2022

The Effects Of Venlafaxine And Voluntary Exercise On Chronic Unpredictable Stress Induced Anxiety

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by

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Presented in Partial Fulfillment of the

Requirements of Independent Study Thesis Research

Supervised by

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Neuroscience Program

2021-2022

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Acknowledgements

First, I would like to thank my advisor, Dr. Amy Jo Stavnezer. She has gone above and beyond to help me generate my project, inspiring my research in her courses my junior year and encouraging me to pursue my passions. She has also guided and supported me throughout my entire project with her expansive depth of knowledge, continuously pushing me to do my best, and help keep me on track.

Similarly, I would like to thank Julie Pringle. She was vital for teaching me how to care for my rodents, helping me implement my procedures, and keeping me company in the rodent lab during long lab days. My procedure would not have worked without her guidance and continued support, thank you.

My rats were also an essential component to the research questions that I was interested in pursuing. I thank them for their service to my project and their sacrifice in the name of scientific discovery.

My IS cohort has also been incredibly supportive in giving me feedback on my writing, keeping me sane, and always providing an ear for any of my troubles. They have even gone out of their way to help me implement my procedure when I couldn't. Specifically, Rajani and Angelo have continuously reassured me and uplifted me in ways that I needed, thank you both.

I would like to thank my family, for always listening to me talk about my research and my passion for neuroscience. To my sister, who paved the way before me in college and was always willing to give me guidance, or a plant if guidance failed. To my brother, who can always make me laugh and who inspires me to be my best role model. To my father, for inspiring my love of learning and exposing me to the wonderful world of science, always encouraging me to ask questions and seek out answers for myself.

Finally, my friends have continuously motivated and cheered me on. Whether it was a quick call or a weekend getaway to Cedar Park, my friends have unknowingly helped me take a step back from my work, reflect on all that I have learned and my growth, and rejuvenated me to continue pursuing this project.

Abstract

Anxiety disorders can impair cognition and emotional control, particularly in the face of novel situations, which is concerning due to the high prevalence of such diagnoses. Exposure to unpredictable chronic stress can provide a way to induce anxiety-like behaviors in rodents by disrupting the stress response systems, including the HPA axis and autonomic nervous system. Pharmacological treatments, including SNRIs like venlafaxine, and voluntary exercise are both anxiolytic treatments that can alleviate the impacts of chronic stress. Using unpredictable chronic stress to mimic the human condition, and exposure to venlafaxine and exercise to mitigate the stress, the open field and elevated plus maze were used to assess anxious-like behaviors in Sprague-Dawley rats. It was hypothesized that chronic stress administration would induce anxious-like behaviors in the rodents and that implementing venlafaxine, exercise, or both would decrease anxiety behaviors. Chronic stress did cause an increase in anxious behaviors measured by the open field and elevated plus maze, supporting the effectiveness of unpredictable chronic stress as an anxiogenic. However, neither pharmacological nor non-pharmacological treatments decreased anxious-like behaviors when administered independently or in combination. Although the effects of treatment type were not found to be significant, it is important to consider various treatment options to best help anxiety symptoms in individuals, as anxiety is pervasive, can be debilitating, and more effective treatments are needed to help.

Introduction

Anxiety

Anxiety is a prevalent psychological diagnosis, with an estimated 31.1% of adults in the United States having some type of anxiety disorder in their lifetime (Kessler et al., 2005). It can present in many types of anxiety-related disorders, including generalized anxiety disorder, social anxiety disorder, panic disorder, posttraumatic stress disorder (PTSD), obsessive compulsive disorder, and phobias (American Psychiatric Association, 2013). It is also common for anxiety to present with another disorder, like depression or ADHD (Kompagne et al., 2008; Tsai et al., 2017). Of patients diagnosed with depression, 85-90% also had a comorbid anxiety disorder diagnosis (Tiller, 2013). Commonly, anxiety is associated with high levels of fear or an abnormal fear response (Etkin & Wager, 2007). Anxiety can impair areas such as the social and economic wellbeing of the person it is affecting. Due to the disruptions that anxiety disorders can cause, it is vitally important to know how to treat these disorders and assist in mitigating the negative effects that anxiety may promote.

One specific anxiety disorder is generalized anxiety disorder (GAD). GAD is the most common anxiety disorder and one of the most prevalent disorders overall. This anxiety disorder has common symptoms, including excessive anxiety and worry that are difficult to control and potentially creates feelings of restlessness, fatigue, concentration issues, irritability, tension, or sleep issues (American Psychiatric Association, 2013). GAD has been involved with impairments in cognitive functions like learning, attention, and memory, while specific brain regions associated with cognitive processes have changed in people with GAD (Mantella et al., 2007; Paulesu et al., 2009). In particular, an overly strong response to any anticipatory cue, either negative or neutral was observed in the amygdala of patients with GAD compared to patients

without GAD (Nitschke et al., 2009). Similarly, GAD is highly comorbid with depression, showing that anxiety is often a precursor for depression (Gorman, 2002). GAD significantly impacts cognitive and behavioral functioning of patients with the disorder.

Other common anxiety disorders include PTSD and social anxiety disorders that can also significantly impact daily functioning. Common symptoms of PTSD include hypervigilance and hyperarousal, dissociation, emotional numbing, and flashbacks or nightmares (Etkin & Wager, 2007). The amygdala has been highly linked with the expression of fear in patients diagnosed with PTSD. With the increase in amygdala activation, there is an increase in both fear response and the intensity of other PTSD symptoms (Shin et al., 2006). In contrast, the involvement of regions of the medial prefrontal cortex (mPFC) were observed to decrease in patients presenting with PTSD (Etkin & Wager, 2007). With less mPFC activation, there is less ability to inhibit the hypervigilance and hyperarousal that come with PTSD, as well as a decrease in conscious decision making. Social anxiety is characterized by avoidance of social situations due to fear of negative perceptions (Michopoulos et al., 2016). Similar to PTSD, there is an observed increase in the activation of the amygdala, but there was no observed decrease in the activity of the mPFC with social anxiety.

Development of an anxiety disorder is highly linked with genetic and environmental factors. Genetic factors may include a family history of anxiety or depressive disorders (Smoller, 2015). When a parent or family member has been diagnosed with anxiety or depressive disorders, the individual themselves are at a higher risk for being diagnosed with an affective disorder. Heritability for anxiety disorders has been estimated at 26% for a lifetime prevalence (Purves et al., 2020). Other research has been able to show that humans are evolutionarily predisposed to viewing the world negatively (Todd et al., 2013). Due to our evolutionary need

for survival, humans needed to be able to focus on the negative in order to remain safe and healthy. This creates higher rates of anxiety as well as other affective disorders, like depression, due to evolutionary factors and past need for survival. Another way for anxiety to develop are environmental stressors, like early life stressors or exposure to negative stimuli into adulthood. Long-term stress exposure also poses a risk for establishing anxiety disorders (Faravelli et al., 2012).

Typically, anxiety is a reaction to stressful situations or unpredictable threats; it can be a beneficial reaction in some situations. For instance, when an animal is exposed to a threat, a rapid response is necessary for survival. Fear has allowed for an adaptive and beneficial response, preparing the animal for responding to threats efficiently (Kalin & Shelton, 1989). Acute stressors can generate a fear response that helps the species remain aware and adequately react to situations. There can be an evolutionary benefit to short-term stress exposure and the resulting fear that it creates. While acute stressors are applied for only a short time frame, there are also long-term stressors that can prolong the fear response leading to the development of anxiety. When the stressor is applied over a longer time period, there is no longer the same evolutionary benefit of a fear response. Instead, long-term, or chronic, stressors can lead to maladaptive responses and potentially anxiety related disorders.

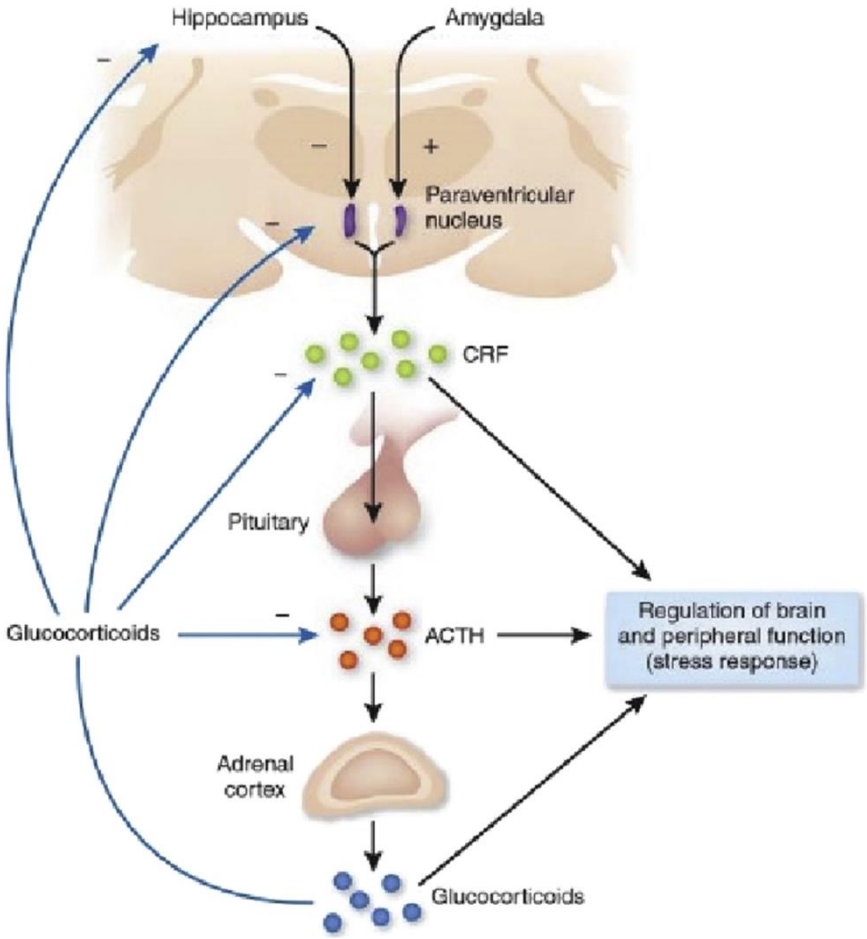
Involvement of the autonomic nervous system is one of the primary ways that an individual can respond to physiological or psychological stressors. Activation of the sympathetic-adreno-medullar pathway (SAM) via stress provides the individual with a rapid response, increasing awareness and vigilance. One of the primary neurotransmitters secreted during SAM activation is norepinephrine. This neurotransmitter is highly involved with feelings of alertness and through the SAM system, is able to impact the autonomic nervous system

activity throughout the body. In response to stress, the sympathetic nervous system dominates inducing signaling pathways that alter cardiovascular processing along with rates of digestion (Godoy et al., 2018). The increase in norepinephrine increases attention, alarm, and excitation through changes to autonomic nervous system activation.

Another way the brain responds to stressors and generates an anxiety response is through the hypothalamic pituitary adrenal (HPA) axis. When exposed to stress, either acute or chronic stress, the HPA axis is activated, causing the release of corticotrophin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH), inducing release of corticoids. Involvement of the HPA axis has been implicated in many affective disorders, including anxiety and depression (Liston et al., 2006). For an acute stressor applied for a brief period of time, the HPA axis will become activated and the secretion of CRH and ACTH will occur. Once the stressor is removed, the HPA axis will shut off, which will shut down the production of corticoids. This will cause the autonomic nervous system to switch between the sympathetic nervous system activation, that is involved in the fight-or-flight stressful levels, to the parasympathetic nervous system, linked to the slowing, rest and digest stage of activity. The HPA is able to self-regulate via a negative feedback system to return to its baseline activity (Stephens & Wand, 2012). The negative feedback can be observed in the hypothalamus as it detects the rise in the glucocorticoids and thereby decreases its output of CRH. This will then decrease the levels of glucocorticoids in the system (Figure 1). The negative feedback mechanism provides an effective, rapid way of returning the system back to normal levels following a stressful event.

Figure 1

HPA Axis Activation and Hormones



Note. Activation of the HPA axis causes the release of corticotropin releasing hormone (CRH, labeled as CRF) that will affect the pituitary causing the release of adrenocorticotropic hormone (ACTH).This hormone goes on to influence the adrenal cortex, which then releases glucocorticoids. The glucocorticoids can help to feedback into the system, thereby shutting down the production of CRH and ACTH, which decreases the stress response. Adapted from (Liyanarachchi et al., 2017).

However, when a stressor is applied chronically, the system becomes overly activated disrupting the balance between specific brain areas. The hypothalamus will overproduce CRH, and the adrenal gland will overproduce glucocorticoids. This will disrupt the balance between the amygdala, hippocampus, and prefrontal cortex (PFC). The amygdala is associated with negative emotional states and habitual learning, whereas the hippocampus is responsible for memory and cognitive learning. The PFC is responsible for decision-making and executive functions, like attention, planning and control. Normally, the brain will rely on the hippocampus for

consolidation and retrieval of memories, and the amygdala will play a minor role. However, when anxiety is present the roles are reversed: the amygdala dominates learning, and the hippocampus activity is minimized. This is seen in the decrease in dendritic density in the hippocampus after repeated stressful events (Davis & Whalen, 2001). Specifically, glucocorticoids that are generated in response to stress have been linked to a decrease in plasticity in specific brain regions, like the hippocampus and the dendritic connections in the hippocampus. While the hippocampal activity is decreased, the amygdala activation is strengthened (Vyas et al., 2002). This shifts the way that learning and memory occur in the brain. A cycle is readily developed that is hard to break as negative emotions and memories are more readily available, creating more stress, continuing the hyperactivation of the amygdala (Stephens & Wand, 2012). Without the negative feedback mechanism to shut down the HPA axis, the stress response is able to continually grow. The chronic exposure to stress also continuously initiates amygdala activation over the hippocampus or PFC. This means that the regular mechanisms for higher level cognitive processes, like learning and memory, are functioning differently than before. The stress system is no longer serving an adaptive response and instead is generating negative consequences to cognitive functioning.

The effects of long-term stress that induces anxiety are widespread in various brain regions due to involvement of hormones and secondary messengers. One primary stress hormone secreted by the hypothalamus is corticotropin-releasing hormone (CRH), that results in the production and release of glucocorticoids from the adrenal gland. Usually, these hormones are able to rapidly increase production during a stressful event over the course of a several minutes, causing activation of the sympathetic nervous system responses by decreasing metabolism and digestion, and increasing cardiovascular rates. Long term exposure to glucocorticoids has been

linked to a decrease in plasticity in the brain and decreased dendritic composition in the hippocampus, which impairs learning and memory (Bondi et al., 2008). CRH is also a major regulator of endorphins found in the hypothalamus, brainstem, and limbic systems (Owens et al., 1993). Endorphins help to regulate pain perception and mood, specifically generating pleasant feelings. CRH and endorphin production that are upregulated during a stressful event through the HPA axis are able to increase sympathetic nervous system activation and decrease the perception of pain, along with various diverse effects throughout the body.

Due to the activation of proteins and secondary messengers, the HPA axis activation can alter synaptic communication, generate intracellular changes, and influence cognitive processes. Brain derived neurotropic factor (BDNF) and cyclic AMP (cAMP) are impacted by an increase in the response from the HPA axis due to stress. BDNF is a neurotrophin that has been linked with the protection of the central nervous system, with high levels in the hippocampus and cerebral cortex (Murakami et al., 2005). This means that BDNF ensures that neurons are healthy, growing properly, and works to maintain the cell as needed. BDNF activates intracellular processes, including extracellular signal-regulated kinase, as well as influencing neuronal survival and synaptic plasticity (Huang & Reichardt, 2001). With acute or chronic stress, BDNF levels decrease, decreasing the protection to the hippocampus, impacting the learning and memory systems in the brain (Radecki et al., 2005). BDNF levels were shown to decrease in the PFC following exposure to chronic stress and repeated activation of the HPA axis (Chen et al., 2008). Cyclic AMP levels were observed to decrease in the hippocampus when stress was applied (Nair & Vaidya, 2006). The secondary molecule is highly involved in changes in neurotransmitter concentrations, receptor concentrations, or cellular processing that happens as a result of receptor activation. cAMP is able to upregulate many intracellular processes, which

have significant and widespread effects on neurons throughout the nervous system. The HPA axis is associated with the increased levels of corticosterone, decreased levels of BDNF, and decreased cAMP responsiveness (Song et al., 2006). Because of the decrease in BDNF and cAMP, stress can negatively impact learning and memory as well as cellular processing.

Similarly, anxiety linked with stress has been shown to decrease the activation of the medial prefrontal cortex (mPFC). The PFC is involved in higher level processing, like executive decision making and control of actions. When chronic stress is present, the PFC has been shown to decrease its activation. A decrease of function of the PFC in chronic stress was observed with lessened dendritic mass by 20% in chronically stressed rodents (Bondi et al., 2008; Liston et al., 2006). Attention shifting and decision-making capabilities thereby decrease, which negatively impacts the anxious human or rodent. Cognitive functioning in the prefrontal cortex decreases in patients with anxiety and depressive disorders. With stress-induced anxiety, abilities, and performance of the PFC, specifically the mPFC, is significantly decreased. This impacts the ability for higher level cognitive functioning to occur.

The activation of the stress system and the hypothalamic pituitary adrenal (HPA) axis are related to the neurotransmitter serotonin (Lapmanee et al., 2013). When cortisol levels in humans or corticosterone levels in rodents are raised in response to stress, the serotonin response is affected in most hypothalamic nuclei. The serotonin neurotransmitter has a higher concentration of 5-HT_2 receptors to bind to in the post-synaptic cell but less 5-HT_{1a} receptors (Barden, 2004). The 5-HT_{1A} receptor is an autoreceptor on the pre-synaptic neuron that will downregulate the amount of serotonin released into the synapse when there is a certain level of serotonin present. In the presence of stress, the 5-HT_{1A} prevent the release of serotonin by inhibiting the pre-synaptic cell (Celada et al., 2013). This restricts the amount of serotonin present in the brain and

contributes to symptoms associated with anxiety. When functioning of the 5-HT_{1A} autoreceptor was measured in mice, mice with fewer receptors present were more resilient to stress and more responsive to different pharmacological treatments (Yohn et al., 2017). On the post-synaptic cell, the 5-HT_{1A} has potential to negatively impact memory encoding, but not memory consolidation. It also reduces the activity of adenylyl cyclase and cAMP, which decreases neuronal excitability (Stiedl et al., 2015). Stress that decreases the levels of serotonin coming from pre-synaptic cells and signaling pathways present in post-synaptic cells that will restrict the communication between neurons and generate an anxiety response. Meanwhile, the activity of 5-HT_2 receptors is highly linked with CRH release inducing a fear and anxiety response. These receptor types for serotonin are affected by the stress response while also inducing some of the impacts of chronic stressors.

Another primary neurotransmitter involved in the HPA axis neuronal response is norepinephrine. Norepinephrine (NE) helps to activate the responses of the HPA axis. When an acute stressor is applied, the release of NE increases which has been linked with an increase in the anxiety behaviors. It increases the amount of corticotropin-releasing hormone (CRH) and acts to signal other neurotransmitters, like GABA and glutamate, in the presence of HPA activation (Levy & Tasker, 2012; Morilak et al., 2005). The increase in the release and subsequent receptor binding of norepinephrine creates the fight or flight response typical of the sympathetic nervous system activation (Guilliams and Edwards, 2010). Norepinephrine increases in response to stress and, as a result, affects the other hormones released and activates the sympathetic nervous system.

Pharmacological Treatments

Many pharmacological treatment methods have been used to help treat anxiety or other closely related affective disorders. One commonly prescribed treatment method is benzodiazepines, which are a class of pharmacological agents that impact neurotransmitters, specifically gamma-aminobutyric (GABA) (Roy-Byrne, 2005; Olfson et al., 2015). This helps improve symptoms of anxiety disorders and sleep problems. However, benzodiazepines are not without issues, sometimes leading to a dependence and misuse of the drug (O'Brien, 2005). The medically related visits due to benzodiazepine usage increased to 7.4% in 2015 from 4.4% in 2010, showing that more individuals were receiving benzodiazepines, using them for a longer period of time, and displaying negative implications of the drug (Agarwal & Landon, 2019). While benzodiazepines have provided an option to decrease anxious behaviors, they also come with risks that can be avoided with other pharmacological agents.

An alternative treatment to benzodiazepines is antidepressants, in particular tricyclics. Tricyclics are able to function by increasing levels of the neurotransmitters serotonin and norepinephrine by blocking reuptake from the synapse. This makes both neurotransmitters remain in the synapse for a longer period of time, meaning they can have more effects on the post-synaptic neuron (Moraczewski & Aedma, 2020). Administration of tricyclic antidepressants has been able to show an impact in decreasing the stress related response in rodent models (Prevot et al., 2019; Song et al., 2006). Tricyclics have proven effective when used for anxiety. However, this drug is able to influence serotonin and norepinephrine to variable rates depending on the type of tricyclic administered, but it lacks specificity to just one neurotransmitter. Because of this, it has been shown to have consistent negative side effects, including dizziness (Trindade et al., 1998), changes in appetite and weight (David & Gourion, 2016), and cardiovascular issues

(Fanoe et al., 2014). Even though tricyclics are able to decrease anxiety effectively, the lack of specificity and multiple adverse effects encourages the use of other pharmacological agents to treat anxiety.

A better tolerated pharmacological treatment are selective reuptake inhibitors, either serotonin or norepinephrine, or both. Typically, these are referred to as selective serotonin reuptake inhibitors (SSRI) or selective norepinephrine reuptake inhibitors (SNRI). SSRIs and SNRIS specifically block the reuptake of the neurotransmitter by the presynaptic neuron, which again means the neurotransmitter lasts longer in the synapse and can have more of an effect on its receptors. SSRIs will increase the levels of serotonin most directly and SNRIs will increase norepinephrine. SSRIs and SNRIs are regularly used to treat anxiety and depression (Bandelow et al., 2015; He et al., 2019; Kornstein et al., 2010). For both humans and rodent models, the SSRI or SNRI needs time in order to be effective. At minimum, it takes two weeks, but can take up to six weeks for the drug to properly impact the neuronal physiology in humans (Bandelow et al., 2017). For a rodent model, short-term administration can actually result in an enhancement of anxiety as well as less consistent and reliable decreases in anxious behaviors in various behavioral tests (Borsini et al., 2002; Rodgers et al., 1997). In human patients diagnosed with anxiety, SSRIs and SNRIs have both been readily prescribed for anxiety and have been shown to improve symptoms of anxiety (Hillhouse & Porter, 2015). SSRIs and SNRIs have been an effective treatment strategy for anxiety. Specifically, increasing the concentrations of serotonin or norepinephrine in the synapse provides a direct effect on neurotransmitters that are highly involved with anxiety and the HPA axis activation. By increasing specified neurotransmitters, the behavioral impacts of anxiety are less prevalent, making the symptoms of anxiety less impactful.

In human and rodent models, SSRIs have been shown to be an effective treatment method for anxiety. SSRIs and SNRIs are one of the primary pharmacological treatment methods commonly prescribed for anxiety in humans. While originally intended for alleviating symptoms of depression, these drugs work well with anxiety as the two disorders are often comorbid. For a rodent model, when measured by various anxiety behavioral tasks, SSRIs generally produced an anxiolytic effect (Borsini et al., 2002). When various chronic stressors were administered to rodents, SSRIs consistently abolished the impacts of the anxiety caused by stress (Leveleki et al., 2006; Rodgers et al., 1997). Reuptake inhibitors, specifically SSRIs, have been shown to be beneficial to treating anxiety in humans as well as rodents over a long-term period.

Considerably less research has been conducted on the effects of SNRIs in rodent models after chronic stress administration induced anxiety. SNRIs have been frequently prescribed and used in humans for treatment of anxiety alongside SSRIs. One experiment conducted by Lapmanee et al. (2013) investigated various pharmacological treatments, including an SSRI and SNRI and found equal effectiveness in decreasing anxiety in anxiety behavioral testing measures for the rodents. Because SNRIs impact the level of norepinephrine, there should be an observed effect when rodents are treated with this drug as norepinephrine is highly linked with stress-induced anxiety and the involvement of the HPA axis. An SNRI treatment method should be as effective as an SSRI, which has been supported in human models, but has been used far less frequently in rodent studies investigating anxiety. Because of the neurotransmitter system that SNRIs impact, there should be an observed anxiolytic effect with SNRI treatments.

Non-pharmacological Treatments

There are many alternative treatments for treating anxiety that do not include a pharmacologically prescribed medication. One of these options is cognitive behavioral therapy,

or CBT. CBT helps patients alter their responses to anxiety or anxiety-causing events. This is done by cognitive restructuring to develop more suitable responses and behavioral exposure to help approach the anxiety-causing event and observe the outcomes (Arch & Craske, 2008). When CBT was implemented, it was observed that 63% of patients no longer met the diagnosis criteria for anxiety disorders after 6 months of treatment (Cartwright-Hatton et al., 2004). CBT is an effective method for reducing anxiety without the need of a pharmacological intervention.

A newly investigated treatment for relieving symptoms of anxiety is cannabidiol, or CBD. This is a component present in the *Cannabis sativa* plant that does not create psychoactive effects and is not addictive. In patients with social anxiety disorder, a CBD intervention was able to reduce anxiety measured in a questionnaire over three weeks (Masataka, 2019). Specifically, CBD is able to activate many different receptor types, including the cannabinoid type 1 receptor and the serotonin 5-HT_{1A} receptor, which are able to have beneficial effects on symptoms of anxiety disorders (Blessing et al., 2015). With activation of the cannabinoid type 1 receptor, fear response changes, such that fear extinction occurs more readily and it protects against fear reconsolidation (Marsicano et al., 2002). Agonists of the 5-HT_{1A} receptor, like CBD, have been shown to prevent the adverse effects of stress (Campos et al., 2012). CBD provides a nonpharmacological, nonprescription method for reducing anxiety through involvement of cannabinoid and serotonin receptors.

An additional alternative treatment is exercise. Exercise is a beneficial activity for the physical health of a person, improving circulation and reducing the risk of diseases like diabetes or osteoporosis (Fentem, 1994). In patients suffering from anxiety related diseases, the risks of heart related diseases and health risks increase. With exercise, the risk of health-related

conditions decreases, which also decreases some of the adverse consequences of anxiety related diseases.

Exercise is one of the potential non-pharmacological treatment options to help with limiting the impacts of stress induced anxiety. When implemented, exercise conditions demonstrate a decrease in the anxious and depressive behaviors in humans (Salmon, 2001). The decrease in anxiety response may be due to lower sympathetic nervous system and HPA axis reactivity, or potentially an increase in the ability to self-regulate and distract from the anxiety causing event. While exercising, the HPA axis is more activated, creating an increase in the heart rate and sympathetic system activation. As the HPA axis is activated for the healthy exercise activity, the individual is primed for HPA axis involvement during acute or chronic stress exposure. As a result, the stress does not activate the HPA axis and the subsequent stress response to the same extent as before. The healthy activation of the HPA axis improves the tolerance for HPA axis activation during stressful events. Exercise can also be implemented as a distractor. The individual is relieved of the anxiety that they experience, instead focusing intentionally on their exercise condition. This helps to relieve HPA activation and reduce anxiety by distracting from the stressor (Anderson & Shivakumar, 2013; McCann & Holmes, 1984). Exercise can provide many psychological benefits when it is implemented in humans that can help to improve symptoms associated with anxiety disorders

Exercise also influences anxiety by altering neurotransmitter levels, BDNF, and endorphins. Exercise specifically impacts the levels of serotonin and norepinephrine, two of the primary monoamine neurotransmitters. While reductions in these neurotransmitters are linked with more anxious and depressive behaviors, the neurotransmitters have been shown to increase in a rodent model after an exercise condition was implemented (Eyre & Baune, 2012; Meeusen

& De Meirleir, 1995). The monoamine neurotransmitters that are increased with SSRIs, SNRIs, or other pharmacological interventions are also increased following exercise administration. This helps the anxious behaviors dissipate. Likewise, the levels of BDNF were found to increase following a physical activity task in a rodent model specifically in the hippocampus (Russo-Neustadt et al., 1999). This may promote hippocampal neurogenesis and a return to regular hippocampal activation following impairment from glucocorticoids and stress system activation. Increasing BDNF levels also improves brain plasticity and helps return the memory systems back to balance (Fuss et al., 2010). The amygdala returns to its normal, baseline level of activation and the hippocampus becomes more activated. Along with influencing neurotransmitter levels and BDNF, exercise also influences endorphins or other endogenous opioids. These endogenous opioids have an effect in mood and emotions and have been observed to be decreased in patients with depression and anxiety (Darko et al., 1992). An increase in endorphins following exercise helps to create a positively affected emotional state and reduce pain, benefitting the mental and physical state of the individual exercising (Thoren et al., 1990). Overall, exercise increases the levels of monoamine neurotransmitters, boosts BDNF levels improving learning and memory, and strongly decreases the impacts of anxiety related disorders.

Non-pharmacological treatments, like exercise, are effective for individuals that are treatment resistant, patients with mild or early symptoms, are low cost, and easy to implement. While pharmacological agents are readily used to treat anxiety, in particular selective serotonin or norepinephrine reuptake inhibitors or benzodiazepines, they have the potential not to work, and create a tolerance and dependance in the individual (Bystritsky, 2006). Non-responsive individuals do not gain any relief from their symptoms, even when receiving the pharmacological treatment. This can push treatment to much more drastic measures, like

electroconvulsive therapies or deep brain stimulation. While electroconvulsive therapies have been shown to be effective at alleviating the symptoms of depression and anxiety, it is usually a last resort measure to help the individual (Beale et al., 1995). Using exercise conditions as an anxiolytic can be done at home or in a gym with less economic burden compared to frequent therapy appointments for CBT. Exercise provides an alternative or adjunctive treatment that is still effective at reducing anxiety with less cost that is relatively easy to implement into a routine.

In a rodent model, exercise shows a comparable reduction in anxious-like behaviors as a pharmacological treatment. For instance, when the rat was chronically stressed via restraint stress and given a wheel to voluntarily run on, the anxious behavior of the rodent was reduced to levels comparable to an SSRI or SSNRI intervention (Lapmanee et al., 2013). Even when physical exercise was forced and not voluntary, rodents showed a comparable effect to an SSRI treatment on reducing anxiety-like behaviors in the rodents (Brenes et al., 2020). Voluntary wheel running did increase levels of BDNF in rodents and also increased the levels of corticosterone for the rodents (Fuss et al., 2010). This shows that there are positive benefits to exercise in the increase in BDNF to help promote neurogenesis. Exercise has been an effective agent implemented in a rodent model that reduces anxiety behaviors and neurogenesis equally effectively as pharmacological interventions, with increased benefits to physical health.

Stress Induction

Similar to humans, rodents can be genetically predisposed to anxiety-like behaviors. One of these genetic models is the Wistar-Kyoto (WKY) rat, that consistently express anxiety- and depressive-like behaviors. The WKY rats have commonly demonstrated low activity in the open field, increased sensitivity to stressors, and changes in learning and memory (Aleksandrova et al., 2019). Along with this, WKY rats present with higher rates of behavioral inhibition, or

withdrawal from novel situations, as measured by an operant box with novel stimuli presentations (McAuley et al., 2009). The WKY rat is able to display symptoms common in anxiety that can be comparable to the human model of anxiety, with an increase in the responsiveness of stress-related hormones (McAuley et al., 2009). Although this model is effective at expressing symptoms of anxiety, it is also readily expressing behaviors associated with depression (Aleksandrova et al., 2019; Gunter et al., 2000; Lahmarme et al., 1997). Although the disorders are often comorbid, it is best to minimize behaviors associated with depression and instead isolate anxiety-like behaviors. Even though the WKY rat is able to exhibit signs of anxious-like behaviors, other models that display more strict anxiety behaviors may provide a better model.

Another method to induce anxiety in rodents is by stress. As anxiety is highly related to the overactivation of the prolonged stress response, the introduction of a stressor to a rodent will activate the HPA axis that can create anxiety. When an individual is exposed to stress repeatedly or is consistently experiencing a high-level of stress for a prolonged period of time, the HPA system cannot regulate itself, which as described earlier, can induce anxiety in humans or anxious-like behaviors in rodents. The overactivation of the HPA axis will increase the production of stress-related hormones, including ACTH and glucocorticoids, that can have broad implications for responding to stressors throughout the body. Stress will also increase the synaptic density of the amygdala, while simultaneously decreasing functioning in the PFC and synaptic density of the hippocampus (Bondi et al., 2008; Davis & Whalen, 2001; Liston et al., 2006; Vyas et al., 2002). With stress, emotional and executive processes are impaired and as a result, anxious-like behaviors can be readily observed.

Acute or chronic stressors can cause anxiety to be induced. With acute stressors, the stress is applied only for a brief period of time, which could include short-term restraint or exposure to a predator. Social avoidance was induced in rats via exposure to an electric shock a day before testing were conducted, showing the induction of a symptom of anxiety disorders in humans (Leveleki et al., 2006). While this investigation was able to replicate one of the primary symptoms of anxiety, it did not investigate if other common markers of anxious-like behaviors in the rodent model. In addition, a drawback of acute stressors is that they only create short-term behavioral outcomes that make it hard to measure or observe (Yin et al., 2016). This means that long-term administration of a pharmacological or non-pharmacological treatment cannot adequately be determined to be effective. Other studies have also been able to determine that acute stressor implementations do not adequately alter behaviors of the rodents. When acute stress was administered to mice by restraint, there was no observed difference in behavior from baseline, non-stressed mice (Prevot et al., 2019). Considering the mixed results with acute stressors, long-term stress options provide a more reliable means to induce anxiety.

Chronic stressors will adequately induce anxious-like behaviors in the rodents by creating more long-lasting, permanent changes in the activation of the stress system. With chronic stress, stressors are applied for a longer period of time, usually 14 days or longer. As with humans, when a rodent is exposed to chronic stress, the HPA axis will be activated for a prolonged period of time. This can create a rapid rise in the levels of glucocorticoids as well as cellular changes in the corticolimbic brain regions (McEwan & Morrison, 2013). When glucocorticoid levels are elevated, it negatively alters the PFC and hippocampus, while in general increasing amygdala activity (Mizoguchi et al., 2003; Stephens & Wand, 2012). This is observed by the dendritic atrophy in the hippocampus matched with more dendritic growth in the amygdala. Exposure to

stressors for a long period of time can create lasting and more permanent changes in dendritic composition and thereby structural changes and activation of various brain regions. Chronic stressors also increase the levels of the stress hormone, corticosterone, which can have effects broadly in the body system (Wood et al., 2003). The chronic stress administration in rodents induces anxious-like behaviors similar to the effects of chronic stress in adults generating symptoms of generalized anxiety disorder (Patriquin & Matthew, 2017). Chronic stress has been able to induce dysregulation in the HPA axis, in particular hormonal disruptions with diverse effects to other regions of the brain and body, to create anxiety-like behaviors similar to those observed in humans.

One commonly employed method for inducing anxiety by exposure to chronic stress is by restraining rodents, called chronic restraint stress. This method can be used to induce stress by placing rodents into restraining tubes such that they can breathe but cannot escape or turn around. The rodents become immobilized which has been shown to create helplessness and anxiety-like behaviors along with dendritic density and cell signaling changes matching physiological alterations due to the HPA axis (Fontella et al., 2005; Müller et al., 2011). Chronic restraint stress is able to create changes in neuronal morphology and generates a fear response in open field investigations (Holmes & Wellman, 2009; Sandi et al., 2001). Other research found that chronic restraint stress decreases the spatial abilities of male rats as measured by a T-maze (Sunanda et al., 2000) and the Y-maze (Wright & Conrad, 2005). Along with that, chronic restraint stress impacts levels of specific neurotransmitters, in particular dopamine and serotonin (Torres et al., 2002). When administered chronically, restraint stress creates prolonged activation of the HPA axis, leading to changes in activation and responsiveness of different brain regions while elevating levels of corticosterone and inducing anxiety for the rodent.

One of the primary challenges in using chronic restraint stress is that the rodents habituate to their stressor due to the ability to predict their stressor. Predictive stressors have been shown to decrease anxiety- and depressive-like behaviors after a prolonged time period. Predictive stressors have been indicated in almost doubling total dendritic length and the volume of dendrites in the hippocampus of rodents compared to the control group without stressors (Parihar et al., 2011). Other investigations determined that predictive chronic mild stress produced an anxiolytic and antidepressant effect over a long-term period, decreasing the anxiety in rodents (Parihar et al., 2011; Suo et al., 2013). In order for anxiety-like behaviors to be properly observed in rodents, the stressors should not be as predictable as repetitive chronic restraint stress.

Unpredictable chronic mild stress will also create anxiety-like behaviors without the rodent becoming too accustomed to the stressor it is receiving. The stressors are varied during the stressing period, with each individual stressor being able to generate an elevated stress response in the rodents. Unpredictable chronic mild stress increases corticosterone levels and changes levels of monoamines as predicted by stress system activation (Cox et al., 2011). It has also been shown to decreases dendritic density in the mPFC and impacts the behaviors of rodents (Liston et al., 2006). Unpredictable chronic mild stress works effectively on Sprague Dawley rodents (Brenes et al., 2020; Liston et al., 2006; Owens et al., 1993). As Sprague Dawley rats are typically considered the normal rodent model of behavior with no predisposition to any one disorder or any behavior indicative of any disorder, it is relatively simple to observe the impacts of stressors on these rodents. Implementing chronic unpredictable stressors to these rodents will readily change their behaviors and show how the rodents were influenced by the stressors. Other

rodent models were seen to be less effective at showing anxiety-like behaviors in general and the behavioral outcomes of stressing techniques would have been harder to obtain (Tsai et al., 2017).

In order to investigate the effects of unpredictable chronic mild stress on the rodents, specific behavioral testing measures need to be conducted. Anxiety-like behaviors are typically measured via open field, elevated plus maze, or novelty suppressed feeding. Both open field and elevated plus maze tests have been used frequently as a measure of anxious-like behaviors in rodents after administration of chronic stress (Burokas et al., 2017; Castros et al., 2012; Huynh et al., 2011; Prevot et al., 2019; Rodgers et al., 1997; Zhu et al., 2014). The open field and elevated plus maze are also commonly used for investigating the effects of pharmacological agents and exercise conditions lowering anxiety-like behaviors of rats after chronic stress administration. Several investigations have been able to show that SSRIs (Borsini et al., 2002; Elizalde et al., 2008; Leveleki et al., 2006) and exercise (Brenes et al., 2020; Fuss et al., 2010; Lapmanee et al., 2013; Lapmanee et al., 2017) conditions help to minimize the anxiety-like behavior of the rodents. For the open field test, rodents are placed into an open field, where distance traveled and location in the maze are measured. For the elevated plus maze, rodents are placed into an elevated plus and again distance traveled, and location are measured for open and closed arms of the test. When a rodent spends more time in the exposed middle portion of the open field or in the open arms of the elevated plus maze, it is displaying less anxiety-like behaviors (Lezak et al., 2017). The open field test and elevated plus maze provide an effective, consistent measure of the anxiety-like behavior found in the rodents, able to indicate the effectiveness of treatment options like SNRI dosage and exercise or the combination of both.

The Present Study

Various investigations have been able to show that voluntary exercise has beneficial outcomes to decreasing the anxiety-like behaviors present and can counteract some of the impacts that anxiety had on an individual. Chronic unpredictable stressors are able to induce behavioral and physiological changes in a rodent model to display anxiety-like behaviors similar to anxiety disorders in humans. SSRIs and SNRIs are also readily used as anxiolytics in human patients and have been proven effective. However, there is less known about SNRIs with a rodent model, even though SSRIs are frequently implemented in investigations and have been shown to decrease anxiety-like behaviors. Because of the lack of investigations of SNRIs in rodent models as well as a need for alternative treatments, such as exercise, this experiment sought to investigate the effects of an SNRI, exercise, or both on anxiety-like behaviors in rodents. At the start, all rodents were given a pretest on the open field to get a baseline measurement of anxiety-like activity. All rodents were then stressed using chronic unpredictable mild stressors to induce chronic anxiety-like behaviors. When the stress administration was complete, one group of rodents received water injections and acted as a control to ensure that the stressor was effective. The remaining rodents were given either a dosage of venlafaxine, an SNRI, a running wheel in their cage, or both the SNRI and running wheel. This means the group of rodents with both an SNRI and exercise were treated pharmacologically and non-pharmacologically at the same time. Following the treatment condition, the rodents were retested in the open field and elevated plus maze to provide insight into the effectiveness of the stress condition and of the treatment administration. Sprague Dawley rats will be used as the rodent model, considering past effectiveness of implementing chronic stress and observing anxious-like behaviors in the rodent model (Brenes et al., 2020; Liston et al., 2006; Owens et al., 1993). The

anxiety-like behaviors in the rodents will be measured via the open field test and elevated plus maze. Both are reliable and effective methods for observing the anxiety-like behavior in rodents for the pre- and post-tests.

Based on previous work and the experimental design, we developed four hypotheses. First, unpredictable chronic mild stress will result in more anxious-like behaviors as measured by the open field test and elevated plus maze as compared to their baseline performance from their pre-test (Cox et al., 2011; Liston et al., 2006). The second hypothesis is that rodents who receive the pharmacological intervention, in this case the SNRI venlafaxine, will show less anxious-like behaviors than rats with no interventions and return to their baseline anxiety levels as observed during the pre-test on open field and elevated plus maze (Lampanee et al., 2013). The third hypothesis is rodents who get access to a running wheel, or a voluntary exercise condition, will show less anxious-like behaviors in the open field and elevated plus maze, and return to their original pre-test anxiety level (Anderson & Shivakumar, 2013; Brenes et al., 2020; Lapmanee et al., 2013). The effect of pharmacological interventions and exercise conditions will be observed to be similar and equally effective treatment options. The final hypothesis is that rodents that experience exercise and pharmacological interventions will present with the largest decrease in anxiety-like behavior measured by the open field test and elevated plus maze compared to the only chronically stressed group as well as both the pharmacological and exercise condition groups (Fuss et al., 2010).

Method

Subjects

Sprague Dawley rats were used (n = 32) from the College of Wooster animal lab. There was mixed sex (F = 23, M = 17) and approximately equal amounts of rodents that were 8 months

and 5 months old (8 months = 18, 5 months = 22). All rodents were pair-housed under a 12:12 hour light-dark cycle. All rodents had access to food and water at all times except under some short-term chronic unpredictable stressor conditions. For each condition, there were approximately equal distribution of rats across sex and age (n = 10 for exercise, SNRI, and both, n = 9 for stress only control).

Open Field Test (OF)

The open field maze was made from white plywood with walls high enough that the rats could not escape (36x36x18 in). Each rat was placed within the start box at the corner of the maze and was released after 10 seconds. Movement was measured via an overhead tracking system for 5 minutes. This test examined general movement, preference for center versus sides, gross locomotive activity and exploration habits. Fecal movements were also recorded during the testing but was not analyzed further as most animals had a score of zero. The perimeter of the open field provides protection with its walls, which will encourage the rats to stay at the perimeter more, particularly when the rodent is anxious. The open field maze was cleaned with a 1:1 solution of Natures Miracle and water. Testing occurred on day 1 and after the chronic stress administration followed by the treatment type.

Elevated Plus Maze (EPM)

The elevated plus apparatus was elevated 21 in. above the floor in a plus shape. The maze is comprised of four arms total, two left open, and two enclosed in 20 in. opaque walls. The arms of the maze were 20 x 4 in. Testing began when the rats were placed in the center of the maze facing an open arm. Testing was conducted with indirect incandescent lighting, and a video camera mounted above the maze. The number of entries into and time spent in each arm was recorded for 5 minutes. The entries and amount of time in the closed arms was indicative of

anxious behavior. Testing occurred twice, before any experimental manipulation and after the chronic stress administration followed by the treatment type.

Chronic Mild Stress Administration

Chronic mild stress administration was modified from Kompagne et al. (2008) and Zhu et al. (2014). The rats were exposed to various stressors including tilted cage, altered light-dark cycle, restraint in a plastic tube, white noise disruption, limited food access, crowded housing, isolated housing, wet bedding, and no nesting materials. Details about each individual stressor is provided below:

- Tilted Cage: tilted at 20° for 4-hours with plank of wood
- Altered Light-Dark Cycle: lighting the rodent cages outside of the typical light cycle that they are kept on either for a few hours (8PM – 10 PM) or overnight
- Restraint: rodents placed into plastic tube for 1-hour such that they could not move but had a mesh or perforated metal opening to breathe comfortably
- White Noise: placing a fan producing consistent noise at 60 dB during the light cycle of the rodents to disrupt their sleep/wake cycles
- Limited Food: rats were given only 1/3 of their normal food amounts overnight to induce physiological distress in the rodents, but was not full food deprivation
- Crowded Housing: rats were placed into cages with twice as many of their normal amount of cage residents of the same sex, or four rats per cage for females and males.
- Isolated Housing: rodents were placed in individual cages without any other rodents with them overnight

- Wet Bedding: 100 mL of water were spilled into the bedding material, such that the bedding became wet, and the rodent was kept in the soiled bedding for 4-hours
- No Nesting Materials: no bedding material in cages overnight

Each stressor was administered at different times in the day with at least an hour between stressors. Chronic stress was administered twice daily, as summarized in Table 1. The stress procedure was administered for 14 days to all the rodents. Animals were monitored for welfare, grooming, eating, and drinking habits, and normal social interactions throughout the stress condition. No animals demonstrated significant distress and had to be removed from the stressors or experiment.

Table 1

Chronic Stress Administration

Time of Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Morning **(8AM – 12 PM)**		Tilted cage 4 h	Crowded Housing 3 h			Restraint in a plastic tube 1 h
Afternoon **(12 PM – 4 PM)**	Restraint in a plastic tube 1 h			Restraint in a plastic tube 1 h	Wet bedding 4 h	
Evening **(4 PM – 8 PM)**	Lights on 2 h	White noise 4 h	Disrupted light-dark cycle, overnight	Limited access to food, overnight	Isolation, overnight	Empty cage/no nesting materials 8 h

Note. Rodents were exposed to various chronic stressors. Administration began on Day 1 and each stressor was administered at least twice in order to expose the rat to stressors for 14 days.

Drug Administration

Stressed rats were given a one daily injection of either 10 mg/kg venlafaxine or water. Venlafaxine was solubilized in water at 20 mg/mL. The administration of venlafaxine or saline persisted for three weeks after the chronic stress administration ceased. The venlafaxine dosage was selected due to its effectiveness on anxiety-like behaviors (Lapmanee et al., 2013).

Voluntary Wheel Running Exercise

One group of rats that did not get administered dosages of venlafaxine or water were provided free access to a running wheel. Another group of rats that received venlafaxine also had a running wheel provided in their cage. The wheel was provided for the rodents for three weeks following the chronic stress procedure.

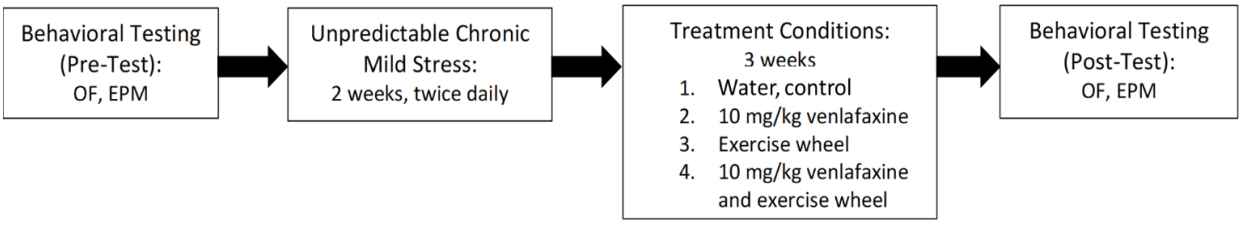
Experimental Design

Rats were first tested on the open field and elevated plus maze in order to get a baseline level of their anxiety and activity levels. This acted as a pre-test before the administration of chronic stress and/or an anxiolytic treatment option. Following the pre-test, the rats underwent a chronic mild stress condition for 14 days. Each stressor was presented for various lengths of time and all rodents received two stressors every day but their final stressor day (see Table 1). The chronic stress condition was intended to induce anxiety behaviors in the rodents.

After the stressors were administered, rodents were either given a water solution, venlafaxine, a running wheel in their cage, or both venlafaxine and a running wheel. Venlafaxine and the exercise condition were intended to provide anxiolytic effects on the stressed rats both separately and together. Each treatment condition was administered for 20 days. Once the three weeks were completed, the rodents were re-tested on the OF and EPM as a post-test behavioral measure. An overview of the experimental procedure is depicted in Figure 2.

Figure 2

Experimental Procedure



Note. Following the pre-test, all subjects underwent chronic mild stress for two weeks, receiving two stressors a day. Then the rodents were sorted into one of four treatment conditions (control/saline, 10 mg/kg of venlafaxine, exercise wheel, or 10 mg/kg venlafaxine and exercise wheel) for a three-week time period. To determine the impacts of the treatment condition, the rats were re-tested on the OF and EPM.

Statistical Analysis

Data were analyzed via a one-way ANOVA for the pre-test with a 2x2 design with the pharmacological agent and exercise as independent variables. A paired samples t-test was run to determine the changes between the pre-test and post-test. Another one-way ANOVA with the same 2x2 design was performed for the post-test conditions in order to determine the effects of treatment on the rodents.

Results

This study aimed to assess the induction of anxious-like behaviors in rodents induced by unpredictable chronic mild stress administration and the mitigation of anxious-like behaviors caused by stress with various treatment types, either venlafaxine, exercise, or venlafaxine and exercise. Anxiety-like behaviors were assessed using the following variables in OF: distance, percent time at the perimeter, and percent time at the center. Similarly, behavior was investigated with the following variables in EPM: distance traveled, percent time in open arms, and percent time in closed arms.

Pre-Test

In order to determine if all animals were displaying approximately equal anxiety-like behaviors before stress and treatment implementations, all rats were tested on the OF and EPM. This provided a baseline of behavior for the rodents as analyzed by a 2x2 ANOVA. There was no significant effect of drug or exercise before the treatments were implemented on any dependent measures for the OF or EPM (all *p*'s > 0.06; Table 2). There was no interaction effect for any variable of the OF (all *p*'s > 0.09; Table 2). There was also no interaction effect between drug and exercise for distance traveled in the EPM ($p > 0.05$; Table 2). There was a significant interaction effect for time spent in the open arms (F (1,36) = 4.73, $p = 0.04$) and time spent in the closed arms (F (1,36) = 4.65, $p = 0.04$) for the elevated plus maze. Tukey post-hoc testing revealed that the group of rodents that would become the stressed group spent significantly more time in the open arms ($M = 41.11$, $SD = 11.99$) than the group that would become the venlafaxine group ($M = 22.54$, $SD = 11.75$; p = 0.03), but there was no significant difference between groups for the closed arms (all p's > 0.32). Because there were no significant main effects on any dependent measure and only significant interaction effects in the time spent in the open and closed arms of the elevated plus maze, all rodents were presumed to be approximately equal before stress and treatment administration.

Table 2

2x2 ANOVA for Pre-Test Measures

		Drug		Exercise		Interaction	
Testing Measure	Df	F	p-value	F	p-value	F	p-value
OF							

Distance Traveled	1, 37	0.40	0.53	0.47	0.50	1.01	0.32
Time in Perimeter	1, 37	1.39	0.25	0.00	0.98	3.11	0.09
Time in Center	1, 37	1.16	0.29	0.42	0.52	0.01	0.93
EPM							
Distance Traveled	1, 36	0.02	0.89	0.10	0.75	2.06	0.16
Time in Open Arms	1, 36	4.03	0.06	1.01	0.32	4.73	0.04 *
Time in Closed Arms	1, 36	0.13	0.72	0.13	0.73	4.65	0.04 *

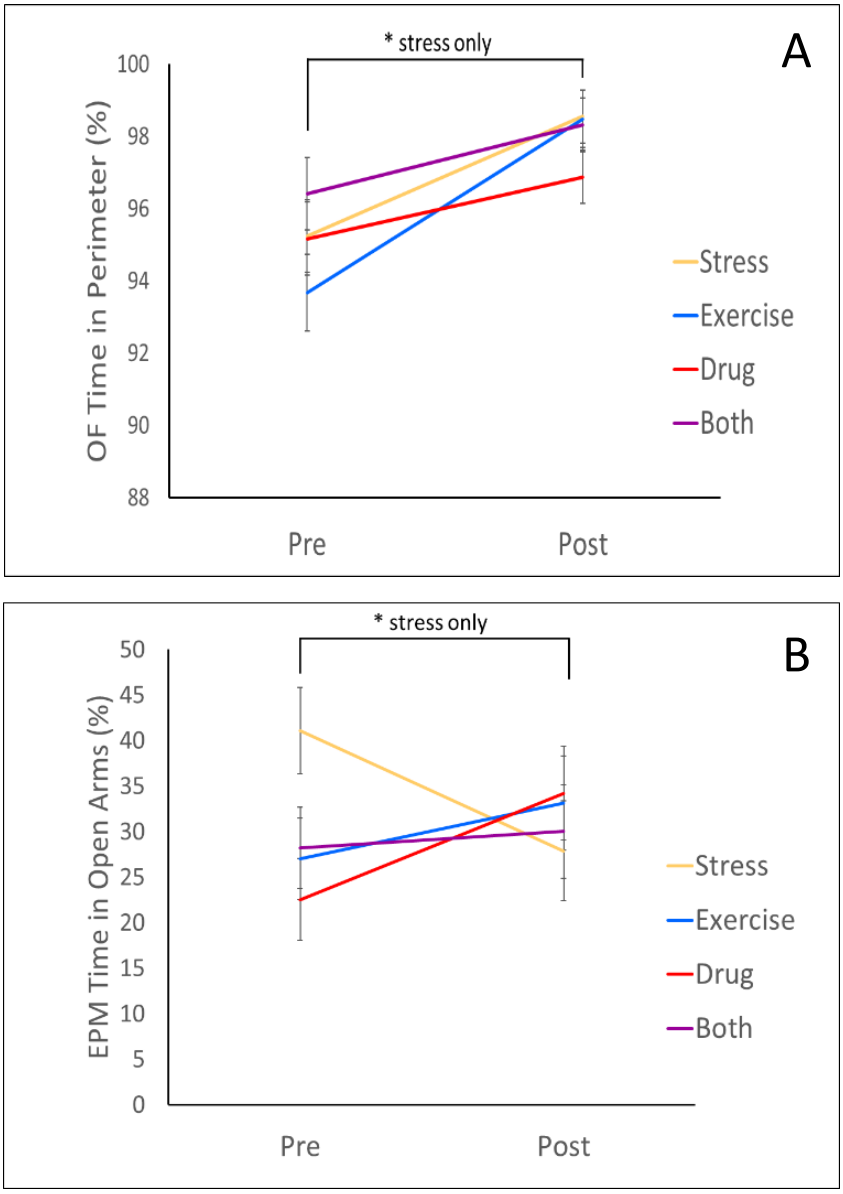
Note. * indicates statistical significance ($p < 0.05$).

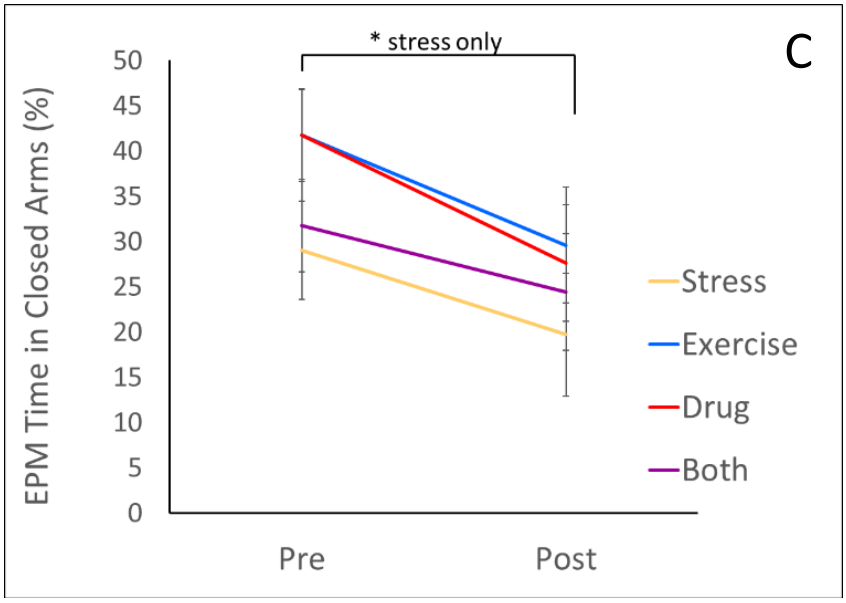
Effects of Unpredictable Chronic Stress

All rodents were then stressed for 14 days. To ensure that chronic unpredictable mild stress was effective at inducing anxiety-like behaviors in the rodents, a paired samples t-test was run to compare pre-tests to post-tests for all dependent measures using only the no treatment condition. This group was exposed to the stressor but received no treatment. There was a significant change from pre- to post-testing on open field distance traveled (t (8) = 3.22, p = 0.01) demonstrating a decrease in activity overall, and time spent in the perimeter (t (8) = 3.71, p = 0.01; Fig. 3A) demonstrating an increase in anxiety, but no differences in time spent in the center of the open field (t (8) = 0.68, p = 0.51). Similarly, there was a significant change in elevated plus maze distance (t (7) = 3.10, p = 0.02) demonstrating a decrease in activity, time spent in the open arms (t (7) = 2.14, p = 0.04; Fig. 3B) demonstrating an increase in anxiety, and time spent in closed arms (t (7) = 2.16, p = 0.03; Fig. 3C) opposite a change in anxiety.

Figure 3

Effects of Stress on Open Field and Elevated Plus Maze





Note. Stress only rodents significantly increased time spent in the perimeter of open field from pre- to post-test (A), showing an increase in anxious-like behavior. Stress only rats also decreased time spent in open arms (B) and decreased time in closed arms (C) , showing less activity and more anxious-like behaviors overall.

Post-Test

Post-testing in the open field and elevated plus maze occurred after the administration of stress for all of the animals and various treatments, including no treatment, venlafaxine, exercise, and venlafaxine and exercise. In order to determine if any treatment had an effect on activity or anxious-like behaviors, a 2x2 ANOVA was performed. There was no significant impact of drug or exercise treatment on any OF measure or any EPM measure following stress induction (all *p*'s > 0.06; Table 3). There was also no interaction effect between drug and exercise for any dependent measure in OF or EPM (all *p*'s > 0.21; Table 3).

Table 3

2x2 ANOVA for Post-Test Measures

		Drug		Exercise		Interaction	
Testing Measure	Df	F	p-value	F	p-value	F	p-value

OF							
Distance Traveled	1, 37	0.29	0.59	0.87	0.36	0.28	0.60
Time in Perimeter	1, 37	1.47	0.23	0.65	0.43	1.29	0.26
Time in Center	1, 37	0.13	0.72	0.31	0.58	1.22	0.28
EPM							
Distance Traveled	1, 37	3.85	0.06	1.07	0.31	1.00	0.33
Time in Open Arms	1, 37	0.12	0.73	0.03	0.87	0.45	0.51
Time in Closed Arms	1, 37	0.00	0.97	0.56	0.46	1.57	0.22

Discussion

The aim of this study was to investigate the effects of various treatments on anxiety-like behaviors caused by chronic stress exposure. It was predicted that unpredictable chronic stress would induce anxious-like behaviors in rodents and that pharmacological interventions and exercise would help to mitigate these effects. It was also expected that the use of both pharmacological treatments and exercise would have the greatest decrease in anxiety-like behaviors caused by stress.

Effects of Chronic Stress Exposure

All of the rodents were exposed to a rotating schedule of stressors for 14 days modified from Kompagne et al. (2008). Rodents in the stress group with no treatment did show an increase in anxious behaviors as measured by the OF. The rodents increased their amount of time at the perimeter of the test, demonstrating higher anxiety after the administration of stress and spent almost all of their time at the perimeter of the field. This meant that the rodents were less likely to explore and more anxious, which is comparable to other investigations that have shown a similar increase in anxiety-like behaviors following chronic stress (Liston et al., 2006; Owens et

al., 1993). There was not a significant change in the time spent at the center of the OF, likely due to a very small percentage of time spent there for both pre- and post-testing.

Previous studies have demonstrated that chronic stress will also increase the anxiety behaviors in the EPM by decreasing time spent in the open arms (Burokas et al., 2017; Prevot et al., 2019). In the current study, the exposure to stress significantly decreased the time spent in the open arms, replicating past research. The open arms have minimal walls that leave the rodent exposed and a decrease in time spent in them demonstrates anxious-like behaviors in the rodents. While rodents in the stress only group did decrease their time spent in the open arms, this pattern was not true for any of the different treatment groups. Because the other three groups received treatment, they may have shown less of a decrease due to unpredictable chronic mild stressors, even showing a minor increase in open arms time (Figure 3B). However, the treatments were not significantly different from the stress only condition during post-testing (Table 3), showing that the treatments did not significantly change the behavior of the rats.

Although the time spent in the open arms decreased as anticipated, the time in the closed arms also decreased, opposite of the expected results (Yohn et al., 2020; Zhu et al., 2014). The closed arms act as a protective space and shelter the rodent, so the anxious rodent should spend significantly more time in them compared to the open arms. One potential cause for the lack of anxious behavior is that chronic mild stress has had mixed effectiveness on anxious-like behaviors in rodents compared to other measures, like depressive-like behavior (Elizalde et al., 2008; Yin et al., 2016). Similarly, the time frame for the chronic stress administration was only 14 days, which is the minimum amount of time that rodents can be stressed chronically (Prevot et al., 2019), meaning that there could be less of an impact on behaviors. Similarly, the rodents in

the three other groups receiving treatments did decrease their closed arm time (Figure 3C), showing a comparable pattern of behavior regardless of receiving treatment.

A more likely cause of the decrease in closed arm time was that the rodents were no longer as active in the post-test as they were in the pre-test. This means that the rodents showed a decrease in movement and investigations into other parts of the maze and were staying frozen in the middle of the EPM for the majority of their post-testing. The freezing in the middle of the maze could demonstrate a fear response in the rodents and demonstrates anxious behaviors in another way. Prior work has shown that freezing in the OF was an indicator of high stress and high anxiety for rodents (Billah et al., 2019). Similar freezing is seen as a result of fear conditioning with other behavioral tests and demonstrates a response to stress (Dias et al., 2009; Stairs et al., 2020). The decrease in closed arm time then might be explained by a fear response, seen as an increase of time in the middle and significant decrease in activity overall, supporting a fear response that is prevalent in anxious-like behaviors.

In sum, we believe the stress did increase the anxious-like behaviors as seen in stress only rats. This supports the hypothesis that chronic unpredictable stress exposure was effective at creating anxious-like behaviors in the rodents as measured by OF and EPM.

Effect of Treatments

Venlafaxine was selected as an anxiolytic drug as it influences both serotonin and norepinephrine transporters as an SNRI, both of which are highly involved in the stress response of the HPA axis and sympathetic nervous system. Pharmacological agents that can influence specific neurotransmitters, like SSRIs, are commonly used to treat depression and anxiety in humans. SNRIs, that are less commonly used, have been shown to be as effective as SSRIs against anxiety (Lapmanee et al., 2013; Kornstein et al., 2010), and what we explored with a

chronic stress model in rodents (Wang et al., 2020). In opposition to the hypothesis, our experiment demonstrated no significant effect of the SNRI on anxiety-like behaviors in the OF or the EPM. There is research in support of the inconsistent results of SNRIs on behavioral measures in rodents (Borsini et al., 2002). This could be linked with specificities in dosage concentrations, timing, and means of administration of the drug. Although the results do not support my hypothesis and show a decrease in anxiety-like measures, they provide more support for the inconsistent effects of SNRIs.

Past literature suggests that the availability of a running wheel provided rodents with access to voluntarily exercise and could even provide enrichment to the environment (Islas-Preciado et al., 2016; Tsai et al., 2017). As exercise provides many benefits, like appropriate activation of the HPA axis (Anderson & Shivakumar, 2013), increasing levels of growth factors (Russo-Neustadt et al., 1999) and neurotransmitter levels (Eyre & Baune, 2012), it was anticipated that exercise would decrease anxiety-like behaviors as supported in previous studies (Duman et al., 2007; Fulk et al., 2004). In opposition to the hypothesis, the non-pharmacological intervention of exercise did not have any significant impacts on behaviors measured on OF and EPM. This literature, however, is not entirely consistent as one investigation found no change in anxiety following exercise (Pietropaolo et al., 2006), and others found an increase in anxiety-like behaviors following exercise (Burghardt et al., 2004; Van Hoomissen et al., 2004). Another potential reason for the lack of effects was the exercise being voluntary, unrecorded, and access to only one wheel per two rodents housed together. This could cause variability in the rodents in the amount of exercise they did and the consistency of their exercise.

With all of the positive implications of both drug and exercise separately, we expected to see an increased effectiveness against anxiety with the administration of both drug and exercise

for the rodent. This means that the rodents exposed to both drug and exercise should be displaying the largest mitigation of anxiety and return to pre-test levels of anxious-like behaviors as measured by the OF and EPM given past research (Fuss et al., 2010). However, the group that experienced both drug and exercise was not statistically different from the stress only group, or the treatment types independently. Again, this fits into the mixed results of both SNRIs and exercise but does refute my primary research hypothesis about the effectiveness of combined treatments.

Limitations and Future Work

Given the lack of effects for both drug and exercise treatments independently and in combination despite the previous literature, certain steps can be taken to rectify the shortcomings of this experiment. One of the primary variations with implementing a drug is determining the proper dosage amount for each rodent. In figuring out what dosage to implement, there was literature supporting varying dosage levels and routes of administration (Lapmanee et al., 2013; Stuart et al., 2013; Zhang et al., 2019) as well as timing of the dosage during stress (Song et al., 2006) or after stress (Leveleki et al., 2006; Prevot et al., 2019). While the wide variety provided a plethora of options, it could be possible that the drug concentration or administration was not potent enough to have an effect on the animals, or that the anxiety induced by chronic stress could not be overcome with only pharmacological intervention following the stressor. Future investigations should explore the various concentrations and timing to determine when the drug is effective. Venlafaxine was also specifically selected in order to investigate a pharmacological agent with mixed effectiveness on chronic stress induced anxiety. It is therefore possible that the pharmacological agent was just not powerful enough and that our results support that the drug did not mitigate anxiety caused by chronic stress. Comparably, the exercise condition did not

work as anticipated given previous work (Duman et al., 2007; Miller et al., 2018). Other studies have been able to demonstrate anxiolytic effects with a forced exercise condition for the rodents (Kim & Leem, 2006), rather than passive access to a wheel and exercising driven by the motivation of the rodent. Similarly, exercise protocols have been administered before the start of stress in some investigations and were able to show an effect of exercise on decreasing anxiety behaviors (Gerecke et al., 2013). Given past research, more active and involved running exercises or changing the order of stress and treatment plans would alter the procedure and potentially provide results in support of the anxiolytic effects of exercise.

Implications

The purpose of my research was to examine different treatment options in order to determine an optimized treatment for anxiety. Chronic stress exposure increases the activation of the HPA axis as well as the sympathetic nervous system, which increases the likelihood of developing an affective disorder, like anxiety and depression. Anxiety can significantly impact daily life and well-being of the individual who has it. Given current events happening in the world, like COVID-19 that acts as a chronic stressor, anxiety levels are increasing. For example, U.S. adults reported symptoms of anxiety from 7.4-8.6% in 2019 that has more than tripled to 28.2-37.2% in 2021 (“Depression and Anxiety Escalate During COVID”). Although my experiment did not adequately support either pharmacological or non-pharmacological treatments, other studies have been able to show promising results in regard to both intervention types. Individuals experiencing anxiety need to have treatment options that are effective for them to help them improve all aspects of their lives. In general, it is vitally important to create effective strategies to mitigate anxiety symptoms, whether this be through pharmacological means, exercise, other non-pharmacological treatments, or some combination of treatment types.

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