the between-subjects variables.

Growth Rate. Growth rate was calculated by dividing rats' total weight gained during the course of the study by the number of total days in the experiment (i.e. 31 days). A two-way, between subjects ANOVA was employed to examine differences between groups, with psychosocial stress and environment serving as the between subjects factors.

Organ Weights. Adrenal, thymus, heart, and kidney weights were harvested from all rats on the last day of testing. All organs were weighed and expressed as milligrams per 100 grams of body weight (mg/100g b.w.). A two-way, between subjects ANOVA was employed to examine differences in body weights with psychosocial stress and EE serving as the between subjects factors.

Results

Fear Memory

Context Test Immobility. For the analysis of immobility during the 5 minute context test, there were no significant main effects of either psychosocial stress, $F(1,31) =$

.129, or environment, $F(1,31) = .292$, and no significant psychosocial stress x environment interaction, $F(1, 31) = .194$ (p values > .05) indicating no significant between-groups differences in immobility to the context associated with the cat exposures (see Figure 1).

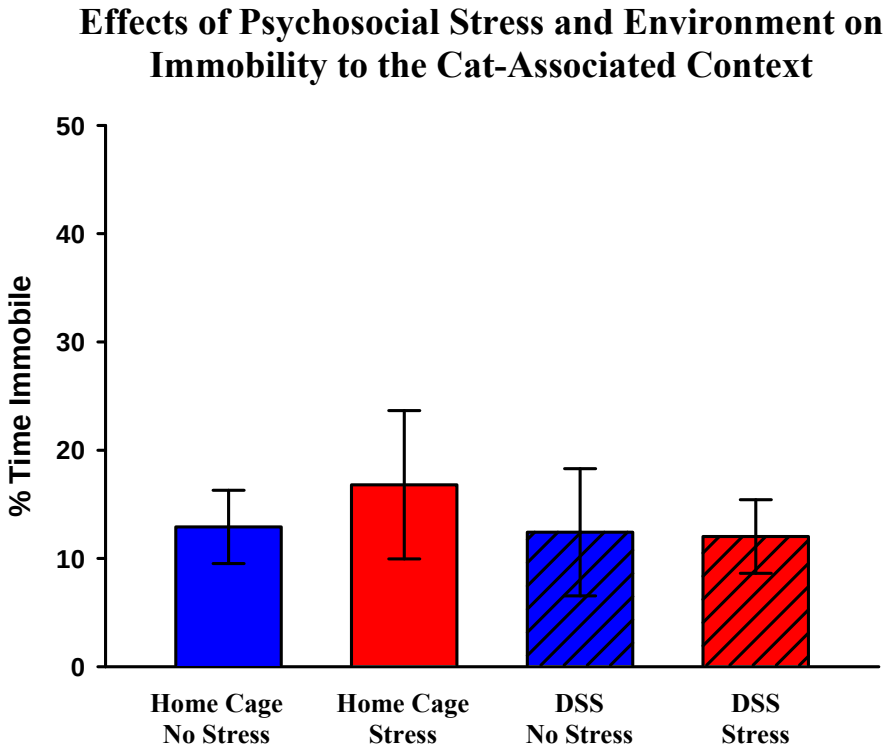

Figure 1. Effects of psychosocial stress and environment on immobility during the context test. The data are presented as mean percent time immobile ± SEM.

Context Test Fecal Boli. As depicted in Figure 2, the analysis of fecal boli produced by rats during the context test revealed no significant main effects of either

psychosocial stress, $F(1,35) = .253$, or environment, $F(1,35) = .999$, and no significant psychosocial stress x environment interaction, $F(1,35) = .999$ (p values > .05).

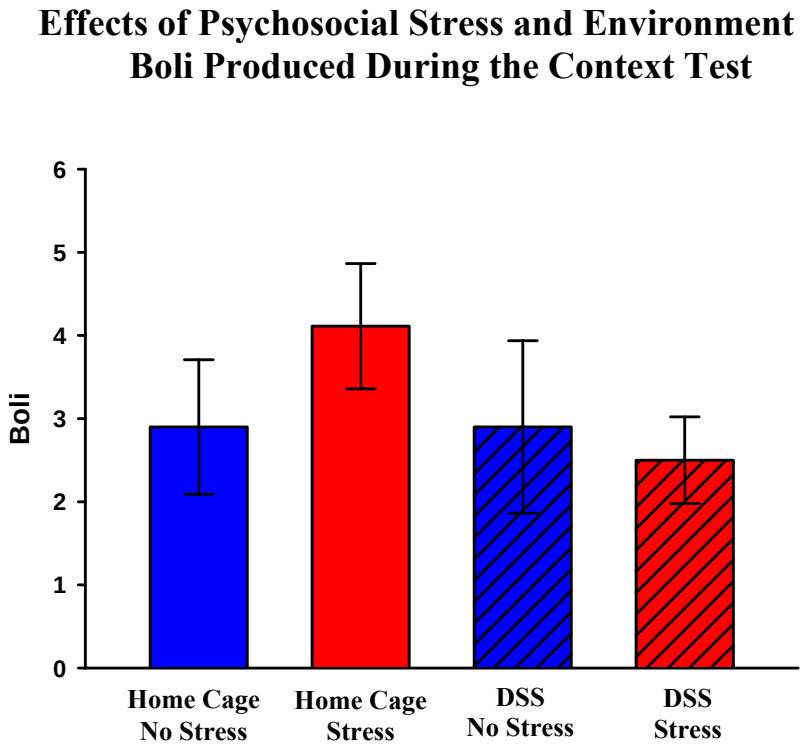

Figure 2. Effects of psychosocial stress and environment on fecal boli produced during the contextual fear test. Data are presented as mean boli produced ± SEM.

Cue Test Immobility. For the analysis of immobility during the cue fear test, there was no significant main effect of tone, $F(1,28) = 1.81$, $p > .05$. There were also no significant main effects of either psychosocial stress, $F(1,28) = 2.77$, or environment,

$F(1,28) = 3.00$ (p values > .05). Analysis did reveal a significant tone x psychosocial stress interaction, $F(1,28) = 5.49$, $p < .05$, as well as a significant tone x environment interaction, $F(1,28) = 5.32$ (p values < .05). There was no significant three-way tone x psychosocial stress x environment interaction, $F(1,28) = 2.50$, $p > .05$. Post-hoc analyses revealed that immobility exhibited by the home cage psychosocial stress group during the tone was significantly greater than before the tone. Also, these animals were significantly more fearful to the tone than all other groups, indicated by significantly higher immobility levels relative to them. Additionally, the data indicated that this significant stress-induced increase in immobility to the tone was prevented with DSS, as shown by the DSS-psychosocial stress group exhibiting significantly less immobility compared to the home cage-psychosocial stress group during the tone. These behavioral data suggest that, in contrast to all other groups, the home cage psychosocial stress animals were more fearful when re-exposed to the salient cue (tone), which was temporally associated with the cat sessions, compared to before the tone. The finding that there were no significant differences in immobility before and during the tone in the DSS-psychosocial stress group suggests that DSS may have ameliorated fear-responses produced by the specific cue temporally associated with cat exposures, as was shown by the home cage-psychosocial stress group. This is further evidenced by data indicating that, relative to non-psychosocially stressed controls, the home cage-psychosocial stress group spent significantly greater time in immobility during the tone, and that this behavioral increase in fear-related memory was prevented with DSS (see Figure 3).

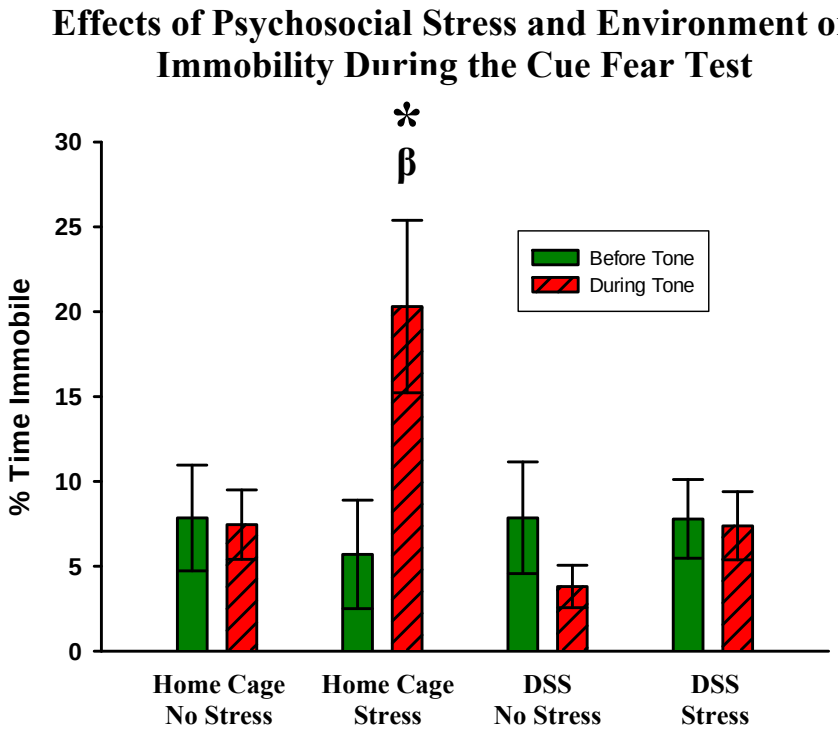

Figure 3. Effects of psychosocial stress and environment on immobility before and during the tone in the cue fear test. Data are presented as mean percent time immobile ± SEM. * = $p < .05$ relative to all other groups; β = $p < .05$ relative to within group immobility before tone

Cue Test Fecal Boli. The analysis of fecal boli produced during the 6 minute cue test revealed a significant main effect of psychosocial stress, $F(1,29) = 33.55$, $p < .05$, but not of environment, $F(1,29) = 1.19$, $p > .05$. Results also indicated a significant

psychosocial stress x environment interaction, $F(1,29) = 20.03$, $p < .05$. Post hoc tests showed that animals in the home cage-psychosocial stress group produced significantly more boli compared to all other groups. Also, this stress-induced increase in boli was prevented with DSS, as evidenced by a significantly lower defecation level in the DSS-psychosocial stress group compared to home cage- no psychosocial stress controls (see Figure 4).

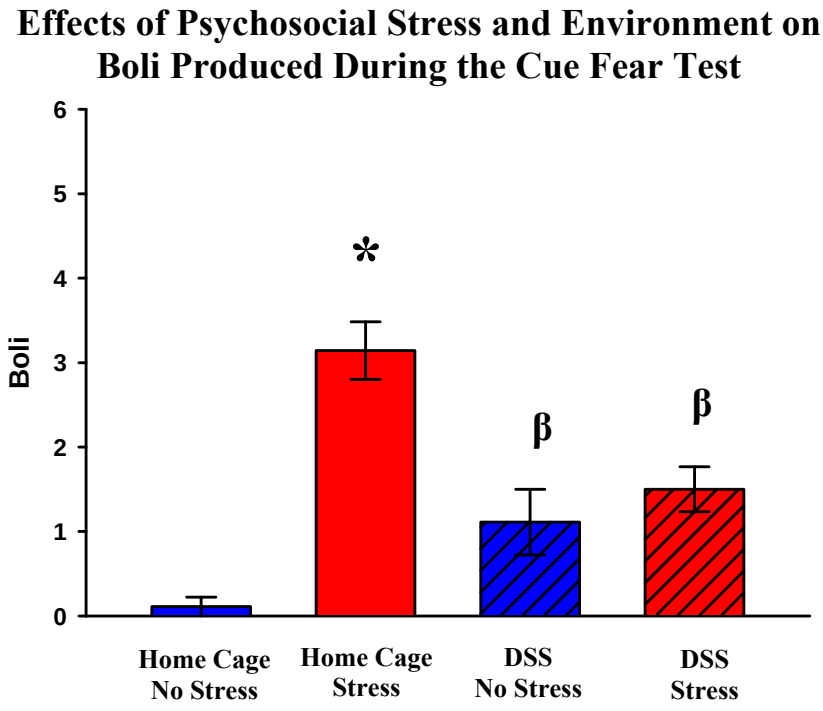

Figure 4. Effects of psychosocial stress and environment on fecal boli produced during the cue fear test. Data are presented as mean boli produced ± SEM. * = $p < .05$ relative to home cage-no psychosocial stress group; β = $p < .05$ relative to home cage-psychosocial stress group.

Elevated Plus Maze

Percent Time in Open Arms. The analysis of percent time spent in the open arms of the apparatus revealed a significant main effect of psychosocial stress, $F(1,28) = 5.86$, environment, $F(1,28) = 33.01$ (p values $< .05$), and no significant psychosocial stress x environment interaction, $F(1,28) = 1.34$, $p > .05$. Planned comparisons indicated that animals in the home cage- psychosocial stress group spent significantly less percentage of time in the open arms compared to the no psychosocial stress controls, $t(12) = 2.33$, $p < .05$ and that the stress-induced increase in anxiety-like behavior was prevented with DSS, $t(14) = -4.84$, $p < .05$ (see Figure 5).

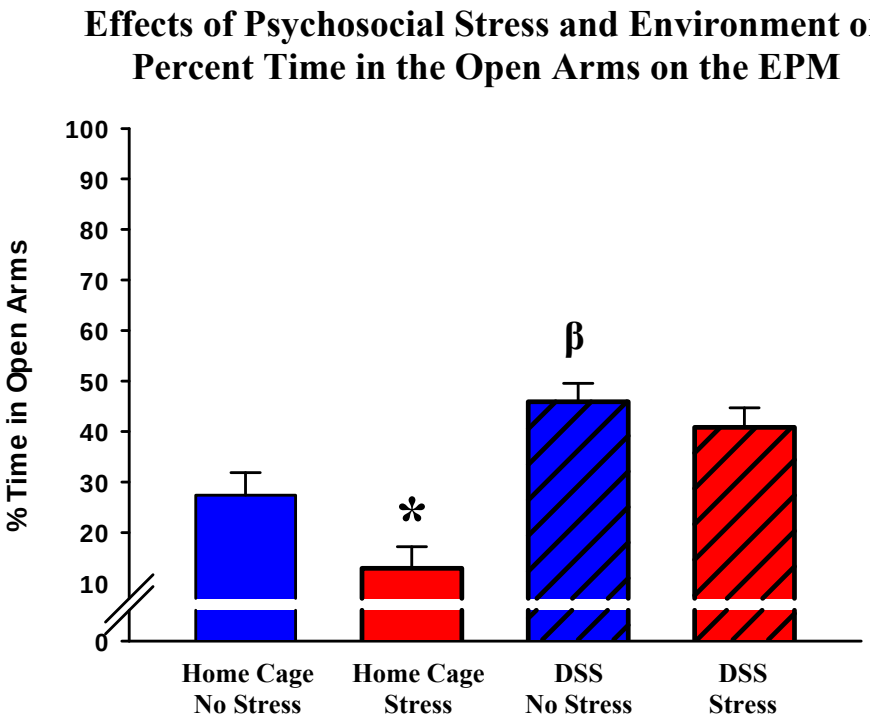

Figure 5. Effects of psychosocial stress and environment on percent time spent in the open arms of the elevated plus maze. Data are presented as mean percent time in open

arms ± SEM. * = p <.05 relative to all other groups. β = $p < .05$ relative to home cage-no psychosocial stress group.

Percent Time in Closed Arms. As shown in Figure 6, the analysis of percent time spent in the closed arms revealed significant main effects of both psychosocial stress, $F(1,30) = 9.23$, and environment, $F(1,30) = 29.34$ (p values < .05). There was no significant psychosocial stress x environment interaction, $F(1,30) = 2.92$, $p > .05$. A planned comparison showed that the home cage-psychosocial stress group spent a significantly greater percentage of time in the closed arms compared to the no psychosocial stress- home cage control group, $t(12) = -3.01$. In addition, this significant increase was prevented with DSS, as shown by the DSS-psychosocial stress group spending significantly less percentage of time in the closed arms compared to the home cage-psychosocial stress group, $t(15) = 4.99$ (p values < .05).

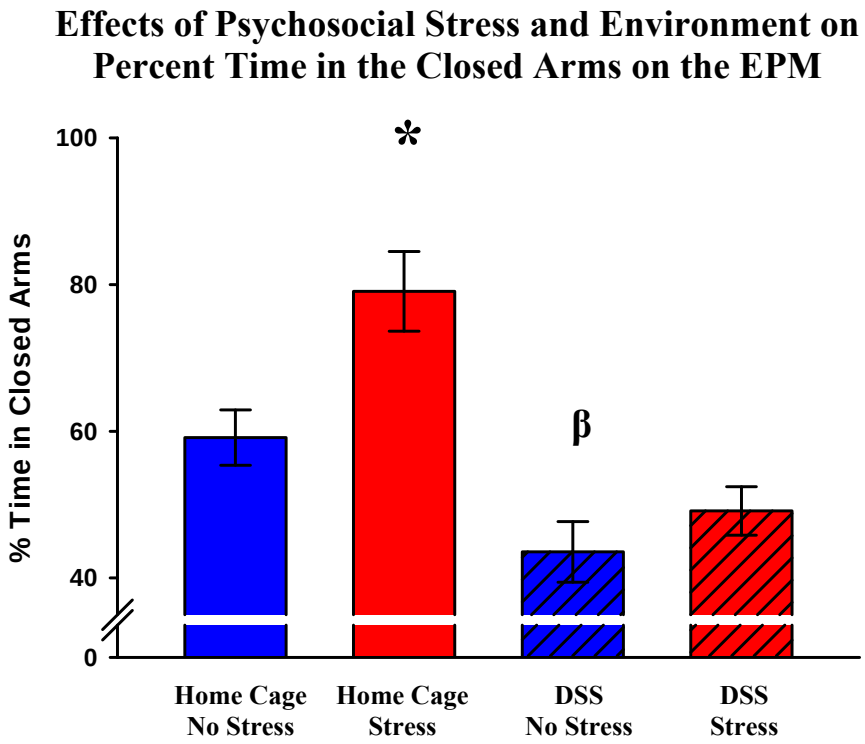

Figure 6. Effects of psychosocial stress and environment on percent time spent in the closed arms on the EPM. Data are presented as mean percent time in closed arms ± SEM. * = $p < .05$ relative to all other groups; β = $p < .05$ relative to home cage- no psychosocial stress.

Movement. Results assessing movement on the EPM revealed no significant main effect of psychosocial stress, $F(1,33) = .722$, $p > .05$. There was, however, a significant main effect of environment, $F(1,33) = 20.71$, $p < .05$, where both DSS groups exhibited significantly more ambulations, suggestive of increased motor activity on the EPM relative to controls. There was no significant psychosocial stress x environment interaction, $F(1,33) = 1.64$, $p < .05$. These findings suggest that DSS produced more movement relative to controls (see Figure 7).

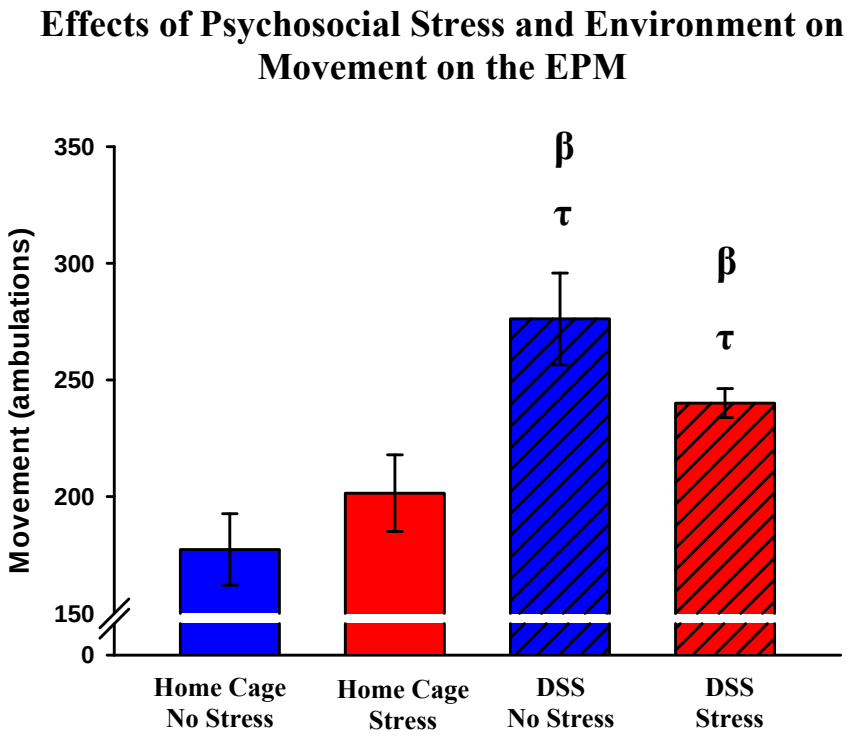

Figure 7. Effects of psychosocial stress and environment on ambulation made on the EPM. Data are presented as mean percent time in closed arms ± SEM. β = $p < .05$ relative to home cage-no psychosocial stress; τ = $p < .05$ relative to home cage psychosocial stress.

Velocity. In order to further investigate movement on the EPM, velocity calculations for each rat were made by dividing the total time spent in the open and closed arms by respective distances travelled. A one-way ANOVA was employed to analyze group differences on behavior on the open versus closed arms. Results did showed an overall significant ANOVA, $F(7,68) = .015$,but no significant within group differences, indicating that groups did not travel at significantly different rates when in

the open and closed arms. Findings did, however, indicate that both the home cage-psychosocial stress, and DSS-no psychosocial stress groups exhibited greater velocities on the open arms of the EPM relative to home cage controls. In addition, DSS seemed to increase velocity in the closed arms compared to home cage- no psychosocial stress controls (see Figure 8)

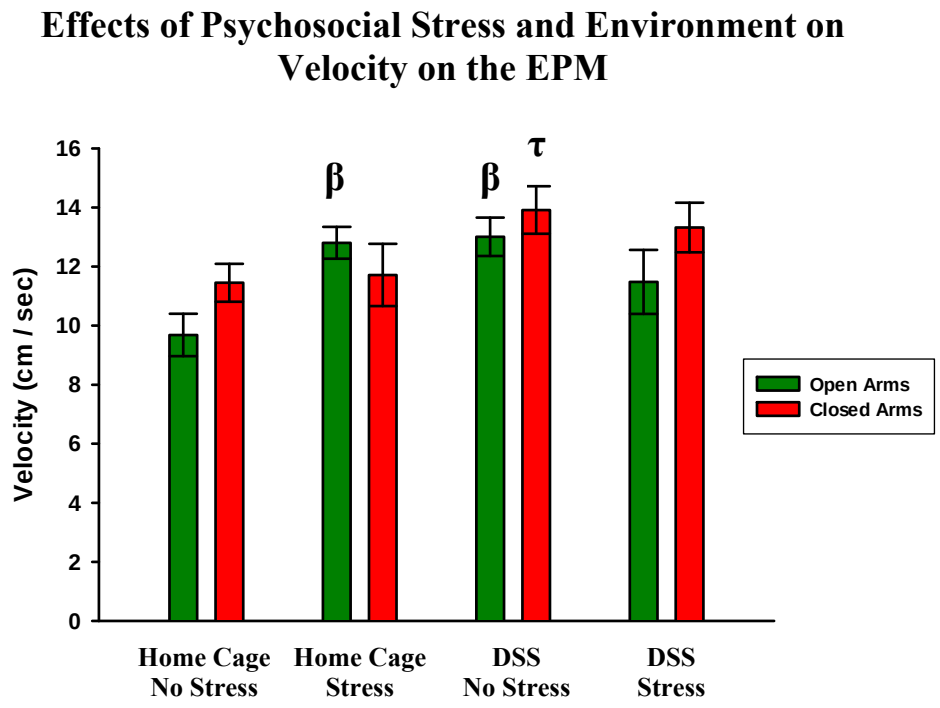

Figure 8. Effects of psychosocial stress and environment on velocity in the open and closed arms on the EPM. Data are presented as mean velocity ± SEM. β = $p < .05$ relative to home cage-no psychosocial stress, open arms; τ = $p < .05$ relative to home cage psychosocial stress, closed arms

Head Dips. Analysis of head dips exhibited during the entire 5 minute test on the apparatus revealed no significant main effect of psychosocial stress, $F(1,30) = .002$, $p < .05$, a significant main effect of environment, $F(1,30) = 35.33$, but no significant psychosocial stress x environment interaction, $F(1,30) = .374$ (p values > .05). These results indicate that both DSS groups exhibited significantly greater head dips on the EPM compared to the home cage groups. These behaviors provide another index which suggests that animals given DSS exposures were more exploratory than those in the home cage conditions (see Figure 9).

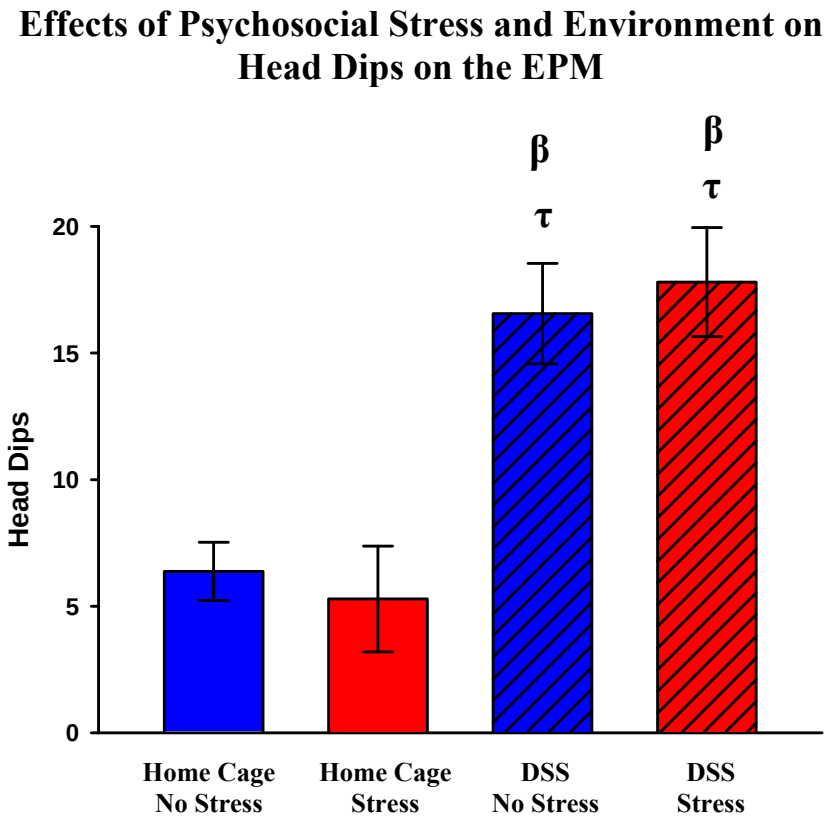

Figure 9. Effects of psychosocial stress and environment on head dips made on the EPM. Data are presented as mean head dips ± SEM. β = $p < .05$ relative to home cage- no psychosocial stress controls. τ = $p < .05$ relative to home cage- psychosocial stress group.

Startle Response

90dB Acoustic Stimuli. At the 90dB stimulus level of the startle response test, there were no significant main effects of psychosocial stress, $F(1,33) = .914$, environment, $F(1,33) = .790$, and no significant psychosocial stress x environment interaction, $F(1,33) = .843$ (p values > .05).

100dB Acoustic Stimuli. For the 100 dB stimulus analysis, there was no significant main effect of stress, $F(1,30) = .220$, a significant main effect of housing, $F(1,30) = .025$, and no significant psychosocial stress x environment interaction, $F(1,30) = 5.58$ (p values > .05).

110dB Acoustic Stimuli. Analysis of the 110 dB auditory stimulus of the startle response test revealed no significant main effect of psychosocial stress, $F(1,28) = 4.57$, a significant main effect of environment, $F(1,28) = 3.61$, $p < .05$, and a significant psychosocial stress x environment interaction, $F(1,28) = 12.76$ (p values < .05). Post hoc tests revealed that animals in the home cage- psychosocial stress group exhibited significantly greater startle responses relative to both home cage groups, but not the DSS-no psychosocial stress group. These data indicate that the psychosocial stress-induced increase in startle response was prevented with DSS, evidenced by animals in the DSS-

psychosocial stress group exhibiting significantly lower startle responses compared to the home cage- psychosocial stress group (see Figure 10).

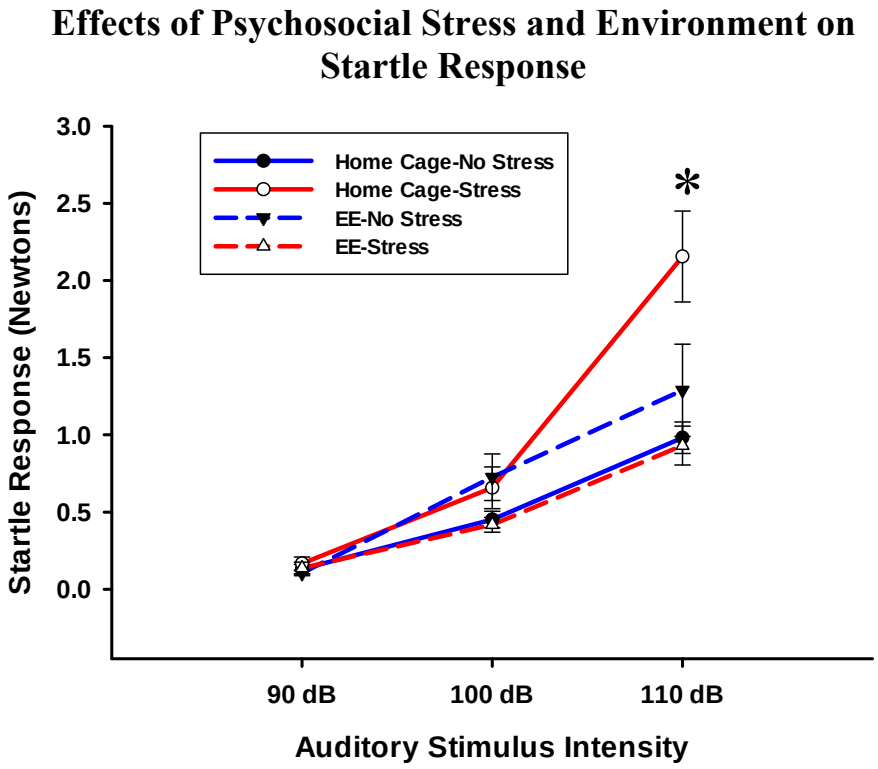

Figure 10. Effects of psychosocial stress and environment on startle response. Data are presented as the mean startle response (Newtons) to the 90, 100, and 110 dB acoustic stimuli ± SEM. * = $p < .05$ relative to all other groups at 110 dB stimulus intensity.

Novel Object Recognition

Ratio Time- 5 Minute Test. Statistical analysis of memory measured by a ratio time revealed no significant main effects of either psychosocial stress, $F(1,25) = .016$ or environment, $F(1,25) = .120$, and the psychosocial stress x environment interaction was also not significant, $F(1,25) = .504$ (p values > .05) indicating no significant differences with regards to the amount of time spent with the novel and familiar objects between groups during the 5 minute test (see Figure 11).

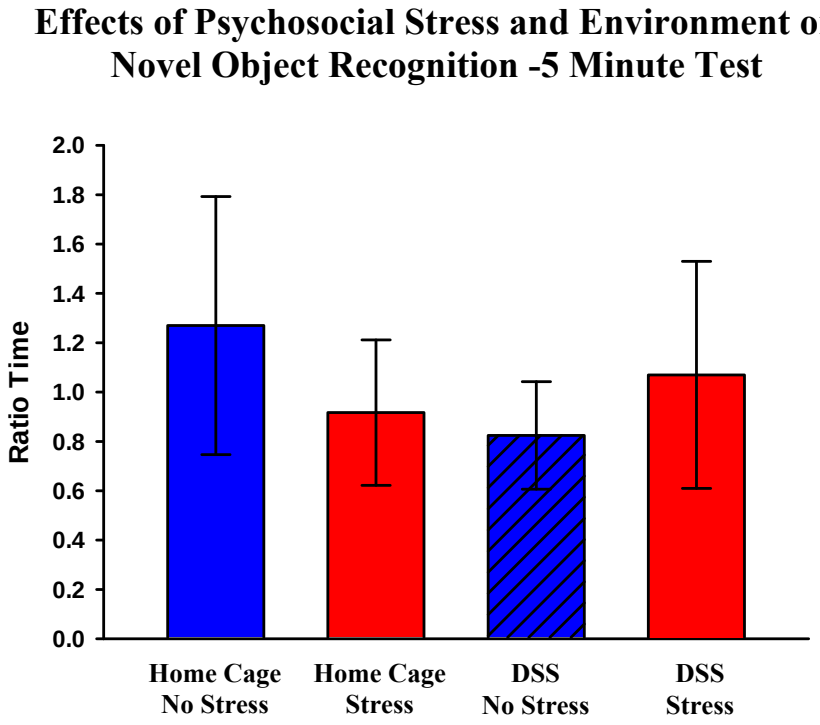

Figure 11. Effects of psychosocial stress and environment on memory, measured by a recognition index in the NOR task. Data are presented as the mean ratio time ± SEM.

Ratio Time- First Minute of Test. Similar to the 5 minute test results, analysis of ratio time during the first minute of the testing period also revealed no significant main

effects of either psychosocial stress, $F(1,25) = .190$ or environment, $F(1,25) = .015$, and no significant psychosocial stress x environment interaction, $F(1,25) = 1.35$ (p values > .05) (see Figure 12).

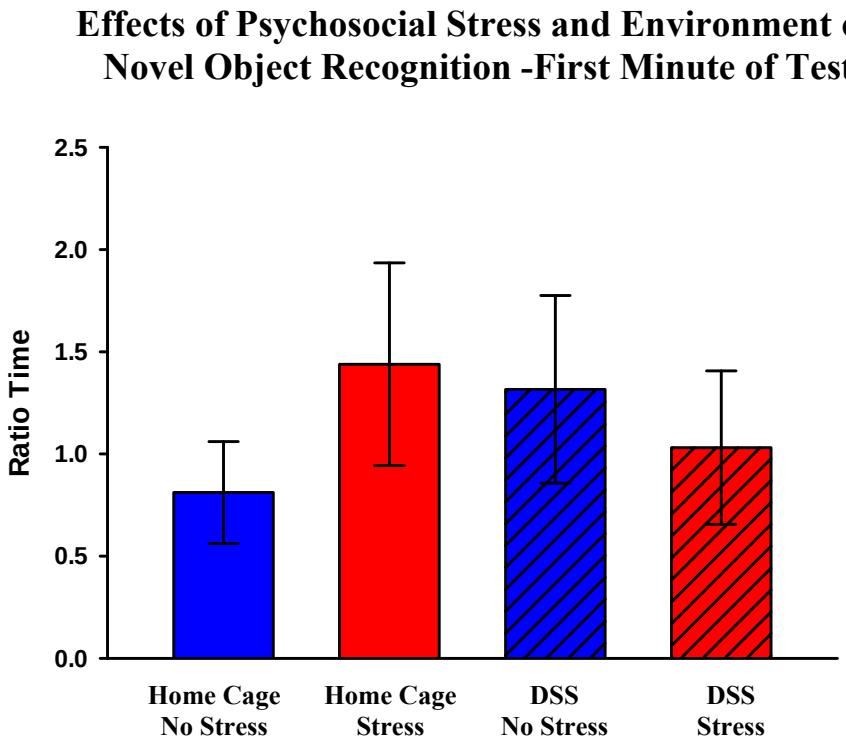

Figure 12. Effects of psychosocial stress and environment on memory, measured by ratio time in the NOR task during the first minute of the test. Data are presented as mean ratio time ± SEM.

Growth Rate

The analysis of growth rate revealed no significant main effects of psychosocial stress, $F(1,33) = .948$ or environment, $F(1,33) = .850$ (p values > .05), but there was a significant psychosocial stress x environment interaction, $F(1,33) = 9.45$, $p < .05$. Post hoc analyses of the data showed a significantly lower growth rate of the home cage-psychosocial stress group compared to non- psychosocially stressed controls. Also, this stress-induced reduction in growth rate was prevented with DSS. This was indicated by the DSS psychosocial stress group demonstrating a significantly higher growth rate compared to their home cage- psychosocially stressed controls. These data indicated that DSS blocked chronic stress-induced reductions in growth rate.

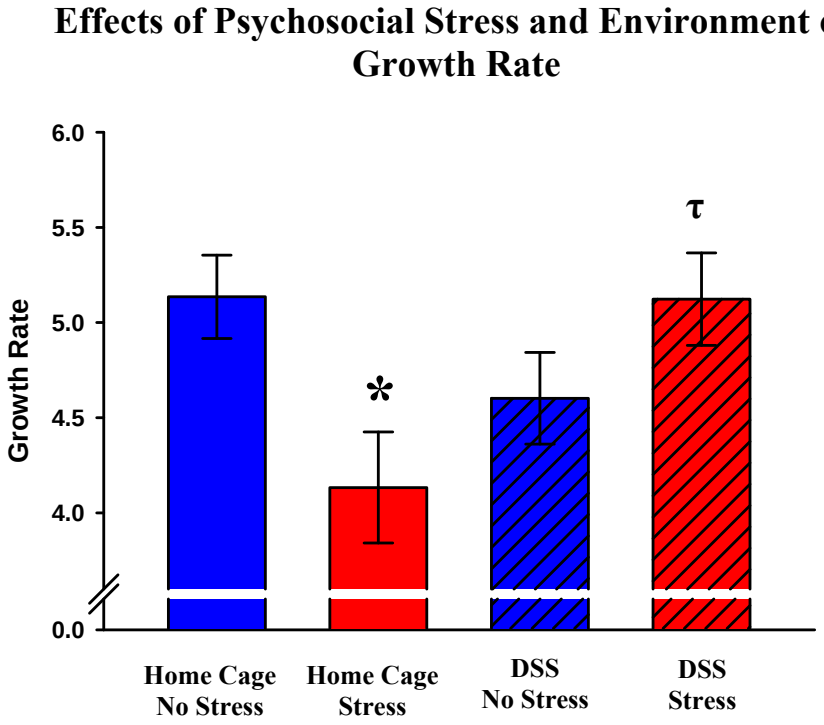

Figure 13. Effects of psychosocial stress and environment on growth rate. Data are presented as mean growth rate ± SEM. * = $p < .05$ relative to home cage- no psychosocial stress controls. τ = $p < .05$ relative to home cage-psychosocial stress group.

Cardiovascular Testing

Heart Rate. Analysis of between group effects on heart rate revealed no significant main effects of psychosocial stress, $F(1,21) = 1.25$, environment, $F(1,21) = .390$, and no significant psychosocial stress x environment interaction, $F(1,21) = .082$ (p values > .05) indicating no significant differences between groups on heart rate (see Figure 14).

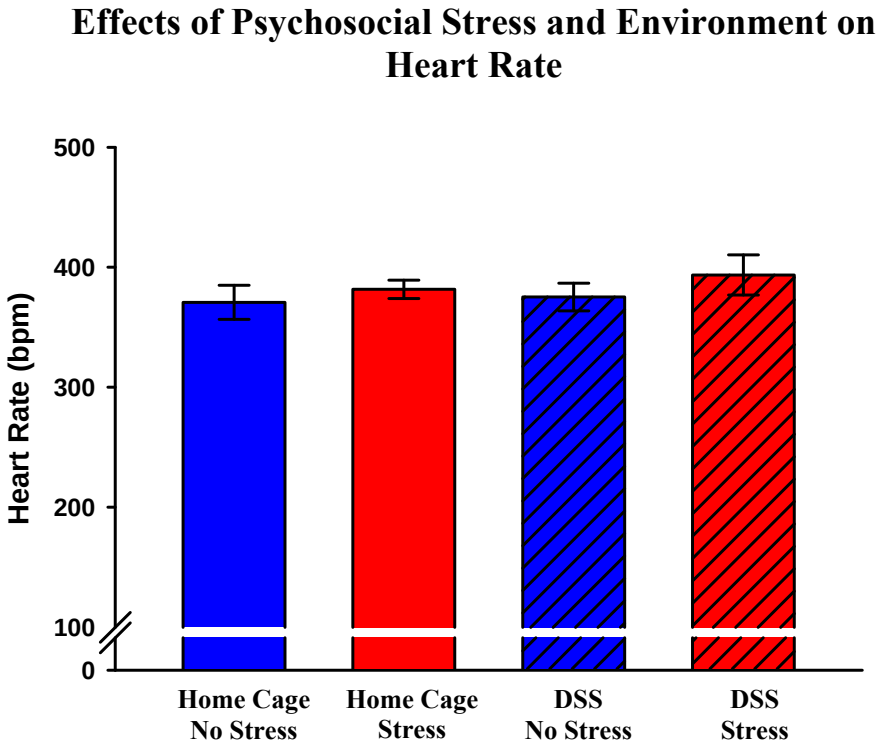

Figure 14. Effects of psychosocial stress and environment on heart rate. Data are expressed as mean heart rate (bpm) ± SEM.

Systolic Blood Pressure. Systolic blood pressure level analysis produced no significant main effects of psychosocial stress, F (1,21) = .693, environment, F (1,21) = .045, and no significant psychosocial stress x environment interaction, F (1,21) = .000 (p values > .05) indicating no significant differences between groups on systolic blood pressure (see Figure 15).

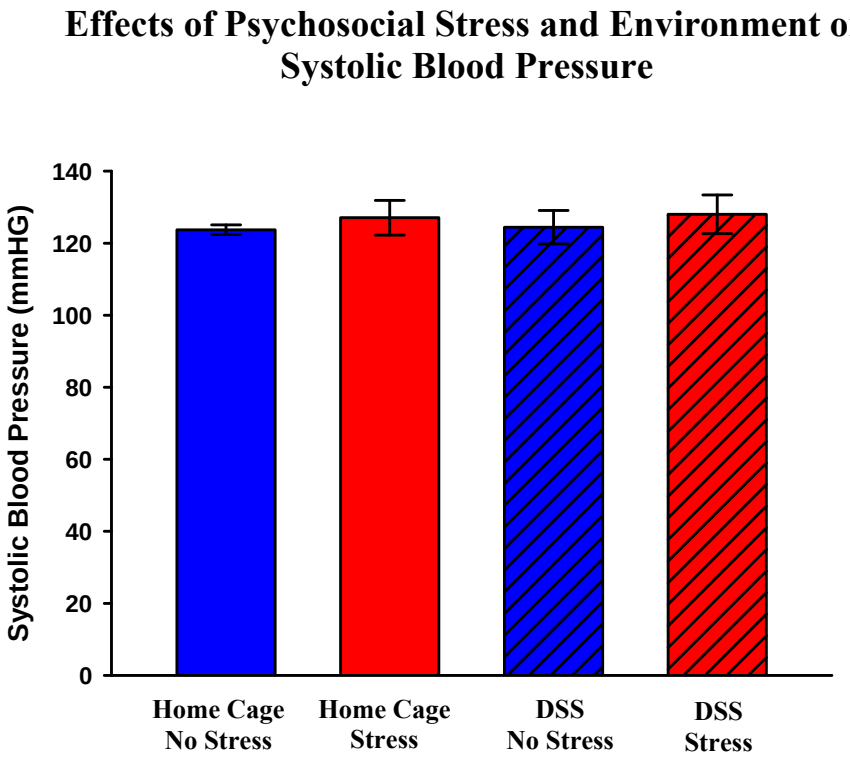

Figure 15. Effects of psychosocial stress and environment on systolic blood pressure. Data are presented as mean blood pressure (mmHg).

Diastolic Blood Pressure. For the analysis of diastolic blood pressure, there was no significant main effects of psychosocial stress, F (1,21) = .044, environment, F (1,21) = .141, and no significant psychosocial stress x environment interaction, F (1,21) = .427 (p values > .05) indicating no significant differences between groups on diastolic blood pressure (see Figure 16).

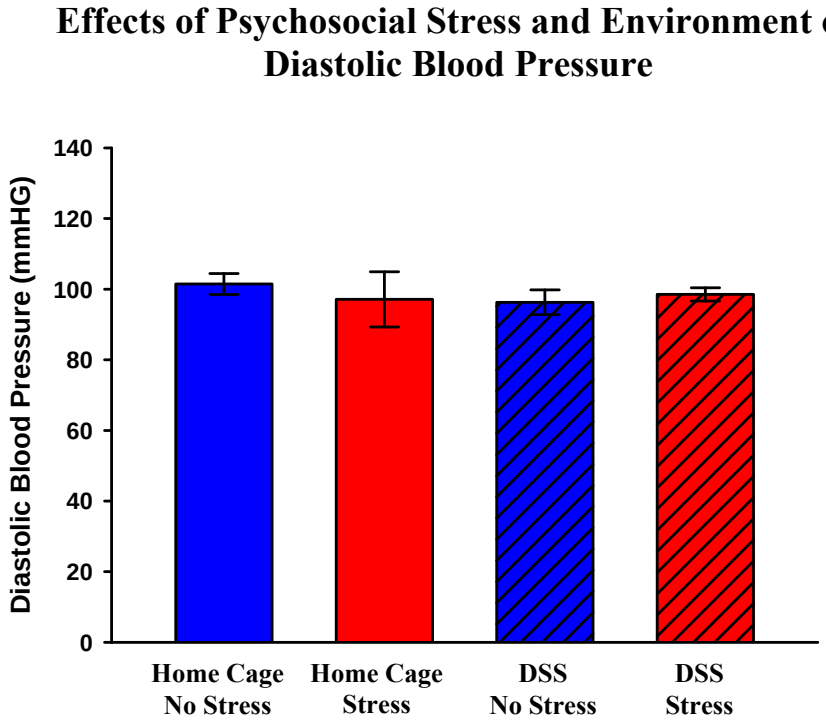

Figure 16. Effects ot psychosocial stress and environment on diastolic blood pressure. Data are presented as mean blood pressure (mmHg).

Organ Weights

Adrenal Gland. Analysis of adrenal weights (see Figure 17), expressed as milligram per 100g body weight, revealed a marginally significant main effect of psychosocial stress, $F(1,31) = 3.68$, $p = .07$, a significant main effect of environment, $F(1,31) = 5.95$, $p < .05$, indicating that the DSS groups had significantly larger adrenal glands than animals in the home cage groups, This result confirms previous findings that stimulating environments produces larger adrenals (Bakos, Duncko, Makatsori, Pirnik, Kiss & Jezova, 2006) compared to animals housed under standard conditions. There was no significant psychosocial stress x environment interaction, $F(1,31) = 2.91$, $p > .05$. A planned comparison performed based on predictions made *a priori* revealed that the home cage- psychosocial stress group had significantly heavier adrenal glands compared to the home cage- no psychosocial stress control group, $t(14) = -4.13$, $p < .01$, confirming previous findings of work by our group which utilized a similar chronic psychosocial stress paradigm (Zoladz et al, 2008).

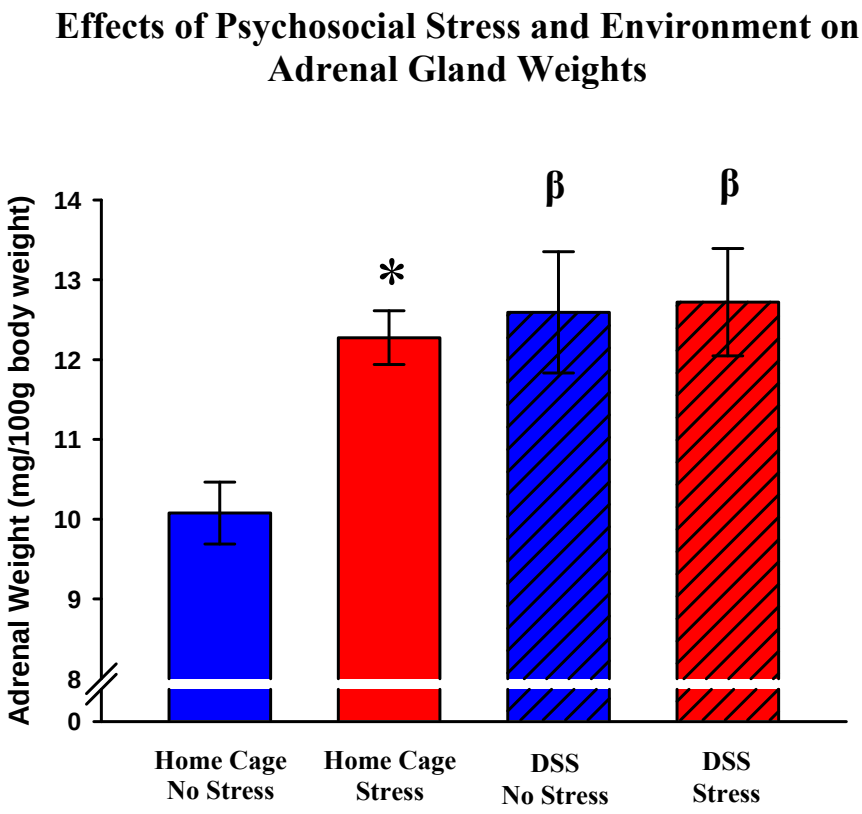

Figure 17. Effects of psychosocial stress and environment on adrenal gland weights. Data are presented as mean adrenal weight (mg / 100g body weight). * = $p < .05$ relative to home cage- no psychosocial stress group (planned comparison). β = $p < .05$ relative to home cage-no psychosocial stress controls (ANOVA main effect of environment).

Thymus Gland. For the analysis of thymus glands (see Figure 18), there were significant main effects of psychosocial stress, F (1,28) = 5.96, and environment, F (1,28) = 7.54 (p values < .05). There was no significant psychosocial stress x environment interaction, F (1,28) = 1.03, $p > .05$. Interestingly, these data indicated that both stress

and DSS produced smaller thymus glands compared to no stress-home cage controls. A planned comparison also revealed that the home cage- psychosocial stress group had lower thymus weights compared to their no psychosocial stress control animals, $t(14) = 2.39$, $p < .05$, confirming previous findings of work by our group which utilized a similar chronic psychosocial stress paradigm (Zoladz et al, 2008).

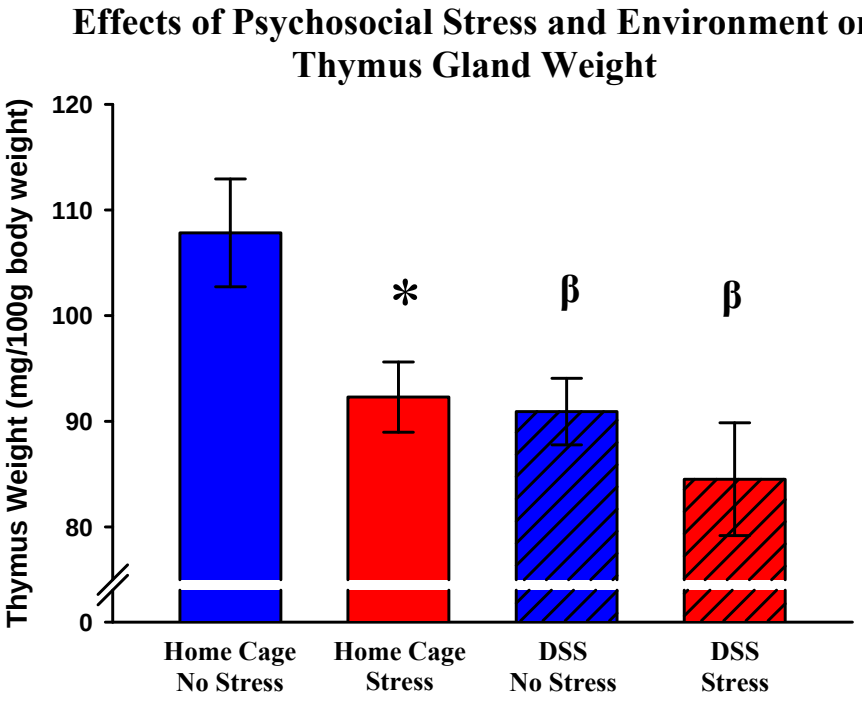

Figure 18. Effects of psychosocial stress and environment on thymus gland weight. Data are presented as mean thymus weight (mg / 100g body weight) ± SEM. * = $p < .05$ relative to home cage- no psychosocial stress group (planned comparison). β = $p < .05$ relative to home cage- no psychosocial stress controls (ANOVA main effect)

Kidney. Analysis of between group effects of kidney weights revealed no significant main effects of either psychosocial stress, $F(1,33) = .646$, environment, $F(1,33) = .086$, and no significant psychosocial stress x environment interaction, $F(1,33) = .398$ (p values > .05) (see Figure 19).

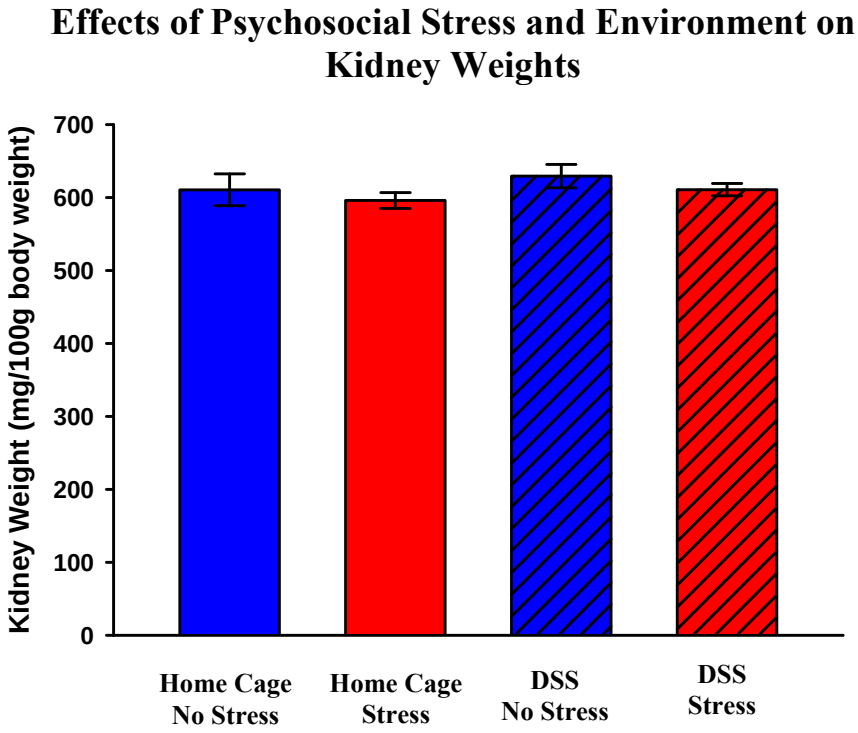

Figure 19. Effects of psychosocial stress and environment on kidney weights. Data are expressed as mean kidney weight (mg / 100g body weight).

Heart. For the analysis of heart weights, there was no significant main effect of psychosocial stress, $F(1,32) = .989$, $p > .05$. There was, however, a significant main effect of environment indicating that, compared to home cage-no psychosocial stress controls, animals in the DSS groups had significantly heavier hearts, $F(1,32) = 4.79$, $p < .05$. There was no significant psychosocial stress x environment interaction, $F(1,32) = 1.43$, $p > .05$ (see Figure 20).

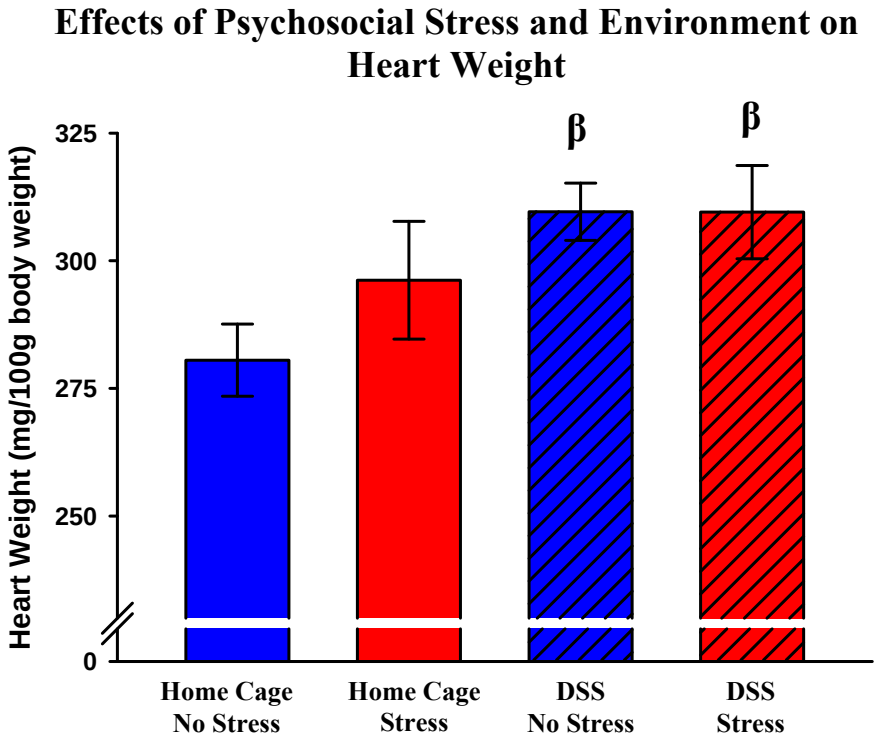

Figure 20. Effects of psychosocial stress and environment on heart weight. Data are expressed as mean heart weight (mg / 100g body weight). β = $p < .05$ relative to home cage- no psychosocial stress control group.

A summary of the above results is shown in the table below. The table includes initial hypotheses of each behavioral and physiological measure, as well as the actual outcome on those measures at testing. An indication as to whether initial hypotheses were satisfied or not with regards to each measure is also given.

Table 1: Measures, Hypotheses, and Outcomes

Behavioral/ Physiological Measure	**Initial Hypothesis**	**Hypothesis Met?**
Contextual Fear Memory	Stress would increase % time immobile to context relative to controls, blocked by DSS	NO
Boli During Context Test	Stress would increase boli production relative to controls, blocked by DSS	NO
Cue Fear Memory	Stress would increase % time immobile to cue relative to controls, blocked by DSS	YES
Boli During Cue Test	Stress would increase boli production relative to controls, blocked by DSS	NO
Anxiety: % time in open arms	Stress would decrease % time spent in open arms of EPM relative to controls, blocked by DSS	YES
Anxiety: % time in closed arms	Stress would increase % time spent in closed arms of EPM relative to controls, blocked by DSS	YES
Ambulations	DSS would increase ambulations relative to controls	YES
Head Dips	DSS would increase Head Dips on the EPM relative to controls	YES
Startle Response	Stress would increase acoustic startle response relative to controls, blocked by DSS	YES @ 110dB
Novel Object Recognition	Stress would decrease time spent with novel object	NO

	relative to controls, blocked by DSS	
Growth Rate	Stress would decrease growth rate relative to controls, blocked by DSS	YES
Heart Rate	No predictions made	
Blood Pressure	No predictions made	
Adrenal Gland Weight	Stress would increase adrenal gland weight, blocked with DSS	NO
Thymus Gland Weight	Stress would decrease thymus gland weight, blocked with DSS	NO
Kidney Weights	Stress would increase kidney weights, blocked with DSS	NO

Discussion

Summary of Major Findings

The major findings of this study showed that providing animals with 2 hours of daily DSS prevented the development of several behavioral and physiological changes analogous to those observed in patients with PTSD. Data obtained from behavioral testing indicated that chronic psychosocial stress produced robust increases in cue fear memory, anxiety, startle responses, and recognition memory, replicating earlier findings from our group utilizing a similar chronic psychosocial stress paradigm (Zoladz et al, 2008). Additionally, DSS prevented all of these psychosocial stress-induced changes in behavior, suggesting that these environmental manipulations were effective in mitigating PTSD-like behavioral outcomes, similar to those found in preliminary work (Seetharaman, Zoladz & Diamond, 2008). Results also revealed that groups subjected to

the chronic psychosocial stress paradigm exhibited physiological changes similar to those observed in previous findings by our group (Zoladz et al, 2008), and others (Magarinos & McEwen, 1995; Vyas et al, 2002) with regards to a significant chronic stress-induced enlargement of adrenal gland weights. Specifically, psychosocially stressed animals were found to have enlarged adrenal glands, which secrete CORT, a main physiological marker of stress relative to non-psychosocial stress controls. Relative to control animals, psychosocially stressed animals also exhibited significantly smaller thymus glands, indicative of inhibited immune function, also observed in previous chronic stress work (Magarinos & McEwen, 1995; Zoladz et al, 2008). Interestingly, data suggested that DSS and stress may produce similar physiological outcomes. For instance, both psychosocial stress and DSS animals had enlarged adrenal glands. Some work has shown that animals provided with environmental enrichment exhibit a significant enlargement of adrenal (Bakos et al, 2006) and reduction of thymus gland weight (Tsai,Pachowsky,Stelzer & Hackbarth, 2002) relative to those housed under standard conditions. Also, similar to psychosocial stress, DSS groups exhibited smaller thymus glands relative to controls, complementing previous reports (Tsai et al, 2002). These findings suggest that stress and DSS may produce physiological outcomes, but differ when it comes to behavioral measures associated with PTSD. That is, this study suggests that, although DSS and stress produce similar physiological profiles, they may be beneficial and detrimental, respectively, in terms of the brain as it relates to behavior. Examining the possible neurobiological and neurochemical mechanisms underlying the efficacy of DSS in preventing the onset of PTSD-like behavioral and physiological

changes seen in this study may provide insight into identifying risk factors of and non-pharmacological treatments for PTSD, and possible directions for future experimental research in this area.

Possible Mechanisms of Action Underlying Findings

Blunted Activation of Stress-Response System. Stress induces the activation of the hypothalamic-pituitary-adrenal (HPA) axis. In this system, CORT is secreted by the adrenal cortex, and is elevated under stress. When CORT is released into the bloodstream, it participates in a negative feedback loop on the HPA axis by binding to glucocorticoid receptors (GRs). This negative feedback mechanism attempts to restore homeostasis in an organism. Previous work has shown that EE produces morphological changes relating to the HPA axis. Specifically, some findings indicated that EE resulted in an up regulation of GR mRNA expression in the hippocampus (Mohammed, Henrikkson, Soderstrom, Ebendal, Olsson & Seckl, 1994; Olsson, Mohammed, Donaldson, Henriksson & Seckl, 1994). An up regulation of GR receptors produced by environmental stimulation may serve to facilitate a more efficient negative feedback control, thereby preventing elevations in CORT (Olsson et al, 1994). This theory is supported by evidence indicating significantly higher baseline CORT levels in mice given EE for 6 weeks but did not significant increases after re-exposure to the context where they received shock (Benaroya-Milshtein et al, 2004) relative to standard housing controls. This finding suggests that EE may produce elevations in basal CORT, but these animals are not as reactive to stress compared to those under standard housing conditions.

Some human work supporting this theory showed that social support suppressed CORT responses to psychological stressors, as well as significantly decreasing cardiovascular and corticosteroid responses to laboratory stressors (DeVries et al, 2003). Intriguingly, an examination of the PTSD literature reveals that patients with the disorder exhibit similar changes. A majority of research indicates that PTSD patients express an increased number of GRs (Rohleder, Love & Bennet, 2004; Stein**,** Yehuda, Koverola & Hanna, 1997b; Yehuda, Boisoneau, Lowy & Giller, 1995), indicative of enhanced negative feedback. These changes observed in PTSD patients correspond to a blunted HPA axis indicated by attenuated CORT levels shortly after trauma, such as in victims of rape (Resnick, Yehuda, Pitman & Foy, 1995), and motor vehicle accidents (Raimonde & Spoonster, 2000). This apparent paradox between observations in PTSD and social stimulation lends itself to the suggestion that diverse environmental experiences, through the repeated introduction of novelty and opportunities to explore, may act as mild chronic stressors. In this fashion, organisms may be better "prepared" for future severe stressors and, in turn, exhibit a reduction of stress-induced responses in behavior (Fox et al, 2006). More work is needed in order to elucidate the differential outcomes of environmental stimulation and chronic stress, including their interactions with the development of PTSD, but may be an underlying mechanism of the behavioral and physiological findings of this study.

Prefrontal Cortex: Extinction Learning and Suppression of Fear-Like Responses.

Neurobiological Changes in the Prefrontal Cortex. Findings of this study indicated that psychosocially stressed animals demonstrated robust fear memory when exposed to the cue temporally associated with the two cat exposures, replicating previous findings by our group utilizing a similar chronic stress paradigm (Zoladz et al, 2008). There were, however, no significant differences between groups upon re-exposure to the context temporally associated with the acute stress sessions. It is important to note that, for the duration of the entire study, *all* groups were transported from the housing room into the laboratory for two hour periods. The difference between groups, with regards to daily manipulations, was that, after transportation from the housing room into the laboratory, the DSS groups were placed in the stimulating apparatus, while the control animals remained in their home cages during this time. Since there were no significant differences between groups in immobility during the contextual fear memory test, there may have been a degradation of the context-cat association over time due to the continuous re-exposure of the psychosocially stressed animals into the laboratory, where the box was located. In addition to the actual box itself, there may have been an association created between the room containing the box and the subsequent cat exposures during fear conditioning training. Upon continuous transportation to the laboratory, which contained the box, there was an extinction of the box-cat association in the psychosocial stress group. In other words, the actual daily transportation of psychosocially stressed animals into the laboratory, in theory, served as extinction trials, in which the association between the conditioned stimulus (box) and the unconditioned stimulus (cat) was, over time, degraded due to extinction-based learning. Previous work

has shown that, over time, re-exposing animals to the CS without presentation of the US extinguishes the conditioned response (Myers & Davis, 2007) which, in this case, was measured by immobility in the box, suggestive of fear. Due to the enhanced ability of DSS to extinguish contextual fear, there were no significant differences in immobility between groups when re-exposed to the training context during testing. There are certain brain structures which play a role in extinction learning. Specifically, The prefrontal cortex (PFC), a frontal lobe structure involved in cognitive flexibility, decision making, and overall executive controls, has also been identified as being part of the neural circuitry of extinction learning. This is evidenced by work showing that damage to the medial prefrontal cortex (mPFC) interferes with the extinction of conditioned fear responses (Lebron, Milad & Quirk, 2004; Morgan, Romanski & LeDoux, 1993; Quirk, Garcia & Gonzalez-Lima, 2006).

In contrast to the contextual fear memory findings, the psychosocial stress demonstrated robust immobility to the cue (tone) temporally associated with the cat exposures relative to home cage- no psychosocial stress controls. Additionally, this increase in fear-related behavior was prevented with DSS, indicated by the DSS-psychosocial stress group being significantly less immobile during the cue relative to home cage-psychosocial stress. These behavioral findings showed that re-exposing psychosocially stresses animals to the cue temporally associated with the cat exposures was effective in producing robust increases in fear to no psychosocial stress controls. The robust cue-based memory demonstrated by the psychosocially stressed animals is indicative of this memory not being subjected to degradation over time since the cue was,

in theory, more salient than the contextual information close in temporal proximity to the cat exposures. The daily transportation into the laboratory may have not reactivated the memory of the cue-cat association as readily as the context. Thus, at testing, the presentation of the cue acted as a more specific, salient reminder of the cat exposures which, in turn, produced the observed behavioral increases in fear. Findings of the cue fear test also revealed that the robust increase in immobility in the psychosocially stressed animals was prevented with DSS, indicated by significantly lower immobility in the DSS-psychosocial stress group relative to their home cage counterparts. The differential expressions of fear-related behavior may be explained by changes occurring in the brain under stress and DSS conditions. Some work has shown dendritic hypertrophy in the amygdala in animals subjected to chronic stress (Vyas et al, 2002), suggestive of enhanced neuronal processing in this structure. Cue-based fear conditioning is predominantly governed by the amygdala relative to the hippocampus, as lesions to the amygdala interfere with the conditioning of fear responses to the cue and context associated with shock, but lesions to the hippocampus only interfere with contextual fear conditioning (Phillips & LeDoux, 1992). The finding, therefore, that chronic psychosocial stress produced robust cue fear conditioning in the current study is not surprising. Some work has suggested that the effects of environmental stimulation may be governed by neurobiological and neurochemical modifications in the functioning of the prefrontal cortex PFC. Examination of studies illustrating such modifications may explain the finding that DSS was effective in preventing the psychosocial-stress induced increase in cue fear. Specifically, some findings indicated that, relative to standard

housing, rats given 3 months of enrichment demonstrated significant increases in dendritic spine density in the mPFC, indicating enhanced neuronal connectivity and enhanced processing of this brain structure (Kolb, Gorny, Soderpalm, & Robinson, 2003). The mPFC has direct projections to the amygdala, which governs emotional behaviors, including in response to stress (McDonald, Mascagni & Guo, 1996). Studies examining the interactions between the two structures have suggested that the mPFC exerts inhibitory projections on the amygdala, such that the stimulation of the mPFC inhibits the demonstration of robust fear-related responses (Zbrozyna & Westwood, 1991; Quirk, Garcia & Gonzalez-Lima, 2006), governed predominantly by the amygdala. In contrast, selective lesions of the mPFC seem to disinhibit amygdala activation, resulting in a perseveration of affective behaviors (Morgan & LeDoux, 1995). Other work also indicates that rats exposed to chronic restraint stress exhibit significant shortening of dendrites in the mPFC in conjunction with dendritic hypertrophy in the amygdala, suggestive of a relative impairment of the mPFC to suppress the response of the amygdala and, in turn, behavioral responses to stress activation (Radley, Sisti, Hao, Rocher, McCall, Hof, McEwen & Morrison, 2004). These studies suggest that, in theory, SES enhanced mPFC inhibitory projections onto the amygdala which, in turn, prevented the expression of cue fear-related behaviors in psychosocially stressed animals given daily SES in the current study. Additionally, enhanced mPFC processing relative to standard housing may have also facilitated increased capabilities to extinguish contextual fear.

Neurochemical Changes in the Prefrontal Cortex. Some recent work has examined neurochemical changes in the PFC which may underlie the actions of environmental stimulation on fear memory. Specifically, EE for 6, 12, and 24 months prevented handling stress-induced increases in dopamine (DA) levels in the PFC compared to standard housing (Segovia, Del Arco, de Blas, Garrido & Mora, 2008). In addition, animals under enriched conditions have been found to express a significant decrease in the functioning of the dopamine transporter in the medial pre-frontal cortex (mPFC) **(**Zhu, Apparsundaram, Bardo & Dwoskin, 2004), and decreased expression of dopamine receptors in the PFC (Del Arco, Segovia, Canales, Garrido, de Blas, Garcia-Verdugo & Mora, 2007) relative to isolated animals. Such examples of enrichment-induced decreases in DA functioning in the mPFC may reflect a desensitization of the brain to stress, as, relative to controls, DA levels are significantly increased in the mPFC in rats exposed to stressors such as intermittent tail shock (Abercrombie, Keefe, DiFrischia & Zigmond, 1989), 30 minutes of tail pressure (Finlay, Zigmond & Abercrombie, 1994), and repeated foot shock (Meiergard, Schenk & Sorg, 1997). It may be that environmental stimulation acts as a form of mild stress through repeated exposures of animals to novelty (Fox et al, 2006; Stairs & Bardo, 2009) which desensitizes the brain to more severe stress and, in turn, ameliorates stress-induced increases in behavior relating to fear and anxiety upon exposure to more severe stressors, as observed in the current study. Decreased dopaminergic functioning in the mPFC of enriched animals, therefore, may be an underlying mechanism which prevents the expression of deleterious stress-induced behavioral changes.

These enrichment-induced neurobiological and neurochemical changes in the mPFC may be candidates for further investigation into the possible mechanisms underlying the effectiveness of DSS in preventing the psychosocial-stress induced increases in cue fear memory observed in the current study. This fits well with the theory that enriched animals seem to, behaviorally speaking, cope better under stressful situations (Fox et al, 2006; Sale, Berardi, & Maffei, 2009). Inhibitory projections of the PFC onto the amygdala may serve to explain several behavioral outcomes of this study. That is, the observation that DSS was effective in preventing, not only psychosocial stress-induced increases in cue fear memory, but also increased startle responses, heightened anxiety, and impaired recognition memory, may all be explained by the enhancement of PFC inhibitory actions on the amygdala.

Conditioned Fear & PTSD

Examining social and environmental factors which facilitate the suppression of conditioned fear responses may be clinically applicable in the treatment of PTSD, where conditioned responses to reminders of patients' trauma are thought to be compromised (Quirk et al, 2006; Yehuda & LeDoux, 2007). There is some evidence in the human PTSD work which shows patients to demonstrate attenuated activation of the mPFC in response to personalized trauma scripts (Shin, McNally, Kosslyn, Thompson, Rauch Alpert et al, 1999; Yehuda & LeDoux, 2007) or combat sounds (Bremner, Staib, Kaloupek, Southwick, Soufer & Charney, 1999; Yehuda & LeDoux, 2007), which may underlie their inability to suppress fear to cues associated with their trauma due to the

suppressed ability of the mPFC to inhibit these amygdala-mediated responses. Enhanced DSS-induced neural processing capabilities of the mPFC could, not only have played a role in facilitating the extinction of contextual fear, but also suppressed the robust increase in psychosocial stress-induced cue fear observed in this study. There are, however, other brain structures that may play a role with regards to the effectiveness of DSS to suppress psychosocial stress-induced increases in anxiety and enhance risk-taking, and exploratory-like behaviors observed in this study.

Nucleus Accumbens: Decreased Anxiety and Increased Exploratory-Like Behavior

Behavioral outcomes of this study showed that psychosocially stressed rats exhibited heightened anxiety, evidenced by significantly less time spent in the open arms of the EPM relative to control animals. This psychosocial stress-induced elevation in anxiety was prevented with DSS. Findings also revealed that animals in the DSS groups exhibited significantly more head dips on the EPM relative to the home cage groups. These behaviors are indicative of greater risk-taking, or exploratory-like behaviors as they subject the animal, in theory, to greater danger in that objects located over the edges of the apparatus are unknown. The nucleus accumbens (NAcc), a brain structure found in the ventral striatum, has been identified as governing the regulation of behaviors related to risk-taking, anxiety, and exploratory-like behaviors, shown in studies, for instance, utilizing animal models of drug abuse (Robinson & Kolb, 1997; Robinson, Gorny, Mitton & Kolb, 2000). Interestingly, similar to drug abuse, environmental stimulation increases dendritic growth in the NAcc relative to standard housing (Kolb et al, 2003), suggesting

enhanced neuronal connectivity and efficiency in functioning of this brain structure. This suggests that, in addition to the PFC, increased neuronal processing in the NAcc may have played a role in the anxiolytic and exploration facilitating effects on behavior which DSS seemed to exert in this study. In the current study, psychosocially stressed animals demonstrated heightened anxiety, which was prevented with DSS. Examinations of changes in the NAcc may in future studies may be of use in identifying possible mechanisms responsible for behavioral measures of anxiety and exploratory-like behavior observed in this study.

Hippocampus: Recognition Memory

In this study, animals were tested for hippocampus-dependent memory in a novel object recognition test. Rats which spent more time with the novel versus the familiar object was taken as an indication of memory for the familiar object. Findings did not reveal, however, any significant differences between groups on this test. An examination of the literature reveals that, relative to standard housing, enriched environments produce significant changes in the hippocampus of animals shown by elevations in physiological correlates of memory including LTP (Duffy et al, 2001), neurogenesis (Van Praag et al, 2000), neurotrophic growth factors (Ickes et al, 2000) and morphological changes, such as enhanced dendritic growth (Faherty et al, 2003; Leggio et al, 2003). The majority of studies in this field provide animals with enriched environments on a twenty-four hour basis. Since, in this study, rats were only given DSS for 2 hours daily, it can be posited that perhaps this time period was not long enough to produce significant changes in

hippocampal morphology affecting memory processing. As a result, this could explain why there were no differences between groups on the NOR hippocampus-dependent task.

Antidepressant-Like Changes in the Brain

There is some evidence which indicates that environmental stimulation may exert its influence by inducing antidepressant-like changes in the brain. Research supports the observation that SES animals were more exploratory and risk-seeking relative to animals housed under standard conditions. Specifically, rats housed under enriched conditions for 30 days expressed significantly higher levels of serotonin receptor (5-HT1A) mRNA in the hippocampus (Rasmuson, Olsson, Henrikkson, Kelly, Holmes, Seckl & Mohammed, 1998), similar to studies examining the effects of anti-depressant administration on 5-HT1A activity. This suggests a possible involvement of the serotoninergic system in the behavioral effects observed in some enrichment studies relating to anxiety and exploration. One study, for instance, showed that chronic treatment with the antidepressant buspirone for 3 weeks significantly increased 5-HT1A mRNA levels in the dentate gyrus of the hippocampus in rats relative to vehicle-treated animals (Chen, Zhang, Rubinow & Chuang, 1995). These studies are complemented by human work indicating that buspirone administration is effective in the treatment of patients with anxiety (Eison & Temple, 1986). The anxiolytic effects of this 5-HT1A agonist were also illustrated in a study showing that rats given intra-hippocampal administrations of buspirone spent significantly more time in the open arms of an EPM relative to controls, indicating an anxiolytic effect of the drug (Kostowski, Plaznik, & Stefanski, 1989). Interestingly, these studies indicate that there may be similar

neurobiological changes produced by such antidepressants and DSS. Examining the similarities between antidepressants and DSS on the brain and behavior may be useful in identifying possible mechanisms underlying DSS, and its ability to prevent the development of elevations in, for instance, anxiety and startle response produced by chronic psychosocial stress observed in this study.

Physiological Testing.

Prevention of Psychosocial Stress-Induced Changes in Organ Weights.

Intriguingly, both DSS and psychosocial stress produced similar physiological outcomes in this study. Animals subjected to the chronic psychosocial stress paradigm expressed significant increases in adrenal gland weights relative to home cage control animals, replicating previous work by our group (Zoladz et al, 2008) and others. Similar to psychosocial stress, findings indicated that SES also produced significant increases in adrenal weights, relative to controls. These results, although interesting, are perhaps not surprising, as stress-induced increases in adrenal weights has been shown in a study examining rats (Bakos et al, 2006), but not in another assessing the effects of EE on adrenal weights of 3 strains of mice (Tsai, Pachowsky, Stelzer & Hackbarth, 2002), suggesting species-specific changes in physiology. These findings are also not particularly surprising considering the evidence which indicates that enriched animals demonstrate significant increases in endogenous glucocorticoids, such as CORT (Tsai et al, 2002;Webster, Tonelli & Sternberg, 2002; Marashi et al, 2003) measured in mice. It should be taken into consideration that, even though DSS animals in this study were not

provided with running wheels, the apparatus may have provided increases opportunities for physical activity, relative to the much smaller home cages. This increased activity level could explain the driving force behind the enlargement of adrenal glands, as well as hearts of DSS animals observed in this study, as physical activity alone has been found to produce significantly larger adrenals and hearts compared to controls (Anderson, Eckburg & Relucio, 2002). Similar to psychosocial stress, DSS produced a robust decrease in thymus gland weight, which play a role in immune function, relative to home cage control animals. Other studies have suggested that EE produces thymic atrophy in female mice (Tsai et al, 2002) similar to stress in male rats (Zoladz et al, 2008), and a reduction in immunological parameters (Marashi et al, 2003) in male mice relative to standard housing. The precise influence of environmental stimulation on immune parameters is complicated in that some studies have shown 3 months of EE to produce significant increases in natural killer cells, indicative of enhanced immune functioning (Benaroya-Milshtein et al, 2004), which facilitate resistance to viral infections, and tumor growth.

Prevention of Psychosocial Stress-Induced Reduction in Growth Rate. Similar to other chronic stress research, and previous findings in our laboratory (Zoladz et al, 2008), the psychosocially stressed animals demonstrated significantly lower growth rates relative to no psychosocial stress control animals. Again, this psychosocial stress-induced reduction in growth rate was prevented with DSS.

Heart Rate and Blood Pressure. Findings suggested that there were no significant differences between groups on heart rate or blood pressure. It was surprising to note that there were no differences between the home cage psychosocial stress group and their no psychosocial stress counterparts. Previous work by our group has shown chronic psychosocial stress to significantly increase blood pressure, and lower heart rate (Zoladz et al, 2008). The lack of significant differences on these parameters may be due to differential methods between the two studies. In this study, all animals were transported from the housing room into the laboratory daily. This differential experience, compared to the work conducted by Zoladz and colleagues, could have affected the outcomes of these measures.

General Conclusions

The purpose of this study was to test the hypothesis that providing rats with social and environmental stimulation would prevent the onset of PTSD-like behavioral and physiological changes in rats exposed to chronic psychosocial stress. Similar to previous findings from our group (Zoladz et al, 2008), I found that rats subjected to the chronic psychosocial stress paradigm, consisting of two acute cat exposures, in conjunction with social instability, produced significant behavioral changes analogous to those observed in patients with PTSD, supporting initial hypotheses and pr. The psychosocially stressed animals exhibited elevations in cued fear, anxiety, and startle response. Also supporting initial predictions, this study showed that these deleterious effects of psychosocial stress-on behavior were prevented in animals provided with DSS. Additionally, I found that

psychosocially stressed animals exhibited significant changes in various physiological measurements. Specifically, rats subjected to the chronic psychosocial stress paradigm had significantly heavier adrenal glands, smaller thymus glands, and heavier hearts. Interestingly, these psychosocial stress-induced changes in organ weights were similar to those observed in rats in the DSS groups. DSS seemed to prevent the detrimental effects of chronic psychosocial stress on behavior, but produced similar changes in organ weights to psychosocially stressed rats relative to home cage -no psychosocial stress controls. Findings of this study seem to support the theory, and other empirical evidence, that DSS may have, in a sense, inoculated rats by facilitating neurobiological and neurochemical changes in the brain, similar to those observed in reports examining the effects of chronic antidepressant administration on analogous changes.

Implications and Clinical Relevance

In this study, DSS was initiated twenty-four hours after the first cat exposure session and continued daily for 2 hour period for the duration of the 31 day chronic psychosocial stress paradigm. This study was designed to mimic studies which examined the effects of pharmacological treatments on PTSD after trauma exposure. Interestingly, findings of this study seem to be similar to preliminary data in our laboratory indicating that chronic treatment with the atypical antidepressant tianeptine blocked the development of PTSD-like behavioral and physiological changes when initiated twenty-four hours after the first cat exposure and ended prior to testing, analogous to methods of DSS exposures in this study.

Clinical studies have examined the effects of various pharmacological treatments on PTSD, with mixed results. For instance, PTSD patients treated with selective serotonin reuptake inhibitors (SSRIs) exhibited significant improvement in depressive-like symptoms but had little improvement on the memory and anxiety-related symptoms of the disorder (Van der Kolk, 2001). Although tricyclic antidepressants have been found to exert positive effects on PTSD-related symptoms (Bisson, 2007), other reports showed that desipramine reduced symptoms of depression in PTSD patients, but, again, was ineffective in alleviating anxiety-related symptoms, central to the disorder (Reist, Kauffman, Haier, Sangdahl, Demet, Chicz-DeMet, 1989). These studies may provide insight into the importance of furthering research examining the effects of non-pharmacological treatments of PTSD, such as group therapy, which, in contrast to drug treatment, do not produce undesired physical side effects. Additionally, non-pharmacological treatments may serve to address the entire profile of symptoms associated with PTSD, in contrast to drug treatments which may only target a few.

Limitations and Future Directions

This study does not distinguish between the efficacy of the social and environmental components of the DSS manipulations. Future studies should attempt to parse out the differential effects of the environmental and social aspect inherent in the DSS manipulations. This could be accomplished by adding more experimental groups. For instance, placing groups of animals together in the apparatus without any objects would perhaps serve to elucidate the effects of purely social stimulation on outcomes

observed in our model. Additionally, objects could be placed in rats' home cages in order to investigate whether environmentally enriching the home cage exerts the same influences relative to placing a group of animals in the large apparatus with objects. Another, perhaps overlooked, limitation in this study are the actual reasons underlying the behavioral and physiological effects observed. Part of the hypothesis of this study was that DSS would serve to neutralize the additive component of daily social instability to the model and, as a result, ameliorate deleterious PTSD-like changes produced by the entire chronic psychosocial stress paradigm. This thought was based on previous findings by our group which showed that the daily social instability component of the model was necessary in order to produce PTSD-like behavioral and physiological changes (Zoladz et al, 2008). It is unknown in this study, however, whether DSS actually served to neutralize the additive effects of social instability manipulations to the model. Future studies should address this question by utilizing between group pseudo-randomization with a unique set of rats. In other words, instead of switching the cage mates of rats within a group on a daily basis, future work should pair each rat with another rat which it has not encountered before, either in the stimulating apparatus, or in its home cage. This may elucidate the true efficacy of DSS to remove or neutralize the detrimental additive effects of daily social instability to the entire chronic psychosocial stress paradigm. It may be important for future studies to examine the influence of DSS on brain morphology and its interactions with chronic stress. Specifically, morphological, and neurochemical changes in the PFC would be interesting to examine in terms of

elucidating possible mechanisms by which DSS exerted its influence in preventing the development of the behavioral and physiological changes observed in this study.

The human social support literature suggests and strong relationship between social support and lower rates of PTSD, and PTSD-related symptoms. Examining the literature closely, however, reveals that it is not necessarily the social support itself which may be exerting its protective influences on PTSD development but, rather, the *nature* of the support individuals receive after being exposed to trauma which may decrease their chances of developing the disorder. For instance, some work suggests that it is not simply the number of social interactions, or number of individuals in one's social network that is important but, rather, the *positive quality* of those social interactions which act to buffer the detrimental effects of stress on health (Cohen & Wills, 1985; Solomon et al, 1987). In other words, the love from a supportive spouse, or from a few family members may be as beneficial compared to the actual social network sizes of individuals in terms of being associated with lower rates of PTSD, and general health after trauma exposure (Cohen & Wills, 1985; Keane et al, 1985; King et al, 1998). In addition, perceived available support (i.e. the perception that an individual is loved and can count on others for support) may be more strongly correlated with long-term health relative to the number of people available in one's network to offer support (Collin & Feeney, 2004). Interestingly, a study conducted on Vietnam veterans indicated that both structural (size and complexity of a given social network) and functional (emotional sustenance) correlated with lowering PTSD in men, but only functional support was predicted lower rates of PTSD in women (King et al, 1998), suggesting possible gender differences with regards to

interactions between stress and social factors on PTSD development after trauma. The assessment of the quality of social relationships in human research is based on individuals' perceptions of the positive nature of their interactions. Being able to empirically assess the quality of social interactions within the constraints of an animal model would be very difficult, if not impossible, based on the simple fact that perceptions cannot be assessed in rats. Utilizing an animal model is useful in that the opportunities for social interactions can be experimentally manipulated, as was done in this study, but does not allow for the drawing of empirical conclusions based on the quality of social relationships experienced by organisms, which may be important to consider in human work.

Concluding Remarks

Findings of this study revealed that providing animals with DSS blocked the development of several PTSD-like responses in adult rats exposed to chronic psychosocial stress. Importantly, this study, in contrast to the majority of studies in this field, showed that providing adult rats with brief, daily stimulation may exert a profound protective influence on deleterious effects of chronic psychosocial stress on the behavior and physiology of rats when initiated shortly after an acute stress experience. These results are also consistent with human research suggesting that social stimulation may confer resistance of a subset of the traumatized population to develop PTSD. This level of analysis in an animal model of PTSD serves to underlie the importance of clinical

research examining social factors in identifying risk factors for PTSD, as well as non-pharmacological treatments (e.g. social support systems) for the disorder.

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