VALIDATING AN ANIMAL MODEL OF PTSD THROUGH OPEN FIELD BEHAVIOR

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In Dedication

to

My brother in heaven, my parents, and my two sisters whom I love dearly

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ABSTRACT

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Post-traumatic stress disorder (PTSD) is a prevalent anxiety disorder that affects people all over the world. Animal models are often used in clinical research to study human disorders; rats and humans share some similar genetic and biological traits. The open field model is a common paradigm for testing locomotor activity and anxiety related behaviors in rats. This study looks at the effect of anxiety-like behaviors in an open field on a rodent model of PTSD. Anxiety-like behaviors were examined after exposure to either restraint stress and predator odor or to no restraint stress and no predator odor. Significant differences were found in three of the open field measures assessed in attempts to validate an animal model of PTSD through open field behavior.

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CHAPTER I

POST-TRAUMATIC STRESS DISORDER

Clinical Syndrome

Post-traumatic stress disorder (PTSD) is a prevalent anxiety disorder characterized by hyper-arousal, disturbing flashbacks and avoidance of intrusive memories of a traumatic situation an individual has experienced (Borghans & Homberg, 2015). For American adults, the lifetime prevalence of PTSD is about 7.8%, and the annual prevalence of PTSD is estimated to be 3.5%, whereas, in other countries, it may vary between 0.5-1.0% (Sareen, 2014; American Psychiatric Association, 2013). This disorder is seen more often in veterans and individuals whose vocations are predisposed to risk, such as firefighters and other emergency personnel. More females than males experience PTSD in their lifetime and for longer durations. This may be due to a higher likelihood of female victimization. Individuals at the highest risk for developing PTSD include military combatants, survivors of rape and other assaultive traumas, and ethnically or politically motivated imprisonment and genocide (American Psychiatric Association, 2013). In these groups, roughly 33-50% will suffer from post-traumatic stress disorder (American Psychiatric Association, 2013). PTSD can develop as a result of threatened danger, terrorist attacks, military combat, witnessing violent crimes, violent encounters, or the death of a loved one.

Symptoms of PTSD include recurrent and involuntary distressing memories or dreams directly related to the traumatic event, dissociative reactions, intense and prolonged psychological distress, physiological reactions to triggers, such as fears and fearful memories, adverse changes in cognition and mood, and notable changes in arousal and reactivity. These symptoms must be ongoing for at least 30 days in individuals in order to be truly diagnosed with post-traumatic stress disorder and cannot be due to drugs or alcohol (American Psychiatric Association, 2013). This disorder can be debilitating, and life can become increasingly difficult to complete normal everyday tasks (American Psychiatric Association, 2013). The stress experienced in PTSD can affect individuals, not only psychologically, but also physiologically.

Comorbidity of PTSD

Post-traumatic stress disorder is frequently comorbid with other mental health disorders as well as substance abuse disorders; epidemiologic samples have shown that the majority of patients suffering from PTSD have at least one comorbid mental disorder. Such mental disorders include anxiety disorder and major depressive disorder. There has been an accumulation of evidence showing that PTSD is correlated with antisocial personality disorder as well as borderline personality disorder (Sareen, 2014). PTSD is highly comorbid with alcohol use disorder and substance use disorder. Many patients suffering with PTSD will self-medicate with alcohol and illicit drugs, likely as emotional numbing agents. However, trying to treat substance use disorders in those with PTSD is difficult; once they discontinue the substance, the symptoms of PTSD and comorbid mental health disorders resurface. This can be too much for the patient to handle at one

time. Approximately 75% of people with PTSD suffer from alcohol use disorder and approximately 20% of people with PTSD suffer with substance use disorder (U. S. Department of Veterans Affairs, 2015). Both alcohol use disorder and substance use disorder can further aggravate the lives of those with PTSD, as they can make relationships, careers, and livelihoods increasingly difficult. Suicidality is also prevalent among those who suffer from PTSD and comorbid mental health disorders (Flory & Yehuda, 2015).

Risk Factors

Research suggests there may be genetically determined features that contribute to the development of post-traumatic stress disorder. One study proposes there are genetically determined features of dopamine (DA) transmission which may promote the development of PTSD following trauma (Segman, et al., 2002). Early traumatic experiences, including prenatal and childhood stress, interfere with the proper development of neurobiological systems. This may increase stress reactivity and vulnerability to developing PTSD in later life (Nemeroff, 2004; Seckl & Meany, 2006). Children who experience trauma throughout their childhood are more likely to respond with heightened stress in far future traumatic situations if the early trauma results in the development of PTSD. PTSD can also exacerbate risk for development of health problems through obesity, sleep disturbances, physical symptoms, and development of comorbid depression and substance use disorders (Sareen, 2014). Another risk factor for developing post-traumatic stress disorder is neuroticism, a personality trait which is characterized by feelings of anger, fear, anxiety, depressed mood, and loneliness (Sareen,

2014). Patients with cognitive vulnerabilities, such as traumatic brain injuries (TBI) who are trauma survivors are also at an increased risk of developing PTSD (Sareen, 2014). Psychological challenges of traumatic brain injuries may create additional risks throughout the lifetime for victimization that promotes the development of PTSD. Poverty and environmental factors, as well as low levels of education, may also contribute to the development of PTSD; children who come from poverty-stricken backgrounds are more likely to develop PTSD than children who do not (American Psychiatric Association, 2013).

Risk facts include pre-trauma risk factors, trauma risk factors, and post-trauma risk factors which may lead to subsequent post-traumatic stress disorder. Pre-trauma factors include "prior trauma exposure, prior mental disorder, personality factors, and genetics"; trauma factors include "perceived fear of death, physical injury, and assaultive trauma"; post-trauma factors include "high heart rate, traumatic brain injury, acute stress disorder, and disability" (Sareen, 2014). If at least one of these factors from each group has been experienced, the person is at higher risk for development of PTSD. However, having positive social support in the posttrauma phase has been shown to protect individuals from the development of PTSD; it enhances resilience in individuals exposed to trauma (Sareen, 2014). Risk factors of exposure to traumatic situations and development of PTSD may lead to higher occurrence of various physical diseases and syndromes, such as cardiovascular disease, diabetes, and chronic fatigue syndrome (Wilson, et al., 2014). Other diseases include bone and joint disease, metabolic disease, and neurological conditions (Sareen, 2014).

Attempts to Treat PTSD

It is important to carefully and sensitively assess individuals who present with PTSD. It is often very difficult for patients to speak about the traumatic event due to their attempts to suppress intrusive memories. Comorbidity and physical health problems must also be carefully assessed in order to plan a beneficial route of treatment. It is also important to very carefully assess individuals with suicidal ideation (SI) and those who have attempted suicide. Even if they are only passively endorsing SI (neither admitting nor denying it) as they will likely be more vulnerable and reluctant to talk. Psychological, as well as pharmacotherapy treatments, are often used in attempts to treat PTSD. Individuals with PTSD must learn to face and manage their stress, or it will become increasingly difficult to treat as time goes by. Cognitive behavioral therapy (CBT), cognitive processing theory (CPT), eye-movement desensitization and reprocessing therapy (EMDR), and prolonged exposure (PE) are all used and have shown to decrease symptoms of post-traumatic stress disorder (Sareen, 2014). CBT is a problem-solving technique that teaches individuals to change their way of thinking so that when they think of their trauma, they can do so in ways that will not affect them negatively. This eventually changes the way they feel about the experience. There is no standard duration for CBT treatment; therapy can last anywhere from 5-20 weeks (Department of Veterans Affairs, 2009). CPT teaches individuals to change their negative thoughts by talking about them in therapy and completing short writing assignments. It teaches individuals helpful ways to understand their thoughts in a rational and healthy manner. It helps them to realize that trauma they have experienced is not

their fault but merely something that they experienced (Sobel, Resick, & Rabalais, 2009). CPT treatment usually lasts approximately 3 months (Department of Veterans Affairs, 2009). EMDR helps individuals to process and understand their trauma by recalling the trauma and tracking back and forth movement with their eyes, or by using "other forms of rhythmic left-right (bilateral) stimulation", the bilateral stimulation can reduce the negative emotions concerning the trauma memory (American Psychological Association, 2017). This treatment has shown to have positive outcomes with individuals feeling empowered having survived their trauma, no longer feeling like a prisoner of the experiences that once distraught them. EMDR therapy usually lasts approximately 1-3 months (U. S. Department of Veterans Affairs, 2017). PE teaches individuals how to gain control of their negative feelings by re-experiencing their trauma and learning to do things that they have avoided since the trauma took place. It teaches them to confront the traumatic memories they have avoided in order to reduce symptoms of PTSD so that they may once again regain control of their lives (U. S. Department of Veterans Affairs, 2009). PE therapy usually lasts approximately 3 months (U. S. Department of Veterans Affairs, 2009).

Pharmacotherapy for PTSD often uses selective serotonin reuptake inhibitors (SSRIs), such as Sertraline (Zoloft), Paroxetine (Paxil) and Fluoxetine (Prozac), as well as serotonin norepinephrine reuptake inhibitors (SNRIs), such as Venlafaxine (Effexor); these medications have shown to be effective in decreasing symptoms of PTSD (Sareen, 2014). Both of these medication types affect the level of naturally occurring neurochemicals, such as serotonin and norepinephrine. Although these medications are

the first and most important medications to be prescribed, they are not effective enough to help with insomnia and nightmares that occur in individuals with PTSD; additional medications may need to be prescribed to help individuals struggling with insomnia and recurring nightmares.

Individuals struggling with alcohol use disorder may also be prescribed naltrexone, an opioid receptor antagonist that helps individuals to abstain from opioids and alcohol. This can be combined with therapy to treat symptoms of PTSD. Additional treatment for alcoholism may be a little more difficult, as alcohol is often used as a numbing agent to suppress negative feelings, thoughts, emotions and intrusive memories resulting from trauma. Benzodiazepines are not viewed as a good treatment for PTSD, as individuals may become dependent on them and feel they can no longer face stressful situations without the medication. Use of benzodiazepines as a numbing agent for reminders of trauma, instead of facing the trauma directly, may lead individuals to never deal with their stress (U.S. Department of Veterans Affairs, 2017). This can make attempts to treat PTSD even more challenging. Although some individuals benefit from therapy and medication and can achieve remission, many individuals do not get sufficient benefit from currently available treatments (Borghans & Homberg, 2015). This is why animal models are pertinent for learning more about underlying mechanisms of post-traumatic stress disorder and preliminary testing of new treatment approaches.

CHAPTER II

Biological Abnormalities in PTSD Individuals

Serotonin's role in the "pathophysiology of posttraumatic stress disorder (PTSD) has been suggested by the overlap in clinical symptoms between PTSD and psychiatric conditions in which a serotonin dysfunction is implicated" (Cicin-Sain et al., 2000). Research indicates that individuals with PTSD have lower amounts of platelet serotonin uptake activity (Sherin & Nemeroff, 2011). Disruption of 5-HT transmission can cause symptoms that contribute to PTSD, such as stress, impulsive actions, increased startle response, hypervigilance, and memories that intrude the mind during normal activities (Sherin & Nemeroff, 2011). The 5-HT2A serotonin receptor, the main target of hallucinogens such as LSD, is, like PTSD, associated with panic reactions, flashbacks, depersonalization and a sense of unreality, some of which are symptoms of PTSD (Mellman et al., 2009). Therefore, the 5HT2A receptor is a particularly interesting target for experimental PTSD treatment.

Most of the norepinephrine (NE) in the central nervous system comes from neurons which project to regions in the brain that are involved in stress responses; norepinephrine can interact with brain circuits to enhance arousal, vigilance and stress, as well as autonomic responses to stress (Sherin & Nemeroff, 2011). Stress can result in the increased release of NE from the sympathetic nervous system, inducing physiological hyper-arousal. Also, the excitatory transmitter glutamate may play a role. Overexposure

to glutamate can lead to the loss of neurons in patients with PTSD (Sherin & Nemeroff, 2011).

Biological alterations have been in found in the hypothalamic-pituitary-adrenal axis with likely implications for brain structure and function. One of the biggest structural abnormalities in the brain is decreased hippocampal volume. The adrenocortical stress hormones may play a complex role in this. On the one hand, administering cortisol to patients shortly after trauma was beneficial. Cortisol impedes memory retrieval and administering cortisol can help decrease chances of developing PTSD (Pitman, 2012). On the other hand, chronic high levels of such stress hormones cause loss of hippocampal tissue, with severe effects on memory function and cognition. Neuroimaging has also revealed decreased volume in regions of the prefrontal brain (Pitman, et al., 2012). Those suffering with PTSD experience increased activation of the insular cortex in response to intrusive memories and triggering stimuli (Pitman, et al., 2012). This may help explain emotional abnormalities in PTSD since insular cortex activation is associated with feelings of illness and malaise.

CHAPTER III

ANIMAL MODELS OF PTSD

The use of animal models in research has been instrumental in discovering new treatments for humans with various illnesses. Many genetic and biological characteristics of rats are similar to those of humans; rat models have at least partially mimicked symptoms of various human illnesses or disorders. This allows for researchers to test the potential of novel medications and other treatments to help combat these illnesses and disorders. Since stressors can work through some similar fear pathways in rats and humans, rodent models of PTSD may be helpful in finding new treatments. Fear reactions in animals are similar in several regards to anxiety-related behaviors in humans; this provides face validity for animal models of anxiety disorders (Palanza, 2001).

Animal Subjects

Different strains of rats are used in animal models of post-traumatic stress disorder and anxiety. In addition, each strain may behave differently depending on the supplier as well as housing conditions at the breeding facility; the same strain from different suppliers may produce different results for the same battery of tests. The three most common strains are the Lewis rat, Sprague Dawley rat, and Wistar rat, all of which are albino. Other models use strains such as Fischer rat, Long-Evans rat, Brown Norway rat, knockout rats, and different types of cross-bred strains. Lewis rats are known to be more docile creatures, but are also more hyper-emotional than other rats; they are also

known to have lower fertility rates. They are easy to handle and habituate to handling. Sprague Dawley rats are used widely in many different types of medical research, such as nutrition, toxicology, oncology and surgery. Sprague Dawley rats are also quite docile animals, but with higher fertility. Like Lewis rats, they are very easy to handle and habituate to being handled. Wistar rats are all also used in medical research for various biological reasons. They tend to be more active than Sprague Dawley rats and Lewis rats. The Wistar rat strain is in the genetic background of the Sprague Dawley strain as well as the Long-Evans strain (Charles River, 2015).

The Fischer rat, Long-Evans rat and Brown Norway rat are all pigmented rat strains. It is noted that pigmented strains are used extensively in research for cognitive purposes, whereas albino strains are commonly used in models of mental health disorders, such as anxiety and schizophrenia (Turner & Burne, 2014). Fisher rats are also used in medical research, such as that dealing with oncology and surgery (Charles River, 2017). Long-Evans rats are used in various research models of behavior and learning (Charles River, 2017). Brown Norway rats are used in research for genetic mapping and mental disorders (Charles River, 2017). They engage in play fighting and tend to be more aggressive than the other rat strains listed, especially when dominance is at the root of the issue. Knockout rats are genetically engineered with a gene that has been turned off for purposes of imitating genetically caused human illnesses and diseases and for testing drugs to treat such diseases.

Types of Stressors

Underwater trauma (UT) is a model that requires the rat to be placed in deep water, for 30 seconds of forced swimming, followed by completely submerging the rat for 30 seconds (Borghans & Homberg, 2015). This model increases subsequent anxiety behavior in the rat. Reminders of underwater trauma induce changes related to memory in brain areas such as the amygdala and hippocampus (Borghans & Homberg, 2015).

Restraint stress (RS) requires that the rat be completely immobilized in either a tubular plastic restraint device or by attachment to a wooden board; time requirements vary. Restraint stress increases fear and anxiety because the rat is unable to move or escape; this also leaves the rat vulnerable to the potentially existing nearby predators. After-effects of restraint stress have been seen in a forced swim test; during the forced swim test, increased immobility is assessed. The restraint stress model has also induced altered activity in the hypothalamic-pituitary-adrenal (HPA) axis (Borghans & Homberg, 2015).

Predator threat is another type of powerful stressor that increases levels of anxiety in rats. This stressor can include something as simple as used cat litter or cotton balls soaked with wildcat urine or being in the physical presence of a natural predator, such as a cat. This produces significant behavioral and physiological changes, such as reduced growth rate, increases in anxiety, interference with endocrine system responses, and exaggerated startle response; these reactions are persistent (Whitaker, et al., 2014). Exposure to predator odor can also produce dysregulation of the HPA axis. Predator threat is typically used alongside other stressors, such as an intermittent auditory stimulus, social instability, and restraint stress (Whitaker, et al., 2014).

Other types of stressors include social stressors, such as housing instability and social instability. Housing instability occurs when rats are changed to new cages daily, being constantly paired with a different rat. Chronic housing instability results in increased anxiety-like behavior as well as HPA dysfunction because housing conditions have become unstable and the rat is reassured by stability (Borghans & Homberg, 2015). Social instability requires the rat to be isolated from all other animals for at least one day at a time. This results in increased contextual freezing as well as impaired fear extinction indicative of PTSD. This type of instability creates higher levels of stress and anxiety in rats than the occurrence of housing instability alone (Borghans & Homberg, 2015).

Fear conditioning consists of the rat learning to associate a neutral conditioned stimulus (CS), such as a tone or setting with an aversive unconditioned stimulus (US), such as exposure to a predator threat or an electric shock. Once the rodent has been exposed to the conditioned stimulus with the aversive unconditioned stimulus then re-exposure to that conditioned stimulus will trigger a conditioned fear response, which will resemble the same fear elicited during exposure to the aversive stimulus. The animal will learn to pair the conditioned stimulus with the aversive one, even if the aversive unconditioned stimulus is no longer present, thus resulting in the conditioned response (CR) of fear. Conditioned fear responses in animals to a conditioned stimulus include freezing, reflex expression, and physiological responses, such as increased heart rate and hormone release as a response to stress.

Electrical foot shock is widely used as a conditioned stressor. Physical shocks, ranging in intensity and duration, are administered to the rat via a metal floor. Auditory

cues are often paired together with foot shocks in order to examine fear recall in the absence of foot shocks in the same environment. If the rat responds to the auditory cue alone, then fear conditioning has been achieved. Rats that are administered electrical foot shocks show reduced locomotion, and repeated exposure increases their anxious behavior. Fear extinction has been impaired with this model of PTSD (Borghans & Homberg, 2015).

Single prolonged stress (SPS) is comprised of three different stressors followed by a period of isolation with no disturbance. Prolonged stress can include immobilizing the rat for a 2-hour time period, followed by a forced swim test for approximately 20-minutes, and ending with the administration of ether until the rat loses consciousness. Upon completing this, the rats are isolated for 7-days and left undisturbed. This 7-day period is thought to allow for the development of PTSD-like symptoms. Use of this model has induced altered HPA function, as well as various PTSD-like behaviors (Whitaker, et al., 2014).

Types of Measures

The elevated plus maze (EPM) is an apparatus that is raised several inches above the floor with two open arms and two closed arms. This model tests instinctive avoidance of unprotected, open areas; confinement to unprotected, open areas elicits physiological reactions of stress in this prey animal. Staying inside of the closed arms away from open areas is a sign of anxious behavior, as the animal is trying to avoid areas of anticipated danger, such as detection by predators. Anxiety that the animal exhibits may be affected by different factors, such as stress exposure, handling, housing

instability, etc. Other variations of the elevated plus maze include the elevated T maze (ETM), which is an apparatus in the shape of a T with only one enclosed arm, and the elevated zero maze (EZM) which is a circular apparatus with two enclosed dark areas on opposite sides of each other (Braun, Skelton, Vorhees, & Williams, 2011).

The light-dark box is an apparatus that consists of two separate compartments that assesses the instinctive aversion of animals to places with bright light. One side of the apparatus consists of a protected, dark area, and the other side consists of an unprotected, brightly lit area. This test assesses the instinctive conflict between the animal's curiosity drive to explore its surroundings and its evasion of brightly lit areas that leave the animal vulnerable to predators (Arrant, Schramm-Sapyta, & Kuhn, 2013).

The acoustic startle response (ACS) model consists of a testing chamber where the animal is exposed to bursts of noise. The subject reacts to these as a threatening stimulus. The force of the animal's startle response is measured by the apparatus, reflecting the animal's degree of anxiety. The acoustic startle response model can include another variation of response – prepulse inhibition. Prepulse inhibition allows for the startle reflex to be depressed by prior exposure to much quieter pre-stimulus noises, such as barely detectable air puffs, before exposure to the much louder noises. Pre-pulse inhibition is also a measure of sensorimotor gating of neurological processes: the ability to filter out unimportant stimuli, from other stimuli that may actually signal danger. Thus, sensorimotor gating helps to prevent stimulus overload and exaggerated fear responses (Geyer & Swerdlow, 2001).

Open Field Measures

The open field (OF) is an apparatus used to assess locomotor activity and eagerness to engage in exploratory behavior of a novel environment while assessing anxiety levels in the animal. The open field is typically split up into two parts, the outer square and the inner square. Higher levels of anxiety can result in thigmotaxic behavior (hugging the walls), decreased locomotor activity, decreased rearing, increasing freezing and immobility, increased defecation, and the occurrence of stretch-attend postures. Lower levels of anxiety will result in increased locomotor activity, increased instances of rearing, and more entries into the inner square. Behavior in the open field can be influenced by various factors, such as housing conditions, illuminance, temperature, sex and age of the rat, rat strain, and extraneous noise during testing conditions. Rats have an innate drive to explore their surroundings, even in the presence of threatening stimuli as well as an aversion to lighted, unprotected areas. These two tendencies conflict with each other and result in anxiety-related behaviors (Campos, Fogaca, Aguiar, & Guimaraes, 2013).

Many behavioral measures can be assessed in open field models of PTSD. These behaviors include grooming, defecation, rearing, freezing, immobility, and thigmotaxis. Grooming is assessed when a rat engages in washing of its forelimbs, hind paws, face, body, and genitals. Self-grooming can be a sign of calming behavior for anxious rats, as it can decrease the impact of stress on the rat. Rapid bouts of grooming are indicative of anxious behavior, whereas normal instances of grooming can take place in low-stress situations. Grooming has been shown to be heightened by novelty (Estanislau, 2012). A high rate of defecation is also indicative of high anxiety, as they result from sympathetic

activation. Thus, highly emotional animals may have increased rates of defecation and low levels of ambulatory activity.

Locomotion is the primary behavior assessed in the open field paradigm – it involves movement around the open field from one to place to another. Rats with increased anxiety tend to have decreased locomotion; anxious animals will have decreased movement in order to avert the attention of a potential predator nearby. Rats engage in decreased locomotion as a safety mechanism to avoid catching the attention of predators. Conversely, rats with lower levels of anxiety will engage in increased locomotion; they will have greater instances of mobility in the open field with higher levels of exploratory activity (Seibenhener & Wooten, 2015).

Rearing occurs when the rat stands on its hind legs in an upright posture. It is typical of risk-assessment behavior and may occur when the rat is investigating its surroundings in an unfamiliar environment, especially one that may allow for the presence of a predator searching for prey. Rearing allows for the animal to sniff the surrounding currents of air from various vantage points or engage in exploratory motivation in order to get a better view of its surroundings (Lever, Burton, & O'Keefe, 2006). It also allows for an animal to update spatial information and become aware of any potential threats so that they may act appropriately. However, highly anxious prey animals are likely to avoid rearing in exposed areas to avoid being seen by predators.

"Freezing" occurs as the result of fear-conditioning when a rat becomes immobile for at least two seconds with no movement other than breathing. Freezing typically occurs when the animal feels unsafe. It can occur in the open arm of an elevated plus

maze or as the result of a conditioned stimulus, such as a tone, that has been paired with an aversive stimulus. This parameter is widely used to indicate a high state of stress. "Immobility" occurs when a rat ceases all movement other than breathing for a period of time; immobility lasts for a longer period of time than that of freezing behavior. Freezing behavior and immobility time increase as levels of anxiety increase. In order to decrease instances of accidental freezing behavior as an experimental artifact, noises outside of the experiment room are to be kept at a minimum (Walf & Frye, 2007).

Thigmotaxis consists of wall hugging and staying near protected areas of an environment to minimize visibility. This behavior is indicative of high stress and anxiety, as the rat uses thigmotaxis as a safety mechanism to stay hidden from light, open areas, and predators. As levels of anxiety increase, so does thigmotactic behavior (Seibenhener & Wooten, 2015). Thigmotaxis allows for the avoidance of areas of anticipated danger. In an open field, the walls of the apparatus can create shadowed areas where the animal feels it can more safely maneuver about.

CHAPTER IV

EXPERIMENTAL HYPOTHESIS

Rats have many genetic and biological characteristics similar to those of humans and sometimes have the ability to mimic symptoms of human illnesses and diseases. Rodent models of human disorders have been useful for testing theories of underlying causes and evaluating potential treatments. A team of behavioral neuroscience researcher at UHCL has been working to create and validate an animal model of PTSD. This model of post-traumatic stress disorder uses three stressors, restraint stress, predator odor threat, and housing instability to create PTSD-like symptoms. After allowing seven days since the two acute stressors for the syndrome to develop, various procedures evaluate the long-term effects of the stressors. These planned tests include the elevated plus-maze (EPM), acoustic startle response test (ACSR), and the open field test (OFT). The present thesis deals with the long-term effects of the combined stressors on open field behavior.

As a result of this model of post-traumatic stress disorder, it is hypothesized that the rats will have a longer latency to enter the inner square, fewer inner square entries, decreased locomotion, decreased rearing, increased bouts of grooming, and increased instances of immobility. All of these measures are indicative of anxiety-related behaviors occurring as long-term consequences of exposure to combined stressors.

CHAPTER V

METHODS

Subjects

Forty male Lewis rats (Envigo, Indianapolis, IN) were originally obtained for this experiment, but due to the university shutdown resulting from Hurricane Harvey, we had to discard data from half of the total rats obtained. For purposes of the data for my thesis, twenty male Lewis rats, weighing 275-325 grams, were used for our experiments. Animals were housed in a climate-controlled housing facility with food and water available *ad libitum*. All rats were maintained in a normal 12-hour light/12-hour dark cycle. The dark phase began at 7:00 p.m. and ended at 7:00 a.m. All procedures were approved by to the Institutional Animal Care and Use Committee (IACUC) at the University of Houston-Clear Lake.

Equipment

Both groups were tested using three types of apparatus: the elevated plus maze (EPM), the acoustic startle response (ACSR), and the open field (OF). The elevated plus maze apparatus is raised 50 centimeters from the ground with two sets of arms, two open arms and two closed arms, with a video camera suspended above the maze to record behaviors; the videos were linked to the AnyMaze software program (Stoelting Co., Wood Dale, IL) for purposes of tracking motion.

The acoustic startle response (ACSR) apparatus consisted of a holding box sitting atop a load cell which measures the largest displacement of force, in grams, caused by sudden movements of each rat. Background white noise consisted of 65 decibels which played while each rat was subjected to thirty pseudo-randomized trials of white noise bursts at 90, 105, and 120 decibel acoustic bursts. Responder X software (Columbus Instruments, Columbus, Ohio) was used on a laptop to record the force output of startle responses during testing.

For this thesis, only data from the open field will be analyzed. The open field apparatus consisted of four enclosed walls measuring 30 x 30 inches (45.7 x 45.7 cm) with an inner square of 18 x 18 inches (with a video camera suspended directly above; videos of each rat was analyzed by AnyMaze software (Stoelting Co., Wood Dale, IL) after testing was completed. AnyMaze, a motion tracking program, was used to record exploration time in the inner square, exploration time in the outer square, total exploration time, distance traveled in the inner square, distance traveled in the outer square, total distance traveled, latency to enter the inner square, and total inner square entries.

Stressed Group

Rats were habituated to the lab one week prior to the experiment beginning. The rats were weighed each day in the holding room. Day 1 and day 2 of the experiment was devoted to gentle handling in the holding room starting at six in the evening to habituate the rats to human contact; Room 6, the stress exposure room, was also wiped down completely after spraying the walls and apparatus. On day 3, the rats were gently

handled accompanied by scruffing. (Scruffing consists of grasping the scruff of the neck in the manner that is employed in subcutaneous injections. This is a control for injection procedures planned for future experiments.) Rats were then habituated to Room 6, designated the stressor exposure room, for 20 minutes before being returned to their cages in the housing room; again the walls and apparatus in Room 6 were sprayed down and sanitized. The rats were habituated to scruffing in preparation of receiving a needle poke on day 7. This was a control for injection procedures, which are planned for future experiments.

On day 4, the stress-exposure day, the rats were for one hour in a small cage and kept locked in a transparent plastic tube to induce restraint stress. This was paired with a predator odor. A cotton ball soaked in 0.5 mL of wildcat urine (PMart, LLC, Sandy Point, ME) was placed directly in front of the snout of each rat for the entire hour, while a computer-generated intermittent tone (2 kHz at 3 second intervals) played continuously. The tone was employed as a fear-conditioned stimulus. Afterwards, each rat was returned to the housing room, but was placed with a new cage mate to introduce housing instability. Again Room 6 was sprayed and sanitized.

For the seven days prior to testing, the stressed group was subjected to chronic housing instability. This consisted of each rat being paired with a new cage mate each day. Rats were never paired with the same cage mate twice. On day 5 the only activity was switching cage mates in the housing room for purposes of housing instability. On day 6, rats were once again habituated to handling, plus scruffing, and then received new cage mates upon return to the housing room. On day 7, the rats were handled for 30

minutes and habituated to the needle poke in the holding room; this was vehicle injection day. They were then habituated to the EPM testing room (Room 16) for 15 minutes, followed by habituation to the ACSR testing room (Room 10) for an additional 15 minutes. Cage mates were then switched. On day 8, the rats were handled for 30 minutes again before again being habituated to the EPM testing room for 15 minutes followed by habituation to the ACSR testing room for an additional 15 minutes again. Cage mates were switched again. On day 9, the rats were handled for 10 minutes, followed by 10 minutes of habituation to the EPM testing room before being returned to the housing room. Paired groups were then moved to the ACSR testing room for 5 minutes of habituation, followed by 5 minutes of habituation to the ACSR apparatus per rat. Cage mates were switched again. On day 10, the rats were handled for 15 minutes and were then given a saline injection in the holding room, as a control for injection procedures in planned subsequent experiments. They were then habituated to the EPM testing room for 10 minutes before being transported back to the housing room. Upon returning to the housing room, paired groups were habituated to the ACSR testing room for 5 minutes, followed by 5 minutes of habituation to the ACSR apparatus per rat. Cage mates were switched again. On day 11, the rats were handled for 10 minutes in the holding room and then habituated to the EPM testing room for 10 minutes before being returned to the housing room. Upon return to the housing room, paired groups of rats were habituated to the ACSR testing room for 5 minutes, followed by habituation to the ACSR for 5 minutes per rat. Cage mates were then switched again.

Testing Procedure – Open Field Test

Day 12 was the testing day for PTSD-like behavior. One hour after the acoustic startle response (ACSR) test, the rats were transferred from the holding room, Room 17, to the open field (OF) room for testing in Room 6. Under low red lighting, approximately 30 lux, the rats were recorded using a video camera that was suspended directly above the open field. The video camera transmitted a video feed to a laptop computer located outside of the testing room. Two observers were located outside of the room in order to observe the video recording while testing took place. They placed the rats into the open field apparatus and removed them upon completion of each test.

The open field apparatus was black in color and the walls were lined with black material; dimensions of the apparatus were 30 in X 30 in with an inner zone of 18 in X 18 in. One hour prior to testing, the apparatus was thoroughly cleaned with 90% Isopropyl alcohol. During testing, rats were placed into the open field for a total of 10 minutes each, but each rat was only video-recorded for 5 minutes each. The same intermittent tone played during the stress procedure (2 kHz at 3 second intervals) was played during the 10 minute duration of open field testing. This was intended as an auditory cue for the conditioned fear induced by wildcat urine and restraint stress on day 4 of the experiment. Once each rat completed the testing phase, the open field apparatus was wiped clean with 90% Isopropyl alcohol and dried.

Control Group

Rats were habituated to the laboratory one week prior to the experiment beginning. The rats were weighed each day in the holding room. Day 1 and day 2 of the experiment involved gentle handling in the holding room starting at six in the evening to

habituate the rats to human contact; Room 6, the stress exposure room, was also wiped down completely after spraying the walls and apparatus. On day 3, the rats were gently handled accompanied by scruffing. Rats were then habituated to Room 6, designated the stressor exposure room, for 20 minutes before being returned to their cages in the housing room; again the walls and apparatus in Room 6 were sprayed down and sanitized. The rats were habituated to scruffing in preparation of receiving a needle poke on day 7.

On day 4, the sham-stress exposure day, control rats were exposed to sham stressors. They were placed in a small cage. The tubular restrainer was present, but the rat was not forced inside. The cotton ball was moistened with saline rather than wildcat urine. The same intermittent tone was played during the hour of sham stress. Afterwards, each rat was returned to the housing room with its original cage mate. The control group experienced no social housing instability during the entire experiment. Again, Room 6 was sprayed and sanitized. Day 5 consisted of weighing the rats; cage mates stayed the same. On day 6, rats were once again habituated to handling. On day 7, the rats were handled for 30 minutes and habituated to the needle poke in the holding room. They were then habituation to the EPM testing room (Room 16) for 15 minutes, followed by habituation to the ACSR testing room (Room 10) for an additional 15 minutes. Afterwards, cage mates were returned to the housing room. On day 8, the rats were handled for 30 minutes again before again being habituated to the EPM testing room for 15 minutes, followed by another habituation to the ACSR testing room for an additional 15 minutes. Cage mates were returned to the housing room. On day 9, the rats were handled for 10 minutes, followed by 10 minutes of habituation to the EPM testing room

before being returned to the housing room. Paired groups were then moved to the ACSR testing room for 5 minutes of habituation, followed by 5 minutes of habituation to the ACSR apparatus per rat. Cage mates were returned to their home cages in the housing room. On day 10, the rats were handled for 15 minutes and were then given a vehicle injection of saline in the holding room. (The injection was intended as a control for injections planned for future planned experiments.) They were then habituated to the EPM testing room for 10 minutes before being transported back to the housing room. Upon returning to the housing room, cage mates were habituated to the ACSR testing room for 5 minutes, followed by 5 minutes of habituation to the ACSR apparatus per rat. Cage mates were then returned to their home cages in the housing room following habituation. On day 11, the rats were handled for 10 minutes in the holding room and then habituated to the EPM testing room for 10 minutes before being returned to the housing room. Upon return to the housing room, paired groups of rats were habituated to the ACSR testing room for 5 minutes, followed by habituation to the ACSR for 5 minutes per rat. Cage mates were once again returned to their original home cages in the housing room.

On day 12, the non-stressed control rats were tested for PTSD-like behaviors in the same manner as the stressed group, described above.

Behavioral Measures and Analysis

Each open field test was run through the AnyMaze (Stoelting Co., Wood Dale, IL) software to track locomotion in the inner square, locomotion in the outer square, total locomotion, and latency to enter the inner square. In addition to the video scoring

software, the following behavioral measures were scored by an observer who was blind to the experimental conditions – rearing, grooming activity in the inner square, grooming activity in the outer square, exploration time, thigmotaxis, immobility time in the inner square, and immobility time in the outer square. Frequency will be counted for number of rearing instances, which refers to the number of times a rat stands on its rear limbs in an upright, vertical position. Frequency was also counted for number of entries into the inner square, instances of immobility in the inner square, instances of immobility in the outer square, grooming activity in the inner square, and grooming activity in the outer square. Behaviors such as exploration of the open field, latency to enter the inner square, time spent in the inner square, time spent in the outer square, time of immobility in the inner square, time of immobility in the outer square, time spent grooming, and thigmotaxis, staying near the walls of protected areas, was measured in time. Distance traveled in the inner square, distance traveled in the outer square, and total distance traveled was measured in distance, tracked by the AnyMaze (Stoelting Co., Wood Dale, IL) software, using meters. Each video recorded was hand-scored at least three times for purposes of eliminating any possible discrepancies. A 2-tailed *t*-test was used to score each behavioral measure. These statistical analyses were performed using SPSS Statistics software (version 22, IBM Corp, Armonk, NY) with an alpha of .05.

CHAPTER VI

RESULTS

Due to once instance of failure in the video camera recording, data for one rat in the stressed group was lost; ending data consists of 10 rats in the non-stressed group and 9 rats in the stressed group.

Rears

The data indicates that there was a drastic decrease in the number of rears in the stressed group than in the non-stressed group (Fig. 1). A *t*-test detected a significant difference. The stressed group of rats had 6.89 ± 2.91 vertical rears, while the non-stressed group of rats had 15.00 ± 1.33 vertical rears; $t(17) = 2.53$, $p = .011$.

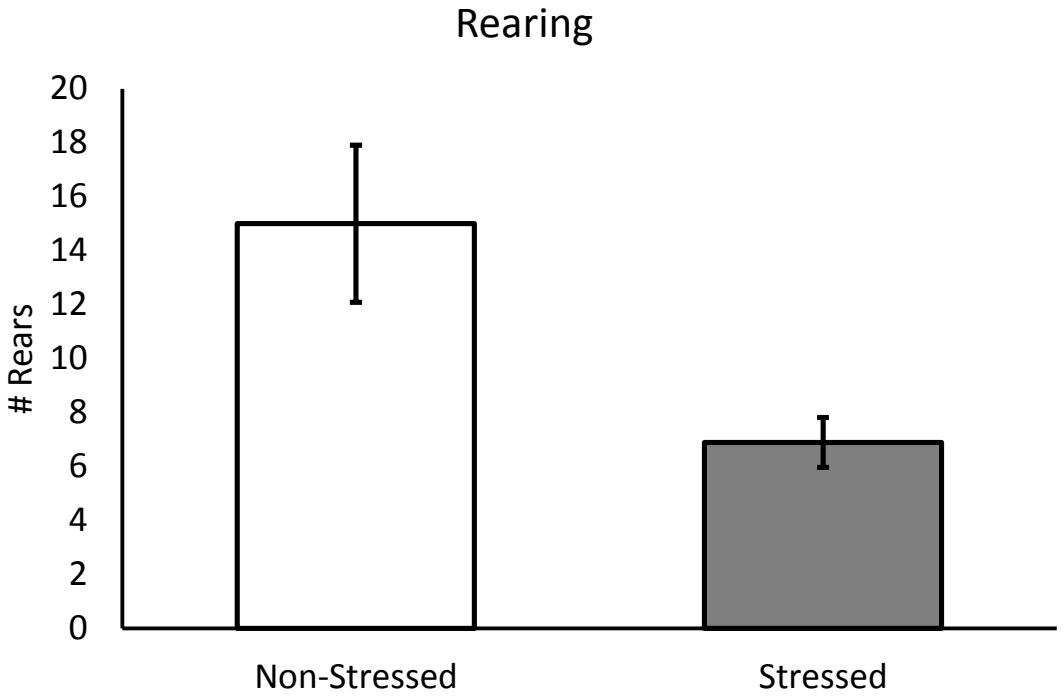

Fig. 1. The effect of three stressors on numbers of rears (mean ± standard error) over five minutes in an open field. The stressed group had been exposed to predator threat and restraint stress followed by a week of housing insecurity (daily changes in cage mates). The non-stressed group was exposed to sham-stressors only, followed by a week of stable paired housing. ** $p = .011$.

Total Distance Traveled

The data indicate that the stressed group of rats showed less horizontal movement, indicative of anxiety in an exposed situation (Fig. 2). The non-stressed group of rats traveled a total distance of 12.53 ± 1.27 meters, whereas the stressed group traveled a total distance of 9.69 ± 1.0 meters. A t-test detected a significant difference; $t(17) = 1.731$, $p = .048$.

Fig. 2. The effect of three stressors on total distance traveled in an open field (mean ± standard error in meters). The stressed group had been exposed to predator threat and restraint stress followed by a week of housing insecurity (daily changes in cage mates). The non-stressed group was exposed to sham-stressors only, followed by a week of stable paired housing. * $p = .048$.

Inner Square Entries

The data indicate that the stressed group of rats had fewer instances of inner square entries than the non-stressed group of rats. The non-stressed group of rats entered the inner square zone of the open field 20.40 ± 2.77 times, whereas the stressed group of rats entered it only 15.11 times (Fig. 3). The difference between these two groups approached significance, $t(17) = 1.43$, $p = .085$.

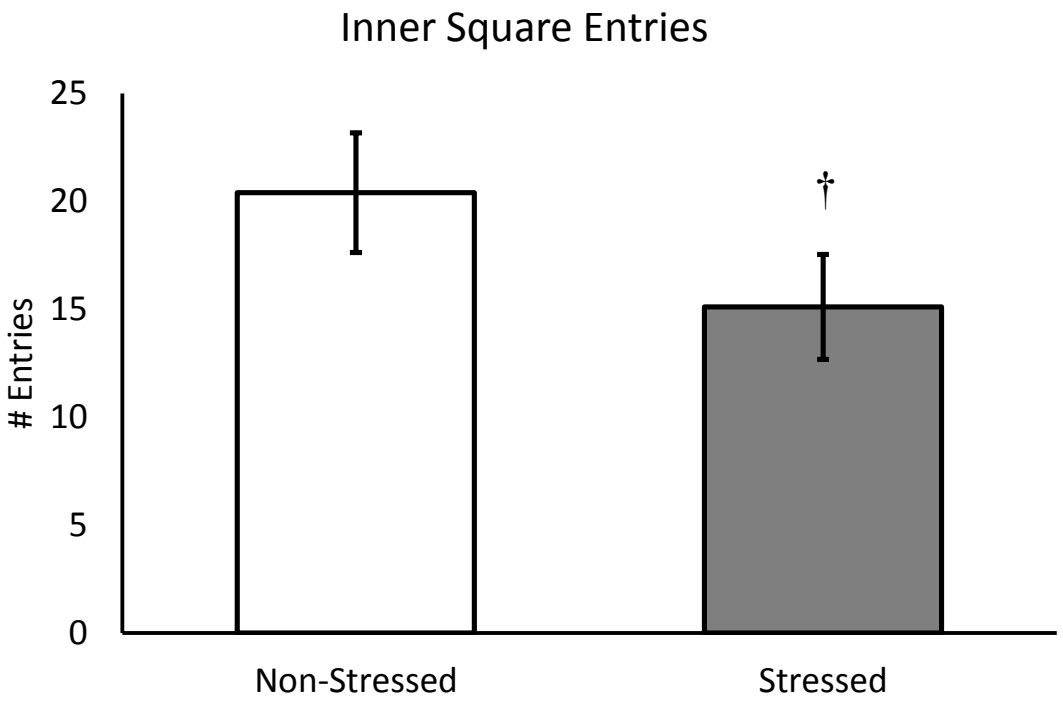

Fig. 3. The effect of three stressors on numbers of entries over five minutes into the more exposed inner square within an open field (mean ± standard error). The stressed group had been exposed to predator threat and restraint stress followed by a week of housing insecurity (daily changes in cage mates). The non-stressed group was exposed to sham-stressors only, followed by a week of stable paired housing. †$.05 < p < .10$.

Other Open Field Measures

Table 1 summarizes the data for various observed variables in the open field test. There were no other statistically significant differences between the stressed group and the non-stressed group found in the remaining variables.

Open Field Measures	Group	N	Mean	Std. Error Mean
Total Distance Traveled (m)*	No Stress	10	12.53	1.27
	Stress	9	9.69	1.00
Outer Square Time (s)	No Stress	10	266.98	5.74
	Stress	9	263.66	6.30
Inner Square Time (s)	No Stress	10	33.29	5.71
	Stress	9	36.34	6.30
Inner Square Entries†	No Stress	10	20.40	2.77
	Stress	9	15.11	2.43
Total Immobility Time (s)	No Stress	10	1.37	0.75
	Stress	9	2.11	1.51
Total Immobility Episodes	No Stress	10	0.30	0.15
	Stress	9	0.56	0.44
Rears**	No Stress	10	15.00	2.91
	Stress	9	6.89	0.93

Table 1. Various open Field Measures In Previously Stressed and Non-Stressed Rats.

** p <.02, * p < .05, † .05< p <.10

CHAPTER VII

DISCUSSION

The validity of this PTSD model was supported by a few of the open field measures, particularly distance traveled in the open field and numbers of rearing episodes. As prey animals, being in a state of high anxiety may make rats reluctant to do anything to attract attention from predators, particularly in an open, exposed area. Thus, anxious or panicky rats will move around less and avoid standing up. The three sorts of stressors appear to have produced such a state of anxiety.

Limitations of the Study

Due to the university closure during Hurricane Harvey, we lost half of our data, and therefore were unable to view the full set of results. Additionally, due to the video camera malfunctioning during the experiment for the stressed group, we lost an entire set of open field data for one animal. The relatively short duration of the experiment might have negatively influenced the results. In other PTSD models, it takes at least a week for the PTSD-like effects to fully develop. If the experiment had lasted longer, we might have observed additional significant differences between treatment groups (Zoladz, Conrad, Fleshner, & Diamond, 2008). Also, with more stress exposure, we might possibly have a more complete PTSD-like pattern. We only subjected the animals to the predator odor and restraint stress once. This may have had a weaker effect than

subjecting them to the stressor twice. It would be interesting to see the results of an animal model with longer duration of the experiment and with more exposures to the acute stressors. Another limitation was a failure in scoring "freezing" behavior. Since we were unable to develop an objective criteria for freezing, it was difficult to score. Ultimately, we were unable to use that data.

In addition, our results were probably affected by a fundamental problem in PTSD research. In both clinical PTSD and animal models, only a minority of the subjects develop a severe syndrome. Thus comparing overall means of traumatized versus non-traumatized subjects is not a highly sensitive way to detect PTSD or PTSD-like behavior. More individualized types of analysis may be needed to identify affected individuals from large samples.

Suggestions for Future Directions

In order to increase levels of anxiety in the rats, Zoladz et al. (2008) suggested incorporating two periods of predator threat exposure. Future research could possibly focus on lengthening the duration of the experiment with implementation of additional exposure to the predator odor threat. Instead of condensing the experiment down to twelve days, then it might be more feasible to make the experiment last longer, giving the full PTSD-like syndrome more time to develop. Also, instead of changing cage mates, it might be worthwhile to singly house the subjects. Beery and Kaufer (2014) found that paired housing nearly eliminated the effects of various stressors.

Once the UHCL rodent model of PTSD is perfected and more fully validated, it could be used for preliminary screening of potential treatments. In particular, the

transmitter serotonin is known to modulate anxiety behaviors differentially via its diverse receptors. In particular, the 5HT2A serotonin receptor is the main target of LSD, and LSD induces some effects such as a sense of unreality, depersonalization and flashbacks that are reminiscent of some PTSD symptoms. Therefore, it would be interesting to test whether 5HT2A receptor antagonists, such as Pimavanserin might reduce PTSD-like behaviors in the rat.

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