AN INVESTIGATION OF POTENTIAL RISK FACTORS RELATED TO STRESS PSYCHOPATHOLOGY

A dissertation submitted to Kent State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

By
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INTRODUCTION

Stress has been implicated as an environmental cause of psychopathology (Bremner, 1999). Posttraumatic Stress Disorder (PTSD) is a stress related anxiety disorder in which a precipitating trauma of either a physical or psychological nature may initiate physiological and behavioral abnormalities that persist beyond the actual event (Servatius, Ottenweller & Natelson, 1995). The disorder manifests a wide array of emotional, physiological and cognitive disturbances in function (Kessler, Sonnega, Bromet, Hughes & Nelson, 1995). People suffering with PTSD exhibit an exaggerated stress response (Kessler et al, 1995) and persistent sympathetic nervous system activation (Pitman, Orr, Forgue, deJong & Clairborn, 1987). Disturbances in cognition and memory have also been observed in the clinical profile of the disorder (Bremner, Vythilingam, Vermetten, Afzal, Nazeer, Newcomer & Charney, 2004). Patients diagnosed with PTSD show decreased hippocampal volume (Bremner, Randall, Scott, Bronen, Seibyl, Southwick, Delaney, McCarthy, Charney & Innis, 1995) and hippocampal dependent memory deficits (Bremner, Scott & Delaney, Southwick, Mason, Johnson, Innis, McCarthy & Charney, 1993). Presumably, a memory of an experienced traumatic event may be processed in a way that produces a sense of current and impending threat from which individuals may then generalize to erroneously perceive normal events, situations, people and activities as threatening and dangerous (Ehlers & Clark, 2000). Given that the hippocampus is essential for the encoding of new information, it is most reasonable to
suggest that hippocampal dysfunction may be responsible for observed cognitive abnormalities (Keenan & Kuhn, 1999).

The hippocampus is well known for the prominent role it plays in learning and memory (Olton, 1983) and damage to the hippocampus has long been associated with impairments in cognitive functions and memory processes (Scolville & Miller, 1957). Chronic stress exposure and elevated corticosterone levels result in a reduced number of hippocampal neurons (Keenan & Kuhn, 1999).

There is compelling evidence that prolonged exposure to stress and the subsequent elevation in corticosteroid release can induce permanent damage to the hypothalamic-pituitary-adrenal (HPA) system (Sapolsky, 1992; Sapolsky, Krey & McEwen, 1985). The HPA axis plays a pivotal role in the mammalian stress response (Walker & Diforio, 1997). The biological stress response is an adaptive mechanism designed to ensure an organism’s ability to react successfully against impending threat (McEwen, 1995). Physiologically, in response to stress exposure, glucocorticoids (stress hormones) are released and play an important role in readying an organism to deal with the stressor. Glucocorticoids are, therefore, crucial for optimal survival (Lupien, Sharma, Nair, Hauger, McEwen, deLeone & Meany, 1998), but in excess, have been shown to negatively affect brain function (Luine, Spencer & McEwen, 1993; Keenan & Kuhn, 1999). Specifically, previous research has documented the deleterious effects of prolonged or heightened glucocorticoid exposure on hippocampal neurons and the subsequent damage, dysregulation and dysfunction associated with that exposure (Sapolsky, 1992).
The hippocampus has a high density of glucocorticoid receptors that modulate HPA axis activity (Sapolsky, 1990) and is responsible for providing negative feedback to the HPA system and shutting down hormone release after activation and response (Sapolsky, 1992). Excessive glucocorticoid release culminates in neurotoxic effects that damage the hippocampus by compromising and impairing the ability of the negative feedback system and interfering with the inhibition of adrenal secretions, thereby exacerbating the effect and resulting in chronic alterations (Sapolsky, 1992; Walker & Diforio, 1997). The induction of hippocampal damage and dysregulation of HPA axis activity results in increasingly elevated levels of stress hormones and places the hippocampus at risk of further damage and cell loss across time.

Clinical research has indicated that the stress response systems in people suffering from PTSD are abnormal (Servatius, Ottenweller & Natelson, 1995). A number of studies cite relevant evidence of similarities between the behavioral disturbances observed in animals subjected to experimental manipulations and symptoms of PTSD (Kolb, 1987; van der Kolk, 1987; van der Kolk, Greenberg, Boyd & Krystal, 1985; Adamec, Burton, Shallow & Budgell, 1999). Adamec and Shallow (1993) cite evidence of the dysregulation of the HPA axis in rats after exposure to a cat and long lasting increases in anxiety-like behavior following a 5-minute inescapable predator exposure. Adamec, Burton, Shallow and Budgell (1999) developed an animal model characterizing lasting changes in affective functioning in rats following predator stress exposure that mimics the alterations in affective functioning observed in human victims of traumatic stress. A specific pattern of elevated plasma stress hormones in rats parallels similar
patterns as those observed in human patients suffering from PTSD (Yehuda, Resnick, Kahana & Giller, 1993; Adamec et al, 1999). Adamec (1997) also reports analogous changes in sensorimotor reactivity, such as exaggerated startle responses and delayed habituation, between human PTSD patients and cat exposed rats. The pharmacological manipulation of glucocorticoid levels has been shown to cause deficits in memory functions in both animals (Bodnoff, Humphreys, Lehman, Diamond, Rose & Meaney, 1995) and humans (Lupien, Fiocco, Wan, Maheu, Lord, Schramek, & Tu, 2005). The similarities between animal symptomology and symptoms suffered by human trauma victims may therefore reflect a common etiology that would make animal models critically instrumental in furthering our understanding of the development of the disorder (Foa, Zinbarg & Rothbaum, 1992).

Diagnostic statistics reporting the high prevalence of PTSD attests to the existing abundance of stress related traumas plaguing society (Breslau, Kessler & Chilcoat, 1998). However, inconsistent with such a high rate of notable trauma inducing stressors is the fact that many trauma survivors do not develop symptoms of the disorder (Yehuda, 1999). Although PTSD is characterized by initial onset symptoms in some individuals, others report few or no symptoms immediately and sometimes not for months or years, if ever, following the trauma (Ehlers & Clark, 2000). It seems plausible to suggest that specific abnormalities, vulnerabilities or preexisting conditions (risk factors) may predispose certain at risk individuals to suffer stress related psychopathology. These risk factors may include specific demographics, health, gender, genetics, familial, social, biological or environmental factors, as well as prior victimizations, the magnitude of the
trauma, personal appraisals and cumulative lifetime stress (Yehuda, 1999). Evidence from human studies suggest that adverse life events could precede the development of specific psychopathologies as well as modulate the impact of subsequent stress exposures, thereby rendering some people more susceptible than others to develop PTSD or stress related disorders (Yehuda, 1999). Therefore, in humans, the exposure to a traumatic event or stressor may result in enduring changes in affective functioning in vulnerable individuals (McFarlane, 1997).

Previous research has documented specific behavioral and cognitive alterations following hippocampal lesions which resemble disturbances observed in schizophrenia, such as deficits in prepulse inhibition and increased locomotor activity (Lipska & Weinberger, 2000; LePen & Moreau, 2002). Given that many psychological disorders reflect problems in memory and cognition likely implicates hippocampal involvement. However, verifiable anatomical damage is not always observed as part of the clinical profile, suggesting that something else may be modulating the resultant functional disruptions. For example, the brain may be more vulnerable to insult during specific developmental stages and the particular sensitivity of limbic-cortical circuits to disruption could be one possibility that may not be as easily detected or recognized as lesion based impairments.

Whereas obvious trauma may result in expected consequences, unrecognized vulnerabilities may also be modulating observed deficits. To examine that possibility, kainic acid (KA) may be useful in promoting the development of potentially obscure risk factors. Kainic acid, a glutamate agonist excitotoxin, has been experimentally shown to
kill neurons by over stimulating them and is presently regarded as a more selective means of producing brain lesions. Other lesioning techniques, such as aspiration, can result in collateral damage to adjacent brain areas. Kainic acid has the potential to alter the hippocampus in rat pups at a sub cellular level without inducing the gross anatomical damage and mortality rates of more invasive lesions while still resulting in a cumulative and consequential biological predisposition to negative stress effects later in life.

During the first two postnatal weeks, systemic administration of kainic acid resulted in selective activation of the hippocampus without high levels of cell death (Howland, Hannesson & Phillips, 2004). Such abnormal neural hyperactivity in corticolimbic circuitry may well be a predisposing factor in schizophrenia (Lipska & Weinberger, 2000) as well as other psychopathologies, but lacks the anatomical alterations observed with hippocampal lesions. Subtle damage at a sub-cellular level might still reflect a compromised hippocampus and could be responsible for observed cognitive deficits in the absence of gross hippocampal lesions.

Previous studies have also shown that exposing rats to chronic restraint stress has resulted in hippocampal alterations at the cellular (McEwen, 1999), molecular (Venero, Tilling, Hermans-Borgmeyer, Schmidt, Schachner & Sandi, 2002) and functional (Pavlide, Nivon, Lucas & McEwen, 2002) levels. In rats, a single stress exposure may produce chronic disruptions in HPA axis response (Marti, Garcia, Valles, Harbuz & Armario, 2001). Research has well documented the fact that the HPA axis can be modified by early life experiences, including environmental factors and early exposure to stress (Levine, 1957; Liu, Diorio, Tannenbaum, Caldji, Francis, Freedman, Sharma,
Pearson, Plotsky & Meaney, 1997; Park, Campbell & Diamond, 2001). Cordero, Venero, Kruyt & Sandi (2003) utilized a two hour restraint stress paradigm investigating contextual fear conditioning and showed that a single exposure of significant duration was sufficient to facilitate fear conditioning.

Gender has also been shown to be a contributing factor in susceptibility and vulnerability to psychopathology (Louvart, Maccari, Lesage, Léonhart, Dickes-Coopman & Darnaudéry, 2006). Sex-differences in HPA reactivity and function have been suggested as the basis of increased vulnerability to stress in women (Young, 1995). Structural and functional differences in the hippocampal formation between males and females have been documented in rats and humans (Kolassa & Elbert, 2007) and may account for differences observed in stress related vulnerability. Women are two times more likely than men to exhibit symptomology consistent with PTSD (Stein, Walker & Forde, 2000) even though men are exposed to more traumatic events (Christiansen & Elklit, 2008). Experimentally, female rats produced higher levels of corticosterone compared to males in response to acute stressors (Kitay, 1961) as well as different patterns of neurochemical alterations after chronic stress exposure (Luine, Beck, Bowman & Kneavel, 2002). However, in evaluating the statistics reported for women suffering with PTSD considerations should account for several factors that may distort the clinical profile. For example, the relative number of traumas suffered by women may outnumber those suffered by men, so an adjustment should be made for the disproportionate number of incidents. Also, a precedent has existed in society that has allowed women more freedom to report trauma and to seek treatment for anxiety based
pathology. Therefore, gender skewed reporting of such incidents may also lead to inaccuracies and distort the actual profile of PTSD and other stress related disorders.

PTSD is clearly a stress related disorder with an array of behavioral manifestations that may be assessed in animal models, the results of which may then be used to further characterize and identify the disorder, as well as to suggest plausible and appropriate interventions to prevent or alleviate suffering in human populations. Animal models must focus on specific assessments aimed at revealing similarities between the clinical profile of PTSD and the abnormalities and deficits observed in animal subjects. Those resemblances include behavioral hyperresponsivity and exaggerated startle as quantifiable and assessable objective signs of a disorder (Servatius, Ottenweller & Natelson, 1995).

The utility of any animal model of anxiety to adequately elaborate on the specific brain mechanisms underlying pathologic anxiety must take into consideration the existence of different types of anxiety and subsequently develop and rely on models that are able to discriminate and identify between conditioned and unconditioned fear, which differentially represent generalized anxiety and panic (Zangrossi & Graeff, 1997). The elevated T-maze has been widely used in behavioral assessments of different types of anxiety (generalized anxiety and panic) that may underlie stress related pathologies and provides a viable animal model to study anxiety related behaviors (Estanislau & Morato, 2005). Inhibitory avoidance is assessed by placing a rat in the closed arm of the maze and recording the latency to leave that arm. Rats are curious by nature and should be inclined, by virtue of that curiosity, to readily explore their surroundings. In the present
study, inhibitory avoidance was evaluated as a measure of generalized anxiety and longer avoidance latencies were interpreted as a heightened level of generalized anxiety impinging upon the rats natural inclination to explore the maze. Following the precedent of Estanislau & Morato (2005), escape was analyzed as a measure relating to panic and was assessed by placing the rat in an open arm of the maze and recording the latency to leave that arm for the protective shelter of the closed arm. A quick escape time (shorter latencies) was assessed as an indication of heightened reactivity related to panic as evidenced by the lack of inclination to explore in a species specific typical fashion.

Also included among the specific behaviors observed in patients suffering from PTSD are exaggerated startle responses and diminished habituation and prepulse inhibition (PPI) of the startle response (Conti & Printz, 2003). In the present study habituation of the startle response and PPI were simultaneously assessed to qualify and quantify the stress response and examine subsequent behavioral and cognitive impairments implicated in PTSD.

The present experiment was designed to contribute to our understanding of the deleterious effects of stress. Most pertinent to this specific line of research is an investigation into specific vulnerabilities and predisposing factors associated with and contributing to stress psychopathology. An investigation into specific preexisting vulnerabilities and an assessment of the potential contribution such risk factors may have on emotional, behavioral and cognitive measures following trauma would make this animal model instrumental in furthering our understanding of the development and maintainance of stress related disorders. Since many studies have reported evidence of
the differential effects of gender in relation to psychopathology the inclusion of both males and females in the study reflects the importance of studying both sexes in animal models. The identification of potential risk factors could potentially contribute to behavioral, cognitive and medical interventions targeted both at the pre and post trauma levels. Given that only a proportion of persons who experience a traumatic event develop PTSD, the identification of specific risk factors would be highly beneficial to both the treatment and prevention of the disorder, as well as other psychopathologies such as depression and panic disorder (Yehuda, 1999). For example, pharmacological interventions that have an effect on glucocorticoid receptors in the hippocampus may lead to effective improvements in the attenuation of neurological insult vulnerability and of the development of symptoms associated with PTSD (Bremner, et al, 2004).

A wide array of putative risk factors may contribute to the development and maintenance of these disorders. The present investigation addressed the potential biological risk factor of brain damage as a plausible vulnerability that could increase one’s susceptibility to stress effects or which could modulate the impact of subsequent stress exposure on emotional, behavioral or cognitive functioning.

One example of brain damaged at risk populations may include infants compromised at birth as a result of suffering acute hypoxia. Such children may be medically vulnerable and susceptible to future developmental psychopathology (O’Dougherty & Wright, 1990). A neurodevelopmental model of schizophrenia, for example, suggests that perinatal hypoxia may serve as the catalyst to a genetic predisposition to develop schizophrenic symptoms (Cannon, 1998) and Cannon, Rosso,
Bearden, Sanchez & Hadley (1999) found that the risk of schizophrenia increased as a function of hypoxia related obstetric complications.

A number of studies also report the occurrence of PTSD resulting from physical illnesses and other miscellaneous conditions, such as cardiac surgery, myocardial infarction, hemorrhage and stroke (Tedstone & Tarrier, 2003). The potential disruption of oxygen supply and subsequent damage to brain cells may result in a biological predisposition and vulnerability. If experimentally substantiated, individuals with a known history of medical illness or surgical complications could then be identified and targeted for preventative measures to avoid the cascade of problems, both behavioral and biological, that could result from a later exposure to a traumatic episode.

Subtle damage that we may be virtually unaware of may accumulate over time resulting in a biological susceptibility. In effect, any number of “situational” or environmental life variables may produce stress and result in some degree of hippocampal damage that may not be apparent but could be a potential contributing factor in individual vulnerability to PTSD or other stress related pathologies.

In the present study a group with induced hippocampal insult (HIPPOCAMPAL INSULT group) was conceptualized to represent the presence of a biological vulnerability. The hippocampal damage represents a specific abnormality or pre-existing condition that could be considered a risk factor in stress related etiology. My conceptualization of this group is based on what is known about the hippocampus and stress. Sapolsky (1992) and others have found and documented that the hippocampus is at risk and vulnerable throughout the lifetime to specific stress related insults. Traumatic
injury to the nervous system (biological risk factor) can occur in any number of ways ranging from an obvious blow to the head and penetrating injuries to surgical complications and consequential anoxia or hypoxia. Conceptually, this group also models people who have not suffered a specific trauma insult like rape or war but may have suffered the less obvious and not as well defined traumas of poverty, neglect, spousal abuse, aging and child abuse. Although we may not yet know the cumulative effects of these more obscure traumas and life experiences, such situational demographics may also predispose us to stress vulnerability and may warrant further investigation.

To represent an obvious trauma exposure minus a preexisting vulnerability a stress alone group (STRESS) was conceptualized. This group models, for example, physically and emotionally healthy individuals who are victim of a well defined and specific trauma inducing experience of either a physical or psychological nature such as a rape, car accident, victimization or even the unexpected death of a loved one.

Representing the synergistic effect of specific trauma on a preexisting vulnerability is the concept of a hippocampal insult plus stress induced group (HIPPOCAMPAL INSULT + STRESS). This group models individuals who may or may not recognize the existing vulnerability and who then are exposed to a trauma inducing situation. For example, individuals who have been compromised due to chronic negative life events ranging from birth complications to poverty and then subsequently suffer a specific stress exposure like rape or war may be at an elevated risk level for suffering stress related pathology. The combined effect of the situational stress exposure
may serve as a primary risk factor that when coupled with a specific trauma incident later in life could promote a problematic outcome.
GENERAL DESIGN

In the general design of the present study forty-eight rats comprised the four groups designed to model varying degrees of vulnerability to negative stress effects. Potential risk factors associated with stress psychopathology were examined. Treatment groups received KA injections, restraint stress induction and three stress reminders. Figure 1 illustrates a flowchart diagram of the experimental manipulations. Rats were tested in the elevated T-maze on measures of inhibitory avoidance and escape, followed by a test session in an automated startle chamber on assessments of startle reactivity, habituation and prepulse inhibition. Full details of all procedures are described in the methods section.
Figure 1. Flowchart diagram of the two primary factors of interest assessed in the four treatment groups, Hippocampal Insult, Hippocampal Insult + Stress, Stress, and Control.
Figure 1.
METHOD

**Subjects.** Six Harlan Sprague-Dawley (HSD) female rats (each with a litter of eight pups) were purchased and scheduled for arrival at the animal lab facility at Slippery Rock University on two consecutive shipment dates. Each shipment consisted of three dams to allow for the staggering of manipulations and testing procedures. Each dam and her litter were individually housed in Plexiglas cages. Food and water was provided ad lib. Lighting was maintained on a 12:12 hour light/dark cycle. The birth date of the pups was designated as postnatal day (PND) 0.

**Apparatus. Elevated T-maze.** The elevated T-maze consists of three arms elevated (71 cm) off the floor. One arm (71.1 cm in length and 10.2 cm wide) is enclosed by walls (10.2 cm high) and stands perpendicular to the two opposite open arms (each 45.7 cm in length) (Poltronieri, Zangrossi & de Barros Viana, 2003) and looks like a capital letter T. The T-maze was developed from the elevated plus-maze to provide information on different types of anxiety by analyzing avoidance of and escape from the open arms of the maze (Zangrossi & Graeff, 1997).

**Automated Startle Chamber.** Animals were tested in an automated startle chamber (SR LAB STARTLE RESPONSE SYSTEM, San Diego Instruments Inc., model # SIC 001202). The testing procedure in the startle apparatus consists of placing the animal in the soundproof chamber that consists of a Plexiglas cylinder (8.2 cm in diameter) resting on a Plexiglas frame (12.5 x 25.5 cm) within a sound attenuated
ventilated enclosure. Acoustic startle is produced by a loudspeaker mounted 24 cm above the cylinder. A piezoelectric accelerometer on the base of the cylinder frame detects and transmits vibrations, which are digitized and recorded by an interface unit and microcomputer.

**Restraint Tubes.** Animals were restrained in a Plexiglas tubular restraint device (Harvard Apparatus rodent restrainer #520292) (21 cm long, 6 cm high, 6.5 cm inside width). Animals were inserted nose first into the receptacle and an adjustable divider was inserted at the tail end of each animal. The divider had a tail opening and could be adjusted to accommodate individual size differences for maximum fit. The adjustments were made to ensure the animals were securely restrained and unable to turn around in the tube, but not so tight as to inflict physical pain.

**Procedure.** Half of each litter was randomly assigned to kainic acid (KA) treatment and the other half to saline treatment. Pups were marked with a Sharpie marker to identify the treatment group assignment of each pup.

On PND 7 pups assigned to the KA treatment (n=24) received an i.p. injection of KA (1.5 mg/kg) while pups assigned to the saline condition (n=24) received an i.p. injection of saline (10 ml/kg) (Howland, Hannesson & Phillips, 2004). Pups were subsequently monitored for a minimum of two hours following the injections for signs of seizure activity and then returned to the dam and home cage.

All pups and dams were left undisturbed (except for weekly cage cleaning and routine remarking of the pups as necessary) until PND 28 at which time the pups were weaned from the dams and housed in groups of two or more according to sex and group
assignment. Forty-eight rat pups were used in the study and randomly assigned to four groups. KA treated pups (n = 24) were randomly assigned to either Hippocampal Insult (HI, n =12) or Hippocampal Insult + Stress (HI + S, n =12) groups. Saline injected pups (n = 24) were randomly assigned to either Stress (S, n =12) or Control (C, n =12) groups. Pups remained undisturbed (with the exception of cage cleaning) from the time of weaning for a period of 32 days (60 days of age).

On PND 60 rats in the Hippocampal Insult + Stress (n = 12) and Stress (n =12) groups were subjected to an immobilization (restraint) stress insult. Each rat was placed in a plastic restraint tube for a period of two hours (Cordero et al, 2003) and then returned to the home cage. During the next 30 days the stressed animals were subjected to three more stress inducing manipulations. Every 10 days (on PNDs 70, 80 and 90) rats were again placed in the restraint tubes for a period of 20 minutes (Park, Hoang, Belluzi & Leslie, 2003) and then returned to the home cage. On PND 91 all rats were pre-exposed to the maze by placing them in one of the open arms for a period of five minutes. Animals remained otherwise undisturbed for this period of 30 days with the exception of the three 20 minute stress manipulations (noted above) and weekly cage cleaning.

**Behavioral Testing. T-maze evaluation:** Beginning at ninety-two (92) days of age (the day after maze pre-exposure) all rats were tested in the elevated T-maze. Rats were assessed on inhibitory avoidance and escape measures consecutively. Each rat was placed in the distal end of the enclosed arm facing the intersection of the open arms. The time taken for the rat to leave this arm (cross over with all four paws) was recorded as a measure of inhibitory avoidance and generalized anxiety in three separate trials. Each
trial was separated by a 30 sec interval. Thirty seconds after the last inhibitory avoidance trial rats were placed at the end of the open arm where they had been previously exposed the day before and the latency to escape the open arm with all four paws was recorded in three consecutive escape trials (separated by 30 second intervals) and analyzed as a measure related to panic. During testing rats were returned to a plexiglass holding cage identical to the home cages during each 30 second interval. A time limit of 300 seconds was employed for both inhibitory avoidance and escape latencies. If a rat failed to exit an arm a value of 300 was recorded for that trial. Testing parameters were adopted from Estanislau and Morato, (2005).

**Habituation and prepulse inhibition evaluation:** the day after T-maze evaluation and forty-eight hours after the final restraint session each rat was tested on measures of startle reactivity, habituation and pre-pulse inhibition of the startle response (PPI). Behavioral testing consisted of a five minute adaptation period to the startle box. A 65dB background noise was presented throughout the test session. Test trials consisted of 16 acoustic startle trials (115dB, 40ms white noise burst), 24 prepulse trials (6 each at 72, 76, 80, and 84dB, 40ms white noise burst preceding the startle stimulus by 100ms) and 24 pre-pulse alone trials. Trial sequence was pseudorandom and a 20s intertrial interval was employed. Peak response amplitude of each animal’s movement in response to the stimulation was recorded for each trial. Startle reactivity was assessed by comparing mean overall response amplitudes. Habituation of the startle response was examined by comparing each animal’s average response amplitude of the first four trials with the average response amplitude for the last 12 trials and average responding across four
blocks of four trials. Prepulse inhibition of the startle response was examined by evaluating each animal’s average response across 24 PPI trials consisting of four levels of stimulus warning intensities (six trials per each intensity level). After test the animal was returned immediately to the home cage and the colony room.

**Histological Evaluation:** After completion of behavioral testing all rats were anesthetized with Nembutal (60mg/kg) and intracardially perfused with 10% buffered formalin. Brains were removed immediately after perfusion. The extracted brains were stored in the formalin solution. Each brain was sectioned using a cryostat (IEC Minotome Microtome Cryostat, International Equipment Company). Sections were mounted on 3 x 1 inch gelatin coated microscope slides (Lab Scientific, Inc). Cresyl violet staining procedure was used for general cellular staining. The staining procedure consisted of the following solutions and sequence: (A) **Solutions:** (1) Cresyl violet: 200mg cresyl violet acetate/150 ml H₂O; (2) Buffer solution ratio: 94 ml. 0.1 M acetic acid (6ml/liter of H₂O) to 6 ml. 0.1M sodium acetate (13.6g/liter); (3) Working solution: 100ml. of buffer solution per 6 – 12 ml. of cresyl solution; (4) Acid alcohol decolorizing solution: 10% acetic acid/ETOH 1:9 and (B) **Staining procedure:** (1) Water rinse; (2) Alcohol (2-5 minutes); (3) Xylenes (10 – 20 minutes); (4) Alcohol (2 – 5 minutes agitated; (5) Water rinse; (6) Cresyl violet solution (2 – 10 minutes); (7) Water rinse; (8) Acid alcohol (to decolorize as needed); (9) Water rinse.

Hippocampal integrity was examined using a compound microscope (LeicaDMLS) at 40X magnification. The pyramidal cell layer of the CA1 field of the
dorsal hippocampus was evaluated. Due to problems with the cryostat some of the brains were not sectioned properly and lost for purposes of cell counts.

**Behavioral Observations:** On PND 7 pups receiving KA injections were monitored for two hours following the injections. Seizure activity was observed. All pups displayed seizure activity of varying degrees. Seizure activity ranged from whole body “shivering” to mild spasms and tick-like movements to more vigorous “swimming” type movements. Seizure activity was intense enough to propel the pups across the cage but not intense enough to warrant intervention. All seizure activity subsided within the two hour monitoring period.

No behavioral abnormalities were observed in any of the dams’ maternal responses to the pups following any of the procedures (handling, marking or injections) or separation.
RESULTS

T-maze results: The means of avoidance, escape and trial latencies were evaluated using a three-way analysis of variance (hippocampal damage x stress x sex). Results revealed a significant effect of sex ($F(1, 40) = 7.46, p < .01$) on the measure of inhibitory avoidance. Figure 2 illustrates the mean response latencies for inhibitory avoidance and escape between male and female rats collapsed across group showing that the male rats spent more time (longer latencies) in the enclosed arm of the maze thereby avoiding the open arms and their natural proclivity to explore. There were no significant differences between male and female rats on the measure of escape ($F(1, 40) = 1.13, p = .30$). Both male and female rats displayed similar latencies (time in seconds) to escape the open arms of the T-maze for the safety of the enclosed arm. Figure 3 depicts the mean response latencies for male and female rats on the measures of inhibitory avoidance (Figure 3a) and escape (Figure 3b) across groups showing an overall trend of heightened male response latencies.

The statistical analysis of inhibitory avoidance and escape latencies in the elevated T-maze failed to reveal a significant difference between the groups. Animals exposed to the hippocampal insult condition showed equivalent responding on the measures of inhibitory avoidance ($F(1, 40) = .729, p = .398$) and escape ($F(1, 40) = .781, p = .382$).
Figure 2. Mean (+ SEM) latencies for male and female rats on measures of inhibitory avoidance and escape in the elevated T-maze. Male rats exhibited statistically significant longer latencies on the measure of inhibitory avoidance. Male rats spent more time in the protective enclosed arm of the maze and avoided the open arms. This finding can be interpreted as a measure of heightened anxiety in the male rats. On the measure of escape, similar latencies were recorded in the time recorded for each animal to leave the open arms for the shelter of the enclosed arm.
Figure 2.
Figure 3a. Mean latencies (+SEM) for male and female rats on the measure of inhibitory avoidance in the elevated T-maze.

Figure 3b. Mean latencies (+SEM) for male and female rats on the measure of escape in the elevated T-maze.
Figure 3a.

![Graph showing latency (sec) for different groups: Hipp Insult, Stress, Hipp Insult + Stress, Control. Males and Females are indicated by black and gray bars, respectively.]

Figure 3b.

![Graph showing latency (sec) for different groups: Hipp Insult, Stress, Hipp Insult + Stress, Control. Males and Females are indicated by black and gray bars, respectively.]

Groups
Animals exposed to the restraint stress condition also showed equivalent responding on inhibitory avoidance ($F(1, 40) = 0.026, p = 0.872$) and escape ($F(1, 40) = 0.436, p = 0.518$).

Figure 4 shows the group means for the measures of inhibitory avoidance (Figure 4a) and escape (Figure 4b) collapsed across sex and illustrates the similar response levels between the groups. No significant effects of the experimental manipulations of kainic acid or restraint stress, hypothesized to serve as potential risk factors of brain damage and trauma exposure and elicit deficits on the behavioral measures of anxiety (inhibitory avoidance) or related to panic (escape) were observed.

An analysis of the mean latencies across trials revealed a significant effect of sex on the third inhibitory avoidance trial ($F(1, 40) = 6.72, p < 0.05$). Male rats exhibited statistically longer latencies on the last inhibitory avoidance trial. Figure 5 depicts the mean trial latencies for male and female rats collapsed across groups on the measure of inhibitory avoidance (Figure 5a) showing an increase in trial latencies across the three trial presentations indicative of an increasing unwillingness to venture out of the enclosed arm of the maze and interpretable as increased anxiety related to the learned fear of the open arms. No other differences across the first and second trials on the measure of inhibitory avoidance or on escape trials were observed. Mean trial latencies for male and female rats collapsed across group on the measure of escape (Figure 5b) show an increase across trials in escape latencies.

Rats on the first escape trial were quick to escape the open arm for the safety of the enclosed arm. By the third trial escape response time was slower indicating that the
Figure 4a. Mean (+SEM) latencies for groups on the measure of inhibitory avoidance in the elevated T-maze.

Figure 4b. Mean (+SEM) latencies for groups on the measure of escape in the elevated T-maze.

No differences between groups on either measure were observed. The experimental manipulations hypothesized to serves as indictors of risk related vulnerabilities to the development of stressed related psychopathology failed to result in group differences on the behavioral assessments of the anxiety related measurements.
Figure 5a. Mean (+SEM) latencies for males and females on measure of inhibitory avoidance across three trials. Male rats exhibited significantly longer latencies on Trial 3 indicative of increased anxiety.

Figure 5b. Mean (+SEM) latencies for males and females on measure of escape across three trials. Trial latencies increased across trials for both males and females supporting desensitization across trials.
animals may have become desensitized to the open arm danger by their willingness to spend more time exploring the open arms.

There were no differences between male and female control rats on measures of inhibitory avoidance \((F(1, 10) = 2.92, p = .12)\) or escape \((F(1, 10) = .43, p = .53)\) in the elevated T-maze. All other interactions (sex x hipp insult, sex x stress, hipp insult x stress and sex x hipp insult x stress) on measures of inhibitory avoidance and escape were not significant.

**Startle results:** An analysis of startle responding was examined using a mixed design analysis of variance (ANOVA) on peak amplitude (hippocampal insult x stress x sex). For each animal startle amplitude was averaged over 16 startle trials and across four blocks each consisting of four trials. Results revealed a significant effect of sex \((F(1, 40) = 22.56, p < .01)\) and a significant effect of stress \((F(1, 40) = 4.98, p < .05)\). No significant effect of hippocampal insult was observed \((F(1, 40) = 2.60, p = .12)\). Main effects are qualified and interpreted by a significant sex by stress interaction \((F(1, 40) = 4.39, p < .05)\). Figure 6 illustrates the differential effect of sex on stress. Mean response amplitudes collapsed across group for male and female rats in each stress condition are shown. Following stress exposure the stressed males showed a drop in startle reactivity compared to the non-stressed males while stress had no effect on the response amplitudes of the females. A significant hippocampal insult x sex interaction \((F(1, 40) = 8.64, p < .01)\) was also observed. The kainic acid postnatal insult also affected males and females differentially. Response rates increased only for males subjected to kainic acid injections.
Figure 6. Mean (+SEM) response amplitudes collapsed across group for male and female rats in stress and control conditions. Male rats exposed to the stress condition displayed decreased startle reactivity.
Figure 6.
Figure 7 displays mean response amplitudes for male and female rats collapsed across group at each level of hippocampal insult. Males show significantly higher reactivity following hippocampal insult compared to males not exposed to the postnatal treatment while female response rates were not significantly different between conditions.

No other interactions (hippocampal insult x stress or sex x hippocampal insult x stress) were significant.

The tests of within-subjects effects showed no effect of block \(F(1, 40) = 1.66, p = .206\) or any significant block x treatment condition interactions. Decreased response amplitudes across blocks illustrate habituation, but it was an expectation of the study that the treatment groups would have shown a failure to habituate across stimulus trial presentations. Figure 8 depicts mean response amplitudes for groups collapsed across sex across 16 startle trial presentations averaged into four blocks (mean response amplitudes of four startle trials per block). Although there were no significant differences between groups the response profile may prove to be of interest. The three treatment groups each showed fairly consistent habituation across Blocks. Kainic acid injections and restraint stress exposure did not disrupt habituation in the treatment conditions. The Stress group displayed sensitization on Block 4. The Control group habituated across the first two Blocks and then displayed sensitization on Block 3 followed by slight habituation on Block 4. The Stress alone group displayed the lowest startle reactivity while the Hippocampal Insult group displayed the highest reactivity scores.
Figure 7. Mean (+SEM) response amplitudes for male and female rats collapsed across group for hippocampal insult and control conditions. Male rats exposed to the postnatal hippocampal insult condition (kainic acid) displayed heightened startle reactivity.
Figure 7.
Figure 8. Mean response amplitudes (+SEM) for groups collapsed across sex on startle amplitude across Blocks. The treatment groups all displayed habituation of the startle response across the first three trial blocks.
Figure 8.

Response Amplitude

<table>
<thead>
<tr>
<th>Block 1</th>
<th>Block 2</th>
<th>Block 3</th>
<th>Block 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hipp Insult</td>
<td>Hipp Insult + Stress</td>
<td>Stress</td>
<td>Control</td>
</tr>
</tbody>
</table>

Trials
(4 acoustic startle trials averaged per block)
Prepulse inhibition of the startle response was evaluated using a three way ANOVA on peak amplitude (hippocampal insult x stress x sex) across varying degrees of stimulus warning intensities (72, 76, 80 and 84 dB). For each warning stimulus level six trials were averaged. Results failed to reveal any significant effect of the treatment conditions for any of the pre-warning stimulus intensity levels. No differences between groups on the inhibition of the startle response following a warning stimulus were observed for the 72 dB condition \( (F(3, 40) = 1.66, p = .19) \), 76 dB condition \( (F(3, 40) = .87, p = .47) \), 80 dB condition \( (F(3, 40) = .305, p = .82) \), or the 84 dB condition \( (F(3, 40) = .41, p = .74) \). Figure 9 depicts group means at each pre-warning stimulus intensity level illustrating overall response patterns. All groups responded to the warning stimulus by displaying response decrements following the warning pre-exposures. The potential risk factors of postnatal hippocampal insult and stress did not result in quantifiable deficits in the animals assigned to the treatment groups. Results did reveal a significant main effect of sex for the 72 dB condition \( (F(1, 40) = 10.34, p = .003) \) and for the 76 dB condition \( (F(1, 40) = 6.71, p = .013) \). Figure 10 depicts the average responses for male and female rats at each level of pre-warning stimulus intensity. Male rats showed higher startle reactivity at the lower warning intensities thereby failing to inhibit responding following the lower level pre-warning stimulus presentations. There was a trend toward significance in the 80 dB condition \( (F(1, 40) = 3.22, p = .08) \). At the highest pre-warning intensity level all animals inhibited the startle response following the 84 dB stimulus presentation \( (F(1, 40) = 1.52, p = .222) \).
Figure 9. Mean (+SEM) response amplitudes for groups collapsed across sex at each pre-warning stimulus intensity level. Overall response pattern indicates that all groups inhibited responding to the pre-warning stimulus.
Figure 9.

Response Amplitude

Prepulse Stimulus Intensity
(6 trials averaged per intensity level)
Figure 10. Mean (+SEM) response amplitudes for male and female rats collapsed across groups for each level of pre-warning stimulus intensity. Male rats failed to inhibit responding at the lower level (72dB and 76dB) stimulus pre-warning stimulus intensities.
Figure 10.

![Graph showing response amplitude vs. prepulse stimulus intensity for males and females. The graph displays a decreasing trend in response amplitude as the prepulse stimulus intensity increases from 72dB to 84dB. The data is based on six trials averaged per intensity level.](image-url)
Histological results: Means of cell counts were evaluated using a two-way analysis of variance (group x sex). Results revealed no differences in hippocampal volume between groups ($F(3, 22) = .61, p = .615$). Although not significant there was a strong trend toward gender differences in brain cell counts ($F(1, 22) = 4.27, p = .051$). Male rats show a trend for higher brain cell numbers in the CA 1 region of the hippocampus which most likely reflects larger sizes of brain in the bigger animals. Mean cell counts for each group and for male and female rats are displayed in Table 1. Cell count analysis is based on only 30 brains (12 males and 18 females) due to problems in sectioning six brains were lost for histological verification. Twelve animals were lost prior to perfusion. Four male rats (two from the Stress alone condition, one from the Hippocampal insult + Stress condition and one from the Hippocampal insult condition) were found in the home cages cannibalized by their cagemates. Two males (one from the Hippocampal insult + Stress condition and one from the Stress condition) were euthanized due to health problems and severe and rapid weight loss. Two males from the Control group were found dead in their cages assumed due to natural causes. Four female rats (one from each group) were euthanized after developing tumors.

Table 1. Mean cell counts in the CA1 field for groups and male and female rats in each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cell Count</th>
<th>Males (n)</th>
<th>Females (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI (n=7)</td>
<td>30.88</td>
<td>33.00 (n=3)</td>
<td>28.75 (n=4)</td>
</tr>
<tr>
<td>HI + S (n=9)</td>
<td>32.85</td>
<td>36.50 (n=4)</td>
<td>29.20 (n=5)</td>
</tr>
<tr>
<td>S (n=8)</td>
<td>30.23</td>
<td>31.67 (n=3)</td>
<td>28.80 (n=5)</td>
</tr>
<tr>
<td>C (n=6)</td>
<td>32.86</td>
<td>33.00 (n=2)</td>
<td>32.75 (n=4)</td>
</tr>
</tbody>
</table>
DISCUSSION

There appears to be a wide array of putative risk factors that may contribute to the development and maintenance of PTSD and other stress related disorders. With only a proportion of persons who experience a traumatic event developing the symptoms of PTSD, the identification of specific risk factors would be highly beneficial to both the treatment and prevention of the disorder, as well as other psychopathologies such as depression and panic disorder (Yehuda, 1999). The proposed study was designed to examine some biological vulnerabilities and predisposing factors associated with, and possibly contributing to, stress psychopathology. Traumatic injury to the nervous system can occur from a number of obvious or subtle influences which may affect individual vulnerability to PTSD and other stress related disorders. This investigation addressed the plausible risk factor of brain damage (induced via kainic acid administration) to assess whether a preexisting condition might increase susceptibility to negative stress effects or could modulate the impact of subsequent stress exposure on emotional, cognitive and behavioral functioning.

I hypothesized that the three treatment groups, representing specified and varying degrees of abnormality, vulnerability or risk would all exhibit substandard assessments on measures of anxiety, panic, startle reactivity, habituation and pre-pulse inhibition compared to the Control group. The Hippocampal Insult + Stress group was further expected, in comparison to the Hippocampal Insult and Stress alone groups, to perform
more poorly on the specific cognitive and behavioral assessments (T-maze evaluation and startle evaluation). The expected results could have been interpreted as an indication of a specific vulnerability or predisposition to the effects of stress and the manifestation of PTSD. An argument could also have been made for the exacerbation of cognitive deficits and/or anxiety based behaviors due to an exacerbating effect of the stress on the preexisting biological vulnerability and suggested as a plausible explanation for why some individuals suffer PTSD while others do not.

The results of the study were inconsistent with the hypotheses. The treatment groups did not differ at test on assessments in the elevated T-maze or the automated startle chamber. Although a power analysis indicated that treatment groups of twenty rats would have been optimal, the variability of the sample was not known in advance. Sex effects were observed on measures of inhibitory avoidance in the T-maze and startle reactivity in the startle chamber. Those results may be discussed in relation to sex specific differential effects on stress response systems.

Although the overall results are inconsistent with the expectations of the study the negative findings may be discussed across several possibilities in an attempt to explain the lack of significant differences between groups. The hypothesis that the Hippocampal Insult (brain damage), Stress, and Hippocampal Insult + Stress groups would all exhibit exaggerated behavioral assessments on measures of anxiety, panic and acoustic startle reactivity compared to Control animals was not supported. Although the treatment parameters were adopted from previous studies (Howland, Hannesson & Phillips, 2004;
Cordero et al, 2003; Park et al, 2003), considerations must include whether the manipulations were sufficient to produce the expected outcomes in the present study.

Kainic acid was systemically injected to selectively compromise subcellular neural circuitry and elicit a preexisting condition of brain damage. Systemic administration of KA has resulted in increased activation of hippocampus without manifesting high levels of cell death (Silveira, Sogawa, & Holmes, 2002) and has been reported to result in functional deficits in conditioned avoidance (de Feo, Mecarelli, Palladini & Ricci, 1986) and spatial learning (Lynch, Sayin, Bownds, Janumpalli & Sutula, 2000). Consistent with other reports, the KA administration in the present study did result in observable seizure activity within the two hours following administration. This outcome is similar to the findings reported by Howland, Hanneson & Phillips (2004) demonstrating that intense corticolimbic induced activity caused a disruption in PPI and denoting the importance of this critical developmental period with regard to insult vulnerability. All pups injected with KA displayed seizure activity of varying degrees supporting the efficacy of the treatment to compromise the circuitry. Although it seems unlikely, it remains possible based on those observations that the kainic acid induced hippocampal insult may not have been sufficient to compromise the hippocampus to the degree necessary to promote future vulnerability.

Stress can result in either adaptive or maladaptive changes in both humans and animals but the effects have been shown to be highly dependent on the intensity of the stress or duration of the exposure (Nelson, 2000). Stress dependent elicited responses appear to follow a temporal pattern of response in which short term stressors are
associated with adaptive enhancements and long term stressors associated with maladaptive alterations (Luine, Beck, Bowman, Frankfurt & MacLusky, 2007). Short periods of restraint stress in particular have been shown to enhance performance on some tasks (Kneavel, Chrisakos, Ferguson & Luine, 2000) while impairing performance on the same tasks when stress periods were lengthened (Mizoguchi, Yuzurihara, Ishige, Sasaki, Chui & Tabira, 2000).

In the present study the stress manipulation may not have been sufficiently intense in either strength or duration to evoke the necessary biological response and may have instead served to enhance performance. Although there is no definitive pattern suggesting that one stressor is more effective in eliciting corticosterone release than another, Bowman, Beck and Luine (2003) discuss how the stress response differs with the use of varying types of stressors. Exposure to moderate and high levels of stress has been shown to have a facilitative effect (Conrad, LeDoux, Magarinos & McEwen, 1999; Cordero et al, 2003) on contextual fear conditioning despite causing a wide array of cellular, molecular and functional impairments in the hippocampus (McEwen, 1999; Sandi, Merino, Cordero, Touyarot & Venero, 2001; Pavlides et al 2002). A single exposure to restraint stress has also been shown to facilitate fear conditioning, elicit sensitization of the HPA axis and produce an elevated corticosterone response (Cordero et al, 2003) without inducing the other alterations in structure and function of the hippocampus (Pavlides et al, 2002).

The use of restraint stress involves both psychogenic and neurogenic properties. It is considered a good candidate for the evaluation of stress induced psychopathology
because it allows for dual components of potential stress response activation to be examined. Restraint stress elicits a mental/emotional affective (psychogenic) response and a biological/neural (neurogenic) response. However, the intensity of any stress exposure and its ability to evoke or predict anxiety is qualified by a subject’s perceptual response following the stress event (Adamec, Kent, Anisman, Shallow & Merali, 1998). Perception of stress severity and the subsequent behavioral reaction is also a clinical feature in the human profile of the disorder (Shalev, Freedman, Peri, Brandes & Sahar, 1997). Humans and rats display differential vulnerability possibly based on perceptual manifestations that may not be realistically assessed in animal models and result in confounding variability beyond what this study could address.

At the time of behavioral testing the rats had reached young adulthood (three months of age). Therefore, another plausible explanation for the lack of observed short term effects may be that an accrual of damage as the animals aged could have resulted in susceptibility that might have become more pronounced over time. The aging process results in a decrement in the number of CA1 cells in the hippocampus (Kerr, Campbell, Applegate, Brodish & Landfield, 1991). Cognitive impairments observed in aged rodents may be the result of stress induced glucocorticoid release and dysregulation across the lifespan accumulating in hippocampal damage with the progression of time (McEwen, 1992, Sapolsky, 1992). Aged rats and humans are susceptible to declines across all physiological systems and it can be assumed that stress may exert a larger degree of impairment in aged subjects (Luine et al, 2007). Therefore the vulnerability to stress effects alone or the synergistic effect of the biological predisposition may have become
more evident with the passage of time. The developmental progression of time specific damages was beyond the scope of this study. Future studies could be designed to assess the chronic effects of trauma induced susceptibility examining age as a vulnerability to psychopathology.

Another factor of interest regarding the lack of differences between groups is the conceptualization and use of the stress re-exposures. The three stress reminders delivered at 10 day intervals following the initial 2-hour stress exposure were designed to serve as situational reminders of the trauma. In addition to the precipitating trauma, exposures to situational reminders have been shown to contribute to the maintenance of fear related disturbances in behavior, the induction of behavioral sensitization and the long term effects associated with PTSD (Pynoos, Ritzmann, Steinberg, Goenjian, & Prisecaru, 1996). Responding to situational or environmental cues with heightened behavioral and physiological responses is a common feature of PTSD. Servatius, Ottenweller, Soldan, Bergen & Natelson (1992) observed exaggerated startle responding and an increase in corticosterone response to stressor re-exposures 10 days after the last stress session in both 3-day stress and 1-day stress paradigms designed to differentiate between specific levels of PTSD symptomology. Exposure to situational reminders has been associated with an increase in the magnitude of the startle response (Pynoos et al, 1996). Although an argument can be made that the effect of the re-exposures may have instead desensitized the animals, in the present study informal behavioral observations are consistent with previous research observations of dramatic behavioral disturbances and the elicitation of aggression. Pynoos et al (1996) describe excessive fighting in mice
resulting in maiming or death consistent with findings well established in the literature of increased aggressive behaviors in humans suffering from PTSD (Bell & Orcutt, 2009). Although no direct observations were made, it appears that the male rats in the present study may have engaged in aggressive related behaviors subsequent to the stress re-exposures and resulting in the deaths of cage mates and cannibalization in some instances. This finding could not be substantiated in the rat literature which focuses exclusively on the maternal cannibalization of rat pups. One study described an incident of wild caught rats wounding and mortally injuring their cage mates and engaging in extreme cannibalism after stress exposure (Boice, 1969). Although atypical, this behavioral evidence may be related to the exacerbating effect of trauma inducing situational reminders in the etiology of PTSD.

Motivational changes following stress exposure also influence behavior and may account for the lack of differences between groups. Tasks using measures of inhibitory avoidance and escape such as those used in the elevated T-maze paradigm involve a manipulation of drive or motivation to either move away from or toward a specific location (Bowman, Beck & Luine, 2003). Stress induced helplessness was illustrated in a classic study by Overmier and Seligman (1967). Dogs exposed to inescapable shock lost the initiative to avoid shock when an escapable situation followed an inescapable exposure. Loss of control resulted in learned helplessness. Any behavioral assessment following stress exposure may involve motivational drive reduction as a by product of the inescapable stress (Bowman Beck & Luine, 2003).
For the purpose of evaluation, acoustic startle may not be as reliable a measure of PTSD as previously thought. Reports in the literature are inconsistent as to the utility of this assessment. Exaggerated startle responses have been observed in only a portion of combat veterans diagnosed with PTSD (Butler, Braff, Rausch, Jenkins, Sprock & Geyer, 1990). Ornitz & Pynoos (1989) report that children suffering from PTSD display decreased rates of startle inhibition, but conversely, equivalent rates of habituation between PTSD and healthy controls have been reported (Shalev et al, 1992). These differences may be based on methodological differences in startle technique, or related to the heterogeneity of PTSD, or be such that objective evidence simply does not support the subjective experience of the disorder and may not be quantifiable with this type of assessment (Servatius, Ottenweller & Natelson, 1995).

Although no group differences were observed in the elevated T-maze, results did reveal a significant effect of sex. Male rats spent more time in the enclosed arm of the T-maze on the measure of inhibitory avoidance (anxiety). The evaluation of startle reactivity also revealed no differences between the groups on measures of habituation and prepulse inhibition. However, a significant Sex x Stress interaction was observed. Following stress exposure, male rats exhibited less reactivity to acoustic startle than the female rats. A significant Sex x Hippocampal insult interaction was also revealed. Male rats in the hippocampal insult condition (brain damage) exhibited increase reactivity compared to female rats.

Gender has been shown to be a contributing factor in susceptibility and vulnerability to psychopathology. Although stress has been shown to elicit profound
alterations of the HPA axis in both males and females (Ehlert, 2001), sex differences in HPA reactivity have been suggested as the basis of increased vulnerability in women (Young, 1995). Stress is mediated by the sexually differentiated HPA axis (Bowman, Beck & Luine 2003). Female rats exhibit higher levels of corticosterone in basal levels and in response to acute stress exposure (Kitay, 1991). Increased corticosterone may be involved in the cognitive outcomes of chronic stress exposure (Conrad, McLaughlin, Harman, Foltz, Wieczorek, Lightner & Wright, 2007). Females release higher levels of corticosterone following stress yet stressed males are more cognitively impaired than females (Luine et al, 2007). Corticosterone levels habituate faster in females and may contribute positively to stress resistance (Bowman, Zrull & Luine, 2001). In the present study, the non-significant trend displayed by the female rats on the measure of escape adds support to previous findings of increased female reactivity and the hypothesized gender expectation.

A plethora of research evidence is available in the literature documenting evidence of the differential effects of stress exposure and sexual differentiation across a number of paradigms. For example, neonatal handling has been shown to elicit opposite effects, with male rats showing decreased CORT and ACTH responses and decreased stress reactivity after handling in comparison to female rats (Shors, Chua, & Falduto, 2001). In associative learning, stressed males learn faster than non-stressed males while stressed females learn slower than non-stressed females (Beck, Jiao, Cominski & Servatius, 2008). Male rats have shown impairments in radial arm maze performance following stress compared to female rats showing enhancements (Luine, Villegas,
Martinez & McEwen 1994). Supporting the differential effects of stress and consistent with other findings in the literature, the results of the present study did reveal some sex differences. Although difficult to explain, such findings may prove to be interesting and relevant to future investigations.

In the present study heightened stress reactivity in male rats was observed on the measure of inhibitory avoidance (anxiety). An interesting finding in the literature reports the identification of an alarm pheromone in male rats has been shown to enhance anxiety related responses including stress induced hyperthermia, defense and risk assessment behavior and acoustic startle (Inagaki, Nakamura, Kiyokawa, Kikusi, Takeuchi & Mori, 2009). However, stressed males displayed decreased acoustic startle reactivity.

Recent studies report that anxiety also adheres to the same temporal pattern of expression as other stress dependent responses and that chronic stress effects on anxiety are also sexually differentiated (Luine et al, 2007). Male rats display increased anxiety compared to females in a measurement of latency to enter an open field after chronic stress exposure and a trend toward increased male anxiety and decreased female anxiety has been previously observed (Luine et al 2007). The finding of the present study in which stressed males exhibited increased anxiety on the measure of inhibitory avoidance is consistent with other findings of heightened anxiety in males following stress exposure. In the human profile of stress related disorders gender differences have been shown to be related to the predictive power of specific risk factors. Anxiety has been shown to be predictive of psychopathology in men, and depression a predictor in women (Christiansen & Elklit, 2008).
Startle reactivity has also been shown to be differentially affected across sex. Females have shown suppressed reactivity and reduced motor responses (Beck & Servatius, 2005) while males have exhibited enhanced startle (Servatius, Ottenweller, Bergen, Soldan & Natelson, 1994; Servatius, Ottenweller & Natelson, 1995) in response to tail shocks. Male rats exposed to a series of inescapable consecutive tail shock exposures have been shown to exhibit exaggerated startle responses compared to suppressed reactivity in equally stressed females (Beck, Brennan & Servatius, 2002). Although the clinical profile of PTSD includes exaggerated startle as a prominent symptom of the disorder, this symptom is not always present in women diagnosed with PTSD (Beck et al, 2008). Some studies even report blunted startle responses in abused women diagnosed with PTSD (Medina, Mejia, Schell, Dawson & Margolin, 2001). A plausible explanation implicates endocrine modulation of startle suppression. Beck et al (2008) examined stress induced changes in sensory reactivity and found that female rats exhibit changes in startle sensitivity and response patterns at certain stages of the estrous cycle. Following stress exposure, Beck et al (2008) observed suppressed startle responses and reduced reactivity as differentially phase specific. Such findings may explain contradictions in the literature that describes evidence for both increased startle (Fullerton, Ursano, Epstein, Crowley, Vance, Kao, Dougall & Baum, 2001) and decreased startle (Medina et al, 2001) reactivity in PTSD profiles and may explain in part the present findings given that qualifying sex differences in endocrine hormone expression was beyond the scope of this study.
The brain damaged males displayed increased acoustic startle reactivity possibly related to the hippocampal insult. Hippocampal involvement has been implicated in multiple disorders. Damage to the hippocampus has resulted in increased startle amplitude (Swerdlow, Lipska, Weinberger & Braff, 1995) and increased sensorimotor gating following hippocampal lesioning has been associated with schizophrenia (Lipska & Weinberger, 2002; LePen & Moreau, 2002). Perinatal hypoxia and the purported damage associated with that insult may increase the risk of schizophrenia as a consequence of hypoxia related obstetric complications (Cannon et al, 1999). Therefore, the increased reactivity observed in the brain damaged males may reflect a compromised hippocampus and deficits associated with schizophrenia or a number of psychological disorders. However the differential sex effect may not be as easily explained.

Ovarian hormones may specifically contribute to observed sex differences in stress response reactivity (Bowman, Beck & Luine, 2003). Regarding the effect of stress on learning performance, research has found sex effects subject to the influence of hormonal differences between males and females (Shors, Beylin, Wood & Gould, 2000). Estrogen has been shown to have a neuroprotective effect that may underlie sex differences in hippocampal changes (Wise, Dubal, Wilson, Rau & Bottner, 2001) and may render females less sensitive to the impairments in memory and alter response to anxiety by acting as an anxiolytic agent (Luine et al, 2007). Luine et al (2007) report that as a consequence of lower estrogen levels young males have displayed heightened anxiety compared to young females. Subsequently, age related changes and higher estrogen may then account for enhancement in aged males displaying less anxiety (Luine
et al, 2007). Acute stress enhances estrogen levels (Shors, Pickett, Wood & Paczynski, 1999) while levels decrease after longer periods of restraint stress (Galea, McEwen, Tanapat, Deak, Spencer & Dhabhar, 1997). Bowman, Zrull & Luine (2001) observed enhancing effects of chronic stress in female rats in the radial arm maze after a 21 day restraint stress exposure while performance degraded after a long stress exposure. Given the observed sex differences in this study it may be plausible to suggest that the female rats could have benefited from an estrogen enhancing effect, especially if the stress exposure failed to meet the criteria for traumatic stress, further increasing the protective and possibly facilitative effects of estrogen.

Another worthy consideration in an attempt to explain the lack of significant effects in support of the original hypotheses is the factor of social support and what role this factor may play in stress related outcomes. We recognize the importance of support systems for humans in the manifestation of stress based disorders and the role that social support plays as a buffer against stress and anxiety based diseases. Research examining the relationship between social support and psychological distress as a symptom of PTSD has found social support associated with low levels of psychological distress (Glass, Flory, Hankin, Kloos & Turecki, 2009). Chronic PTSD has been linked to many preexisting factors such as the type and severity of the trauma, level of impairment associated with symptoms and lack of social support (Owashi & Perkonigg, 2007). It is not as clear as to what role support plays in animal models of psychopathology. In the present investigation the element of double housing may have confounded the results by providing an enhancing level of social support to the animals. Evidence suggests that
maternal response mechanisms may play a beneficial role in negating the detrimental effects of stress. Isolation rearing has produced pronounced neurochemical and behavioral abnormalities including the disruption of habituation and prepulse inhibition of acoustic startle (Geyer, Wilkinson, Humby & Robbins, 1993). Brief maternal isolation has also been shown to produce impaired habituation and greater response amplitudes of acoustic startle in the absence of altered prepulse inhibition (Finamore & Port, 2000). Maternal stimulation might therefore have been a factor pertinent to the enhancement of the pups stress response. The pups were marked with Sharpie marker to differentiate between groups. Although no direct data was able to be collected on the effect of maternal grooming in a control group what was observed was the dams’ diligence in cleaning the pups’ markings so efficiently that daily marking was required. This may have constituted excessive maternal attention that could have proven beneficial in alleviating the biological stress response.

In conclusion, the lack of significant evidence in support of the original hypothesis, and failure to find differences between the treatment groups or the identification of putative risk factors in PTSD appears to support instead the already inconsistent and contradictory findings in the literature. Speculatively one can question any number of methodological issues in treatment and testing parameters or physiological assessments and developmental progressions that were beyond the scope of the present study. In spite of the failure of these findings to suggest a suitable animal model of PTSD, the results may be useful in providing some insights or interesting anomalies to our understanding of stress psychopathology and may better serve as a model of
generalized anxiety. Given the various contradictory findings concerning sex differences in stress reactivity, future investigations into gender specific mechanisms in stress related disorders is necessary to elucidate the probability of sexually differentiated risk factors in the expression and maintenance of PTSD and other stress related disorders.
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