EFFECTS OF THE 5-HT_{2A} RECEPTOR INVERSE-AGONIST PIMAVANSERIN ON A RODENT MODEL OF POST-TRAUMATIC STRESS DISORDER

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Dedication

to

The friends and family that I have gained and lost along the way.

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ABSTRACT

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Post-Traumatic Stress Disorder (PTSD) is a chronic and debilitating psychological disorder that manifests in individuals that are witness to or directly exposed to intense life-threatening situations. Exposure to such events can result in a cluster of symptoms that include intrusive memories, avoidance, persistent anxiety, and hyper-reactivity. While PTSD in returning veterans bought this disorder to public attention, it is widespread in the civilian population, with a majority of cases reported in females. The most common pharmacological treatments for PTSD and related anxiety disorders are the class of drugs known as Selective Serotonin Reuptake Inhibitors (SSRIs). However, many individuals diagnosed with PTSD are resistant to such treatments, and some patients experience aversive side effects. To remedy this problem more selective therapeutic approaches are required.

The neurobiological correlates related to PTSD appear to be quite complex. However, the up-regulation and activation of the Serotonin 2A Receptor sub-type (5-

$HT_{2A}R$) is implicated in the formation, maintenance and symptoms of PTSD. Our research group developed a rodent model of PTSD in female Lewis rats, utilizing chronic isolation in conjunction with acute episodes of restraint stress and predator threat as the stressors. The current experiment set out to test the hypotheses that our rodent model of PTSD would increase the expression of several PTSD-like behaviors, as assessed on the Elevated Plus Maze (EPM), Conditioned Place Aversion Test (CPA), Acoustic Startle Response Test (ACSR) and Open Field Test. In addition, we wanted to determine whether a 1mg/kg injection of Pimavanserin, a selective 5-HT_{2A} serotonin receptor inverse agonist, could attenuate the behavioral symptoms resulting from our PTSD model.

Findings from our experiments suggested that our model was able to produce several robust phenotypes resembling PTSD. Additionally, Pimavanserin-treated animals demonstrated greatly reduced PTSD-like behaviors, further suggesting that 5-$HT_{2A}R$ plays a role in the expression of several characteristics of PTSD.

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CHAPTER I:

BACKGROUND

The Clinical Disorder of PTSD

Definition and Principle Features

Post-traumatic stress disorder (PTSD) is a serious and persistent condition that may arise when an individual is exposed to severely traumatic or life-threatening situations. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), the traumatic event must involve exposure to actual or threatened death, serious injury or sexual violence. In this context, exposure is narrowly defined as directly experiencing or witnessing the traumatic event or learning that a trauma has occurred to a relative or close friend. In addition, PTSD may also be the result of repeated exposure to aversive details of a traumatic event (DSM-V, 2013). Under this definition of exposure, the DSM-V explicitly omits the perceived exposure to traumas via television, movies, pictures, or electronic mediums. The choice to limit the definition of is likely because these modes of exposure might create a construct too broad to be useful (DSM-V, 2013; Lancaster, Teeters, Gros & Back, 2016).

Individuals who develop PTSD respond to a traumatic experience with intense fear, helplessness or horror. In addition to a history of trauma, patients deal with a chronic pathopsychological profile defined by a cluster of distressing symptoms. Patients contend with distress brought on by repeatedly reliving their trauma through intrusive, flashback memories (Speckens, Ehlers, Hackmann & Clark, 2006; Solomon & Mikulincer, 2007; Ehlers et al., 2010). The intrusive flashback memories are frequently precipitated by the presence of cues associated with the traumatic event. Therefore, PTSD patients often strongly avoid stimuli such as places, people, thoughts, or activities associated with the trauma (Solomon & Mikulincer, 2007). However, in many cases, stimuli may generalize beyond just the trauma-associated cues, so that individuals perceive threatening or fearful stimuli in contexts that were previously neutral (Mahan & Ressler, 2011; Homberg, 2012; VanElzakker et. al, 2014). PTSD patients also

develop several additional debilitating symptoms, including persistent anxiety, exaggerated startle response, cognitive impairments, diminished extinction of conditioned fear, sleep disturbances, hyperarousal, and feelings of depersonalization and unreality (Brewin et al., 2000; DSM-V, 2013; Zoladz & Diamond, 2013). Furthermore, because individuals with PTSD re-experience their traumas and actively avoid memory triggers of the event, symptoms of the disorder come to negatively affect their everyday functioning (Solomon & Mikulincer, 2007; Schnurr & Lunney, 2008).

Epidemiology. Exposure to a traumatic experience is not uncommon. Estimates suggest that around 90% of the general population reports exposure to at least one or more traumatic events within their lifetime. The most commonly reported negative experiences include sexual or physical assault, war-related events, vehicle accidents, or traumas related to natural disasters (Kilpatrick, et al. 2013). Several reports claim that anxiety disorders and PTSD are some of the most pervasive and prevalent psychiatric maladies among the general population (Kessler, Chiu, Demler, & Walters, 2005; Kessler et al., 2012).

Many individuals who experience a traumatic event will present with severely acute stress reactions such as hyper-reactivity, problems with memory and cognition, and/or mood and sleep disturbances for days or weeks after exposure to trauma. However, studies have suggested that these are normal responses to such situations and will typically resolve on their own with time (Santiago et al., 2013). Nevertheless, up to 30% of individuals impacted by trauma-related events will continue to experience the constellation of symptoms for greater than one month, thus meeting the criteria for a PTSD diagnosis (Breslau, Davis, Andreski & Peterson, 1991; Kilpatrick, et al. 2013). National prevalence rates suggest that around 8.3% of Americans are affected within a lifetime, with women being twice as likely as men to be impacted by the disorder (DSM-V, 2013; Kilpatrick, et.al. 2013). Furthermore, reports suggest that up to 50% of individuals diagnosed with PTSD will continue to meet diagnostic criteria after 3years (Perkonigg, Kessler, Storz, & Wittchen, 2000).

Risk factors. Several studies have investigated the risk factors associated with PTSD. One major risk factor is a history of trauma-related incidences. Modeling this history as a type of dose-response relationship suggests that, as the number of traumatic exposures increases, the likelihood of PTSD also increases (Kilpatrick, et al. 2013). Similarly, the duration and intensity of the exposures are linked to the severity of PTSD symptoms (Dohrenwend et al., 2006; Zoladz & Diamond, 2013). Numerous other pre- and post-trauma risk factors are also implicated in the development of PTSD. These include female gender and social, intellectual, and educational disadvantages. Additionally, individual characteristics such as negative emotional attentional bias, anxiety sensitivity and a family history of psychopathology have been associated with the development of PTSD (Brewin et al., 2000; Schmidt, Kaltwasser & Wotjak, C. T. 2013; Sareen, 2014). Lastly, genetic and epigenetic factors linked to cortisol regulation and several neurotransmitter systems have been implicated in contributing to the expression of PTSD (Mellman et. al., 2009; Sarapas et. al., 2011; Castro-Vale et al., 2016; Lueken et. al., 2016; Sabban et al., 2018).

Biological and Neurological Correlates of PTSD

As previously suggested, PTSD stems from a complex etiology. Many lines of evidence suggest multiple neuroanatomical structures and systems may become altered as a result of experiencing trauma. Much of the current research has focused on the systems that regulate stress response, such as the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis, as well as pathways related to emotional learning and memory. Leading theories suggest that trauma induces acute activation of the stress response systems. This, in turn, increases the induction of aberrant conditioned fear, while impairing fear extinction. This may occur through known interactions of stress response mechanisms with limbic structures and systems that mediate emotional memories (Fuchs, Flugge & Czeh, 2006; Tronel & Alberini, 2007; Mitra, Ademec &Sapolsky, 2009; Morrissey, Mathews & McCormick, 2011; Wilson, Ebenezer, McLaughlin & Fancis, 2014; Sabban et al., 2018). Unabated, the emotional memory centers produce signals that lead back to the activation of the stress response system. Thus, it is

believed that dysregulation in these two systems can influence one another in a circular manner. This may mediate the persistent expression of symptoms such as avoidance, hyper-reactivity and persistent anxiety (Mahan & Ressler, 2011; Morrissey, Mathews & McCormick, 2011; Finsterwald & Alberini, 2014).

Sympathetic Nervous System and the Hypothalamic-Pituitary-Adrenal (HPA) Axis

The sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA)-axis are comprised of several neuronal and neuroendocrine structures that conjointly regulate the body's response to stress. Though SNS arousal is immediate, the HPA-axis effects are slower-acting but longer lasting as it is the primary neuroendocrine component of the stress response system (Hill, 2013). When a stressful stimulus is present, information is taken in from various sensory modalities and processed through the amygdala, a region of the brain known for processing fear-inducing stimuli. Projections from the amygdala send signals to the lateral hypothalamus, which then rapidly innervate the sympathomedullary pathway (SAM). Through the SAM pathway, the SNS stimulates the adrenal medulla to release epinephrine (EPI) and norepinephrine (NE) into the bloodstream. This generates a cascade of biological changes that engage the body's fight or flight response (Hill, 2013; Bear, Connors & Paradiso, 2016).

In synchronous fashion, the amygdala also sends excitatory signals to the paraventricular nucleus of the hypothalamus (PVN). Corticotrophin-releasing factor (CRF) is then released into the hypophyseal portal, a micro-vascular circulation system, which mediates the interaction between the hypothalamus and the anterior pituitary (Gross et al., 1993). In turn, CRF stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the systemic circulation. ACTH then provokes the secretion of glucocorticoids, primarily cortisol (in humans) or corticosterone (in rodents), produced by the zona fasciculata of the adrenal cortex (Herman et al., 2016). Upon release, these hormones travel through the bloodstream and interact with systems that modulate energy redistribution and memory formation (Hill, 2013; Finsterwald, Steinmetz, Travaglia, & Alberini, 2015). In addition, stimulation of glucocorticoid receptors

located in the hippocampus leads to negative feedback inhibition of the HPA-axis, preventing the subsequent release of glucocorticoids (de Kloet et al., 2005; Bear, Connors & Paradiso, 2016).

Several lines of research have demonstrated that individuals diagnosed with PTSD exhibit several abnormalities in these systems. With regards to the SNS, a study performed by Southwick et al. (1999) examined the effects of yohimbine in healthy controls and patients with PTSD. In healthy controls, administration of yohimbine leads to a pleasurable affect. This is because yohimbine is an $\alpha 2$ adrenergic receptor antagonist. Antagonizing the $\alpha 2$ receptor increases the release of NE, which can produce a pleasurable stimulating effect. However, PTSD patients exhibited flashbacks, anxiety and increased sympathetic arousal. This suggests that NE may already be heightened in PTSD patients or that they are more sensitive to the release of NE. Either explanation suggests dysregulation in the SNS and may explain the exaggerated SNS related symptoms exhibited by those afflicted with PTSD (Zoladz & Diamon, 2013). Moreover, corroborating evidence for the systemic elevation of NE has been found. Geracioti et al. (2001) investigated the concentration levels of NE in the cerebrospinal fluid of PTSD patients compared to healthy controls. The results suggested that not only were NE levels higher in PTSD patients but those levels in PTSD patients were also correlated with the severity of symptoms.

Most of the research suggests that disruption in the HPA-axis is preceded by abnormally low basal levels of cortisol in PTSD patients (Yehuda, 2009). This could suggest adrenal insufficiency. However, this notion is countered by evidence that shows that PTSD patients exhibit a highly reactive HPA-axis response when anticipating stressful stimuli or when confronted with acute laboratory stressors (Zoladz & Diamond, 2016). Therefore, an alternative explanation for the reduced basal levels of cortisol holds that there is disruption in the HPA-axis leading to exaggerated negative feedback inhibition (Schoner et al., 2017). This enhanced feedback inhibition could be due to preexisting gene mutations or trauma-induced epigenetic changes producing increased expression of glucocorticoid receptors and/or receptor hypersensitivity (Yehuda, 2009; Castro-Vale et al., 2016). Evidence for these hypotheses is

found in studies that administered the synthetic glucocorticoid dexamethasone which lead to enhanced suppression of ACTH and cortisol release (Zoladz, Fleshner & Diamond, 2012). In addition to the sympathetic and autonomic dysregulation to the body's stress response accompanying PTSD, memory circuitry and other brain regions can be similarly affected (Sherin & Nemeroff, 2011).

Emotional Learning and Memory

A major symptom experienced by individuals diagnosed with PTSD involves reliving their trauma through intrusive, flashback memories which precipitate feelings of fear. Moreover, these feelings of fear may also generalize to other stimuli that were once considered safe, leading to the manifestation of avoidance behaviors, feelings of anxiety and hyperarousal, suggesting that fear is atypically regulated in PTSD (Mahan & Ressler, 2012). Experimentally, fear conditioning and extinction are ways to study the process by which individuals may develop PTSD. This is because conditioning and extinction easily lend themselves to studying how people or animals learn to predict dangerous, fearful or safe stimuli in the environment (Goswami, Rodríguez-Sierra, Cascardi, & Paré, 2013). The brain regions implicated in the processing and subsequent dysfunction of fear conditioning and extinction are the amygdala, hippocampus and prefrontal cortex (PFC). In addition to their regulation of neuroendocrine function, they are also associated with learning and memory and likely play a further role in the development and maintenance of PTSD (Blanchard et al., 2001; Mahan & Ressler, 2012; Parsons & Ressler, 2013; Goswami, Rodríguez-Sierra, Cascardi, & Paré, 2013; VanElzakker et al., 2014).

The role of the amygdala, as previously mentioned, is involved in processing fearful stimuli. It serves this function in part by modulating fear learning and the expression of anxiety (Mahan, Kerry & Ressler, 2012). This functional relationship is demonstrated in case studies in which there was a loss of function in the amygdala which produced reductions in fear learning and the ability to express fear in the presence of fearful stimuli (Feinstein et al., 2013). However, neuroanatomical animal studies have observed amygdala hypertrophy, or enlargement,

by means of increased dendritic spine densities in response to traumatic stress (Fuchs,Flugge & Czeh, 2006; Mitra, Adamec & Sapolsky, 2009). This suggests that the resultant changes in plasticity to this area of the brain may enhance the perception of fear in humans. Indeed, evidence for this conjecture is found in functional neuroimaging studies, which show patients with PTSD exhibit hyper-activation within the basolateral amygdala (BLA) in response to stimuli associated with trauma (Etkin & Wagner, 2007).

Though the hyperactivation found in the amygdala is in part due to aberrant synaptic connections, the PFC and the hippocampus also play a role in modulating its activity. With regards to the PFC, projections from the PFC feed into the amygdala and provide top-down control of its activation (Mahan & Ressler, 2012). In addition, the PFC is implicated in the process of fear leaning extinction through its modulatory effects on the amygdala. However, in patients with PTSD, there is marked volume loss compared to healthy individuals, which is believed to be a result of glucocorticoid-induced hypotrophy (Etkin & Wagner, 2007; Sherin & Nemeroff, 2011). This, in turn, expresses itself as hypofunctioning of this brain region in fMRI studies, which suggests a diminished capacity to exert its inhibitory influence on amygdala activation (Etkin & Wagner, 2007; Mahan & Ressler, 2012; Parsons & Ressler, 2013). Given the PFC involvement in extinction, its hypofunctioning alludes to a possible reason why individuals with PTSD exhibit extinction deficits toward stimuli related to the trauma (Goswami, Rodríguez-Sierra, Cascardi, & Paré, 2013).

Finally, the hippocampus is a region of the brain associated with regulating stress response systems but is also implicated in declarative memory and contextual fear conditioning (Mahan & Ressler, 2012; Parsons & Ressler, 2013; VanElzakker et al., 2014). In patients with PTSD, similar to the PFC, the hippocampus is also smaller in volume and demonstrates a reduction in activity levels (Etkin & Wagner, 2007; Sherin & Nemeroff, 2011). These abnormalities are likely due to increased exposure to glucocorticoids which have been shown to reduce the length of apical dendrites and stunt dendritic arborization (Fuchs, Flugge & Czeh, 2006; Finsterwald & Alberini, 2014). Due to the deficits in dorsal hippocampal functioning, the

stress response system is prone to becoming active and is unable to initiate the negative feedback inhibition loop, leading to further hypotrophy. Furthermore, in PTSD patients, hippocampal dysfunction may impair its normal role in fear extinction and in its ability to discriminate safe from dangerous contexts (Goswami, Rodríguez-Sierra, Cascardi, & Paré, 2013). It is possible that theses deficits lead to overgeneralized and prolonged fear. This might be reflected in the symptoms of intrusive memories, avoidance behaviors, in addition to feelings of anxiety and hyperarousal.

The Serotonergic System

Essential to the function of the systems described above is the role that neurotransmitters play in regulating their activity. Serotonin, or 5-hydroxytryptamine (5-HT), is one such neurotransmitter. Serotonin is produced and distributed through a diffuse modulatory system originating from cells located in the raphe nuclei, two structures that lie bilateral to the midline of the brain stem (Bear, Connors & Paradiso, 2016). From this domain, many efferent projections lead to brain regions including the prefrontal cortex (PFC), amygdala, hippocampus, and hypothalamus (Stam, 2007; Xiang et al., 2017). Due to its vast connections, serotonin has been found to influence learning, arousal, anxiety, fear, neuroendocrine function and the regulation of mood, all of which seem to be augmented in patients with PTSD (Sherin & Nemeroff, 2011; Homberg, 2012; Wilson, Ebenezer, Mclaughlin & Fancis, 2014).

Many studies investigating biological risk factors for PTSD have implicated genes that affect 5-HT neurotransmission. For example, polymorphisms in genes associated with the serotonin transporter protein (5-HTT) have been identified (Adamec, Holmes & Blundell, 2008; Mellman et. al., 2009; Borghans & Homberg, 2015). Specifically, short allele variants of the polymorphic promoter region of 5-HTT (5HTTPLPR) have been associated with a global reduction in 5-HTT. As 5-HTT is the mechanism by which serotonin is removed from synapses, functional loss of the protein has been found to decrease 5-HT re-uptake (Adamec, Holmes & Blundell, 2008; Mellman et. al., 2009). Individuals with this genetic disposition appear to be more susceptible to stress reactions and exhibit trait anxiety. This may reflect reductions in 5-

HTT expression in emotional regulatory centers of the brain such as the PFC and amygdala (Adamec, Holmes & Blundell, 2008). Furthermore, a link between the 5HTTPLPR and increased amygdala activity has been observed in fMRI studies (Mellman et. al., 2009).

Despite the links between 5-HT neurotransmission and PTSD, the exact role of 5-HT in this disorder is not fully understood. This is because the effects of 5-HT on stress and affective responses are widely dependent on the intensity of stress exposure, which brain regions are innervated by 5-HT, and the receptor subtypes through which 5-HT functions (Sherin & Nemeroff, 2011). Sixteen different 5-HT receptors have been identified in the brain. These are further subdivided into seven distinct classes, 5-HT_1 through 5-HT_7 (Homberg, 2012; Murnane, 2019). Due to the vast number of receptors, attempts at identifying the exact function of individual receptors has been quite difficult.

Nevertheless, the 5-HT_2 family of receptors (comprised of 5-$HT_{2A,2B,2C}$) as well as the 5-HT_{1A} receptor have been implicated in the regulation of stress and anxiety responses in rodent studies (Graeff, Guimarães, De Andrade, & Deakin, 1996; Adamec, Bartoszyk, & Burton, 2004; Adamec, Creamer, Bartoszyk & Burton, 2004; Weisstaub et al., 2006; Adamec, Holmes & Blundell, 2008; Jiang et al, 2011). Findings from these studies suggest that activation of 5-HT_2 receptors in the amygdala, prefrontal cortex and hippocampus induces an anxiogenic response. Conversely, activation of 5-HT_{1A} receptors has been demonstrated to have an anxiolytic effect (Sherin & Nemeroff, 2011; Xiang et al., 2017). Additionally, activating the 5-HT_{1A} receptor has been found to suppress associative learning in fear conditioning paradigms and facilitate fear extinction (Homberg, 2012). Furthermore, increased expression of the 5-HT_2 receptor family and down-regulation of 5-HT_{1A} receptors in the emotion regulation centers have been observed in rodents exposed to chronic stress (Adamec, Holmes & Blundell, 2008; Sherin & Nemeroff, 2011). Taken together, dysregulation of 5-HT neurotransmission may lead to altered expression levels of the 5-HT receptors. This, in turn, may modulate the corticolimbic circuity's response to stress. Additionally, this may facilitate plastic changes that increase the

salience of traumatic memories and may account for the generalized fear responses in PTSD patients (Adamec, Holmes & Blundell, 2008).

5-HT$_{2A}$ receptor. The 5-HT$_{2A}$ receptor has garnered much attention in PTSD research as it is highly expressed in the prefrontal cortex and limbic regions of the brain, such as the amygdala and the hippocampus (Weisstaub et al., 2006). Furthermore, expression levels of 5-HT$_{2A}$ have been found to be affected by stress (Murnane, 2019). The 5-HT$_{2A}$ receptor has been directly and indirectly implicated in modulating emotional memory, as well as mediating anxiety and defensive responses (Graeff, Guimarães, De Andrade, & Deakin, 1996; Weisstaub et al., 2006; Homberg, 2012; Aznar & Klein, 2013; Murnane, 2019). Many PTSD patients present with dissociative symptoms that might be under the control of the same mechanisms implicated in other psychiatric conditions, such as schizophrenia, which has known associations with the expression of the 5-HT$_{2A}$ gene (Mestre, Zurowski & Fox, 2013).

The 5-HT$_{2A}$ receptor was first discovered and identified as the main target for psychedelic drugs such as psilocybin, mescaline and lysergic acid diethylamide (LSD). The hallucinogenic effects of these drugs are mediated by 5-HT$_{2A}$ receptor stimulation in the PFC (Hanks & Gonzalez-Maeso, 2012). Furthermore, 5-HT$_{2A}$ is one of the main excitatory receptors in the brain and regulates dopamine and glutamine release (Murnane, 2019). The5-HT$_{2A}$ subtype is a G-protein coupled receptor. Its activation has been demonstrated to have downstream effector sites required for long term potentiation, which mediates brain plasticity and strengthens synaptic connections needed for conditioning (Aznar & Klein, 2013). Behavioral studies have found that activation of 5-HT$_{2A}$ receptors enhances associative learning in contextual and cued conditioning studies (Homberg, 2012; Murnane, 2019). Chronic pharmacological treatment with selective serotonin reuptake inhibitor medications (SSRIs) in fear conditioning paradigms induces a down-regulation of 5-5-HT$_{2A,}$ which is associated with diminished fear conditioning (Homberg, 2012). Based on these findings, it has been suggested that increased expression of 5-HT$_{2A}$ in the PFC in response to trauma may sensitize the cells to fire excitatory signals to the amygdala. This, in turn, activates the circuits related to fear, which may lead to a feedback loop

that disengages the inhibitory effects of the PFC on amygdala activation. The consequences of this are hyperactive neural circuitry prone to activation in response to environmental cues directly or indirectly associated with trauma (Murnane, 2019). This may be reflected in the symptoms of PTSD, such as generalized fear, avoidance, hyper-reactivity and deficits in fear extinction.

In addition to 5-HT_{2A} receptor's relationship with emotional memory, it is also implicated in mediating anxiety and defensive responses (Graeff, Guimarães, De Andrade, & Deakin, 1996; Weisstaub et al., 2006; Aznar & Klein, 2013; Murnane, 2019). However, the exact nature of this relationship is clouded, as much of the research in this domain has produced mixed results. Dhonnchadha, Bourin & Hascoët (2003), found an anxiolytic-like effect in animals that were administered DOI, a selective 5-HT_{2A} agonist, 30 minutes prior to behavioral tests on Elevated Plus Maze (EPM). However, 5-HT_{2A} Knock-Out (KO) mice were found to have reduced anxiety responses in the EPM, Open Field and Light-Dark test. This effect was reversed by the conditional Knock-In (KI) of 5-HT_{2A} receptors in the PFC (Weisstaub et al., 2006). Another study examined the effects of EMD 281014, a selective antagonist of 5-HT_{2A}, in a rodent model of PTSD. It was found that prophylactic treatment with the drug attenuated the effects of the stressor, whereas post-stressor administration had no effect on anxiety-like behaviors. Finally, Jiang et al. (2011) found that a single injection of MDL 11,939, another potent 5-HT_{2A} antagonist, prior to or just after three days of restraint stress paired with tail shocks, attenuated the expression of an exaggerated acoustic startle response 10 to 30 days after stress exposure.

The conflicting results on 5-HT_{2A}'s role in modulating anxiety may partially be explained by Deakin and Graeff's (1991) hypothesis of the anticipatory mechanisms of defense. This theory posits that there are different neuroanatomical structures and different 5-HT_2 receptors that are activated by 5-HT in response to different types of stressors. In sum, the idea suggests that 5-HT_{2A} receptors mediate the activity of the prefrontal cortex, amygdala and SNS response only when proximal or conditioned aversive stimuli are encountered which produce an

anxiogenic effect. Conversely, in response to unconditioned stimuli, activation of 5-HT_{2A} mediates an anxiolytic effect through a different set of anatomical regions. If true, antagonizing 5-HT_{2A} receptors may produce anxiolytic responses to conditioned stimuli while producing an anxiogenic response to unconditioned stimuli. As individuals with PTSD are predisposed to a generalized fear response in relation to trauma, it's possible that antagonism of 5-HT_{2A} may aid in reducing symptoms of the disorder. Furthermore, in studies that introduced the 5-HT_{2A} antagonist prophylactically, it is possible that 5-HT_{2A} antagonism may inhibit the consolidation of the traumatic and stressful memory, thereby, attenuating the behavioral effects of exposure.

Further work is needed to understand the role that 5-HT_{2A} receptors play in PTSD. Nevertheless, the overall data suggest that the 5-HT_{2A} receptor might possibly be a prime target for pharmacological interventions to alleviate some symptoms of PTSD.

Current Pharmacological Treatments

To date, selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed medications for pharmacological treatment of PTSD. Sertraline (Zoloft) and Paroxetine (Paxil) are currently the only FDA approved drugs for this disorder, though other SSRIs and serotonin/norepinephrine reuptake inhibitors are often used off-label. SSRIs are demonstrated to be effective in treating some symptoms of PTSD (Sareen, 2014). However, their response rate in PTSD patients is roughly 60%, with only up to one-half of patients being treated achieving complete remission (Alexander, 2012).

The mechanism of action through which almost all SSRIs function is by inhibiting 5-HTT, which blocks the reuptake of the neurotransmitters in the synapse, making serotonin more available for binding (Marek & Carpenter, 2003). However, the exact therapeutic mechanism through which SSRIs function is not fully known, as they are not 5-HT receptor specific. Thus, the therapeutic efficacy of these drugs in many patients is unsuccessful, and the nonspecific effects can lead to aversive side effects (Kolar & Kolar, 2016). However, combination or stand-alone therapies using 5-HT_2-related drugs have been found to be therapeutic in treatment-resistant and refractory patients with PTSD. It is believed that the increased efficacy of these

treatments is mediated through the inhibition of 5-HT_{2A} receptors and activation of other 5-HT receptors (Marek & Carpenter, 2003; Quesseveur, Nguyen, Gardier & Guiard, 2012). Such findings, in addition to those presented previously, have guided the goals of our experiments, as we aimed to implicate the role of the serotonin system through specific receptor subtype targeting in a model of PTSD.

Pimavanserin

Patients with Parkinson Disease-induced psychosis present with symptoms such as hallucinations, delusions, depression, and anxiety. Pimavanserin is a drug that has been FDA approved for the use of treating this disorder. The drug has also been demonstrated to potentially aid in the treatment of insomnia and depression (Marek, Martin-Ruiz, Abo, & Artigas, 2005; Teegarden, Al Shamma, & Xiong, 2008). Based on its therapeutic profile, Pimavanserin may be a good candidate for the treatment of PTSD. This is because many of the symptoms of PTSD are potentially mediated through the same neurobiological substrates that are targeted by this drug.

Biochemical studies of this drug suggest it is a highly selective 5-HT_{2A} receptor inverse agonist. It has been demonstrated to have a pKi of 9.3, with a 40-fold selectivity over 5-HT_{2C} receptors and no functional activity at 5-HT_{2B} or other receptors (Mestre, Zurowski & Fox, 2013; Melzer & Roth, 2013). This suggests it may also act as a molecular probe to aid in clarifying the role of serotonin receptor subtypes in creating the symptoms of PTSD.

CHAPTER II:

ANIMAL MODELS OF PTSD

Importance of Animals in Research

Research dedicated to PTSD is multifaceted and approached from many scientific disciplines. While much can be gleaned from studying patients with PTSD, there are many limitations to taking this approach. For one, the acquisition of PTSD in humans is incidental and trauma exposure comes in many forms. This presents a problem for researchers, as there is no way to directly observe the induction of PTSD in real time. Secondly, and this goes without saying, it would be quite unethical to induce PTSD in healthy individuals (Borghans & Homberg, 2015). Thirdly, although clinical research is important for implementing novel pharmacological treatments, every novel compound that is tested in human beings begins with the use of cultured cell models, and once cell viability is determined, then the compound is segued to utilize in animal models. This is done to test the compound for its safety profile, potential efficacy in treatment and to further elucidate the underlining biological mechanism of the disorder (Zoladz & Diamond, 2016).

Due to the methodological and ethical constraints, investigators have been limited in their ability in understanding the biological mechanisms of PTSD. Therefore, preclinical animal research offers several advantages. For instance, it allows for the planned study of risk factors such as gender, social support, and severity of trauma exposure that contribute to the development of PTSD. It also allows for rapid cost-effective preliminary evaluation of potential pharmacological treatment options for PTSD. Finally, it permits researchers to intervene at different time points relative to trauma exposure, allowing for a deeper understanding of critical periods for intervention (Zoladz & Diamond, 2016).

Methods of PTSD Animal Research

Animal models play a pivotal role in PTSD research and are considered a powerful tool. When modeling PTSD in the rodent, it is of vital importance that the model meets three criteria for validation. First, animal models should demonstrate face validity. In the case of preclinical modeling of PTSD, this means animals should demonstrate biological and behavioral indicators that mimic characteristics found in human PTSD patients. Though a one to one correspondence between human symptoms and animals does not exist, many behavioral measures have been designed to be analogs for symptoms exhibited by PTSD patients (Cohen, Matar & Zohar, 2013; Borghans & Homberg, 2015). Two, construct validity should also be met. As previously discussed, the development of PTSD in humans is incidental and may develop as a result of exposure to a wide heterogeneous set of traumatic experiences. Therefore, animal models allow for the controlled introduction of stressful stimuli analogous to those that lead to human PTSD. Three, predictive validity in animal models can be achieved through determining whether the same pharmacological interventions can reduce the persistent effects of the traumatic experiences in both PTS patients and animal models. For instance, this could be tested by treating PTSD patients and trauma-exposed animals with various selective serotonin reuptake inhibitors to potentially reverse the effects of stress (Borghans & Homberg, 2015). Validity of a model might also be confirmed by testing the effects of compounds that affect known biological mechanisms linked to PTSD. Table 1 presents a summary of various different stress models in rodents that can fit these criteria.

Animal Strain Differences in Models of PTSD

Individual differences play a large role in the developmental course of PTSD. This also applies to rodents, as they have large variations in genetic lineage. There are many different strains of rat available to researchers. The most common strains used in PTSD research are Sprague-Dawley, Wistar, Fischer and Lewis rats. Several investigators have demonstrated that biological and behavioral differences are found between these strains when animals are exposed to a contrived traumatic event. For instance, Cohen et al. (2006) exposed animals to a predator

scent paradigm and found that Lewis rats presented with lower corticosterone production and greater stress-induced increases in anxiety-like behaviors than Sprague-Dawley and Fischer F344. Furthermore, the results suggested that the prevalence rates for extreme behavioral responses (EBRs) resulting from the stressor were two-fold higher among Lewis rats (50%) compared to Sprague-Dawley (25%), and five times greater than Fischer rats (10%). Other investigators have replicated this finding and expanded on it to show that highly stress-reactive Lewis rats also exhibit deficits in fear extinction as compared to stress resistant animals (Goswami et al., 2010).

Sex differences. Sex differences have been found to be a risk factor for the development of PTSD in humans. Prevalence rates suggest that women are twice as likely as men to develop PTSD with in the general population (Brewin et al., 2000; Kilpatrick, et al. 2013). However, in rodents, it has been suggested that the prevalence rates for exhibiting PTSD-like phenotypes are not different between males and females (Cohen and Yehuda, 2011). Nevertheless, multiple lines of evidence suggest that male and female rats do respond to stressors deferentially (Adamec, Holmes & Blundell, 2008; Weintraub, Singaravelu & Bhatnagar, 2010). For instance, in 2006, Adamec et al. showed that female mice exhibited a higher acoustic startle response over males after being exposed to predator threat. In Kokras and Dalla's (2014) review of sex differences in PTSD models, they suggest that stress-induced behavioral differences do seem to exist between males in females. However, the differences depend on stress conditions and the behaviors being assessed. They conclude that males tend to show higher rates of freezing behaviors in fear conditioning models while females express more anxiety related behaviors when subjected to more acute stressors.

Stressors in Animal Models

Physical stressor. According to the DSM-V (2013), the core features associated with a traumatic experience must involve the perceived threat of death or serious injury. Ergo, researchers have come up with several physical stressors that have been shown to induce PTSD-like behaviors in rodents.

Foot shock. Foot shock is a method typically used in studying the processes of fear conditioning and extinction. As previously described, fear conditioning involves the pairing of an unconditional stimulus (US) with a conditional stimulus (CS). This results in an unconditional response (UR) when presented with the CS alone. Extinction occurs when repeated presentation of CS leads to a diminished UR. Protocols vary in the number, duration, and amplitude of the inescapable shock exposures. Nevertheless, experiments using these stressor protocols have found long-lasting behavioral changes suggestive of enhanced fear conditioning, avoidance, increased anxiety and hyperarousal (Borghans & Homberg, 2015). However, many studies were unable to produce evidence suggestive of HPA-axis and SNS dysfunction (Borghans, & Homberg, 2015; Whitaker, Gilpin & Edwards, 2015; Schoner et al., 2017). This suggests that basic fear condition induces a state fundamentally different from PTSD.

Another model of PTSD that utilizes foot shock is the stress-enhanced fear learning paradigm (Rau et al., 2005). Unlike fear conditioning, animals are not conditioned to associate a cue with the shock. Instead, animals are subjected to either no shock or a sequence of foot shocks at random intervals. The following day, subjects are introduced to a new environment in which they either receive no shock or a single shock. On day three of the protocol, animals are placed back in the environmental context they were exposed to on the preceding day. Animals are then evaluated for freezing behavior, which is suggestive of rodent fear memory. Animals that undergo this procedure have been found to be resistant to extinction and can exhibit freezing behavior for up three months (Rau et al., 2005). However, further investigation is needed to determine whether stress-enhanced fear learning can elicit other PTSD-like symptoms in rodents. One noted advantage of using this model is its ability to demonstrate that non-associative sensitization of fear learning can have long-lasting effects (Whitaker, Gilpin & Edwards, 2015).

Restraint stress. Restraint stress, as the name implies, involves restraining an animal either in a plastic restraint tube or immobilizing the animal by attaching the limbs and head to a wooden board. Restraint sessions may take place either in a single session or over repeated days

and can last anywhere between 15 min. to 2 hrs. each. Numerous studies have demonstrated that animals exposed to either acute and chronic restraint sessions exhibit increased anxiety-like behaviors as evaluated in the elevated plus maze (EPM), open field test and other anxiety measures (Campos et al.,2013; Schoner et al., 2017). Restraint stress has been shown to demonstrate non-associative learning sensitization and time-dependent sensitization to other stressors such as the forced swim test (Borghans, & Homberg, 2015; Whitaker, Gilpin & Edwards, 2015). Finally, restraint stress has also been reported to increase negative feedback inhibition of the HPA-axis (Borghans, & Homberg, 2015).

Single prolonged stress. Single prolonged stress (SPS) involves exposing animals to a battery of individual physical stressors in rapid succession. Animals are first restrained for two hours, followed by a 20 min. forced swim and then rendered unconscious by ether inhalation. Studies utilizing this regimen have demonstrated a wide variety of PTSD-like characteristics including; increases in anxiety behaviors, acoustic startle response, HPA-axis negative feedback and enhanced freezing behaviors (Toledano & Gisquet-Verrier, 2014).

Social stressors. Much like humans, rats are social animals and sensitive to disruptions in their ecological social environments. Impoverished or disrupted social support have been suggested as contributing risk factors for the development of PTSD. Researches have applied several methods that disrupt rodent's social networks and produce stressful habitats. Much of the time, chronic social stressors are used in conjunction with physical stress as they have been found to increase the response to the more acute forms of stress (Zoladz, Conrad, Fleshner & Diamond, 2008; Borghans & Homberg, 2015).

Early life stress, social isolation, and housing instability. Early life development is a sensitive period that is easily influenced by environmental factors (McCormick, 2010). Integral to the development of many mammalian animals, rats included, is the parent-child dyad. Some early life stress models take advantage of this fact and introduce stress to pre-weaned pups through maternal separation. The procedure calls for dams to be isolated from their pups for 1 to 3 hours a day on postnatal days 2 to 14 (Whitaker, Gilpin & Edwards, 2014). Maternal

separation from pre-weaned pups has been found to have a profound impact on later biological and behavioral functions in response to stress (Schoner et al., 2017).

Another isolation method used to induce stress in older animals is chronic social isolation. Under these conditions, animals are kept in isolation anywhere from 1 day to 8 weeks. Adolescent animals subjected to social isolation have been found to exhibit long-lasting deficits in fear conditioning and extinction behaviors, as demonstrated by increased freezing time in response to both contextual and auditory cues (Morrissey, Mathews & McCormick 2010). Additionally, chronic social isolation during this period has been implicated in increasing anxiety-like behaviors and HPA-axis functioning (McCormick, 2010). Though the latter has been primarily linked to depressive-like biomarkers, depression is a highly comorbid condition to PTSD (Sareen, 2014). Moreover, Weintraub, Singaravelu & Bhatnagar (2010) found evidence that this may be a sex-dependent phenomena as male rats subjected to early life stress were found to have lower corticosterone responses when challenged with an acute stressor.

Adult rodents have also been shown to be negatively affected by social isolation. Ieraci, Mallei & Popoli (2016), found social isolation produced an increase in several anxiety-like behaviors in the open field test. In addition, animals displayed biological indicators of HPA-axis hypo-functioning and decreases in several gene markers essential for normative neuroplastic changes in both the hippocampus and prefrontal cortex.

One of the other social stressors commonly used in PTSD research is housing instability. This protocol consists of randomly pairing different cage mates daily for a period of 31 days. This model was found to have little effect on measures of anxiety unless paired with two episodes of inescapable predator exposure (Zoladz, Conrad, Fleshner & Diamond, 2008).

Psychological stressors. While physical stressor models of PTSD commonly employ methods that may cause pain to animals e.g. foot shocks, psychological stressor models of PTSD attempt to more naturalistically induce a PTSD-like phenomenon in rodents through non-physical means such as predator threat and social stress (Goswami, Rodriguez-Sierra, Cascardi & Pare, 2013).

Predator stress and predator threat. Predator stress models of PTSD expose rodents to a natural predator such as a cat. This model has been incredibly reliable at producing long-lasting increases in anxiety-like behaviors as tested on the EPM, Acoustic startle response and the hole board test (Ademec, Walling & Burton, 2004). However, predator threat models that utilize predator odor stress are not only more practical than predator stress but have also been highly effective at modeling the characteristics of human PTSD. Though laboratory rats are have never experienced aversive interactions with predators, predator cues have still been found to elicit profound biological and behavioral responses. The most commonly used odors for producing these effects are materials impregnated with the odor of cat fur or urine or dihydro 2,5 trimethylthiazoline (TMT), a synthetic compound derived from fox anal secretions (Dielenberg &McGregor, 2001; Takahshi, 2014). Exposure to TMT has been demonstrated to be a potent aversive US to rodents. However, it has been suggested that fur-derived odors are more effective at inducing cued and contextual avoidance and anxiety-like behaviors in rats (McGregor, Schrama, Ambermoon & Dielenberg, 2001; Blanchard et al. 2002; Blanchard, Griebel & Blanchard, 2003). A study examining the effects of feline odor produced by different cats on rodent behavior demonstrated that exposure to scents obtained from both male and female cats evoked long term conditioned responds in the subjects. However, only exposure to male cat odors precipitated a long-lasting unconditioned anxiogenic effect as evaluated on the EPM (Munoz-Abellan, Amario, & Nadal, 2009).

As noted above, predator urine is another stressor used to induce a PTSD like response in rodents. Perhaps the most well-known protocol utilizing this stimulus involves exposing rats to soiled cat litter for 10 min. (Cohen, Matar & Zohar, 2013). One week later, animals are tested on the elevated plus maze (EPM) and the acoustic startle response (ACSR). The use of this protocol has demonstrated that heterogeneity exists in animal responses to this stress when tested for anxiety-like behaviors. Some animals exhibit extreme behavioral responses (EBRs), minimal behavioral responses (MBRs) or partial behavioral responses (PBRs) (Cohan & Zohar, 2004; Cohen, Mator & Zohar, 2013). The advantages of this procedure are two-fold; one, it

demonstrates that this model comes close to mirroring, in rodents, the variability in response similar to that found in the human population. Two, it allows for comparisons to be made between high and low responding animals, which may allow researchers to elucidate risk factors involved in the development of PTSD.

Predator-based psychosocial stress model. The predator-based psychosocial stress model (PPS) developed by Zoladz et al. (2008) is comprised of acute physical stressors component and a chronic social stress component. Rodents are first exposed to unavoidable predator stress (restraint + a cat) on two occasions separated by 10 days. In addition, animals undergo chronic housing instability for a total of 31 days. Three weeks after the second set of acute stressors, animals are tested for biological and behaviors irregularities. The model was explicitly designed to imitate several core risk factors associated with the etiology of PTSD in humans. For instance, during the one-hour predator exposures animals are restrained. This was hypothesized to be ethologically analogous to the lack of control felt by individuals experiencing a traumatic event. The second acute stress exposure is carried out at a different time of day than the first. This is done for three reasons. One, it introduces a sense of unpredictably to trauma exposure. Two, the second exposure acts as a reminder of the traumatic experience, reminiscent of reliving of the trauma. Three, it also increases the likelihood of inducing a PTSD-like phenotype, as a history of early trauma is associated with an increase in the development of PTSD. Additionally, the chronic housing instability disrupts social interaction which is another known predictor for the development of PTSD (Brewin et al., 2000; Kilpatrick, et al. 2013; Zoladz & Diamond, 2013; Zoladz & Diamond, 2016).

This model has been extensively studied and has been shown to produce long-lasting increases in anxiety, startle response, avoidance behaviors, cardiovascular reactivity, and impaired cognition. In addition, it has also demonstrated HPA-axis and SNS dysfunction, such as lower basal corticosterone levels, increased suppression of glucocorticoid secretion in response to the dexamethasone test and an exaggerated response to yohimbine (Zoladz, Conrad, Fleshner & Diamond, 2008; Zoladz, Fleshner & Diamond, 2012). Furthermore, Wilson et al.

(2014) discovered that this model alters neurotransmitter levels in the brain. Results suggested that there were marked decreases of 5-HT and increases of NE levels in the hippocampus and prefrontal cortex. Finally, post-trauma treatment with common pharmacological agents used to treat PTSD differentially attenuated PTSD-like symptoms produced by this rodent model (Zoladz, Fleshner & Diamond, 2013).

Table 1.
Rodent Stress Models

Stressor Type	Model	Method	Behavioral and Psychological Responses
Physical			
	Foot Shock	Stress enhanced fear learning or sensitization, fear conditioning by means of inescapable or unpredictable foot shock	Enhanced fear conditioning, ↑anxiety behaviors, ↑hyperarousal, ↓extinction, ↑freezing behaviors (Rau et al., 2005; Whitaker, Gilpin & Edwards, 2015)
	Restraint Stress	Acute or chronic use of restraint by tube or wooden board	↑Anxiety behaviors, ↑negative feedback inhibition HPA-axis, non-associative learning sensitization (Campos et al., 2013; Schoner et al., 2017; Borghans, & Homberg, 2015)
	Single Prolonged Stress	Exposure to a battery of individual physical stressors in rapid succession (e.g. restraint, forced swim test and then ether inhalation)	↑Anxiety behaviors, ↑acoustic startle response, ↑negative feedback inhibition HPA-axis, ↑freezing behaviors (Toledano & Gisquet-Verrier, 2014)
Social			
	Early Life Stress	Maternal separation of pre-weaned pups for 1-3 hrs per day on post-natal days 2-14	Impacts on biological and behavioral reactions to stress in lifespan, ↓corticosterone responses in males (Whitaker, Gilpin & Edwards, 2014; Schoner et al., 2017; Weintraub, Singaravelu & Bhatnagar, 2010)
	Social Isolation	Single housing animals for up to 8 weeks	Deficits in extinction and fear conditioning behaviors, ↑freezing behaviors, ↑Anxiety behaviors, ↑HPA-axis (Morrissey, Mathews & McCormick, 2010;)
	Housing Instability	Random pairing of individual cage mates for up to 31 days	↑Anxiety behaviors when paired with repeated inescapable predator threat (Zoladz, Conrad, Fleshner & Diamond, 2008)
Psychological			
	Predator Odor Stress	Exposure to materials bearing the odor of cat fur, predator urine or dihydro 2,5 trimethylthiazoline (TMT) that can serve as potent aversive US	Cued and contextual avoidance and anxiety-like behaviors, extreme behavioral responses (Dielenberg &McGregor, 2001; Takahshi, 2014; McGregor,Schrama, Ambermoon & Dielenberg, 2001; Blanchard et al. 2002; Blanchard, Griebel & Blanchard, 2003; Cohen, Matar & Zohar, 2013)
	Predator-based Psychosocial Stress	Acute physical stressors paired with chronic social stress (e.g. unavoidable predator stress and housing instability)	↑Anxiety behaviors , ↑startle response, ↑avoidance behaviors, cardiovascular reactivity and impaired cognition, lower basal corticosterone levels, increased suppression of glucocorticoid secretion (Zoladz et al., 2008; Zoladz, Fleshner & Diamond, 2012)

Measuring PTSD-Like Behaviors in Rodents

As previously discussed, animal models of PTSD should demonstrate face validity. That is, they should demonstrate biological and behavioral indicators such as increases in general anxiety, avoidance, and reactivity, all characteristics found in human PTSD patients. However, the expression of these behaviors may be vastly different in humans and rodents. Therefore, researchers have developed ethologically relevant measures that may be translated to human symptoms of PTSD (Cohen, Matar & Zohar, 2013; Borghans & Homberg, 2015).

Elevated plus maze. The elevated plus maze (EPM) is a well-established standardized model used for measuring anxiety-like behaviors in rodents. It takes advantage of rodent's ethological unconditioned responses to novel and perceptually dangerous environments. Rodents have a propensity for novel exploration. However, as they are prey animals, they display risk assessing behaviors in open environments and prefer safer enclosed spaces. The elevated plus maze capitalizes on this by creating the conditions for an approach-avoidance conflict (Campos et al., 2013).

The maze is comprised of four elevated arms in the shape of a plus sign. Two open arms are set across from each other and perpendicular to two closed arms. The arms of the maze are connected by a small, open center platform. To begin the test subjects are placed on the center platform facing an open arm. They are then allowed to explore the maze freely for 5 min and behaviors are recorded. Measures recorded for this test include: entries into the open and closed arms, time spent on the open and closed arms and distance traveled in the maze. Additional, behavioral measures such as freezing, rearing, head dipping, and stretch attended postures are also commonly recorded (Komada, Takao & Miyakawa, 2008). Typically, anxiety is evaluated as the time spent in the open and closed arms, so that animals that spend more time in the open arms are considered to be less anxious than those that spend more time in the closed arms.

Open field test. Much like the EPM, the open field is another ethologically relevant assay used to assess unconditioned approach-avoidance conflicts. The apparatus itself consists of a large open space, surrounded by four walls, and is typically demarcated by two square

zones. The zones consist of inner and outer square segments. The inner zone of the open field represents an open, unprotected environment. In contrast, the outer zone is in close proximity to the walls of the apparatus, which signals safety to the animals. Measures such as locomotor activity in the two zones, as well as exploratory and anxiety-like behaviors are recorded. Anxiety-like behaviors are indicated by thigmotaxis and suppression of rearing behavior, in addition to entries and time spent exploring the inner zone of the open field (Seibenhener & Wooten, 2015).

As the outer zone is in close proximity to the walls of the apparatus this allows for the evaluation of thigmotaxic behavior. Thigmotaxis is a safety mechanism displayed in many prey animals. It consists of wall hugging, which produces a more protected environment that minimizes visibility to predators (Lamprea, Cardenas, Setem, & Morato, 2008). Conversely, rearing behavior and inner zone exploration, from the ethological perspective, would permit more visibility to prey. Thus, it is generally considered to be indicative of exploratory behaviors. It is suggested that animals with lower levels of anxiety will exhibit increased locomotor activity, increased instances of rearing, and will display more entries to and time spent in the inner zone (Lever, Burton, & O'Keefe, 2006; Seibenhener & Wooten, 2015).

Freezing behavior, defined as the sudden immobility of an animal, can also be observed in the open field. Typically freezing behavior is evaluated in response to a conditioned stimulus, such as a light or a tone, which has been paired with an aversive stimulus. Thus, after a fear conditioning paradigm has been implemented animals can be tested for freezing behaviors in response to the conditioned stimulus. Freezing behavior is believed to be associated with anxiety-like behaviors and hyper-reactivity (Curzon, Rustay & Browman, 2009).

Conditioned place aversion. Conditioned Place Aversion (CPA) is another fear conditioning paradigm in which an unconditioned aversive is paired with neutral contextual cues. In PTSD research the aversive stimulus is typically electrical shocks though some investigators have also used a predator odor stimulus or restraint stress (Murua & Molina, 1990; Blanchard et al., 2001; Muñoz-Abellán et al., 2009; Whitaker et al., 2016). Though the contextual cues used

in this assay vary among researchers, one common apparatus used is the CPA chamber which consists of a start box that leads into a larger chamber that is divided into two predetermined zones. The zones are differentially marked by distinct visual cues on the floors and wall of the chamber. Prior to conditioning, animals first undergo a bias preferences test. This is done to determine if animals have a preference for one-half of the chambers and to allow them to acclimate them to the apparatus. If both zones of the CPA are found to be explored equally, the box is considered unbiased and animals can be conditioned in either side of the box (Cunningham, Gremel & Groblewski, 2006). During conditioning, animals are confined to one zone of the CPA chamber and exposed to the aversive stimulus. Repeated pairing of the context with the aversive has been shown to increase avoidance and risk assessment behaviors to the contextual environment when tested in the absence of the aversive stimulus (Blanchard et al., 2001; Muñoz-Abellán et al., 2009; Zoladz, Fleshner and Diamond, 2012; Whitaker et al., 2016).

Acoustic startle response. The startle reflex is an unconditioned reaction to a loud noise. Auditory information is transduced from the hair cells of the inner ear and transmitted to the medulla, which feeds signals to the pons. The pons then sends signals to the muscles that cause them to tense up and contract, resulting in a jumping-like sensation indicative of surprise or fear (Kalat, 2016). The intensity and sensitivity of this response can be modulated by the anxiety state of the individual which can reflect hyper-arousal. Hyper-arousal, as indicated by increased startle response, is reported in patients with PTSD and is therefore used as a diagnostic criterion (Brewin et al., 2000; DSM-V, 2013). Several researchers have modeled this diagnostic procedure in animals as well (Rassmussen, Crites & Burke, 2008; Zoladz, Conrad, Fleshner & Diamond, 2008; Cohen, Matar & Zohar, 2013).

Measurement of acoustic startle is typically performed in sound-attenuated startle chamber containing a speaker and a holding box that sits atop a load cell or accelerometer that measures the largest displacement of force induced by the rat's sudden movement in response to an abrupt noise. During testing, a background white noise, 10 dB above general room noise, is used to mask any incidental sounds in the testing room and to sensitize animals to the white

noise (Geyer & Swerdlow, 1998). Animals are then presented with a sequence of randomized white noise bursts a range of decibel (dB) levels (Chabot & Taylor, 1992; Conti & Printz, 2003; Zoladz, Conrad, Fleshner & Diamond, 200; Smith et al., 2011). However, some protocols call for one constant dB stimulus that is presented in random time intervals (Nalloor, Bunting & Vazdarjanova, 2011). The output measure is then collected by a software program and digitally converted into a response curve as a function of dBs. Analysis of the response curve is usually performed on the largest positive peak of the amplitude wave, as this is indicative of the strength of response to the stimulus (Geyer & Swerdlow, 1998).

Rationale for the UHCL Rodent Model of PTSD

Our research group has developed a novel psychosocial animal model of PTSD in female Lewis rats. This model was highly influenced by the work of Zoladz and associates, as well as Cohen and associates. Our model utilized chronic isolation in conjunction with two acute episodes of restraint stress and predator odor exposure. Each element of the model was crafted to replicate several risk factors involved in the etiology and persistence of PTSD symptoms in people. We combined a perceived life-threatening stress experience through two sessions of unavoidable predator odor stress. Rats were confined within a plastic tube to prevent them from retreating from the odor and to add the element of restraint stress. Additionally, pretreatment with chronic single-housing modeled the early deprivation of psychosocial support found in some PTSD victims. All these procedures mimic well-described risk factors for PTSD (Brewin et al., 2000; Kilpatrick, et al. 2013; Zoladz & Diamond, 2013; Zoladz & Diamond, 2016).

The choice to use female Lewis rats was based on the finding that this strain is more likely than others to exhibit EBRs when exposed to predator threat stress (Cohen et al. 2006). Furthermore, the sex of the animal was explicitly chosen based on the assumption that females would produce a more robust response to the stress regimen. This was hypothesized because prevalence rates of PTSD among human females are twice as high compared to males (Kilpatrick, et.al. 2013). In addition, female rats have been suggested to be more reactive to acute stressors (Kokras & Dalla, 2014).

There were several reasons to impose chronic social isolation two weeks before acute stress exposure and continued through the remainder of the experiment. One, lack of social support, before or after a trauma, is a risk factor found in human epidemiological studies (Brewin et al., 2000; Schmidt, Kaltwasser & Wotjak, C. T. 2013; Sareen, 2014). Two, we believed that the two-week isolation period prior to the first acute stress exposure would lead to time-dependent sensitization, which would increase the impact of the acute stress sessions. Three, many studies investigating the effects of chronic social isolation, alone or in conjunction with acute stress, have shown that it can lead to increased anxiety-like behaviors (Campos, Fogaca, Aguiar, & Guimaraes, 2013; Borghans, & Homberg, 2015; Schöner et. al., 2017).

Restraint stress during acute stress exposure was implemented to mimic the sense of helplessness that trauma victims experience. In addition, it has been demonstrated as an effective physical stressor that enhances anxiety-like behaviors and can cause biological alterations in the stress response systems (Campos et al., 2013; Borghans & Homberg, 2015; Schoner et al., 2017). Wildcat urine and a cat collar impregnated with the odor of a cat were used during the acute stress sessions to more closely replicate the experience of being exposed to an actual predator (a cat). We speculated that the success of Cohen and associates work may stem from the fact that the soiled cat litter used in their experiments may also contain fur and other traces of predator odor which may have an intensifying effect. Furthermore, predator odor stress models utilizing well-used cat collars have repeatedly induced lasting anxiogenic effects (Dielenberg, & McGregor, 2001; Blanchard, Griebel, & Blanchard, 2003; Cohen & Zohar, 2004; Muñoz-Abellán et. al., 2009).

Based on these considerations, we hypothesized that our model would elicit long-lasting heightened anxiety and hyper-reactivity. Additionally, we hypothesized that this would be reflected in increased acoustic startle response, avoidance of the exposed arms of the elevated plus-maze and defensive behavior in the open field.

CHAPTER III:

THE EXPERIMENT

Effects of the 5-HT$_{2A}$ Receptor Inverse-Agonist Pimavanserin on a Rodent Model of Post-Traumatic Stress Disorder

Experimental Design and Hypothesis

The purpose of the current experiment was to test whether our model of PTSD would be able to induce a PTSD-like phenotype in female Lewis rats. In addition, we wanted to determine if a 1mg/kg dose of Pimavanserin could ameliorate any behavioral effects produced by our stress protocol. The current experiment utilized a between-subjects design. Experimental animals were exposed to stressor conditions that included long-term social isolation (chronic stressor) and two exposures to a combination of restraint and predator threat (acute stressors). Testing was performed ten days after the second acute stress exposure. On testing day, animals were either given an injection of saline or a 1 mg/kg dose of Pimavanserin and evaluated on the EPM, CPA, ACSR and open field apparatus to assess various rodent behaviors associated with a PTSD-like phenotype. Figure 1 shows a general timeline of events the animals were exposed to.

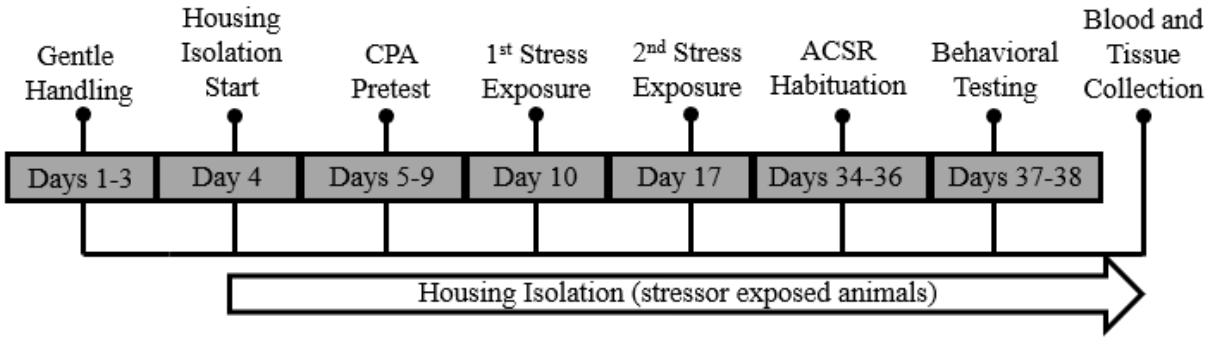

Figure 1. PTSD model procedural timeline

We hypothesized that this combination of stressors would: 1) increase anxiety-like behaviors in the EPM, 2) increase avoidance behaviors in the CPA box, 3) increase ACSR reactivity, 4) increase thigmotaxic and anxiety-related behaviors in the open field test and 5) Pimavanserin would prevent these effects. Taken together, such findings would suggest that this

combination of stressors can provoke a robust, persistent PTSD-like phenotype in female Lewis rats and that activation of the 5HT2aR may play a role in the expression of these behaviors.

Methods

Animals

Thirty-seven female Lewis rats, weighing 180-200 grams, 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/dark cycle. The dark phase began at 6:00 p.m. and ended at 6:00 a.m. All procedures were approved by to the Institutional Animal Care and Use Committee (IACUC) at the University of Houston-Clear Lake.

Sham/Stress Procedures

Chronic isolation housing conditions. Upon arrival to the lab, animals were group housed and given seven days to acclimate to the lab before any manipulations took place. On days 1-3 of the protocol animals were weighed daily and subjected to gentle handling. On day four, animals were randomly assigned to one of three group's:

No Stress - Saline controls (n = 12), experimental Stress - Saline controls (n = 12) and experimental Stress - Pimavanserin treated (n = 13) conditions. Non-stressed control animals were paired with cage mates and subjected to standard housing conditions and procedures. Experimental stress animals were isolated in single housed cages, enrichment materials were removed, and precautions were taken to limit animal exposure to other animals and experimenter handling. Housing conditions remained consistent throughout the duration of the experiment.

Conditioned place aversion pretest. The Conditioned Place Avoidance Chamber (CPA) (Columbus Instruments, Columbus, Ohio) consists of a start box that leads into a larger chamber that can be divided into two predetermined zones. The zones are marked by distinct visual cues on the floors and wall of the chamber divided in the middle. On day 10 of the protocol, animals were weighed and then screened for biased preference for one chamber of the conditioned place avoidance boxes (CPA). This was done to determine which side of the box animals would be conditioned to avoid.

One hour prior to testing, the testing chamber was thoroughly cleaned with 90% isopropyl alcohol. Testing took place under red lighting conditions, approximately 35 lux. Animals were placed in the start box and allowed to explore both halves of the box freely for 5 min. Time started when the rat stepped into the CPA chamber. Activity was recorded by a video camera suspended over the chamber and connected to a computer. AnyMaze software (Stoelting Co., Wood Dale, IL) was then used to score the total amount of time spent in either marked zone. No preferences were found for any animal tested.

Cue and context fear conditioning & acute sham/stress exposure. On days 17 and 27 of the protocol, animals were weighed, given an injection of saline and then subjected to a fear conditioning paradigm to induce anxiety-related behaviors, avoidance behaviors, and heightened states of arousal and reactivity. To accommodate both non-stressed and stress groups being run on the same day, non-stressed controls were run first with the stress group then run after a ten-minute delay.

Two conditioned place avoidance boxes were used. Animals were placed in one side of the box for 5 min. A ventilated petri dish containing cotton balls soaked in either saline (for controls) or wildcat urine (for experimental animals) were affixed to the wall of the chamber. In addition, a 1/2 in strip of cat collar either worn by a cat (stressed groups) or never worn (non-stressed groups) was attached to the petri dish. This was done so the animals would associate the predator odors to the context of the box. During the final 30 sec the animals were in the chamber a 2 kHz tone was played at ~ 70 dB to act as a cued conditioned auditory stimulus.

Immediately following the tone, control animals and the petri dish were transferred to a small cage with an animal restrainer present. Animals in this condition were not restrained but allowed to sit without disruption for one hour. Conversely, experimental stress animals were placed in the restrainers, securely enough to prevent body movement, and the predator odor stimulus was placed immediately in front of the rat's head by the head. Animals were restrained for one hour. Roughly thirty minutes into the sham/restraint session the 2 kHz tone was played once again. The tone was played one last time 30 sec. before the end of the sham/restraint

session. Animals and the petri dish were then transferred back to the CPA box for an additional 25-minutes so the animals would have time to consolidate the memory associated with the stressful event and the box. CPA boxes and restrainers were wiped down with 10% bleach and dried between animals. Figure 2 demonstrates the time course of the sham/stress procedure.

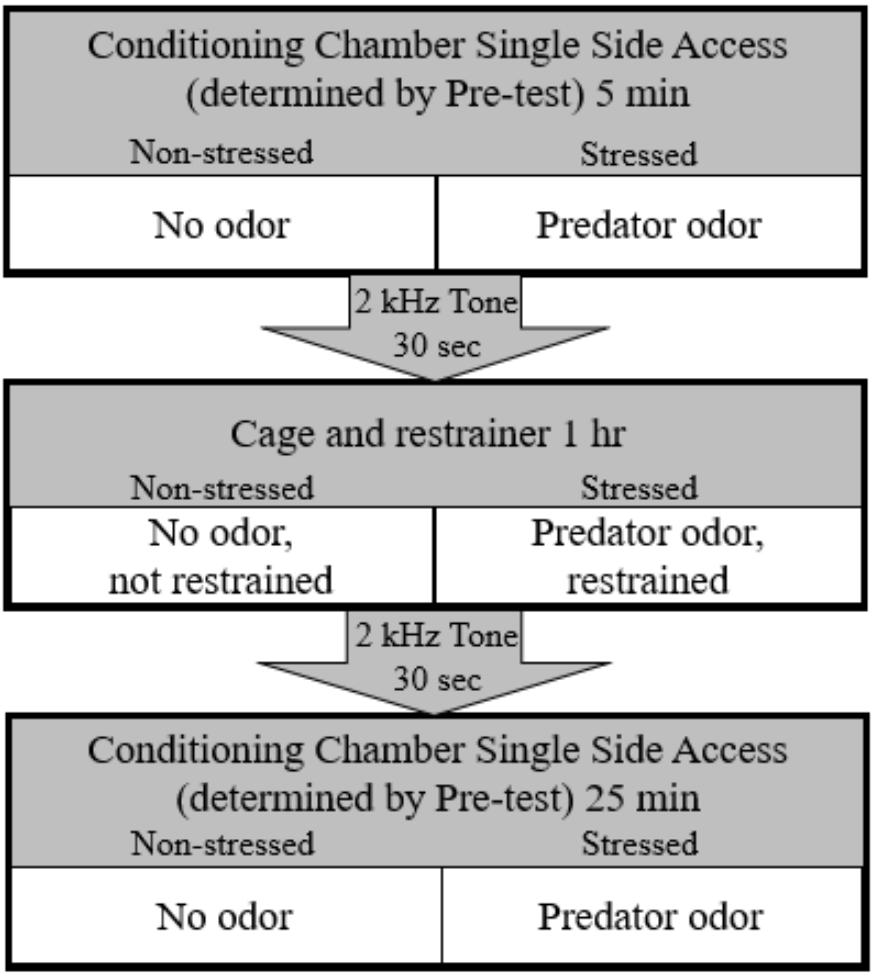

Figure 2. Procedure for sham/stress days

Procedure for sham/stress days 17 and 27Conditioning to sequential mild stressors performed on two different days10 days apart. Rats that were in the non-stress group were exposed to each condition without being restrained or exposed to the predator odor. Rats were placed in the Conditioned Place Avoidance (CPA) Chamber (opposite to preferential side obtained in pre-test) for 5 min, then into cages with restrainers present. Stress group animals were restrained and exposed to the predator odor for 1 hr. Then each animal is placed back into the CPA Chamber for 25 minutes. All animals experienced a 2kHz tone played for 30 sec two times within the procedure, once 30 sec before being placed into the cage with the restrainer and again while in the cage with the restrainer.

Testing

On days 37 and 38 of the protocol, animals underwent testing on four different dependent measures: The Elevated Plus Maze (EPM), Acoustic Startle Response Test (ACSR), Conditioned Place Aversion (CPA) and the Open Field Test. All four tests measure different aspects of anxiety-related behaviors commonly thought to resemble PTSD-like symptoms in the rat. Behavioral testing sequences over the two testing days were counter-balanced across groups and behavioral testing sequences over the two testing days. In addition, animals were allowed a 1 hr cool-down period between tests. These steps were taken to control for potential confounding order effects and carryover effects that might influence subsequent behavioral tests.

All tests were run between 6:00 p.m. and 12:30 a.m. This period was within the animal's dark cycle to minimize sleepiness as a cause of low performance on the measures. At the beginning of each evening, animals were weighed and given a subcutaneous injection of either saline or a 1 mg/kg dose of Pimavanserin one hour prior to the first test. Figure 3 shows a schematic representation of the testing day schedule.

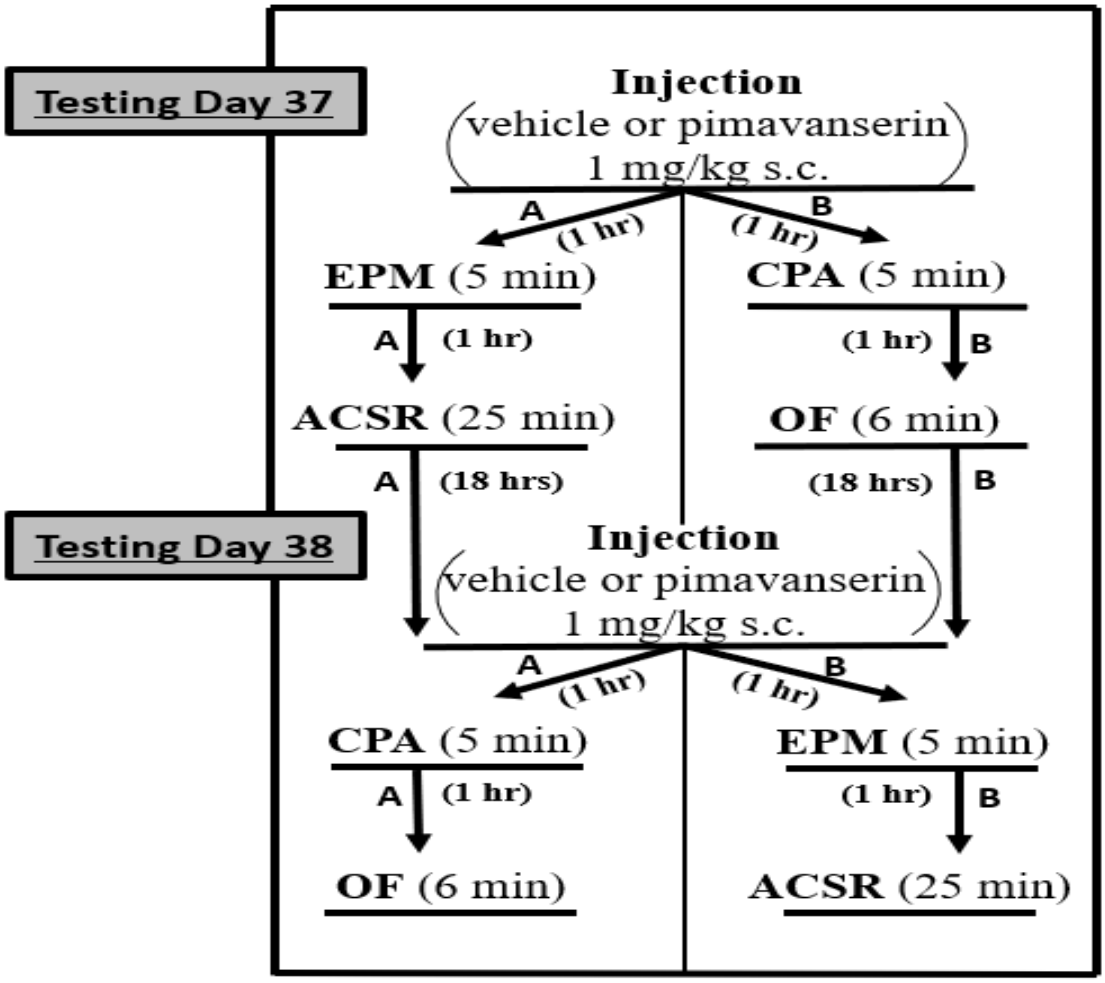

Figure 3. Testing day procedures

EPM and CPA tests are run simultaneously in two different rooms due to limited testing equipment. To keep times after injections similar between groups, animals are divided into testing groups A and B to complete two behavioral tests per day with 18 hours between the last test and the next day's injection.

Elevated plus maze. The EPM consists of a platform raised 50 cm from the ground with two sets of perpendicular arms adjoined at a center space. Two arms are laid out opposite of each other with open platforms and the other two arms are laid out similarly but enclosed with walls. A video camera is set up above the maze to record behaviors. Dimensions of the EPM can be seen in Figure 4.

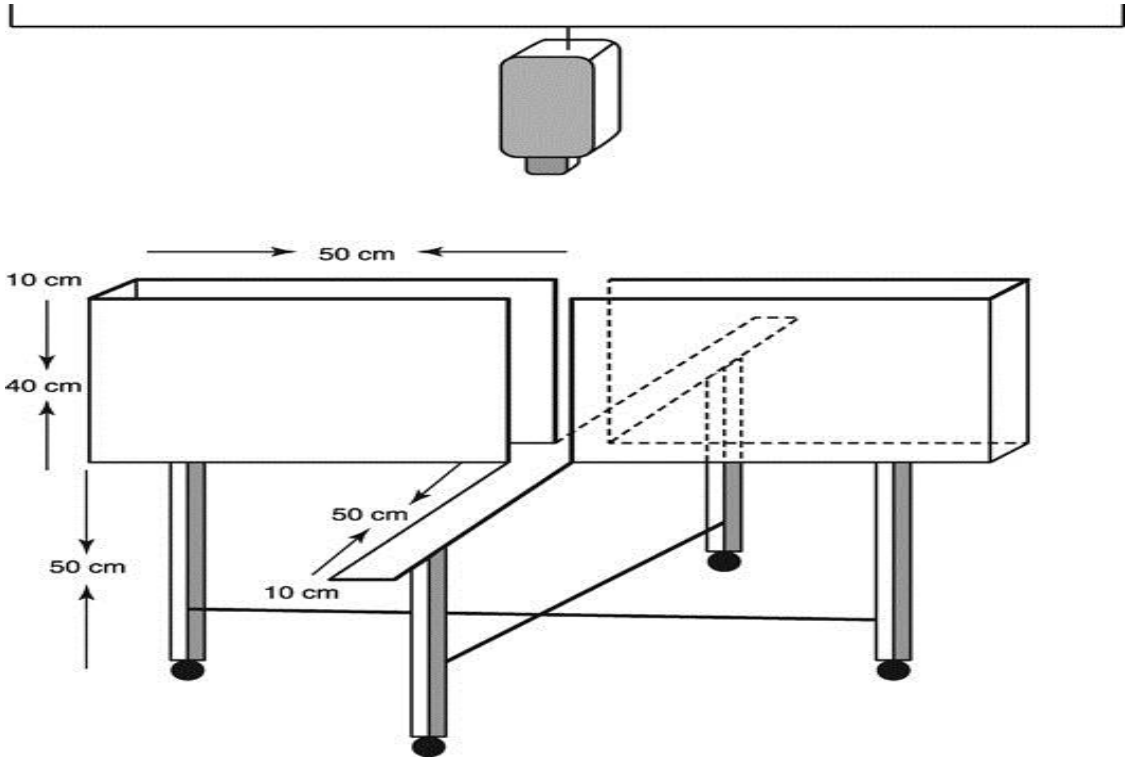

Figure 4. Basic design of the elevated plus maze (Cohen, Matar and Zohar, 2013).

Testing occurred under low red lighting (~35 lux). The maze was thoroughly cleaned with 90% Isopropyl alcohol 1 hour before testing. Animals were placed in the middle of the plus maze facing an open arm and allowed to explore the maze for five minutes. After each individual trial, the maze was wiped down with 10% bleach and allowed to dry.

Rat activity was recorded by a video camera suspended over the maze linked to a computer running the AnyMaze software (Stoelting Co., Wood Dale, IL). The software recorded the total amount of time spent in the open, closed and center areas of the maze. One observer

was present in the testing room to observe the recording on a computer, place the rat back on the maze in the event it should fall off, and to remove the animal from the maze once the trial was completed. In the testing room, a curtain was used to separate the maze portion of the room from the observer at the computer to minimize observer presence and interference.

Conditioned place aversion. Similar to the CPA-pretest, **t**he testing chamber was thoroughly cleaned with 90% Isopropyl alcohol 1 hour before testing. Testing took place under red light conditions adjusted to ~35 lux. Animals were placed in the start box and timing was started once a rat stepped into the CPA chamber. The test duration was 5 min, after each trial, boxes were cleaned with 10% bleach. Behavior in the chamber was recorded by video camera and animals were scored using the AnyMaze software for time spent in each zone. The scores were compared to pre-test scores to determine if stress interventions affected behaviors. Scores were then averaged across groups to evaluate differences.

Acoustic startle response. To measure acoustic startle, testing was performed in a sound-attenuated startle chamber (Columbus Instruments, Columbus, Ohio) with a holding box that sits atop a load cell that measures the largest displacement of force (in grams) induced by the rat's sudden movement. Calibration of the load cell required a 1500 g weight and a 750 g weight. This was based on the maximum expected force that can be exerted by a 300 g rat. Calibration of the speakers was based on the user manual's recommendations. A background white noise of 65 dB was played while each animal was presented with 30 pseudo-randomized trials of white noise bursts at 90, 100, and 110 dB acoustic bursts (40 milliseconds each), at 30 sec intervals. The output measure was recorded using Responder X software (Columbus Instruments, Columbus, Ohio) on a laptop computer.

On each testing day, the testing chamber was thoroughly cleaned with 90% Isopropyl alcohol 1 hour before testing. The animals were placed in the ACS chamber and allowed to acclimate to 65 dB white noise for a period of 5 min. During the final 30 sec of the acclimation period, the 2 kHz tone was played to act as a trigger memory for the acute stressors. After the acclimation period, animals were exposed to three 120 dB white noise bursts at 15 sec intervals

to act as baselines. Then animals were subjected to 10 trials of 90, 100, & 110 dB white noise bursts in a pseudo-random order for a total of 30 trials. Total time of the test was 22 min. In between each test, the chamber was wiped clean with 10% bleach and allowed to dry.

As mentioned, the peak amplitude is the measure of the largest displacement of force (in grams) from calibrated zero. This measure is the sum of the subject's mass and the startle response. The subject's mass (in grams) was then subtracted from the peak amplitude to determine the animal's actual acoustic startle response. The startle response force was then averaged for each of the 3 trial conditions to find the mean score of the animal per trial level. The mean scores of the trials were then averaged across all animals in the group to determine the group's startle response per level of dB exposure.

Open field test and cued fear memory test. The open field apparatus was a 76 cm x 76 cm chamber with an inner zone of 46 cm x 46 cm; the outer zone was 15 cm. Adjacent to the walls. One hour prior to testing, the open field apparatus was cleaned with 90% Isopropyl alcohol. Testing occurred under low red lighting (~35 lux). Testing was broken up into two three-minute epochs. Testing lasted for a total of six minutes.

Each rat was placed in the bottom left-hand corner of the open field facing the same direction at the start of testing. During the last 3 mins of testing, the 2 kHz auditory stimulus was introduced to act as an auditory cue to elicit memories of the stress exposure. This allowed us to evaluate the freezing behavior of the rodents when presented with the conditioned fear stimulus. The trials were recorded via a video camera suspended over the open field. The video camera was linked to a computer which allowed an observer to monitor the test from outside of the room. After each rat completed their six-minute testing phase the open field was cleaned with 10% bleach and allowed to dry.

From the video recordings, the following thigmotaxic and activity behavioral measures were scored using the Any Maze software; exploration time of the inner square, distance traveled in the inner and outer square, and entries into the inner square. Additionally, an observer, blind

to the experimental conditions, scored the number of rearing episodes during the first three minutes and freezing behaviors during the last three minutes of the video.

Statistical analysis. All statistical analysis set $\alpha = 0.05$. Weight data were analyzed using the independent samples *t*-test to investigate differences between the stress and non-stress groups. One-Way ANOVAs followed by Tukey's *post-hoc* comparisons were used for the behavioral measures captured in the EPM, CPA and Open field tests to determine significant differences among the three groups. Analysis of the ACSR data were performed using a mixed model ANOVA with decibel level as the repeated measure variable followed by Tukey's *post-hoc* comparisons to evaluate the between-subject effects of experimental condition. Furthermore, appropriate effect size measures were included to reflect the magnitude of the differences between the group means and can be interpreted as the percent of variability accounted for by group differences.

Results

Weight Data

Weight data were collected every day animals underwent a procedure. The mean growth weigh ratio was calculated by subtracting the weight of the animals at the start of the experiment from the weight recorded on day 36 and divided by the total number of days in between. One animal was dropped from the analysis due to a complication with food administration.

To determine if animal growth rates differed between non-stressed ($n = 12$) and stressed ($n = 24$) animals independent sample *t*-tests with corrected degrees of freedom were used to compensate for the unequal samples sizes. Figure 5 shows the mean growth ratio of the groups.

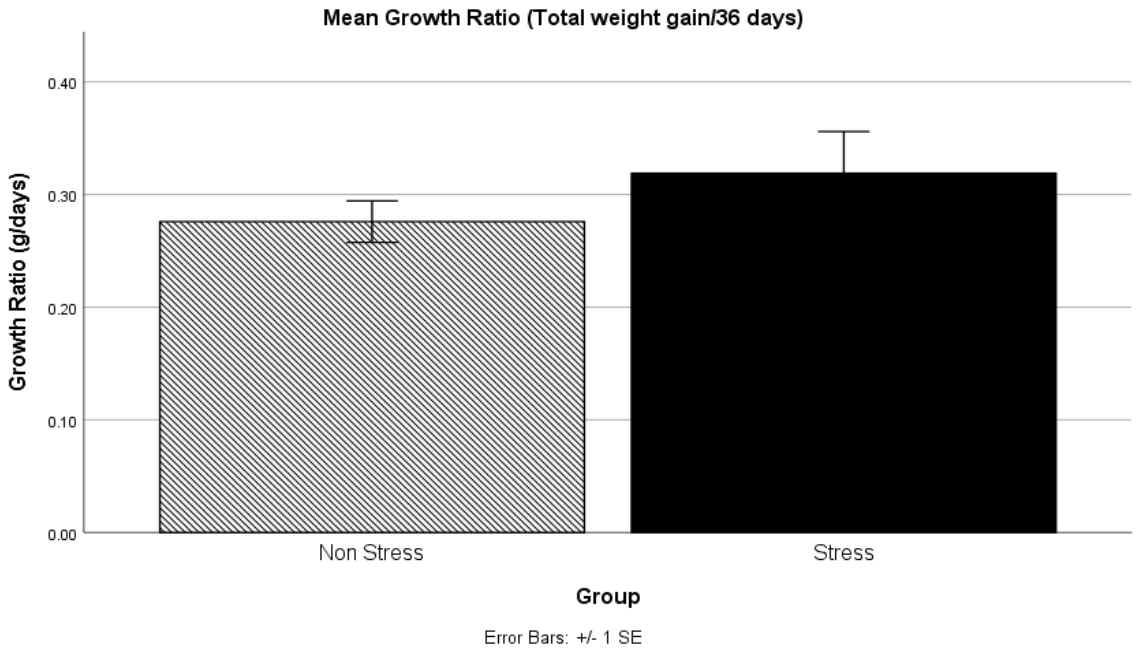

Figure 5. Growth weight ratio (mean ± SEM) of stressed and unstressed rats calculated on day 36.

No significant differences were found between stress and non-stressed animals as $t(31.715) = -1.038$, $p = .37$, $d = .316$.

Elevated Plus Maze

Six animals were removed from the analysis due to falling off the maze during their trial. This left group sizes of; No Stress-Saline ($n = 11$), Stress-Saline ($n = 11$) and Stress-Pimavanserin treated ($n = 10$). One-Way ANOVA revealed a significant difference for open arm time between the groups as $F(2,29) = 6.635$, $p = .004$, $\eta_p^2 = .314$. Tukey's post-hoc comparisons indicate significant differences exist between the No Stress-Saline group and the Stress-Saline group ($p = .029$). Significant differences were also found between the Stress-Pimavanserin treated animals and Stress-Saline controls ($p = .005$). No differences were found between No Stress-Saline treated animals and Stress-Pimavanserin treated animals ($p = .708$). No significant differences were found between any groups for time spent in the center square as $F(2,29) = 2.168$, $p = .133$, $\eta_p^2 = .1\ 3$. An increase in mean closed arm time was observed for the Stress-Saline group ($M = 169.59 \pm 19.51$) compared to No Stress-Saline controls ($M = 131.36 \pm$

9.72) and Stress-Pimavanserin treated animals (M = 125.9 ± 12.82). However, no statistically significant differences were found between groups as $F(2,29) = 2.635$, $p = .089$, $\eta_p^2 = .1\ 54$. Figure 6 displays the means of open arm time, center square time and closed arm time across group.

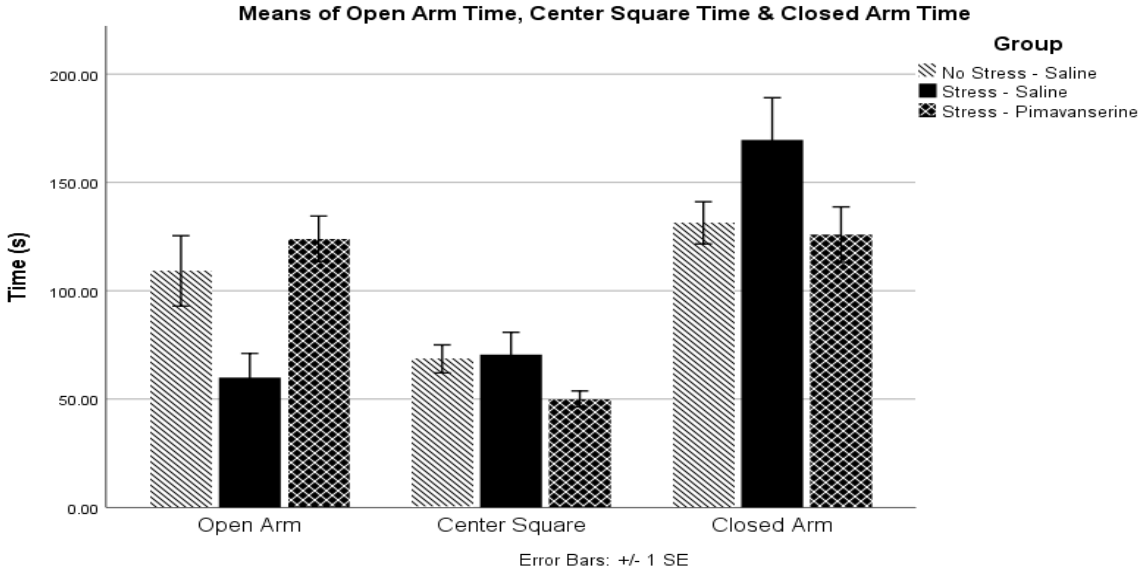

Figure 6. Graphical representation of the measures from the EPM Seconds (Mean ± SEM) spent in the open arms, closed arm and center of an elevated plus-maze.

Conditioned Place Avoidance

Avoidance in the Conditioned Place Avoidance test was defined as the amount of time spent in the side of the box in which animals were exposed to the predator odor stress. One-Way ANOVA detected no significant differences between groups as $F(2,34) = .072$, $p = .931$, $\eta_p^2 = .004$. Figure 7 displays the mean time spent in the stress associated side of the CPA chamber by condition.

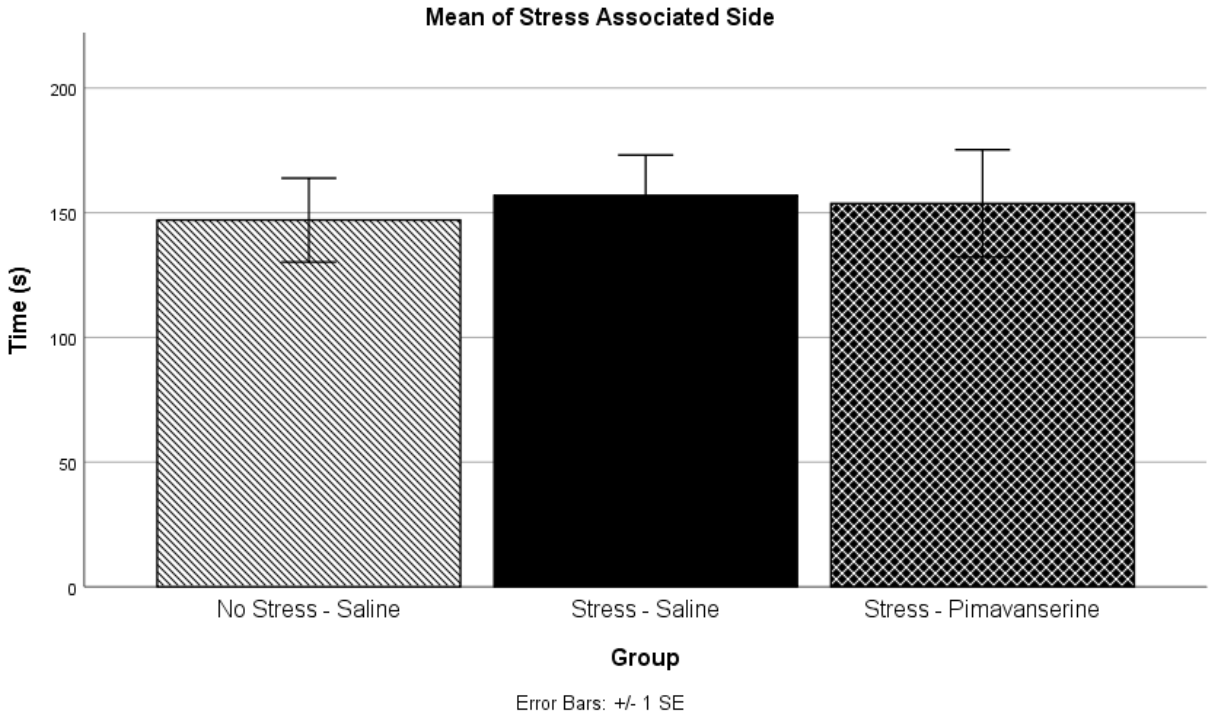

Figure 7. Time spent, represented as seconds (mean ± SEM), in the stressor-associated side of the conditioned place preference apparatus chamber.

Acoustic Startle Response

Two animals were removed from the analysis due to outlier status as they were found to deviate by 2 *SD* from the mean of their groups. Group numbers were adjusted to; No Stress-Saline (n = 12), Stress-Saline (n = 12) and Stress-Pimavanserin treated (n = 11). Results of a One-Way ANOVA on acoustic startle response at the 90 dB stimulus level revealed no differences between the groups ($p > .05$). This was probably due to the stimuli being too weak to elicit a response from the animals tested and therefore was dropped from the analysis.

A Mixed Model ANOVA was run on the 100 dB and 110 dB stimulus level across groups. A main effect for stimulus level was detected as $F(1,32) = 48.191$, $p < .001$, $\eta_p^2 = .601$. This was expected as it was hypothesized that animals would respond in a positive linear fashion as sound stimuli increased. No interaction effects between dB and condition were detected as $F(2,32) = 1.796$, $p = .182$, $\eta_p^2 = .101$. This was also predicted as there should be a similar trend between groups in response to the sound stimuli. However, a main effect for treatment group was found to be statistically significant as, $F(2,32) = 4.702$, $p = .016$, $\eta_p^2 = .227$. Tukey's *post-*

hoc comparisons revealed a statically significance difference between No Stress-Saline treated animals and Stress-Saline treated animals (p = .035). Differences were also detected between Stress-Saline treated animals and Stress-Pimavanserin treated animals (p = .003). No differences were found between No Stress-Saline treated animals and Stress-Pimavanserin treated animals (p = .992). Figure 8 shows the mean acoustic startle response by group and across dB level.

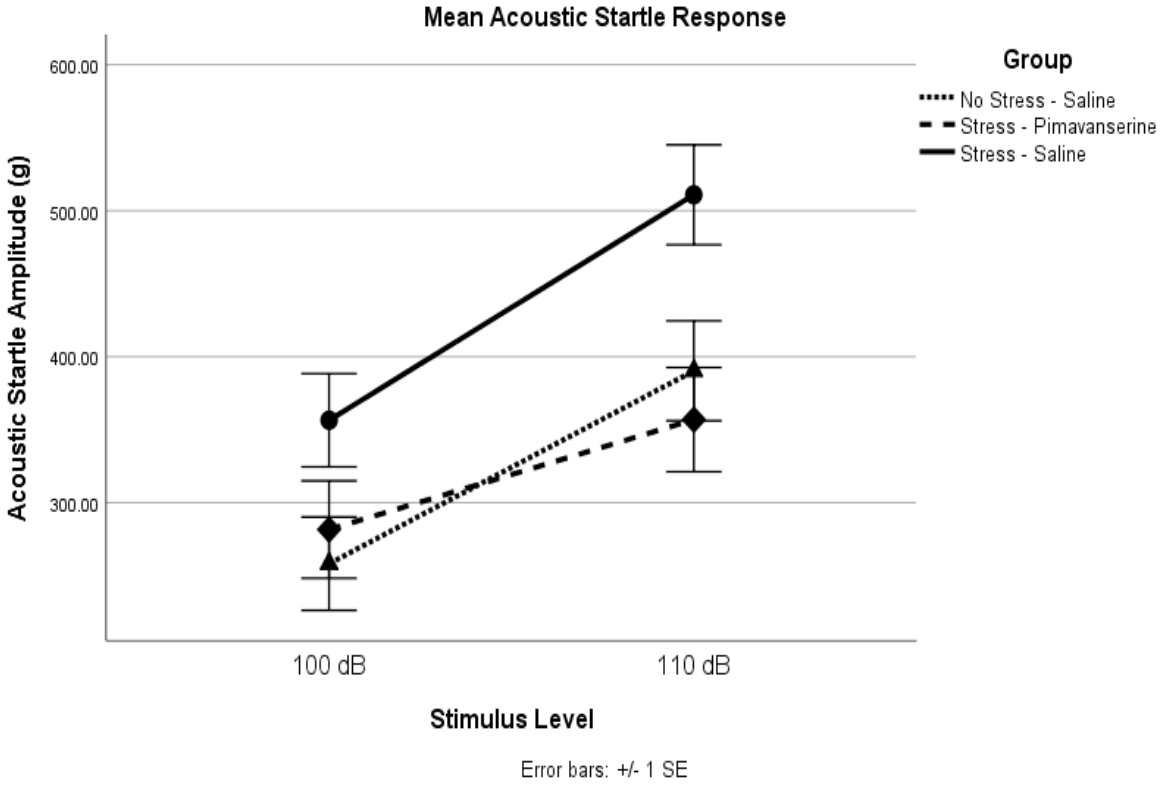

Figure 8. Strength of the acoustic startle response, represented as grams (M ± SEM), to 100 and 110 decibel stimuli.

Open Field Test

Inner square time. Figure 9 displays the means of time spent in the inner square of the open field. A slight reduction in time spent exploring the exposed inner square of the open field was observed for the Stress-Saline animals (M = 32.52 ± 5.58) compared to the No Stress-Saline controls (M = 50.125 ± 7.82) and Stress-Pimavanserin treated animals (M = 47.42 ± 5.33). However, One-Way ANOVA analysis revealed no statistically significant differences between the groups as, $F(2,34) = 2.222$, p =.124, η_p^2 = .227.

Figure 9. Graphical representation of the measures from the OF test central square time Means were calculated from time spent in the centrally defined (inner) square per testing group.

Locomotor activity. Analysis of locomotor activity was defined as the total distance traveled in the open field. One-Way ANOVA for the total distance traveled revealed no statistically significant difference between the groups as, $F(2,34) = 1.488$, $p = .249$, $\eta_p^2 = .078$. To further investigate exploratory behavior of the outer and inner zones of the open field One-Way ANOVAs were run on the distance traveled in each zone. No statistically significant differences were detected for outer zone distance traveled as, $F(2,34) = .388$, $p = .687$, $\eta_p^2 = .022$. However, a statically significant difference was observed for inner square distance traveled as, $F(2,34) = 3.225$, $p = .05$, $\eta_p^2 = .159$. Despite the significant ANOVA result, Tukey's *post-hoc* comparisons found no significant difference among the compared groups ($p > .05$). Notwithstanding, because a medium effect size was detected and the ANOVA was significant, the less sensitive Fishers LSD was used to further investigate the possible differences between groups. The results of the Fisher LSD suggested that differences exist between the Stress-Saline group and the Stress-Pimavanserin group ($p = .022$). No differences were found between the No

Stress-Saline and Stress-Pimavanserin groups (p = .693). Finally, comparisons between the No Stress-Saline and Stress-Saline groups only approached significance (p = .059). Figure 10 displays the means of the total distance traveled by the three treatment groups, as well as, the means of the outer and exposed inner partitions of the open field.

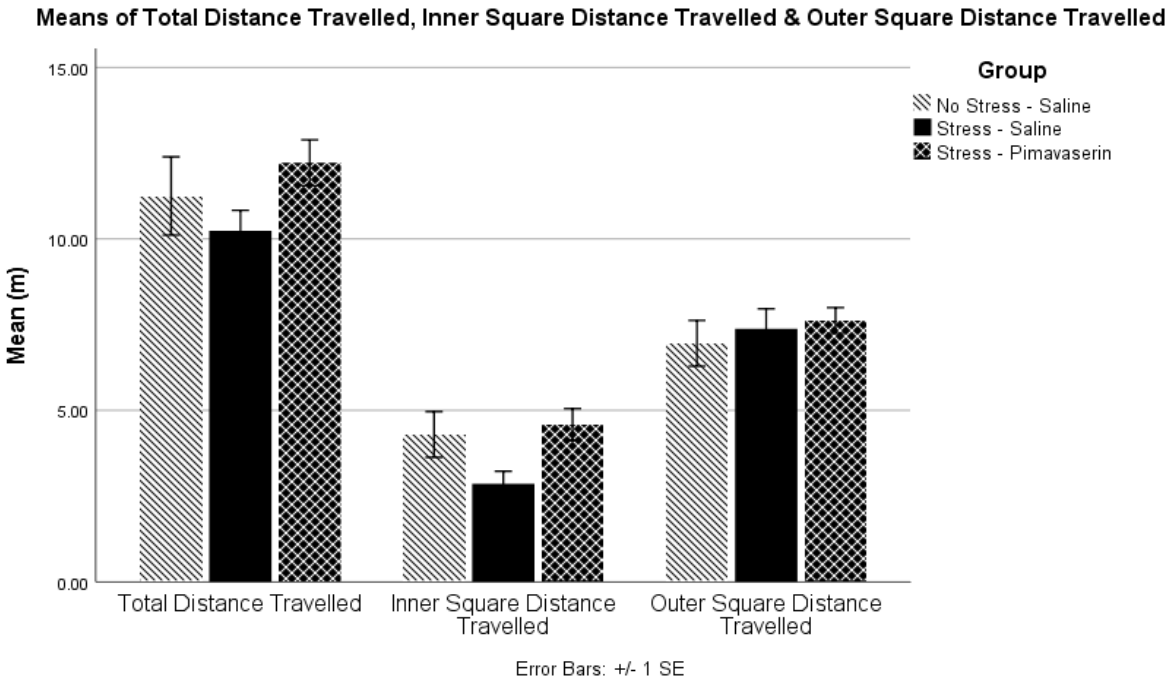

Figure 10. Total distance traveled, represented in meters (Mean ± SEM), as well as distance traveled in the inner and out sectors of the open field.

Rearing behavior. Rearing behavior was defined as an instance when the animal stood on its hind legs in an upright posture. Comparisons were made on the mean frequency of rearing episodes across groups. The results of the One-Way ANOVA revealed that a statistically significant difference was present as $F(2,34) = 7.527$, p =.002, η_p^2 = .307. Tukey's *post-hoc* comparisons revealed no significant differences existed between No Stress-Saline and Stress-Pimavanserin treated animals (p = .933). However, statistically significant differences were discovered between No Stress-Saline and Stress-Saline groups (p = .006), as well as, Stress-Saline and Stress Pimavanserin treated animals (p =.004). Figure 11 displays the mean episodes of rearing observed by the three treatment groups.

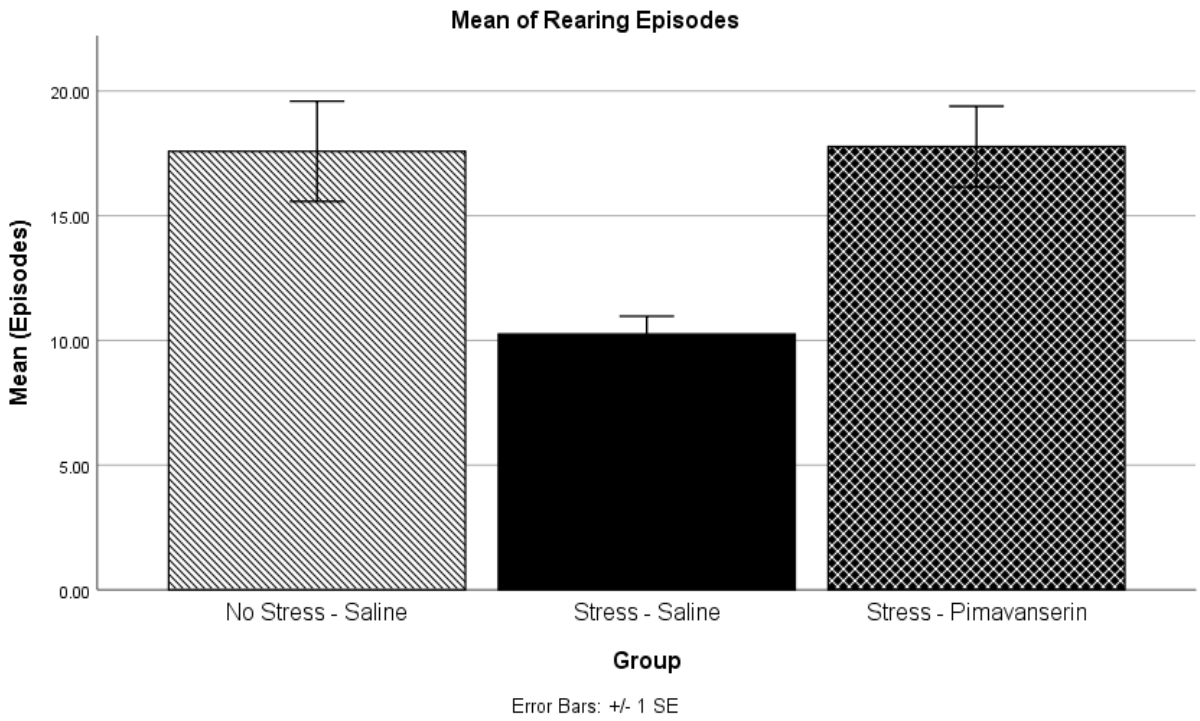

Figure 11. Number of rearing episodes (mean ± SEM) in an open field.

Conditioned freezing behavior. During the last three minutes of the open field test animals were exposed to 10 trials of the 2 kHz tone used as the CS played on days when the animals underwent acute stress exposure. Conditioned freezing behavior was defined as the sudden immobility of an animal in response to the tone. One-Way ANOVA detected a statistically significant difference among the group means on the number of freezing episodes in response to the tone as, $F(2,34) = 9.611$, $p < .001$, $\square p2 = .361$. To determine where the differences existed Tukey's post-hoc comparisons were run. Analysis revealed a statistically significant difference between No Stress-Saline and Stress-Saline groups ($p = .001$). A statistically significant difference was also observed between Stress-Saline and Stress-Pimavanserin groups ($p = .002$). No significant differences were found between No Stress-Saline and Stress-Pimavanserin groups ($p = .998$). Figure 12 shows the group means for episodes of freezing behavior in response to the tone.

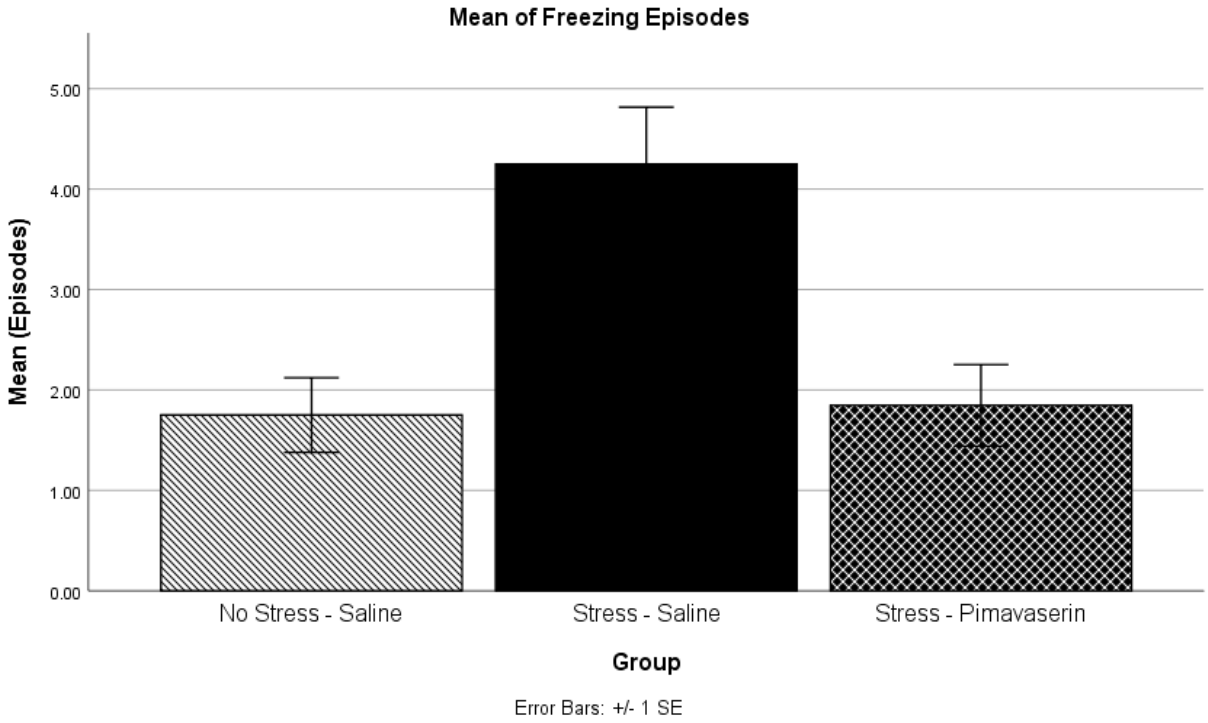

Figure 12. Number of freezing episodes (Mean ± SEM) during 10 exposures to a stress - associated tone.

Discussion

The current experiment was conducted with two aims in mind. The first aim was to investigate whether chronic isolation in conjunction with two episodes of restraint and predator scent stress could induce a PTSD-like phenotype in the female Lewis rats. Second, we wanted to determine if a 1mg/kg dose of Pimavanserin could ameliorate any behavioral effects produced by the stress protocol.

Overview of the Results

Concerning the first aim, there was an overall pattern of results. On all behavioral measures except conditioned place aversion, there were persistent differences between the stressed/saline-injected rats and the non-stressed/saline injected rats. A number of these differences were significant. Animals subjected to the stress protocol demonstrated an increase in anxiety-like behaviors as measured in the EPM and the Open field test. The results of the ACSR test suggested that stressed animal also presented with an increase in anxiety-like

behaviors and hyper-reactivity. Finally, freezing episodes in response to a conditioned stimulus suggested that stress animals were more sensitive to the CS's relationship to the traumatic event, implying a persistent fear memory for the event.

Though this last finding is countered by the failure of stressed animals to demonstrate contextual avoidance, it is possible that this discrepancy may be due to hormonal effects in the rodents. This supposition is based on the findings that suggest female rats have reduced long-term retention of contextual fear relative to males. Furthermore, this difference is not found in cued conditioning (Gresack, Schafe, Orr, & Frick, 2009). Corroborating evidence for this speculation is reflected in a study that implicated estradiol shifts in female rats to be related to enhancing fear extinction (Maeng et al., 2017). An alternative explanation for the results is that we were simply unable to properly induce contextual avoidance due to methodological variations we made from other context fear conditioning protocols. Nevertheless, taken together the overall results suggest that our model was able to produce several characteristics related to a PTSD-like phenomenon in the animals.

Concerning the second aim of the study, the scores of the stressed/Pimavanserin group closely resembled the scores of the non-stressed controls on all behavioral measures. In short, 1 mg/kg Pimavanserin largely or entirely reversed the persistent effects of the stressors. These effects might conceivably be due to central nervous system depression or a global reduction of activity levels induced by Pimavanserin. However, this seems unlikely, since the Pimavanserin-treated animals actually had the highest overall activity level (greatest distance traveled) in the open field. In addition, the persistent effects of stressor exposure did not seem to result from debilitated animals, since stressed animals actually showed a non-significant trend toward faster growth over the long course of the experiment.

Limitations and Future Directions

Despite the promising evidence that we were able to produce a PTSD-like phenotype in the animals, there are several limitations to this study regarding the model. To begin, it is not entirely clear how persistent the effects of the model are, as animals were only tested at one time

point after the second acute stress exposure. Subsequent tests are needed to determine the overall time course of the effect. Moreover, because a combination of one chronic stressor with two acute stressors was employed, it was not possible to separately assess the relative impact of the different stressors. Future studies could incrementally introduce the different stressors to determine if the current results were due to the confluence of the stressors or isolated to a particular procedure. It remains unknown whether or not our model produced other behavioral and biological abnormalities related to PTSD. Further studies are needed to determine if this model also produces cognitive deficits or endocrine dysfunctions in addition to structural and molecular changes. It should be noted that the effects on stress hormones are currently being investigated by our group. Finally, the use of female Lewis rats specifically in this study opens the door for subsequent studies examining strain, sex and hormonal difference in response to the stressor paradigm.

Concerning the introduction of Pimavanserin as a therapeutic agent, there are also several limitations. One, we only evaluated one dose of Pimavanserin. Further studies are needed to determine if the effect of Pimavanserin in this model is dose-dependent. Two, we only used one 5-HT_{2A} antagonist. It has yet to be determined whether other 5-HT_{2A} inverse agonists, such as volinanserin, will have a similar effect on this PTSD model. However, plans for this experiment are currently under development. Three, we only used one sex of rat. A follow-up study is needed to determine if the effects of Pimavanserin are sex-dependent. Four, we only administered the drug over one particular time-course relative to the administration of stressors. Future studies are needed to determine if administering Pimavanserin shortly before or after traumatic stimuli will prevent subsequent persistent PTSD-like effects. Finally, future studies are needed to determine what long-term effects chronic Pimavanserin administration would have on persistent PTSD-like behaviors.

Possible implications of the study

The results suggest that the activation state of the 5-HT_{2A} serotonin receptor is critical to modulating PTSD-like effects in our rodent model. The 5-HT_{2A} inverse agonist Pimavanserin is

already an FDA-approved drug for psychosis in Parkinson's Disease. It has been found by the FDA to have a generally acceptable side-effect profile. Therefore, if further evidence supports its efficacy in animal models of PTSD, initial clinical trials might be contemplated. Also, the role of the 5-HT_{2A} receptor might be evaluated in animal models of other emotional disorders involving anxiety and hyper-reactivity.

REFERENCES

Adamec, R, Walling, S., & Burton, P. (2006). Long-lasting, selective, anxiogenic effects of feline predator stress in mice. *Physiology & Behavior*, *83*(3), 401–410. https://doi.org/10.1016/j.physbeh.2004.08.029

Adamec, R., Bartoszyk, G. D., & Burton, P. (2004). Effects of systemic injections of vilazodone, a selective serotonin reuptake inhibitor and serotonin 1A receptor agonist, on anxiety induced by predator stress in rats. *European Journal of Pharmacology*, *504*(1–2), 65–77. https://doi.org/10.1016/j.ejphar.2004.09.009

Adamec, R., Creamer, K., Bartoszyk, G. D., & Burton, P. (2004). Prophylactic and therapeutic effects of acute systemic injections of EMD 281014, a selective serotonin 2A receptor antagonist on anxiety induced by predator stress in rats. *European Journal of Pharmacology*, *504*(1–2), 79–96. https://doi.org/10.1016/j.ejphar.2004.09.017

Adamec, R., Holmes, A., & Blundell, J. (2008). Vulnerability to lasting anxiogenic effects of brief exposure to predator stimuli: Sex, serotonin and other factors—Relevance to PTSD. *Neuroscience & Biobehavioral Reviews*, *32*(7), 1287–1292. https://doi.org/10.1016/j.neubiorev.2008.05.005

Alexander, W. (2012). Pharmacotherapy for post-traumatic stress disorder in combat veterans. *Pharmacy and Therapeutics*, *37*(1), 32–38.

Aznar, S., & Klein, A. B. (2013). Regulating prefrontal cortex activation: An emerging role for the 5-ht2a serotonin receptor in the modulation of emotion-based actions? *Molecular Neurobiology*, *48*(3), 841–853. https://doi.org/10.1007/s12035-013-8472-0

Bear, M. F., Connors, B. W., & Paradiso, M. A. (2016). *Neuroscience: Exploring the brain* (Fourth edition.). Philadelphia: Wolters Kluwer.

Bentefour, Y., Bennis, M., Garcia, R., & M'hamed, S. B. (2015). Effects of paroxetine on PTSD-like symptoms in mice. *Psychopharmacology*, *232*(13), 2303–2312. https://doi.org/10.1007/s00213-014-3861-2

Blanchard, D. C., Griebel, G., & Blanchard, R. J. (2003). Conditioning and residual emotionality effects of predator stimuli: some reflections on stress and emotion. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *27*(8), 1177–1185. https://doi.org/10.1016/j.pnpbp.2003.09.012

Blanchard, R. J., Yang, M., Li, C.-I., Gervacio, A., & Blanchard, D. C. (2001). Cue and context conditioning of defensive behaviors to cat odor stimuli. *Neuroscience & Biobehavioral Reviews*, *25*(7), 587–595. https://doi.org/10.1016/S0149-7634(01)00043-4

Borghans, B. & Homberg, J. R. (2015). Animal models for posttraumatic stress disorder: An overview of what is used in research. *World Journal of Psychiatry*, *5*(4), 387. https://doi.org/10.5498/wjp.v5.i4.387

Breslau, N., Davis, G. C., Andreski, P., & Peterson, E. (1991). Traumatic events and posttraumatic stress disorder in an urban population of young adults. *Archives of General Psychiatry*, *48*(3), 216–222.

Brewin, C. R., Andrews, B., & Valentine, J. D. (2000). Meta-analysis of risk factors for posttraumatic stress disorder in trauma-exposed adults. *Journal of Consulting and Clinical Psychology*, *68*(5), 748–766.

Campos, A. C., Fogaca, M. V., Aguiar, D. C., & Guimaraes, F. S. (2013). Animal models of anxiety disorders and stress. *Revista Brasileira de Psiquiatria*, *35*(suppl 2), S101–S111. https://doi.org/10.1590/1516-4446-2013-1139

Castro-Vale, I., van Rossum, E. F. C., Machado, J. C., Mota-Cardoso, R., & Carvalho, D. (2016). Genetics of glucocorticoid regulation and posttraumatic stress disorder--What do we know? *Neuroscience and Biobehavioral Reviews*, *63*, 143–157. https://doi.org/10.1016/j.neubiorev.2016.02.005

Chabot, C. C., & Taylor, D. H. (1992). Circadian modulation of the rat acoustic startle response. *Behavioral Neuroscience, 106*(5), 846-852. http://dx.doi.org/10.1037/0735-7044.106.5.846

Cohen, H., Matar, M. A., & Zohar, J. (2014). Maintaining the clinical relevance of animal models in translational studies of post-traumatic stress disorder. *ILAR Journal*, *55*(2), 233–245. https://doi.org/10.1093/ilar/ilu006

Cohen, H., Zohar, J., Gidron, Y., Matar, M. A., Belkind, D., Loewenthal, U., … Kaplan, Z. (2006). Blunted hpa axis response to stress influences susceptibility to posttraumatic stress response in rats. *Biological Psychiatry*, *59*(12), 1208–1218. https://doi.org/10.1016/j.biopsych.2005.12.003

Cohen, Hagit, & Yehuda, R. (2011). Gender differences in animal models of posttraumatic stress disorder. *Disease Markers*, *30*(2–3), 141–150. https://doi.org/10.1155/2011/734372

Cohen, Hagit, & Zohar, J. (2004). An animal model of posttraumatic stress disorder: the use of cut-off behavioral criteria. *Annals of the New York Academy of Sciences*, *1032*(1), 167–178. https://doi.org/10.1196/annals.1314.014

Cohen, Hagit, Matar, M. A., & Zohar, J. (2013). Animal models of post-traumatic stress disorder. *Current Protocols in Neuroscience*, *64*(1), 9.45.1-9.45.18. https://doi.org/10.1002/0471142301.ns0945s64

Conti, L. H., & Printz, M. P. (2003). Rat strain-dependent effects of repeated stress on the acoustic startle response. *Behavioural Brain Research*, *144*(1–2), 11–18. https://doi.org/10.1016/S0166-4328(03)00061-5

Crocq, M. A., & Crocq, L. (2000). From shell shock and war neurosis to posttraumatic stress disorder: a history of psychotraumatology. *Dialogues in clinical neuroscience*, *2*(1), 47-55.

Cunningham, C. L., Gremel, C. M., & Groblewski, P. A. (2006). Drug-induced conditioned place preference and aversion in mice. *Nature Protocols*, *1*(4), 1662–1670. https://doi.org/10.1038/nprot.2006.279

Curzon, P., Rustay, N. R., & Browman, K. E. (2009). Cued and contextual fear conditioning for rodents. In *Methods of Behavioral Analysis in Neuroscience*. https://doi.org/10.1201/NOE1420052343.ch2

de Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience*, *6*(6), 463–475. https://doi.org/10.1038/nrn1683

Deakin, J. F., & Graeff, F. G. (1991). 5-HT and mechanisms of defence. *Journal of Psychopharmacology (Oxford, England)*, *5*(4), 305–315. https://doi.org/10.1177/026988119100500414

Dielenberg, R. A., & McGregor, I. S. (2001). Defensive behavior in rats towards predatory odors: a review. *Neuroscience & Biobehavioral Reviews*, *25*(7–8), 597–609. https://doi.org/10.1016/S0149-7634(01)00044-6

Dohrenwend, B. P., Turner, J. B., Turse, N. A., Adams, B. G., Koenen, K. C., & Marshall, R. (2006). The psychological risks of vietnam for u.s. Veterans: a revisit with new data and methods. *Science (New York, N.Y.)*, *313*(5789), 979–982. https://doi.org/10.1126/science.1128944

Ehlers A. (2010). Understanding and treating unwanted trauma memories in posttraumatic stress disorder. *Zeitschrift fur Psychologie*, *218*(2), 141-145.

Etkin, A., & Wager, T. D. (2007). Functional neuroimaging of anxiety: A meta-analysis of emotional processing in ptsd, social anxiety disorder, and specific phobia. *American Journal of Psychiatry*, *164*(10), 1476–1488. https://doi.org/10.1176/appi.ajp.2007.07030504

Feinstein, J. S., Buzza, C., Hurlemann, R., Follmer, R. L., Dahdaleh, N. S., Coryell, W. H., … Wemmie, J. A. (2013). Fear and panic in humans with bilateral amygdala damage. *Nature Neuroscience*, *16*(3), 270–272. https://doi.org/10.1038/nn.3323

Finsterwald, C., & Alberini, C. M. (2014). Stress and glucocorticoid receptor-dependent mechanisms in long-term memory: From adaptive responses to psychopathologies. *Neurobiology of Learning and Memory*, *112*, 17–29. https://doi.org/10.1016/j.nlm.2013.09.017

Finsterwald, C., Steinmetz, A. B., Travaglia, A., & Alberini, C. M. (2015). From memory impairment to posttraumatic stress disorder-like phenotypes: The critical role of an

unpredictable second traumatic experience. *The Journal of Neuroscience*, *35*(48), 15903–15915. https://doi.org/10.1523/JNEUROSCI.0771-15.2015

Freidman, J. M. (n.d.) history of ptsd in veterans: Civil war to dsm-5 - ptsd: national center for ptsd. Retrieved March 4, 2019, from https://www.ptsd.va.gov/understand/what/history_ptsd.asp

Fuchs, E., Flugge, G., Czeh, B. (2006). Remodeling of neuronal networks by stress. *Frontiers in Bioscience*, *11*(1), 2746. https://doi.org/10.2741/2004

Geracioti, T. D., Baker, D. G., Ekhator, N. N., West, S. A., Hill, K. K., Bruce, A. B., … Kasckow, J. W. (2001). CSF norepinephrine concentrations in posttraumatic stress disorder. *The American Journal of Psychiatry*, *158*(8), 1227–1230. https://doi.org/10.1176/appi.ajp.158.8.1227

Geyer, M. A., & Swerdlow, N. R. (1998). Measurement of startle response, prepulse inhibition, and habituation. *Current Protocols in Neuroscience*, *3*(1), 8.7.1-8.7.15. https://doi.org/10.1002/0471142301.ns0807s03

Goswami, S., Cascardi, M., Rodriguez-Sierra, O. E., Duvarci, S., & Pare, D. (2010). Impact of predatory threat on fear extinction in Lewis rats. *Learning & Memory*, *17*(10), 494–501. https://doi.org/10.1101/lm.1948910

Goswami, S., Rodríguez-Sierra, O., Cascardi, M., & Paré, D. (2013). Animal models of post-traumatic stress disorder: face validity. *Frontiers in Neuroscience*, *7*, 89. https://doi.org/10.3389/fnins.2013.00089

Graeff, F. G., Guimarães, F. S., De Andrade, T. G., & Deakin, J. F. (1996). Role of 5-HT in stress, anxiety, and depression. *Pharmacology, Biochemistry, and Behavior*, *54*(1), 129–141.

Gresack, J. E., Schafe, G. E., Orr, P. T., & Frick, K. M. (2009). Sex differences in contextual fear conditioning are associated with differential ventral hippocampal extracellular signal-regulated kinase activation. *Neuroscience*, *159*(2), 451–467. https://doi.org/10.1016/j.neuroscience.2009.01.009

Gross, P. M., Joneja, M. G., Pang, J. J., Polischuk, T. M., Shaver, S. W., & Wainman, D. S. (1993). Topography of short portal vessels in the rat pituitary gland: a scanning electron-microscopic and morphometric study of corrosion cast replicas. *Cell and Tissue Research*, *272*(1), 79–88. https://doi.org/10.1007/BF00323573

Hanks, J. B., & González-Maeso, J. (2013). Animal models of serotonergic psychedelics. *ACS Chemical Neuroscience*, *4*(1), 33–42. https://doi.org/10.1021/cn300138m

Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., … Myers, B. (2016). Regulation of the hypothalamic-pituitary-adrenocortical stress response. *Comprehensive Physiology*, *6*(2), 603–621. https://doi.org/10.1002/cphy.c150015

Hill, J.E. (2013). Contributions of individual differences in stress reactivitiy to post-

Homberg, J. R. (2012). Serotonergic modulation of conditioned fear. *Scientifica*, *2012*, 1–16. https://doi.org/10.6064/2012/821549

Ieraci, A., Mallei, A., & Popoli, M. (2016). Social isolation stress induces anxious-depressive-like behavior and alterations of neuroplasticity-related genes in adult male mice. *Neural Plasticity*, *2016*, 1–13. https://doi.org/10.1155/2016/6212983

Jiang, X., Zhang, Z., Zhang, S., Gamble, E. H., Jia, M., Ursano, R. J., & Li, H. (2011). 5-HT $_{2A}$ receptor antagonism by MDL 11,939 during inescapable stress prevents subsequent exaggeration of acoustic startle response and reduced body weight in rats. *Journal of Psychopharmacology*, *25*(2), 289–297. https://doi.org/10.1177/0269881109106911

Kessler, R. C., Chiu, W. T., Demler, O., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of twelve-month DSM-IV disorders in the National Comorbidity Survey Replication (NCS-R). Archives o f General Psychiatry, 62(6), 617-627.

Kessler, R. C., Petukhova, M., Sampson, N. A., Zaslavsky, A. M., & Wittchen, H.-U. (2012). Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. *International Journal of Methods in Psychiatric Research*, *21*(3), 169–184. https://doi.org/10.1002/mpr.1359

Kilpatrick, D. G., Resnick, H. S., Milanak, M. E., Miller, M. W., Keyes, K. M., & Friedman, M. J. (2013). National estimates of exposure to traumatic events and ptsd prevalence using dsm-iv and dsm-5 criteria. *Journal of Traumatic Stress*, *26*(5), 537–547. https://doi.org/10.1002/jts.21848

Kolar, D., & Kolar, M. (2016). Critical review of available treatment options for treatment refractory depression and anxiety - Clinical and ethical dilemmas. Medicinski Pregled, 69(5-6), 171-176.

Kokras, N., & Dalla, C. (2014). Sex differences in animal models of psychiatric disorders. *British Journal of Pharmacology*, *171*(20), 4595–4619. https://doi.org/10.1111/bph.12710

Komada, M., Takao, K., & Miyakawa, T. (2008). Elevated plus maze for mice. *Journal of Visualized Experiments : JoVE*, (22). https://doi.org/10.3791/1088

Lamprea, M. R., Cardenas, F. P., Setem, J., & Morato, S. (2008). Thigmotactic responses in an open-field. *Brazilian Journal of Medical and Biological Research = Revista Brasileira De Pesquisas Medicas E Biologicas*, *41*(2), 135–140.

Lancaster, C. L., Teeters, J. B., Gros, D. F., & Back, S. E. (2016). Posttraumatic stress disorder: Overview of evidence-based assessment and treatment. *Journal of Clinical Medicine*, *5*(11). https://doi.org/10.3390/jcm5110105

Leedy, G. M., Barrows, L. F., & Clark, S. (2013). Effects of social housing on hippocampal dendrites and behavior in ovariectomized rats. *Brain Research Bulletin*, *92*, 69–75. https://doi.org/10.1016/j.brainresbull.2012.11.006

Lever, C., Burton, S., & O'Keefe, J. (2006). Rearing on hind legs, environmental novelty, and the hippocampal formation. *Reviews in the Neurosciences*, *17*(1–2), 111–133.

Lueken, U., Zierhut, K. C., Hahn, T., Straube, B., Kircher, T., Reif, A., … Domschke, K. (2016). Neurobiological markers predicting treatment response in anxiety disorders: A systematic review and implications for clinical application. *Neuroscience & Biobehavioral Reviews*, *66*, 143–162. https://doi.org/10.1016/j.neubiorev.2016.04.005

Maeng, L. Y., Cover, K. K., Taha, M. B., Landau, A. J., Milad, M. R., & Lebrón-Milad, K. (2017). Estradiol shifts interactions between the infralimbic cortex and central amygdala to enhance fear extinction memory in female rats. *Journal of Neuroscience Research*, *95*(1–2), 163–175. https://doi.org/10.1002/jnr.23826

Mahan, A. L., & Ressler, K. J. (2012). Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. *Trends in Neurosciences*, *35*(1), 24–35. https://doi.org/10.1016/j.tins.2011.06.007

Marek, G. J., Carpenter, L. L., McDougle, C. J., & Price, L. H. (2003). Synergistic action of 5-HT2A antagonists and selective serotonin reuptake inhibitors in neuropsychiatric disorders. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *28*(2), 402–412. https://doi.org/10.1038/sj.npp.1300057

Marek, G. J., Martin-Ruiz, R., Abo, A., & Artigas, F. (2005). The selective 5-HT2A receptor antagonist M100907 enhances antidepressant-like behavioral effects of the SSRI fluoxetine. *Neuropsychopharmacology*, *30*(12), 2205–2215. https://doi.org/10.1038/sj.npp.1300762

Matar, M. A., Zohar, J., & Cohen, H. (2013). Translationally relevant modeling of PTSD in rodents. *Cell and Tissue Research*, *354*(1), 127–139. https://doi.org/10.1007/s00441-013-1687-6

McCormick, C. M. (2010). An animal model of social instability stress in adolescence and risk for drugs of abuse. *Physiology & Behavior*, *99*(2), 194–203. https://doi.org/10.1016/j.physbeh.2009.01.014

McGregor, I. S., Schrama, L., Ambermoon, P., & Dielenberg, R. A. (2002). Not all 'predator odours' are equal: cat odour but not 2,4,5 trimethylthiazoline (TMT; fox odour) elicits specific defensive behaviours in rats. *Behavioural Brain Research*, *129*(1–2), 1–16. https://doi.org/10.1016/S0166-4328(01)00324-2

Mellman, T. A., Alim, T., Brown, D. D., Gorodetsky, E., Buzas, B., Lawson, W. B., … Charney, D. S. (2009). Serotonin polymorphisms and posttraumatic stress disorder in a trauma

exposed African American population. *Depression and Anxiety*, *26*(11), 993–997. https://doi.org/10.1002/da.20627

Meltzer, H. Y., & Roth, B. L. (2013). Lorcaserin and pimavanserin: emerging selectivity of serotonin receptor subtype-targeted drugs. *The Journal of Clinical Investigation*, *123*(12), 4986–4991. https://doi.org/10.1172/JCI70678

Mestre, T. A., Zurowski, M., & Fox, S. H. (2013). 5-Hydroxytryptamine 2A receptor antagonists as potential treatment for psychiatric disorders. *Expert Opinion on Investigational Drugs*, *22*(4), 411–421. https://doi.org/10.1517/13543784.2013.769957

Mitra, R., Adamec, R., & Sapolsky, R. (2009). Resilience against predator stress and dendritic morphology of amygdala neurons. *Behavioural Brain Research*, *205*(2), 535–543. https://doi.org/10.1016/j.bbr.2009.08.014

Morrissey, M. D., Mathews, I. Z., & McCormick, C. M. (2011). Enduring deficits in contextual and auditory fear conditioning after adolescent, not adult, social instability stress in male rats. *Neurobiology of Learning and Memory*, *95*(1), 46–56. https://doi.org/10.1016/j.nlm.2010.10.007

Muñoz-Abellán, C., Armario, A., & Nadal, R. (2010). Do odors from different cats induce equivalent unconditioned and conditioned responses in rats? *Physiology & Behavior*, *99*(3), 388–394. https://doi.org/10.1016/j.physbeh.2009.12.008

Muñoz-Abellán, C., Daviu, N., Rabasa, C., Nadal, R., & Armario, A. (2009). Cat odor causes long-lasting contextual fear conditioning and increased pituitary-adrenal activation, without modifying anxiety. *Hormones and Behavior*, *56*(4), 465–471. https://doi.org/10.1016/j.yhbeh.2009.08.002

Muñoz-Abellán, C., Rabasa, C., Daviu, N., Nadal, R., & Armario, A. (2011). Behavioral and Endocrine Consequences of Simultaneous Exposure to Two Different Stressors in Rats: Interaction or Independence? *PLoS ONE*, *6*(6), e21426. https://doi.org/10.1371/journal.pone.0021426

Murnane, K. S. (2019). Serotonin 2A receptors are a stress response system: implications for post-traumatic stress disorder. *Behavioural Pharmacology*, *30*(2 and 3-Special Issue), 151–162. https://doi.org/10.1097/FBP.0000000000000459

Murua, V. S., & Molina, V. A. (1990). Desipramine and restraint stress induce odor conditioned aversion in rats: suppression by repeated conditioning. *Psychopharmacology*, *102*(4), 503–506. https://doi.org/10.1007/BF02247132

Nalloor, R., Bunting, K., & Vazdarjanova, A. (2011). Predicting impaired extinction of traumatic memory and elevated startle. *PLoS ONE*, *6*(5), e19760. https://doi.org/10.1371/journal.pone.0019760

Nic Dhonnchadha, B. Á., Bourin, M., & Hascoët, M. (2003). Anxiolytic-like effects of 5-HT2 ligands on three mouse models of anxiety. *Behavioural Brain Research*, *140*(1–2), 203–214. https://doi.org/10.1016/S0166-4328(02)00311-X

Parsons, R. G., & Ressler, K. J. (2013). Implications of memory modulation for post-traumatic stress and fear disorders. *Nature Neuroscience*, *16*(2), 146–153. https://doi.org/10.1038/nn.3296

Perkonigg, A., Kessler, R. C., Storz, S., & Wittchen, H.-U. (2000). Traumatic events and post-traumatic stress disorder in the community: prevalence,risk factors and comorbidity. *Acta Psychiatrica Scandinavica*, *101*(1), 46–59. https://doi.org/10.1034/j.1600-0447.2000.101001046.x

Quesseveur, G., Nguyen, H. T., Gardier, A. M., & Guiard, B. P. (2012). 5-HT2 ligands in the treatment of anxiety and depression. *Expert Opinion on Investigational Drugs*, *21*(11), 1701–1725. https://doi.org/10.1517/13543784.2012.719872

Rasmussen, D. D., Crites, N. J., & Burke, B. L. (2008). Acoustic startle amplitude predicts vulnerability to develop post-traumatic stress hyper-responsivity and associated plasma corticosterone changes in rats. *Psychoneuroendocrinology*, *33*(3), 282–291. https://doi.org/10.1016/j.psyneuen.2007.11.010

Rau, V., DeCola, J. P., & Fanselow, M. S. (2005). Stress-induced enhancement of fear learning: An animal model of posttraumatic stress disorder. *Neuroscience & Biobehavioral Reviews*, *29*(8), 1207–1223. https://doi.org/10.1016/j.neubiorev.2005.04.010

Sabban, E. L., Serova, L. I., Newman, E., Aisenberg, N., & Akirav, I. (2018). Changes in gene expression in the locus coeruleus-amygdala circuitry in inhibitory avoidance ptsd model. *Cellular and Molecular Neurobiology*, *38*(1), 273–280. https://doi.org/10.1007/s10571-017-0548-3

Santiago, P. N., Ursano, R. J., Gray, C. L., Pynoos, R. S., Spiegel, D., Lewis-Fernandez, R., … Fullerton, C. S. (2013). A systematic review of PTSD prevalence and trajectories in DSM-5 defined trauma exposed populations: intentional and non-intentional traumatic events. *PloS One*, *8*(4), e59236. https://doi.org/10.1371/journal.pone.0059236

Sarapas, C., Cai, G., Bierer, L. M., Golier, J. A., Galea, S., Ising, M., … Yehuda, R. (2011). Genetic markers for ptsd risk and resilience among survivors of the world trade center attacks. *Disease Markers*, *30*(2–3), 101–110. https://doi.org/10.3233/DMA-2011-0764

Sareen, J. (2014). Posttraumatic stress disorder in adults: impact, comorbidity, risk factors, and treatment. *Canadian Journal of Psychiatry. Revue Canadienne De Psychiatrie*, *59*(9), 460–467. https://doi.org/10.1177/070674371405900902

Schmidt, U., Kaltwasser, S. F., & Wotjak, C. T. (2013). Biomarkers in posttraumatic stress disorder: Overview and implications for future research. *Disease Markers*, *35*, 43–54. https://doi.org/10.1155/2013/835876

Schnurr, P. P., & Lunney, C. A. (2008). Exploration of gender differences in how quality of life relates to posttraumatic stress disorder in male and female veterans. *Journal of Rehabilitation Research and Development*, *45*(3), 383–393.

Schöner, J., Heinz, A., Endres, M., Gertz, K., & Kronenberg, G. (2017). Post-traumatic stress disorder and beyond: an overview of rodent stress models. *Journal of Cellular and Molecular Medicine*, *21*(10), 2248–2256. https://doi.org/10.1111/jcmm.13161

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the open field maze to measure locomotor and anxiety-like behavior in mice. *Journal of Visualized Experiments : JoVE*, (96). https://doi.org/10.3791/52434

Sherin, J. E., & Nemeroff, C. B. (2011). Post-traumatic stress disorder: the neurobiological impact of psychological trauma. *Dialogues in Clinical Neuroscience*, *13*(3), 263–278.

Solomon, Z., & Mikulincer, M. (2007). Posttraumatic intrusion, avoidance, and social functioning: A 20-year longitudinal study. *Journal of Consulting and Clinical Psychology*, *75*(2), 316–324. https://doi.org/10.1037/0022-006X.75.2.316

Southwick, S. M., Bremner, J. D., Rasmusson, A., Morgan, C. A., Arnsten, A., & Charney, D. S. (1999). Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. *Biological Psychiatry*, *46*(9), 1192–1204.

Speckens, A. E. M., Ehlers, A., Hackmann, A., & Clark, D. M. (2006). Changes in intrusive memories associated with imaginal reliving in posttraumatic stress disorder. *Journal of Anxiety Disorders*, *20*(3), 328–341. https://doi.org/10.1016/j.janxdis.2005.02.004

Stam, R. (2007). PTSD and stress sensitisation: a tale of brain and body Part 1: human studies. *Neuroscience and Biobehavioral Reviews*, *31*(4), 530–557. https://doi.org/10.1016/j.neubiorev.2006.11.010

Starcevic, A., Petricevic, S., Radojicic, Z., Djulejic, V., Ilankovic, A., Starcevic, B., & Filipovic, B. (2016). Glucocorticoid levels after exposure to predator odor and chronic psychosocial stress with dexamethasone application in rats. *The Kaohsiung Journal of Medical Sciences*, *32*(5), 235–240. https://doi.org/10.1016/j.kjms.2016.04.011

Takahashi, L. K. (2014). Olfactory systems and neural circuits that modulate predator odor fear. *Frontiers in Behavioral Neuroscience*, *8*. https://doi.org/10.3389/fnbeh.2014.00072

Teegarden, B. R., Al Shamma, H., & Xiong, Y. (2008). 5-HT(2A) inverse-agonists for the treatment of insomnia. *Current Topics in Medicinal Chemistry*, *8*(11), 969–976.

Toledano, D., & Gisquet-Verrier, P. (2014). Only susceptible rats exposed to a model of PTSD exhibit reactivity to trauma-related cues and other symptoms: An effect abolished by a single amphetamine injection. *Behavioural Brain Research*, *272*, 165–174. https://doi.org/10.1016/j.bbr.2014.06.039

Traumatic stress disorder vulnerability and resilience. Dissertations (2009 -). Paper 271. https://epublications.marquette.edu/dissertations_mu/271/

Tronel, S., & Alberini, C. M. (2007). Persistent disruption of a traumatic memory by postretrieval inactivation of glucocorticoid receptors in the amygdala. *Biological Psychiatry*, *62*(1), 33–39. https://doi.org/10.1016/j.biopsych.2006.09.009

Tsai, S.-F., Huang, T.-Y., Chang, C.-Y., Hsu, Y.-C., Chen, S.-J., Yu, L., … Jen, C. J. (2014). Social instability stress differentially affects amygdalar neuron adaptations and memory performance in adolescent and adult rats. *Frontiers in Behavioral Neuroscience*, *8*. https://doi.org/10.3389/fnbeh.2014.00027

VanElzakker, M. B., Kathryn Dahlgren, M., Caroline Davis, F., Dubois, S., & Shin, L. M. (2014). From Pavlov to PTSD: The extinction of conditioned fear in rodents, humans, and anxiety disorders. *Neurobiology of Learning and Memory*, *113*, 3–18. https://doi.org/10.1016/j.nlm.2013.11.014

Weintraub, A., Singaravelu, J., & Bhatnagar, S. (2010). Enduring and sex-specific effects of adolescent social isolation in rats on adult stress reactivity. *Brain Research*, *1343*, 83–92. https://doi.org/10.1016/j.brainres.2010.04.068

Weisstaub, N. V. (2006). Cortical 5-ht2a receptor signaling modulates anxiety-like behaviors in mice. *Science*, *313*(5786), 536–540. https://doi.org/10.1126/science.1123432

Whitaker, A. M., Farooq, M. A., Edwards, S., & Gilpin, N. W. (2016). Post-traumatic stress avoidance is attenuated by corticosterone and associated with brain levels of steroid receptor co-activator-1 in rats. *Stress*, *19*(1), 69–77. https://doi.org/10.3109/10253890.2015.1094689

Whitaker, A. M., Gilpin, N. W., & Edwards, S. (2014). Animal models of post-traumatic stress disorder and recent neurobiological insights. *Behavioural Pharmacology*, *25*(5–6), 398–409. https://doi.org/10.1097/FBP.0000000000000069

Wilson, C. B., Ebenezer, P. J., McLaughlin, L. D., & Francis, J. (2014). Predator exposure/psychosocial stress animal model of post-traumatic stress disorder modulates neurotransmitters in the rat hippocampus and prefrontal cortex. *PLoS ONE*, *9*(2), e89104. https://doi.org/10.1371/journal.pone.0089104

Wilson, C. B., McLaughlin, L. D., Ebenezer, P. J., Nair, A. R., Dange, R., Harre, J. G., … Francis, J. (2014). Differential effects of sertraline in a predator exposure animal model of post-traumatic stress disorder. *Frontiers in Behavioral Neuroscience*, *8*. https://doi.org/10.3389/fnbeh.2014.00256

Xiang, M., Jiang, Y., Hu, Z., Yang, Y., Botchway, B. O. A., & Fang, M. (2017). Stimulation of anxiety-like behavior via erk pathway by competitive serotonin receptors 2a and 1a in post-traumatic stress disordered mice. *Neuro-Signals*, *25*(1), 39–53. https://doi.org/10.1159/000481791

Yehuda, R. (2009). Status of glucocorticoid alterations in post-traumatic stress disorder. *Annals of the New York Academy of Sciences*, *1179*, 56–69. https://doi.org/10.1111/j.1749-6632.2009.04979.x

Zoladz, P. R., & Diamond, D. M. (2013). Current status on behavioral and biological markers of PTSD: A search for clarity in a conflicting literature. *Neuroscience & Biobehavioral Reviews*, *37*(5), 860–895. https://doi.org/10.1016/j.neubiorev.2013.03.024

Zoladz, P. R., & Diamond, D. M. (2016). Psychosocial Stress in Rats: Animal Model of PTSD Based on Clinically Relevant Risk Factors. In C. R. Martin, V. R. Preedy, & V. B. Patel (Eds.), *Comprehensive Guide to Post-Traumatic Stress Disorders* (pp. 1531–1551). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-08359-9_58

Zoladz, P. R., Conrad, C. D., Fleshner, M., & Diamond, D. M. (2008). Acute episodes of predator exposure in conjunction with chronic social instability as an animal model of post-

traumatic stress disorder. *Stress*, *11*(4), 259–281. https://doi.org/10.1080/10253890701768613

Zoladz, P. R., Eisenmann, E. D., Rose, R. M., Kohls, B. A., Johnson, B. L., Robinson, K. L., … Huntley, M. R. (2018). Predator-based psychosocial stress model of PTSD differentially influences voluntary ethanol consumption depending on methodology. *Alcohol*, *70*, 33–41. https://doi.org/10.1016/j.alcohol.2018.01.004

Zoladz, P. R., Fleshner, M., & Diamond, D. M. (2012). Psychosocial animal model of PTSD produces a long-lasting traumatic memory, an increase in general anxiety and PTSD-like glucocorticoid abnormalities. *Psychoneuroendocrinology*, *37*(9), 1531–1545. https://doi.org/10.1016/j.psyneuen.2012.02.007

Zoladz, P. R., Fleshner, M., & Diamond, D. M. (2013). Differential effectiveness of tianeptine, clonidine and amitriptyline in blocking traumatic memory expression, anxiety and hypertension in an animal model of PTSD. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *44*, 1–16. https://doi.org/10.1016/j.pnpbp.2013.01.001