THE EFFECTS OF PIMAVANSERIN ON CORTICOSTERONE LEVELS IN A RODENT MODEL OF POSTTRAUMATIC STRESS DISORDER

by

Kaitlyn M. Gibbons, B.S.

THESIS

Presented to the Faculty of

The University of Houston-Clear Lake

In Partial Fulfillment

Of the Requirements

For the Degree

MASTER OF SCIENCE
in Psychology

THE UNIVERSITY OF HOUSTON-CLEAR LAKE

DECEMBER, 2020

THE EFFECTS OF PIMAVANSERIN ON CORTICOSTERONE LEVELS IN A RODENT MODEL OF POSTTRAUMATIC STRESS DISORDER

by

Kaitlyn Mae Gibbons

APPROVED BY

_____
Georgina Moreno, Ph.D., Chair

_____
David H. Malin., PhD., Co-Chair

APPROVED BY THE COLLEGE OF HUMAN SCIENCES AND HUMANITIES

_____
Christal Seahorn, Ph.D., Interim Associate Dean

_____
Samuel Lyndon Gladden, Ph.D., Interim Dean

Dedication

To my family, whose unwavering support has allowed to me to continue my education and accomplish my goals. Your contributions have not gone unnoticed and I am forever grateful.

Acknowledgements

Thank you to all who volunteered their time and effort to help complete this project. Dr. Moreno, thank you for your guidance and support throughout my graduate career. Learning from you has been such a rewarding experience. Dr. Malin, thank you for sharing your expertise and for providing me with this opportunity, I am so grateful. Ping Sun Tsai, thank you so much for leading me through this project from beginning to end. This would not have been possible without you and I cannot thank you enough for your efforts. You have been a vital part of my graduate career and I cannot wait to see all that you accomplish in your future.

I would also like to give a special thank you to my friends and family for always feigning interest in my rodent discussions and keeping me motivated these past two years.

Finally, thank you to those at ACADIA Pharmaceuticals for your continued donations and funding provided to our research program. Your contributions are greatly appreciated.

ABSTRACT

THE EFFECTS OF PIMAVANSERIN ON CORTICOSTERONE LEVELS IN A RODENT MODEL OF POSTTRAUMATIC STRESS DISORDER

Kaitlyn M. Gibbons
University of Houston-Clear Lake, 2020

Thesis Chair: Georgina Moreno, Ph.D.
Co-Chair: David H. Malin, Ph.D.

PTSD can affect individuals that have experienced or witnessed a traumatic event, resulting in numerous physical and psychiatric symptoms. Selective serotonin reuptake inhibitors (SSRIs) and behavior therapy are often applied to treat PTSD symptoms. Although they are rarely administered, atypical antipsychotic medication has beneficial outcomes in those suffering from severe PTSD symptoms. This study aims to investigate the effects of the antipsychotic drug, pimavanserin, on corticosterone in a rodent model of post-traumatic stress disorder. This study also aims to provide further validity to an existing rodent model of PTSD and the procedures commonly utilized to induce stress. The current model of PTSD is a result of social isolation and a repeated stress exposure procedure, which subjected the rodents to predator odor while restrained. Forty-eight

female Lewis rats were included in this blinded study. Rodents were randomized to one of four equally sized groups: control group (sham-stress), pimavanserin high dose stressed group, pimavanserin low dose stressed group, or no treatment stressed group. The effects of isolation and two stress inducing events were measured by comparing corticosterone levels between the control group and stressed groups, independent of treatment. Additionally, the influence of pimavanserin on corticosterone levels and potential dose-dependent effects were measured. It was hypothesized that corticosterone levels would increase after rodents were subjected to single housing. Additionally, it was predicted that a decrease in stress hormone levels in the blood samples collected following the stress exposure for all groups, except for the non-stressed control group, would be observed. It was also hypothesized that higher levels of corticosterone would be measured among subjects treated with the antipsychotic drug, pimavanserin. Finally, results revealing dose dependent effects were predicted. It was hypothesized that a higher level of corticosterone would be observed in those treated with a higher dose of pimavanserin. Significant differences were also observed within subjects in the corticosterone concentrations collected on Day 8, Day 21, and Day 47. Specifically, stress hormone levels collected on Day 21 differed significantly from levels obtained on Day 8 and Day 47. These findings suggest a temporary effect of social isolation on stress response. Significant differences were observed between groups in corticosterone concentrations in the blood samples collected on Day 55, 24 days after stress exposure. These findings indicate a dose-dependent effect, as subjects that received the higher dose of pimavanserin produced the lowest corticosterone levels.

Outcomes of this study will expand existing literature regarding the use of antipsychotics to treat PTSD symptoms and measures commonly utilized to induce stress in rodents.

TABLE OF CONTENTS

List of Figures xi

CHAPTER I: INTRODUCTION 1

Contextualizing the Problem 1
Purpose of the Study 3

CHAPTER II: BACKGROUND 4

Posttraumatic Stress Disorder 4
Development of PTSD 5
Hypothalamic-Pituitary-Adrenal Axis 6
Rodent Models 7
Cognitive Based Treatments for PTSD 8
Drug Treatments for PTSD 9
Selective Serotonin Reuptake Inhibitors 10
Atypical Antispychotics 11
Pimavanserin 11
Previously Conducted Research 12
Current Research 13

CHAPTER III: METHODS 14

Experimental Methods 14
Animals 14
Stress Exposure 15
Needle Poke and Injections 15
Tail Vein Blood Collection 16
Cardiac Puncture 17
Plasma Corticosterone Assay and Data Analysis 18

CHAPTER IV: RESULTS 20

Corticosterone Levels Prior to Drug Treatment 20
Effects of Single Housing on Corticosterone Levels 21
Effects of Pimavanserin on Corticosterone Levels 24 Days Post-Stress Exposure 22

CHAPTER V: DISCUSSION 24

Limitations of the Study 26
Future Research 27

REFERENCES 30

LIST OF FIGURES

Figure 1 Experimental Timeline 18
Figure 2 Corticosterone Levels for All Groups at Three Time Points..... 21
Figure 3 Corticosterone Levels Collected on Day 55 23

CHAPTER I:
INTRODUCTION

Contextualizing the Problem

Posttraumatic stress disorder (PTSD) is a persistent psychiatric disorder that can develop after experiencing a traumatic event, such as natural disasters, war combat, vehicle accidents and violent assaults. This disorder can present both psychiatric and physical effects and is more prevalent in women than men (Schnurr et al., 2007). Symptoms of PTSD typically include troubling thoughts and feelings related to the traumatic experience, emotional instability, and hyper arousal (American Psychiatric Association, 2013). Hallucinations, primarily auditory verbal in nature, are also commonly observed in those with PTSD, with previous studies finding a prevalence of 50-67% among diagnosed military veterans (McCarthy-Jones et al., 2015). For some, PTSD symptoms present themselves quickly after the traumatic experience, while others may not experience symptoms until months or years later (Bryan et al., 2013). Overlaps in PTSD diagnosis criteria and symptoms with other mood and anxiety disorders in the Diagnostic and Statistical Manual of Mental Disorders (DSM) have resulted in widespread comorbidity with other conditions (Gros et al., 2012). This comorbidity has led to inconsistent diagnosis and has presented a challenge for researchers seeking to identify prevalence rates of the disorder among various populations. The variability among experimental methodology in PTSD studies, as well as similarities between

symptoms of PTSD and other psychiatric disorders has led to prevalence rate variability (Richardson et al., 2010).

Anti-depressants and cognitive-behavioral therapy (CBT) are typical forms of PTSD treatment, commonly used in combination (White, 1983). Selective serotonin reuptake inhibitors (SSRIs) are the most common medications utilized to treat depression and anxiety symptoms of PTSD (Asnis et al., 2004). While SSRIs are effective in improving mood and decreasing anxiety in individuals with PTSD, they do not directly treat hallucinations. Further, research has found evidence that these antidepressants can actually produce complex visual hallucinations (Cancelli et al., 2004). Antipsychotics are utilized to treat schizophrenia, a disorder that produces hallucinations, delusions and interferes with cognitive processes (Ross et al., 2006). Antipsychotics are also prescribed to treat hallucinations for those with Parkinson's disease and have been found to improve long-term progression of hallucinations when an early treatment approach is taken (Goetz et al., 2008). Findings from relatively recent research have revealed that atypical antipsychotic medications have promising effects on severe PTSD symptoms (Pae et al., 2008). However, there is a necessity for greater randomized control studies to further investigate the possible usefulness of such medications as well as their side effects (Ahearn et al., 2011).

Presently, there is a novel type of medication with some antipsychotic effects. Pimavanserin is a selective inverse agonist of the $5HT_{2A}$ serotonin receptor. In contrast with most antipsychotic medications, it has no observed effect on dopamine receptors. This study will investigate its effects on an animal model of PTSD. Considering the

evidence of the benefits of antipsychotics on multiple diseases with overlapping symptoms that occur in PTSD in combination with the lack of research on their potential effects on PTSD, it is necessary to investigate this further.

Purpose of the Study

The current study focuses on the effects of pimavanserin on stress hormone levels following repeated stress exposures. Previous research conducted at UHCL utilized pimavanserin in a rodent model of PTSD and observed a reduction of behavioral PTSD-like behavior patterns, where pimavanserin reduced startle response and avoidant behavior in the open field and elevated plus maze anxiety tests (Malin et al., under revision.) Malin and colleagues (under revision) also observed significant differences in corticosterone levels between stressed and non-stressed rodents at the end of the experiment. The current study aims to further validate the modified PTSD model by observing effects of stressors on corticosterone over time. This study also seeks to assess the effects of pimavanserin on stress hormone levels and potential dose-dependent effects.

CHAPTER II:
BACKGROUND

Posttraumatic Stress Disorder

Posttraumatic stress disorder, previously referred to as "shell shock", was initially believed to occur primarily in war combat veterans. It is now understood that this psychiatric disorder can affect any individual that has experienced or witnessed a traumatic event (American Psychiatric Association, 2013). The traumatic experience can vary in severity and provides actual or threatened death or physical injury to the individual or others, resulting in a psychological reaction. PTSD occurs when the trauma leads to subsequent impairments in an individual's behavioral and psychological function (Wilson, 2004). Individuals with PTSD often re-experience the traumatic event, leading them to actively avoid potential reminders. Additionally, those with PTSD experience difficulty in emotional regulation, resulting in emotional numbing and hyperarousal (Monson et al., 2012). The DSM-5 identifies four categories of PTSD symptoms: intrusion symptoms (intrusive thoughts and flashbacks), avoidance behaviors, alterations in cognition and mood, and changes in arousal and reactivity. In order to be diagnosed, the symptoms must occur for over a month and lead to significant functional impairments (American Psychiatric Association, 2013). Research estimates that 60% of men and 50% of women have experienced a traumatic event during the course of their life that could lead to PTSD (Pacella et al., 2013). However, most individuals will not go on to develop the disorder, resulting in a lifetime prevalence estimated at 8.3% (Kilpatrick et al., 2013).

Characteristics of the traumatic experience influence the likelihood that PTSD will develop in an individual. Furthermore, individual risk factors such as gender, intelligence levels, socioeconomic status, previous trauma exposure, and genetic variations impact an individual's susceptibility to the disorder (Lancaster et al., 2016).

Development of PTSD

Animal models have provided considerable insight into the process of PTSD development, revealing an interaction between stress hormone production and the process of memory consolidation. Additionally, research has identified the facilitated acquisition of an extinction-resistant conditioned fear response as a fundamental aspect of PTSD development (Pitman et al., 2007). The research literature has focused on the body's central stress system, the hypothalamic-pituitary- adrenal axis (HPA), in an effort to identify functional abnormalities that may be causing PTSD. It is believed that irregularities in sympathetic-adrenal-medullary and HPA axis function play a vital role in the development of the disorder. Specifically, a functional interaction between adrenergic response and HPA axis activity in response to trauma may lead to an amplified emotional recall of the experience and an eventual inability to produce adequate levels of primary stress hormones, such as cortisol (Shalev et al., 2008). High levels of corticotrophin releasing hormone (CRH) in cerebrospinal fluid (CSF) and low levels of cortisol have also been observed in baseline data gathered for adults with PTSD. HPA axis feedback regulation along with hormone receptor mechanisms are thought to be responsible for these atypical baseline levels. Unfortunately, studies utilizing pharmacological and non-pharmacological challenge test methodology to asses HPA axis function in PTSD have

reported mixed results in regard to CRH and cortisol levels. As a result, researchers are unable to propose uniform conclusions regarding the effects of HPA axis and adrenal function on stress response. Additional trials with larger sample sizes are encouraged (De Kloet et al., 2006).

Hypothalamic-Pituitary-Adrenal Axis

The HPA is a multi-structural system made up of portions in the hypothalamus, pituitary gland, and adrenal gland. In the event of stress, CRH is released by hypophysiotropic neurons in the paraventricular nucleus of the hypothalamus to hypophysial portal vessels. CRH then binds to receptors on pituitary corticotropes in the anterior pituitary gland, triggering the release of adrenocorticotropic hormone (ACTH). ACTH travels to the adrenal cortex, causing the production and release of glucocorticoids, hormones that are responsible for HPA axis function regulation. An increase in glucocorticoids then causes glucocorticoid receptors to inhibit HPA axis activity in the hypothalamus and pituitary gland by way of a negative feedback mechanism (Smith et al., 2006). In addition to HPA axis inhibition, glucocorticoid feedback mediates activity in the hippocampus and prefrontal cortex structures of the limbic system, further influencing stress response. Ultimately, the negative feedback mechanism is important in returning the brain to homeostasis following a traumatic experience. Abnormalities in the negative feedback mechanism have been seen to play an essential role in disorders related to stress, specifically, depression and PTSD. For individuals with PTSD, an amplified glucocorticoid negative feedback mechanism is observed, resulting in low levels of cortisol and interfering with brain homeostasis

(Herman et al., 2012). Literature has identified numerous brain regions subsequently affected by enhanced glucocorticoid feedback and low levels of cortisol, including the amygdala and prefrontal cortex. While these associations are thought to influence PTSD symptom development, they have not been thoroughly investigated and the neurological process of PTSD development is still not clearly understood (Friedman et al., 2007).

Rodent Models

As previously mentioned, animal models of stress and fear response have been essential for understanding PTSD. This is primarily due to the similarities in the central stress response system of humans and rodents. Numerous rodent models have been designed to induce features analogous to human PTSD. These features include fearfulness, avoidant behavior, hyperresponsiveness, persistence, and resistance to extinction. These models have enhanced literature concerning symptom development and presentation, as well as the effects of pharmacological medications on PTSD. Current animal research on this topic aims to provide insight regarding biological mechanisms at the root of the disorder (Schöner et al., 2017).

To model PTSD development, rodents are exposed to appropriate stressors that can evoke lasting observable behavior responses, as described above. Commonly used stressors include unpredictable foot shock, immobilization, exposure to predator odor, and exposure to an actual predator. Efficiency of these stressors is established by the production of PTSD-like symptoms, including exaggerated responses to sudden stimuli, impaired fear extinction, and changes in HPA axis response. Further validity of rodent models of stress is achieved through symptom decrease following pharmacological

treatment known to be somewhat effective in humans with PTSD, along with consistent conclusions among related studies (Lisieski, et al., 2018). Finally, rodent models fill gaps in human PTSD research by providing the opportunity to gather measurements, behavioral and biological in nature, prior to and after stress exposures.

Cognitive Based Treatments for PTSD

Cognitive-behavioral therapy (CBT) is primarily utilized to reduce PTSD symptoms. The CBT intervention aims to identify and alter the negative thoughts and beliefs associated with the traumatic experience in an effort to reduce negative effects (Frueh, 2012). Research has identified prolonged exposure (PE) as a highly effective CBT treatment for PTSD, providing lasting benefits among individuals (Powers et al., 2010). This exposure therapy is derived from Pavlov's fear conditioning and extinguishing research conducted in the 1920s. This technique involves repeated exposure procedures to create and eventually terminate an association between a stimulus and a conditioned fear response (Lancaster et al., 2016). PE consists of both imaginal and in vivo exposure, requiring the individual to continuously confront the memories and trauma related triggers in a safe environment to modify negative thinking patterns and reduce fear response (Foa et al., 2004). Cognitive processing therapy (CPT) is another equally effective cognitive intervention commonly used in PTSD treatments. CPT is a 12-session therapy that focuses on deconstructing maladaptive beliefs and assumptions associated with the traumatic event. Initially, CPT requires the client to first describe the traumatic experience in detail. Therapists then identify the negative beliefs in the written or verbalized statement and provide the client with methods to revise them and produce

positive and constructive thoughts instead (Resick et al., 2002). Currently, CBT interventions are the most recommended evidence-based treatment that effectively reduce PTSD symptoms long term (Frueh, 2012).

Drug Treatments for PTSD

When analyzing the brain function of an individual suffering from PTSD, one can expect to see numerous abnormalities, including surplus activation in the amygdala in response to a perceived threat. This hyperactivity subsequently influences other areas of the brain interfering with memory consolidation, learning processes, fear reaction, and avoidance behavior. PTSD research has primarily focused on the brain processes responsible for the production of stress response and fear, leading to an abundance of literature regarding adrenergic and hypothalamic-pituitary-adrenal (HPA) mechanisms (Friedman et al., 2007). Additionally, because the variety of symptoms those with PTSD endure suggest that multiple neurobiological systems are involved, extensive research regarding numerous neurotransmitters related to stress response, fear, and emotional regulation has been conducted. These studies have revealed complex associations between multiple neurological abnormalities and PTSD symptoms, creating difficulty in the development of a pharmacological treatment (Albucher et al., 2002). Furthermore, individual risk factors biological in nature that may influence subsequent development of the disorder, in addition to symptom variability, add to the challenge of identifying proper treatment.

Selective Serotonin Reuptake Inhibitors

Initial research on pharmacological interventions for PTSD symptoms involved tricyclic antidepressants (TCAs) and irreversible monoamine oxidase inhibitors (MAOIs), while modern day studies focus on the effects of SSRIs and serotonin antagonist reuptake and inhibitors (SARIs). Findings of numerous randomized clinical trials (RCTs) regarding the reduction of core PTSD symptoms and long-term efficacy of these treatments have established SSRI's as the primary medication utilized to manage PTSD (Stein et al., 2011). When administered, SSRIs block the reuptake of serotonin at the pre-synaptic receptor, leading to an increase in the amount of serotonin available in the synaptic cleft. Understanding this mechanism and its subsequent effects has led to the use of SSRIs as an efficient form of treatment for major depressive and anxiety disorders (Asnis, et al., 2004). The scientific basis for this treatment includes the comorbidity of PTSD with other mood and anxiety disorders. Additionally, research that has identified an association between abnormalities in serotonin receptors and common PTSD symptoms suggests that SSRIs are a reasonable treatment choice for PTSD (Cooper et al, 2005). However, the exact treatment mechanisms of SSRIs on PTSD symptoms are not yet understood and patient response in RCTs is relatively inconsistent. Recent efforts have been made to gain further understanding into SSRIs influence on different neurological systems, as well to identify predictors for the likelihood one will develop PTSD after a traumatic experience. This area of expanding literature aims to generate a treatment specifically for PTSD and decrease patient response variability (Bernardy & Friedman, 2015).

Atypical Antipsychotics

Atypical antipsychotics typically interfere with serotonin $5HT_{2A}$ receptors as well as the dopamine D_2 receptor. Presently, they are primarily used to treat schizophrenia and bipolar disorder. However, growing literature has attempted to increase the usefulness of atypical antipsychotics on a wider range of disorders, including PTSD (Maher et al., 2012). Beneficial outcomes have been observed in research concerning the use of atypical antipsychotic agents to combat symptoms of PTSD. Although these medications are not first line treatments, and are typically administered to those resistant or unresponsive to antidepressants, atypical antipsychotics have produced favorable effects on more severe PTSD symptoms, such as dissociation, paranoia, and psychosis. These effects are believed to be a result of the agent's unique 5-HT_2 receptor antagonism mechanism combined with the more usual D_2 blocking capability. Lack of RCTs in this area of research, along with side effects associated with atypical antipsychotics, have kept this treatment from being commonly prescribed. Further investigation on atypical antipsychotics' method of action and its effects on individuals with severe treatment-resistant PTSD is warranted (Friedman et al., 2007).

Pimavanserin

Parkinson's disease is an incurable degenerative disease that inhibits central nervous system function and commonly causes motor function impairments. As disease progression occurs, additional non-motor symptoms arise and contribute to the worsening of life quality (Hacksell, et al., 2014). Psychosis, distinguished by hallucinations and delusions, affects 20-40% of individuals battling Parkinson's disease. Severity and stage

of the disease, age, and additional medical problems have been identified as risk factors that influence the onset of psychosis in Parkinson's patients (Zahodne et al., 2008).

Parkinson's disease is caused by a deficiency of dopamine in the brain. Therefore, it is risky to treat Parkinsons' psychosis with antipsychotic drugs, which almost universally block dopamine receptors. The atypical antipsychotic pimavanserin (Nuplazid; ACP-103), is a serotonin receptor inverse agonist with selectively high affinity at 5-HT_{2A} serotonin receptors. It was developed and manufactured by Acadia Pharmaceuticals, Inc. Presently, pimavanserin is used as an FDA-approved medication to treat psychosis in patients with Parkinson's disease. Some studies have revealed significant improvements in psychotic symptoms, such as hallucinations and delusions (Meltzer et al., 2010). At this time, research is being conducted to increase the usefulness of pimavanserin on disorders with overlapping symptoms, such as Alzheimer's disease and schizophrenia (Combs, et al., 2017). Comorbidity in psychological symptoms between these diseases and PTSD has also led to the current research.

Previously Conducted Research

Previous research conducted at UHCL by Malin and colleagues (under revision) developed a modified rodent model of PTSD to assess the effects of pimavanserin on multiple behavioral measures of anxiety. Single housing, restraint, and exposure to predator odor was used to induce PTSD symptoms in female Lewis rats. Acoustic startle response, elevated plus maze, and open fields tests were conducted before and after stress exposure procedures to measure changes in anxiety behavior. Results of this study revealed a significant difference in anxiety behavior among stressed rodents following

the stress exposure procedure. A significant reduction in anxiety behavior following administration of pimavanserin was also found. Corticosterone levels were collected at the end of the experiment and significant differences were observed between stressed and non-stressed groups.

Current Research

Supplemental research is needed to provide a better understanding of the effects of antipsychotic medication on PTSD symptoms. This research aims to fill this gap in the literature by analyzing possible dose-dependent effects of pimavanserin on stress hormone levels, as indicated by corticosterone, the main rodent adrenocortical stress hormone, in a rodent model of PTSD. Further, the research aims to provide additional validation for the stress inducing procedures commonly utilized with rodents. It is hypothesized that the stress exposures will be an effective stress inducing method, resulting in a short-term increase in the rodent's corticosterone levels. It is also predicted that, as seen with many PTSD patients, there will be a longer-term depletion of adrenocortical stress hormones (Yehuda et al., 2006). It is further hypothesized that administration of pimavanserin at least partially reverse this depletion. Finally, it is predicted that this pimavanserin effect will be dose-dependent.

CHAPTER III:

METHODS

Experimental Methods

Animals

Female Lewis rats (n = 48) were included in the study and equally divided into four treatment groups: stressed and later injected with saline only, stressed and later injected with 0.3 mg/kg pimavanserin, stressed and injected with 1.0 mg/kg pimavanserin, and a comparison group that was sham-stressed and later injected with saline only. Lewis rats were selected for this study because previous research has revealed they present high levels of anxiety and strong responses to stress (Ramos et al., 2002; Cohen et al., 2006). Females were chosen due to the higher rates of PTSD observed among human women, and female rodent's heightened response to predator threat (Ditlevson & Eklit, 2012; Adamec et al., 2006). All protocols were approved by University of Houston–Clear Lake Animal Care and Use Committee and were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Subjects were housed in 12-hour light, 12-hour dark cycles with continuous access to food and water. Rodents were given four days for habitual assimilation prior to experimental procedures. Researchers then gently handled rodents for four consecutive days. During habituation and handling procedures, rodents were group housed. For the initial 8 days, rodents were kept in group housing. On Day 8, after 7 days in the laboratory, rats were placed in single housing and opaque separators were installed on each side of the cages, preventing the animals from being able to see neighboring rats.

Stress Exposure

Two stress exposures were conducted on the evening of Day 21 and Day 31, beginning at approximately 6:00 pm. Red lights were utilized throughout the laboratory during this procedure. Animals were first placed in a conditioned place preference (CPA) box for five minutes. Subjects in the control group were exposed to a petri dish containing a cotton ball soaked in saline and a small piece of an unworn cat collar. All other cohorts were exposed to a petri dish with a cotton ball soaked in reconstituted wildcat and a portion of a long-worn cat collar. The predator odor was supplied by PMart, Sandy Point, ME. The dish was attached by Velcro to the side of the CPA box. After five minutes, the petri dish and the rodents were moved to a 47 X 41 X 21 cm clear, plastic container for the stress exposure procedure. Rodents in the control group were placed in the container with a tubular restrainer apparatus but were not restrained. Animals in the remaining groups were immobilized in the restrainer with the predator odor placed immediately in front of their snouts. Animals underwent the stress-inducing or sham stress procedure for approximately one hour and were then relocated back to the CPA box for twenty minutes. Subjects were immediately returned to single housing following the stress exposure protocol.

Needle Poke and Injections

To habituate the animals to the injection process, researchers performed a needle poke on all rodents on the evening of Day 21. No substance was administered during this procedure. Subcutaneous injections were performed on Days 48 and 49 for all rodents. Researchers sterilized to injection site with alcohol preparation pads and gently grasped

the rodent's skin, separating it from the muscles. An injection needle was inserted, air bubbles were expelled, and the appropriate contents were administered. Subjects in the sham-stress group and the stressed-no treatment group were administered saline only. Animals in the high and low dose pimavanserin groups received 1.0 mg/kg and a 0.3 mg/kg of pimavanserin, respectfully. Pimavanserin was supplied by ACADIA Pharmaceuticals, San Diego, CA. One-hour post-injection, multiple behavioral tests for anxiety and hyperactivity were performed. The resulting behavioral data is beyond the scope of the current thesis.

Tail Vein Blood Collection

The baseline tail vein blood collection was conducted at the end of habituation, on Day 8 and began at 7:00am. Researchers securely wrapped rodents in a thin towel and placed them in a horizontal position. Tails were completely exposed and submerged in warm water for 1 minute in an effort to increase blood flow and vein visibility. A sterile butterfly needle and syringe were then prepped with a heparin solution to reduce blood clotting. Once the lateral tail vein was identified, the syringe was pulled back to create negative pressure within the syringe and needle was placed into the vein. Up to 1.0% of the animal's total circulating blood volume was collected, based on the rat's current weight. Once the appropriate blood volume was obtained, the needle was removed, and pressure was held at the site until bleeding ceased. Injection sites were cleaned, and animals were monitored for infection or irritation. Blood samples were immediately placed on ice and centrifuged at 5,000 rpm for 15 minutes. Blood and plasma were then separated and stored at a temperature of -80^0 C. Additional blood collections were

performed on Day 21, 11 hours before the first stress exposure, Day 47, 16 days after the second stress exposure, Day 50, 2 days after the first subcutaneous injection, and Day 51, 2 days after the second and final subcutaneous injection (See Figure 1). Identical tail vein blood collection protocol was followed. In accordance with accepted tail vein blood draw protocol, needle insertions began near the tip of the tail, moving upwards for each subsequent blood draw.

Cardiac Puncture

At the end of the experiment on Day 55, cardiac puncture was performed on each subject. Rodents were first placed in an isoflurane container until respiration slowed and movement ceased. Upon removal from the container, an anesthesia mask delivered isoflurane and medical oxygen. To test for depth of anesthesia, rats were placed on their backs and a toe pinch was conducted. The rat's skin and surgical tools were sterilized with alcohol prior surgery. A cut was made through the skin and abdominal wall of the rodent. A needle was inserted into the heart and blood was collected with a syringe. Decapitation subsequently served as the secondary method of euthanasia. Blood samples from the cardiac puncture procedure were immediately placed on ice and centrifuged at 5,000 rpm for 15 minutes. Blood and plasma were then separated with pipettes and stored at a temperature of -80^0 C.

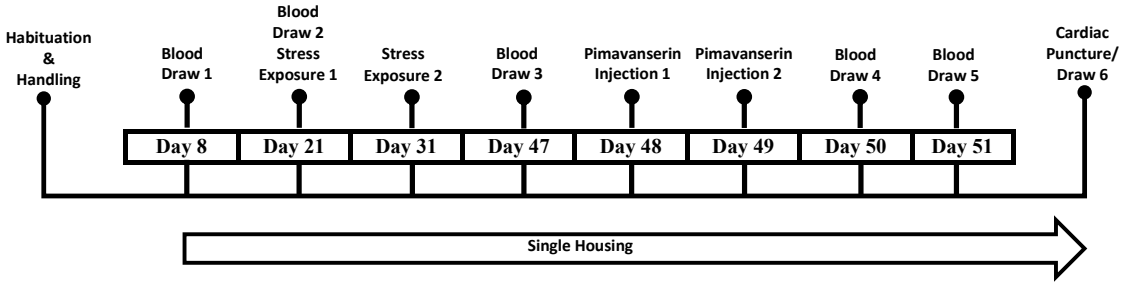

Figure 1

Experimental Timeline. Visual representation of the experimental timeline for each group. Individual group differences in experimental timelines are described in text.

Plasma Corticosterone Assay and Data Analysis

Corticosterone ELISA kits (501320) were provided from Cayman Chemical (Ann Arbor, MI). Assays were run in accordance with Cayman Chemical instructions for blood samples collected on Day 8 (Blood Draw 1), Day 21 (11 hours before the first stress exposure, Day 47 (16 days after the second stress exposure), and Day 55 (cardiac puncture). This assay identifies the concentration of corticosterone, a steroid hormone produced in response to stress. By measuring the constant corticosterone tracer concentration allowed to bind to corticosterone antiserum, this assay can reveal corticosterone concentrations, as these concentrations are inversely proportional.

Serum samples were purified prior to the conduction of assay protocol. Plasma and methylene chloride were added to a test tube and mixed. After the substances separated, methylene chloride was removed from the test tube and evaporated with nitrogen. Once sample purification was complete, mouse anti-rabbit IgG was added to the appropriate plate wells and able to attach. Corticosterone antiserum was then added and bound to the mouse anti-rabbit IgG. Corticosterone from the blood samples and corticosterone tracer were added to the appropriate wells and competed for the corticosterone antiserum. The plate was washed multiple times to expel unbound reagents. Ellman's reagent containing the AChE substrate was then added, resulting in an observable change in substance color. Spectrophotometry was utilized to measure the

strength of the color in each well, which was proportional to the level of corticosterone tracer in the well. The inversely proportional corticosterone levels were then be obtained.

Corticosterone levels were obtained and one-way ANOVAs and repeated measure ANOVAs with a between group variable and a repeated measure were conducted. These statistical analyses were run to identify differences in stress hormone levels within and between the four cohorts across multiple time points. Additionally, the analyses allowed researchers to test the effectiveness of the atypical antipsychotic in addition to potential dose dependent effects on corticosterone levels. All analyses tested for violated assumptions and corrected for any violations that occurred.

CHAPTER IV:

RESULTS

Corticosterone Levels Prior to Drug Treatment

An analysis determined differences in corticosterone levels between groups across the duration of the study prior to drug treatment. This included Day 8 (just prior to single housing), Day 21 (11 hours before the first stress exposure), and Day 47(16 hours after the second stress exposure)., A 4 (condition at the time of subsequent cardiac puncture) x 3 (day) mixed model ANOVA was performed with group as a between subject factor and time as a within subject factor. After correcting for sphericity, outcomes from the repeated measure ANOVA indicated a significant difference within subjects for corticosterone levels measured across the three time points, $F(6,60) = 2.57$, $p = .028$. Tukey's post hoc tests showed the corticosterone levels collected on Day 21($M = 2306$, $SE = 216$), after isolation, were significantly higher than levels obtained on Day 8 ($M = -1556$, $SE = 265$), $t = -5.87$, $p = <.001$, and Day 47 ($M = 1665$, $SE = 265$), $t = 6.28$, $p = <.001$. However, this ANOVA revealed no significant differences across conditions, $F(30,3) = 0.03$, $p = .992$ (See Figure 3). In short, the groups did not significantly differ prior to drug treatment on Day 55.

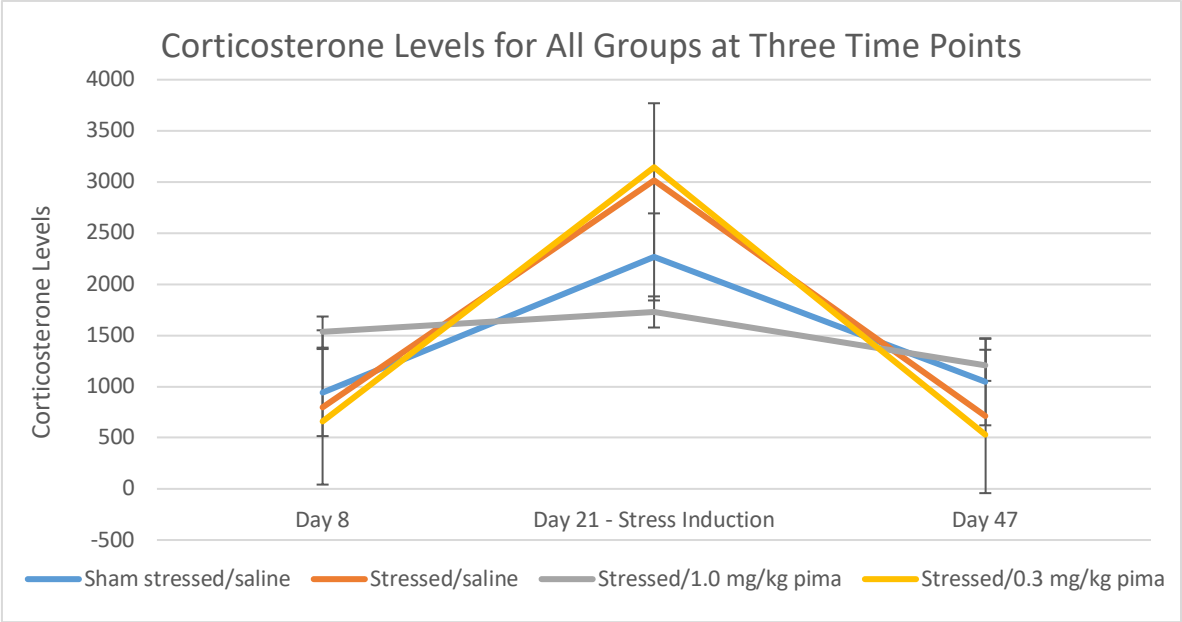

Figure 2
Corticosterone Levels for All Groups at Three Time Points. Visual representation of the mean corticosterone levels for each treatment group at the time of cardiac puncture; sham-stressed/saline, stressed/saline, stressed/1.0 mg/kg pima, stressed/0.3 mg/kg pima, in blood samples collected on Day 8, Day 21, and Day 47. Error bars represent standard deviations. Significant findings are described in text.

Effects of Single Housing on Corticosterone Levels

Since all subjects received identical treatment until the evening of the first stress exposure on Day 31, a paired samples t-test was performed to identify the effects of isolation on corticosterone levels in all subjects. There was a significant difference in the corticosterone levels obtained after isolation (M = 2447, SD = 1383) compared to baseline (M = 1024, SD = 1109), $t(33)$ = -4.37, p = <.001. Potential dose dependent effects of pimavanserin were indicated by the significant difference observed in the previously discussed one-way ANOVA conducted with corticosterone levels collected on

the final day, Day 55. However, as shown in Figure 2, the elevated levels on day 21 had subsided to near/baseline levels on day 47.

Effects of Pimavanserin on Corticosterone Levels 24 Days Post-Stress Exposure

A one-way ANOVA was run to compare corticosterone levels for each of the four groups (sham-stressed/saline, stressed/saline, stressed/1.0 mg/kg pimavanserin, and stressed/0.3 mg/kg pimavanserin) in blood samples collected through cardiac puncture on Day 55, the final day of the experiment (Figure 2). These samples were analyzed separately from the remaining samples because a different blood collection method was performed. Concentrations derived from wells with inadequate amounts of blood sample or samples with coefficient variable values greater than 80% were excluded from the analysis. The one-way ANOVA showed a significant difference between groups in corticosterone concentrations in the blood samples collected on Day 55 $F(3,40) = 3.21$, $p = .033$, with a relatively large effect size, Cohen's $d = 5.5$. Tukey's post hoc test revealed that the corticosterone levels collected on Day 55 for the stressed/saline group ($M = 451$, $SD = 301$) were higher than the stressed/1.0 mg/kg group ($M = 183$, $SD = 111$), and this difference was significant, $t = .022$, $p = .033$ (See Figure 2). There was a significant negative linear trend of hormone levels as a function of pimavanserin dose. This finding indicates a dose-dependent effect of high dose pimavanserin on corticosterone levels. Significant differences in corticosterone levels collected on Day 55 were not observed between the sham-stressed/saline, stressed/1.0 mg/kg pima, and stressed/0.3 mg/kg pima groups.

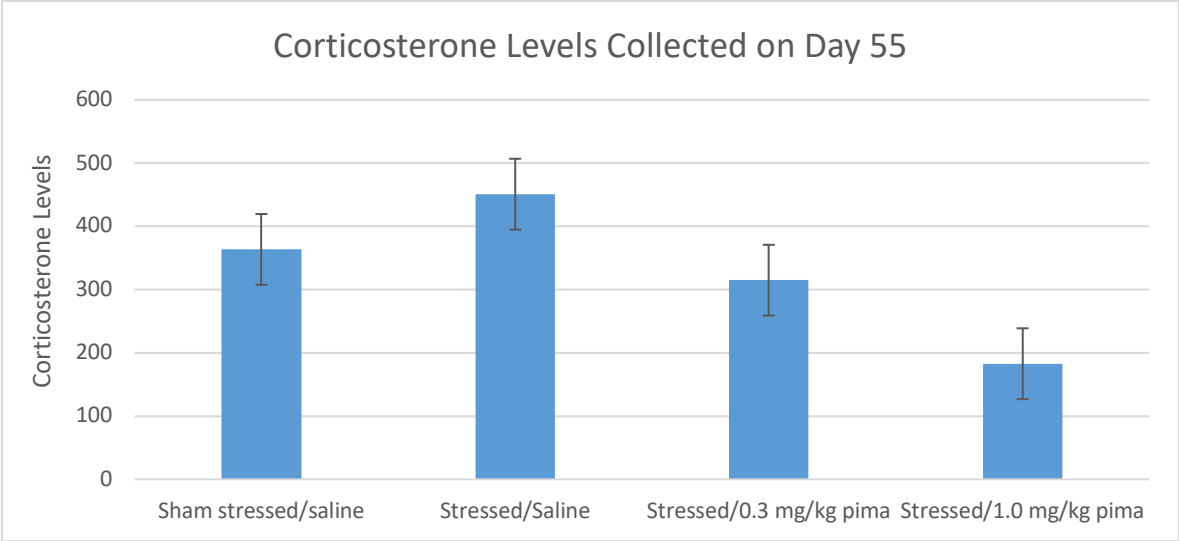

Figure 3

Corticosterone Levels (means ±standard error) collected by cardiac puncure on Day 55. Visual representation of the mean corticosterone levels for each group in blood samples collected on Day 55 through cardiac puncture procedure. Significant findings are described in text.

CHAPTER V:

DISCUSSION

The significant difference in corticosterone levels obtained at the end of the experiment among groups indicated an effect of pimavanserin on stress hormone levels. Lower levels of corticosterone were observed in both stressed/1.0 mg/kg pima and stressed/0.3 mg/kg pima groups. The corticosterone concentrations in subjects that were exposed to stressors and received 0.3 mg/kg of pimavanserin were just below corticosterone levels for the sham-stressed/saline group, suggesting that the medication completely reversed the delayed effects of the stress exposure procedures. Concentrations for rodents in the stressed/1.0 mg/kg pima group were even lower when compared to stressed/saline group levels, suggesting the treatment reversed the effects of both the stress exposure procedure and the stress caused by isolation or other factors. This finding supports the hypothesis of a dose dependent effect of pimavanserin on corticosterone levels. Although reductions in stress hormone levels were observed in both treatment groups, a greater reduction was found in subjects treated with a higher dose of pimavanserin. This is in contrast to the data collected before drug treatment, where there were no significant differences among the four groups.

The differences observed on Day 55 corticosterone concentrations among rodents in the stressed/saline group compared to the remaining groups (i.e. sham-stressed/saline, stressed/1.0 mg/kg pima, and stressed/0.3 mg/kg pima), also provide evidence of the effects of the stressors on stress hormone levels. Although it was predicted that subjects in the stressed/saline group would produce lower levels of corticosterone, higher levels of

corticosterone were observed for subjects in the stressed/saline group. This finding still supports the hypothesis that that stress exposure procedures, in conjunction with single housing, have an influence on rodent stress response.

The significant increase in corticosterone levels collected on Day 21, after isolation, compared to baseline corticosterone levels gathered on Day 8, further indicate an effect of single housing on stress response. Specifically, these findings revealed that subjecting rodents to isolation induced stress and led to an increase in stress hormone production. These results are consistent with previous animal models analyzing the effects of social isolation on corticosterone levels (Kamal et al., 2014). The observable increase in corticosterone concentrations among all four groups post-isolation in the current study also support the hypothesis regarding the effects of single housing on stress hormones and provides additional compelling evidence of the association between social isolation and stress response.

The decrease in corticosterone levels for stressed/saline, stressed/1.0 mg/kg pima, and stressed/0.3 mg/kg pima groups collected on Day 47, after stress exposures but before saline/pimavanserin subcutaneous injections, supported initial predictions. These lower levels of corticosterone may be a result of stress exposure effects on HPA-axis function and stress hormone production. However, the stressed/1.0 mg/kg pima group had higher levels of corticosterone when compared to the sham-stressed/saline group. This finding did not support the hypothesis, as it was believed that sham-stressed/saline rodents would have the highest corticosterone levels prior to subcutaneous injections.

Overall, results from this study were able to provide additional information regarding the effects of the stress inducing methods utilized on corticosterone levels as well as dose dependent effects of pimavanserin. Together, these findings suggest that the social isolation and exposure to acute stressors are appropriate techniques to influence stress hormone production in rats. These findings also support previous research conducted at UHCL, which relied on behavioral measures of anxiety to indicate treatment effects, in regards the beneficial effects of pimavanserin on corticosterone levels. Results from the current study in addition to findings of the previous study, present a pattern that suggest $5HT_{2A}$ inverse agonists, like pimavanserin, may have value for treating long-lasting consequences of traumatic stress. Additionally, both studies are consistent with the hypothesis that activation of the $5HT_{2A}$ serotonin receptor influences aftereffects of trauma, such as PTSD.

Limitations of the Study

This study would have benefitted from the analysis of corticosterone levels obtained on Day 50 and Day 51. These blood draws were performed 1-2 days after all stress exposure procedures and subcutaneous injections of either saline, 0.3 mg/kg of pimavanserin, or 1.0 mg/kg of pimavanserin. Without concentrations from these timepoints, immediate effects of pimavanserin on corticosterone levels cannot be derived. Additionally, the effects of the stress inducing procedures on corticosterone levels during this period could not be measured. It is possible significant differences in stress hormone levels were present at this time between and within the cohorts. Results from these blood

draws would likely enhance the overall findings as well as provide further validation for the PTSD model.

Including a cohort that remained group housed and did not experience any stress exposure would have also informed the overall findings. Data from this cohort could have served as a comparison tool to identify the effects of isolation over the course of the experiment. While effects of isolation on corticosterone levels were observed, a control group with subjects group house could provide additional evidence of this relationship.

Finally, numerous corticosterone concentrations in the ELISA assays were flagged and removed from the analysis. It is possible that issues in blood collections, sample purification, and/or in the production of the assay led to incorrect measurements. Without accurate corticosterone concentrations for all subjects at each time point, it was difficult to make explicit conclusions for the study.

Future Research

Currently, there is no data for corticosterone levels obtained shortly after the administration of pimavanserin or saline. However, samples were collected in the days following the subcutaneous injections. Future work will assay the corticosterone levels for these timepoints. Outcomes from this data could reveal information about the more immediate effects of pimavanserin on corticosterone levels. Analyzing changes in stress hormone levels over time, post administration of pimavanserin, could also identify information about the half-life of the medication.

Future research combining findings of this study with the results of the previously conducted study utilizing the same model, should be conducted to identify correlations

among corticosterone level variations and performance on behavioral tests for anxious and avoidant behavior, such as startle response, elevated plus-maze, and open field tests. If similar changes are observed in corticosterone levels and anxious behavior for subjects throughout the procedure, the modified PTSD model could be further validated.

Additional research analyzing the effects of atypical antipsychotic medication on stress response is essential. The dose dependent effects of pimavanserin on corticosterone levels observed in the study are an indication that further research is warranted to better understand dose effectiveness as well as its long-lasting effects. Although they are not the typical treatment for PTSD, atypical antipsychotics may have promising benefits for those suffering more severe symptoms. Moreover, $5HT_{2A}$ inverse agonists, like pimavanserin, could possibly serve as an alternative for those resistant to commonly used treatments. Studies utilizing larger sample sizes should be performed to gain a more accurate understanding of the effects of pimavanserin on stress response. Moreover, studies should administer the treatment immediately after the stress exposure in order to identify whether or not early treatment can reduce the initial development of PTSD symptoms. Studies administering pimavanserin prior to stress exposures should also be conducted in an effort to establish pimavanserin as a preventative treatment that may inhibit the subsequent development of PTSD following a traumatic event.

Findings of the current research supported existing literature regarding the effects of social isolation on stress response. Moreover, in light of the 2020 Coronavirus (COVID-19) Pandemic, research seeking to identify the long-term effects of social isolation on additional neurological functions is supported. This area of study could

identify the possible chronic symptoms individuals are likely to experience, as a result of mandated restrictions on social gatherings. Further, many are currently experiencing social isolation in combination with multiple stressful events. Subsequent studies utilizing this model of PTSD, which included multiple stressors, may provide information on the effects of numerous stress inducing situations on neurological function.

REFERENCES

Adamec, R., Head, D., Blundell, J., Burton, P., & Berton, O. (2006). Lasting anxiogenic effects of feline predator stress in mice: sex differences in vulnerability to stress and predicting severity of anxiogenic response from the stress experience. *Physiology & behavior*, *88*(1-2), 12–29.

Ahearn, E. P., Juergens, T., Cordes, T., Becker, T., & Krahn, D. (2011). A review of atypical antipsychotic medications for posttraumatic stress disorder. *International clinical psychopharmacology*, *26*(4), 193-200.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders: DSM-5.* Washington, D.C.: American Psychiatric Association.

Asnis, G. M., Kohn, S. R., Henderson, M., & Brown, N. L. (2004). SSRIs versus non-SSRIs in post-traumatic stress disorder. *Drugs*, *64*(4), 383-404.

Bernardy, N. C., & Friedman, M. J. (2015). Psychopharmacological strategies in the management of posttraumatic stress disorder (PTSD): what have we learned?. *Current Psychiatry Reports*, *17*(4), 20.

Cancelli, I., Marcon, G., & Balestrieri, M. (2004). Factors associated with complex visual hallucinations during antidepressant treatment. *Human Psychopharmacology: Clinical and Experimental*, *19*(8), 577-584.

Cohen, H., Zohar, J., Gidron, Y., Matar, M. A., Belkind, D., Loewenthal, U., Kozlovsky, N., & Kaplan, Z. (2006). Blunted HPA axis response to stress influences susceptibility to posttraumatic stress response in rats. *Biological psychiatry*, *59*(12), 1208–1218.

Combs, B. L., & Cox, A. G. (2017). Update on the treatment of Parkinson's disease psychosis: role of pimavanserin. *Neuropsychiatric disease and treatment*, *13*, 737.

Cooper, J., Carty, J., & Creamer, M. (2005). Pharmacotherapy for Posttraumatic Stress Disorder: Empirical Review and Clinical Recommendations. Australian & New Zealand Journal of Psychiatry, 39(8), 674–682.

De Kloet, C. S., Vermetten, E., Geuze, E., Kavelaars, A. M. A. A., Heijnen, C. J., & Westenberg, H. G. M. (2006). Assessment of HPA-axis function in posttraumatic stress disorder: pharmacological and non-pharmacological challenge tests, a review. *Journal of psychiatric research*, *40*(6), 550-567.

Ditlevsen, D. N., & Elklit, A. (2012). Gender, trauma type, and PTSD prevalence: a re-analysis of 18 nordic convenience samples. *Annals of general psychiatry*, *11*(1), 26.

Foa, E. B., & Rauch, S. A. (2004). Cognitive changes during prolonged exposure versus prolonged exposure plus cognitive restructuring in female assault survivors with posttraumatic stress disorder. *Journal of Consulting and Clinical Psychology*, *72*(5), 879.

Friedman, M. J., Keane, T. M., & Resick, P. A. (Eds.). (2007). *Handbook of PTSD: Science and practice*. Guilford Press.

Frueh, B. (2012). *Assessment and treatment planning for PTSD*. John Wiley & Sons.

Goetz, C. G., Fan, W., & Leurgans, S. (2008). Antipsychotic medication treatment for mild hallucinations in Parkinson's disease: Positive impact on long-term

worsening. *Movement Disorders: Official Journal of the Movement Disorder Society*, *23*(11), 1541-1545.

Gros, D. F., Price, M., Magruder, K. M., & Frueh, B. C. (2012). Symptom overlap in posttraumatic stress disorder and major depression. *Psychiatry research*, *196*(2-3), 267-270.

Hacksell, U., Burstein, E. S., McFarland, K., Mills, R. G., & Williams, H. (2014). On the discovery and development of pimavanserin: a novel drug candidate for Parkinson's psychosis. *Neurochemical research*, *39*(10), 2008-2017.

Herman, J. P., McKlveen, J. M., Solomon, M. B., Carvalho-Netto, E., & Myers, B. (2012). Neural regulation of the stress response: glucocorticoid feedback mechanisms. *Brazilian journal of medical and biological research*, *45*(4), 292-298.

Kamal, A., Ramakers, G. M. J., Altinbilek, B., & Kas, M. J. H. (2014). Social isolation stress reduces hippocampal long-term potentiation: effect of animal strain and involvement of glucocorticoid receptors. *Neuroscience*, *256*, 262-270.

Kilpatrick D., Resnick H., Milanak M., Miller M., Keyes K., Friedman M. National estimates of exposure to traumatic events and PTSD prevalence using DSM-IV and DSM 5 criteria. J. Trauma. Stress. 2013;26:537–547. doi: 10.1002/jts.21848

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), 105. doi:10.3390/jcm5110105

Lisieski, M. J., Eagle, A. L., Conti, A. C., Liberzon, I., & Perrine, S. A. (2018). Single-prolonged stress: a review of two decades of progress in a rodent model of post-traumatic stress disorder. *Frontiers in psychiatry*, 9, 196.

Maher, A. R., & Theodore, G. (2012). Summary of the comparative effectiveness review on off-label use of atypical antipsychotics. *Journal of Managed Care Pharmacy*, *18*(5 Supp B), 1-20.

Malin, D. H., Cambell, J.R.*, Tsai, P. * Moreno, G.I., Chapman, H.L., Burstein, E.S., & Ward, C.P. (under revision). Pimavanserin reverses multiple measures of anxiety in a modified rodent model of traumatic stress.

McCarthy-Jones, S. & Longden, E. (2015). Auditory verbal hallucinations in schizophrenia and post-traumatic stress disorder: common phenomenology, common cause, common interventions? *Frontiers in Psychology, 6*. doi:10.3389/fpsyg.2015.01071

Meltzer, H. Y., Mills, R., Revell, S., Williams, H., Johnson, A., Bahr, D., & Friedman, J. H. (2010). Pimavanserin, a serotonin 2A receptor inverse agonist, for the treatment of Parkinson's disease psychosis. *Neuropsychopharmacology*, *35*(4), 881-892.

Monson, C., & Fredman, S. (2012). *Cognitive-behavioral conjoint therapy for PTSD harnessing the healing power of relationships*. Guilford Press.

Pacella, M. L., Hruska, B., & Delahanty, D. L. (2013). The physical health consequences of PTSD and PTSD symptoms: A meta-analytic review. *Journal of Anxiety Disorders, 27*(1), 33-46. doi:10.1016/j.janxdis.2012.08.004

Pae, C. U., Lim, H. K., Peindl, K., Ajwani, N., Serretti, A., Patkar, A. A., & Lee, C. (2008). The atypical antipsychotics olanzapine and risperidone in the treatment of posttraumatic stress disorder: a meta-analysis of randomized, double-blind, placebo-controlled clinical trials. *International clinical psychopharmacology*, *23*(1), 1-8.

Pitman, R. K., & Delahanty, D. L. (2007). Conceptually Driven Pharmacological Approaches. *Recognition and Prevention of Major Mental and Substance Use Disorders*, 371.

Powers, M., Halpern, J., Ferenschak, M., Gillihan, S., & Foa, E. (2010). A meta analytic review of prolonged exposure for posttraumatic stress disorder. *Clinical Psychology Review,* 30(6), 635-641. doi:10.1016/j.cpr.2010.04.007

Ramos, A., Kangerski, AL., Basso, PF., Da Silva Santos, J.E., Assreuy, J., Vendruscolo, L.F., & Takahashi, R. N., 2002. Evaluation of Lewis and SHR rat strains as a genetic model for the study of anxiety and pain. *Behavioural Brain Research*, *129*(1-2), 113-123. doi:10.1016/s0166-4328(01)00337-0

Richardson, L. K., Frueh, B. C., & Acierno, R. (2010). Prevalence estimates of combat related post-traumatic stress disorder: critical review. *The Australian and New Zealand journal of psychiatry*, *44*(1), 4–19.

Ross, C. A., Margolis, R. L., Reading, S. A., Pletnikov, M., & Coyle, J. T. (2006). Neurobiology of schizophrenia. *Neuron*, *52*(1), 139-153.

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.

Schnurr, P. P., Friedman, M. J., Engel, C. C., Foa, E. B., Shea, M. T., Chow, B. K., ... & Turner, C. (2007). Cognitive behavioral therapy for posttraumatic stress disorder in women: A randomized controlled trial. *Jama*, *297*(8), 820-830.

Shalev, A. Y., Videlock, E. J., Peleg, T., Segman, R., Pitman, R. K., & Yehuda, R. (2008). Stress hormones and post-traumatic stress disorder in civilian trauma victims: a longitudinal study. Part I: HPA axis responses. *International Journal of Neuropsychopharmacology*, *11*(3), 365-372.

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in clinical neuroscience*, *8*(4), 383.

Stein, D. J., & Ipser, J. C. (2011). Pharmacotherapy of PTSD. *Post-Traumatic Stress Disorder,* 149-162. doi:10.1002/9781119998471.ch4

White, N. S. (1983). Posttraumatic stress disorder. *Hospital & community psychiatry*, *34*(11), 1061-1062.

Wilson, J. P. (2004). PTSD and Complex PTSD: Symptoms, Syndromes, and Diagnoses. In J. P. Wilson & T. M. Keane (Eds.), *Assessing psychological trauma and PTSD* (pp. 7–44). The Guilford Press.

Yehuda, R. (2009). Status of glucocorticoid alterations in post-traumatic stress disorder. *Annals of the New York Academy of Sciences,* 1179, 56-69.

Zahodne, L. B., & Fernandez, H. H. (2008). Pathophysiology and treatment of psychosis in Parkinson's disease. *Drugs & aging*, *25*(8), 665-682.