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The Effects Of The Alpha1a Adrenergic Receptor In Modulating Psychiatric Symptoms

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THE EFFECTS OF THE ALPHA1A ADRENERGIC RECEPTOR IN MODULATING PSYCHIATRIC SYMPTOMS

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Jason A. Power

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Acknowledgements

I would like to acknowledge my committee and to all who have supported me along the way.

Most Importantly…

To my grandmother; Guadalupe Estrada. May you rest in peace.

Thank you for believing in me, Grandma. For not giving up on me. I am alive because of your help. I am on my way to accomplishing my goals, Grandma. I will make you proud.

Abstract

Everyone experiences disruptive psychiatric symptoms such as depression and anxiety to a certain degree at some point in their life. Despite the familiarity amongst individuals with mental illnesses, the etiology and current treatments for psychiatric disorders are poorly understood. It is known that neurogenesis occurs due to chronic antidepressant treatment which inhibits neurotransmitter reuptake. The surplus of neural transmission is known, but the reason for the length of time required for symptom improvement is suspected to be linked to neurogenic activity. Antidepressants that focus on blocking both serotonin and norepinephrine offer a quicker onset of symptom relief than drugs focused on only blocking only serotonin. In this study, the adrenergic system, primarily the alpha1A adrenergic receptor, is investigated through agonizing the receptor over acute and a month-long treatment. It was hypothesized that agonizing the alpha1A adrenergic system produces an antidepressant effect because of neurogenesis. Results indicate no significant difference in depressive and anxious symptom improvement between the treatments. No induction of somatic symptoms was performed. As such, a 24-hour restraint model was constructed and developed for the induction of psychiatric symptoms in mice. This study indicates that an approved 24-hour restraint can be utilized to assess the activity of the alpha1A-adrenergic receptor in somatic symptom improvement in combination with additional treatment.

Chapter 1

Introduction

Anxiety

Anxiety disorders are different from fears experienced by individuals but share some resemblances. Fear arises because of immediate danger whereas anxiety is the anticipation of fear in the future. The phrase “fight or flight” is used to express both anxiety and fear. Fighting the danger is to “fight” either directly or indirectly and to “flight” is to run from the immediate danger or to anticipate running. The anticipation is what fuels the anxiety. Panic attacks can come when the threat becomes too excessive and is a way for the body to shut down. Anxiety disorders can come in multiple forms and are commonly comorbid with other forms of psychological disorders. These types of anxiety include Separation Disorder, Selective Mutism, Specific Phobia, Social Anxiety Disorder, Panic Disorder, Agoraphobia, Generalized Anxiety Disorder, Substance/Medication-Induced Anxiety Disorder, Anxiety Disorder Due to Another Medical Condition, Other Specified Anxiety Disorder and Unspecified Anxiety Disorder (American Psychiatric Association. & American Psychiatric Association. DSM-5 Task Force, 2013).

Separation Disorder

The prevalence rate of what is known as "separation anxiety" is 0.9%-1.9% in adults and 4% in children within the United States. Separation anxiety is the inability of an individual to be alone from an attachment figure(s). In their childhood, children can experience the fear of being alone such as during bedtime when the child requires an attachment figure(s) to remain in their proximity for them to fall asleep. Separation anxiety can even progress into adulthood from childhood or it can begin in adulthood. In adulthood, similar symptoms are experienced but the actions are different. One may be incapable of staying away from the attached figure and must always require the knowledge of their exact location. This may often appear as insecurity and clinginess, but this is simply the person's Separation Disorder.

Selective Mutism

Selective Mutism is an anxiety disorder that occurs prior to the age of 5 years but is not majorly noticeable until the school years. Selective Mutism is a form of Social Anxiety that causes the child to not be able to speak due to the feeling of embarrassment, or other perceived social judgment. Selective Mutism hinders a child's ability to function optimally in school and restricts from assessment of the child's reading ability. However, the children can express themselves in different ways such as pointing, grunting, and writing. Mutism tends to go away as the child "outgrows" it. When outgrown, there is a major chance that Social Anxiety Disorder will emerge.

Specific Phobia

Specific phobias affect 7%-9% in the United States with 6% in European countries. They occur from negative experiences that provoke anxiety when reintroduced to similar environments.

Social Anxiety Disorder

Social Anxiety Disorder is the fear of social interactions where a person may be exposed to judgmental situations. The fear includes showing that one may exhibit signs of anxiety toward others in social situations. As a result, those suffering from social anxiety may avoid social situations entirely, thus further fueling their social anxiety. The anxiety when out of proportion inhibits the life of the person suffering.

Panic Disorder

An abrupt intense fear peaks within minutes and includes palpitations, sweating, trembling, shortness of breath, chest pain, chills, fear of dying, and much more of considered a panic attack. Sufferers of panic attacks tend to then develop a fear of “fear” (panic attack) itself. The person with panic disorder tends to avoid situations that they believe will trigger their panic attacks which can cause a panic attack.

Agoraphobia

Agoraphobia is the fear of being alone, in crowded spaces, in open spaces, or even standing in a line. Like Panic Disorder, sufferers will avoid these situations to prevent

manifesting their symptoms. Their avoidance lasts a minimum of 6 months and causes mental distress. The distress can be so intense that the person will refuse to leave their home or drive their car. They may require medical support to simply bring them food to survive. If panic attacks do occur during Agoraphobic symptoms, a dual diagnosis can be given i.e., Agoraphobia and Panic Disorder.

Generalized Anxiety Disorder

Generalized Anxiety Disorder (GAD) is the persistent anxiety and worry for over 6 months which lasts most days than not. The person has a hard time controlling their worry and feels restless, they are easily fatigued, have difficulty concentrating, are irritable, have muscle tension, and disturbed sleep such as hyper and hypo-insomnia. GAD includes worries about their health or the health of others. Job performance even when their performance is not questionable, and much more. In the U.S, 2.9% of adults are found to have GAD with females being twice as likely to develop GAD than men.

Substance/Medication-Induced Anxiety Disorder

Substance/Medication-Induced Anxiety Disorder is when a patient has anxiety due to illicit substance use or abuse of a substance that is illegal. To differentiate, anxiety may be brought on during withdrawal or intoxication. To differentiate this diagnosis from that of other anxiety disorders is to determine the substance use prior to diagnosis of an anxiety disorder. Examples of substances that can induce anxiety in patients are caffeine and other stimulants.

Anxiety Disorder Due to Another Medical Condition

When a pathological medical condition is present, patients may develop anxiety over their condition. The anxiety must meet the criteria for other anxiety disorders but cannot be better explained by the criteria of the cause of the disorder itself. Instead, the pathological illness must be the onset of anxiety.

Other Specified Anxiety Disorder

Another Specified Anxiety Disorder is when the criteria for other anxiety disorders are hardly met to the extent that they do not fully meet the diagnostic criteria. For example, the patient may not worry more days than not but does worry slightly less more days than not. This criterion does not apply to GAD but still resembles GAD to an extent.

Unspecified Anxiety Disorder: The patient shows significant distress from anxiety, but their symptoms do not resemble any anxiety diagnosis. This is typically found and observed within emergency rooms.

Depression

Depressive disorders are mood disruptions that bring sadness or anger to one's life. Differentiating the forms of depression depends on the duration and occurrence of the symptoms and the types of symptoms being addressed. Each form of depression must require the patient to experience distress in their life and must receive a diagnosis that is not bipolar which involves a pattern with a period of depression followed by a period of happiness.

Disruptive Mood Dysregulation Disorder

Disruptive Mood Dysregulation Disorder is a diagnosis that must be diagnosed between the ages of 6 and 10. Children with this disorder experience extreme temper outbursts that are inconsistent with the situation being experienced. These outbursts must occur at least 3 times per week and the person must be angry most of the day.

Major Depressive Disorder (MDD)

MDD or an MDD episode is when a person feels depressed continuously for 2 weeks with more days than none. During and after this time, the person may feel a sense of guilt, shame, worthlessness, sadness, emptiness, hopelessness, and discouraged. Common symptoms of MDD include changes in appetite, energy, sleep, weight, and loss of interest. When depressed, a person may eat more or less than they usually do, but it is most common that the person will eat less than they did before, thus affecting their weight. Insomnia typically accompanies MDD as well. The most common type of insomnia that is faced by the affected ones is when the person wakes up during the night and cannot fall asleep. But either hypo or hyper insomnia also can occur. Lastly, a loss of interest in things that once used to be pleasurable, commonly connected to one's energy level, is the symptom that initially indicates depression.

Persistent Depressive Disorder (Dysthymia)

Dysthymia is a form of depression that is similar to MDD but lasts longer than 2 years in adults and 1 year in adolescents.

Premenstrual Dysphoric Disorder

The symptoms of PDD must begin to occur within the week prior to menses, improve during menses, and are minimal or absent after menses. These symptoms may/can include mood swings, anger, depressed mood, tension, and anxiety. For diagnosis, these symptoms must have caused distress in one's life and must have occurred for a year.

Substance/Medication-induced Depressive Disorder

Depression induced by a substance is when a person becomes depressed during intoxication and withdrawal. It is important that the person meets the criteria for depression and that symptoms do not last longer than 1 month following intoxication. If depression lasts longer than 1 month, the diagnosis must be re-evaluated.

Depressive Disorder Due to Another Medical Condition

Several medical conditions can cause one to become depressed due to the pathology of a separate medical condition such as a handicap.

Other Specified Depressive Disorder

Also known as a “short-duration depressive episode” is a form of depression in which the physician tells the patient the type of depression that the patient is experiencing and the duration of it as to why it’s not another Other Specified Depressive Disorder.

Unspecified Depressive Disorder

Unspecified Depressive Disorder is a type of depression that does not meet the criteria for any form of depression. Although it meets a few criteria for types of depression but not the full criteria for a diagnosis. This is seen commonly in emergency rooms of a hospital.

Animal Models of Depression and Anxiety

24-hour restraint

Utilization of restraints, mice restrained for 24 hours mimic the symptoms of victims suffered from traumatic events. Based on prior studies, reports showed that those who suffered traumatic events experienced neurological changes which enhanced the likelihood of a mood disorder, such as post-traumatic stress disorder (PTSD), substance abuse, and depressive disorders. The 24-hour restraint model was developed to simulate earthquake victims trapped under rubble (Chu et al., 2016). Denial of movement for 24 hours was found to increase psychological symptoms both short term and long term. Utilization of the 24-hour restraint is an attempt to explore the possibility of creating a depressive/anxious phenotype.

Chronic Mild Stress Model

The CMS model induces a depressive and anxious phenotype by subjecting mice to a set number of mild stressors (Hao et al., 2019; Murrough et al., 2015). Stressors include an empty cage for 8 hours, 8 hours of a tilted cage, 8 hours of wet bedding within the cage, 12hr illumination during the night, 5 minutes of water immersion up towards the mouse's belly, 15 minutes of tail pinching, cage crowding, and 8 hours of food deprivation. Mice undergoing the CMS must also remain single-caged to counteract the social behavior of mice. Denial of social interaction of mice undergoing CMS adds an additional factor towards inducing depression. Single caged mice remain in the colony room where they can smell and see each other. These stressors are induced singularly throughout the remaining 4 weeks on alternating days.

Behavioral Tests and Assessment

The Elevated Zero and Plus Maze

Used to evaluate anxiety levels by placing a mouse on an elevated circular/cross-shaped platform with open and closed sections of a maze. Mice with lower levels of anxiety will spend more time in the open areas as compared to mice with higher levels of anxiety. The duration of each test is 5 minutes per mouse with a single trial. This test involves minimal stress.

The forced swim test (FST)

The FST involves placing a mouse in a cylinder container filled with water. The mouse is required to swim for a total of 6 minutes. The scoring of this test is to assess the amount of

time the mouse stays mobile. The more mobile the mouse is, it correlates with lower the levels of depressive symptoms. Only one trial is done per mouse and this test is associated with high-stress levels in the mouse.

Tail Suspension Test

Like the forced swim test, this one assesses depressive symptoms in mice. Mice are hung by their tails on an elevated apparatus and the amount of time they remain mobile is assessed. The duration of this test is 6 minutes, requires 1 trial per mouse, and causes high stress to the mouse.

Open Field Test

An illuminated box where mice are placed in the center of the open box. Mice are tested for 10 minutes on their exploration of the box. Mice who wander for the least amount of time are seen to have higher anxiety than more exploratory mice.

Sucrose Test

Utilization of 2 water bottles: 1 with pure water and another with 2% sucrose. Mice are introduced to the novel bottles prior to the sucrose test so that they do not fear the object. Following what is known as the pre-sucrose test, mice are placed in individual housing where the 2 water bottles will be present on the opposite ends of the cage. After 12 hours, the bottle locations are exchanged, and the quantity of liquid is evaluated after an additional 12 hours. The sucrose test takes 24 hours to complete.

Noradrenergic System's effect on psychological disorders

It's been long studied that serotonin (5-HT) was responsible for the pathogenesis of psychological disorders such as depression, anxiety, bipolar, schizophrenia, and more disorders. Newer classes of antidepressants have emerged that not only affect the 5-HT pathways (anxiety, obsessions, and compulsions) but also now activate the NE system. NE deficiency has been associated with decreased alertness, low energy, problems of inattention, concentration, and cognitive ability (Briley & Chantal, 2011).

A meta-study was done to compare the antidepressants of both SSRIs compared to SNRIs to see which has the greatest effect on anxiety disorders both in efficacy and dropout rates. Each drug (SSRI and SNRI) dosage was converted into Imipramine dosages for comparison. A sample of the dosages used in the study can be found in Table 1 (Jakubovski et al., 2019). It was found that those taking SSRIs had a greater reduction in their anxiety symptoms than those taking SNRIs. And, even with increasing dosages, faced greater side effects and the onset of symptom improvement took 4 weeks. Side effects faced by taking SSRIs included sexual dysfunction, insomnia, nausea, and nervousness. For SNRIs, the side effects are like SSRIs, however, the sexual dysfunction isn't as prominent which is a major factor for patients (mainly men). Despite the improvements in anxiety levels of both SSRIs and SNRIs, the dropout rates of SSRIs increased with dosage increase whereas SNRI dropout rates did not continue with dosage increase but the symptom improvement plateaued for anxiety

improvement (Jakubovski et al., 2019). For SNRIs, symptom improvement was found to reach clinical efficacy at a 2-week mark as compared to the 4-week mark when compared to

Table 1. Antidepressant Dosages, Class and Diagnoses Examined.
A sample from the study which indicated each antidepressants used, dosages of the antidepressants, number of patients in the study as well as the duration of each study.

References	N	Diagnosis	Class	Medication	Dose	Duration of Study	Imipramine Equivalent Dose
Allgulander, Mangano et al. (2004)	370	SAD	SSRI	Sertraline	150	12	180
Allgulander, Dahl et al. (2004)	389	GAD	SNRI	Venlafaxine	225	12	225
			SSRI	Paroxetine	50	12	250
Allgulander et al. (2001)	541	SAD	SNRI	Venlafaxine	37.5, 75, 150	24	37.5, 75, 150
Allgulander (1999)	92	GAD	SSRI	Paroxetine	50	12	250
Asakura et al. (2007)	265	SAD	SSRI	Fluvoxamine	150, 300	10	150, 300
Baldwin et al. (2006)	681	GAD	SSRI	Escitalopram	5, 10, 20	12	33.3, 66.6, 133.2
			SSRI	Paroxetine	20	12	100
Baldwin et al. (1999)	290	SAD	SSRI	Paroxetine	50	12	250
Ballenger et al. (1998)	278	PD	SSRI	Paroxetine	10, 20, 40	10	50, 100, 200
Black et al. (1993)	55	PD	SSRI	Fluvoxamine	300	8	300
Book et al. (2008)	42	SAD	SSRI	Paroxetine	60	16	300

SSRIs. As psychological disorders are found in patients with decreased hippocampi sizes, a reduction in symptoms at a 2-week mark indicates the plausibility that the addition of the adrenergic pathway stimulates the neurogenic pathway, thus reducing symptoms within 2 weeks rather than in the case of 5-HT which takes 4 weeks to see any form of neurogenesis (Sen et al., 2008).

Neurogenesis

As science has evolved, we now know that this is not true. However, the rate at which neurogenesis does occur is low. Neurogenesis occurs at a higher rate within the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampus (S. M. G. Braun & Jessberger, 2014). Figure 1 displays how new neurons formed in the hippocampus come from the SGZ and transform into granule cells (smallest neurons). SGZ contains neural stem cells (NSCs) which migrate through pathways through a rostral migratory stream and direct neuroblasts (embryotic cells) which nerve fibers come from to the olfactory bulb where they mature into interneurons. NSCs are self-renewing and generate neurons, astrocytes (star-shaped glial cells), and oligodendrocytes which provide strength and support to axons within the CNS (Research Institute et al., 2009).

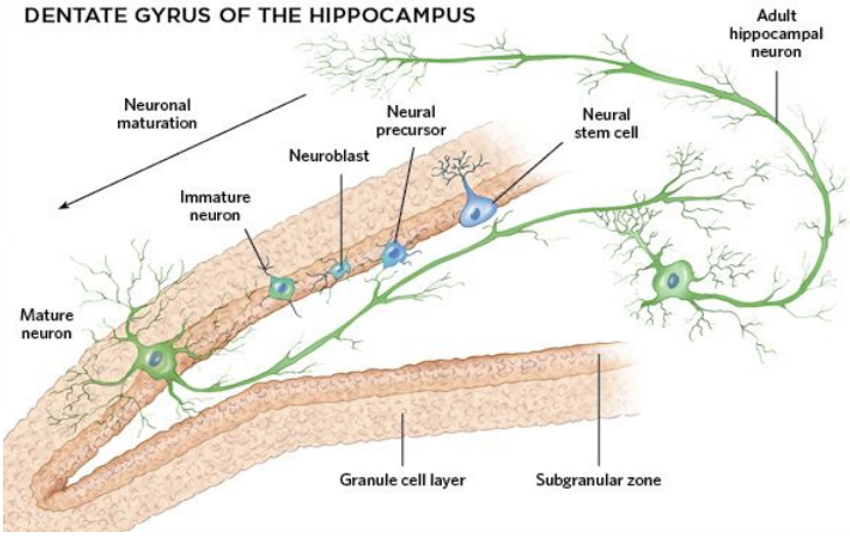


Figure 1. Neurogenesis Process Pathway.

The hippocampus has the largest turnaround rate of neurogenesis. The hippocampus is known for mood, learning and memory which makes hippocampi neurogenesis essential.

For example, a study was done to examine the levels of proliferating neurons before and after exercising and found that exercising boosted the amount of Bromodeoxyuridine

(BrdU) generated by granule neurons (Liu & Nusslock, 2018). It's been discovered that neurogenesis occurs in areas of the brain such as the hippocampus, amygdala, and hypothalamus with the brain-derived neurotrophic factor (BDNF) being a key molecule in modulating neurogenesis. BDNF binds to tropomyosin receptor kinase B (TrkB) which is largely expressed on the surface of hippocampal neurons. This binding gives significant regulatory control. Decreased levels of BDNF may lead to compromised memory, neurodegeneration, and other cognitive impairments such as those found in Alzheimer's disease, epilepsy, depression, and anxiety (S. M. G. Braun & Jessberger, 2014).

To further indicate the importance of BDNF in neurogenesis, the study done by Liu and Nusslock indicated that BDNF knockout (KO) mice had reduced stem cell proliferation in the subgranular zone and significantly more of the stem cells that had proliferated died. Also, TrkB which BDNF binds to, caused the KO mice to have reduced proliferation and differentiation (Liu & Nusslock, 2018).

The importance of BDNF was shown when overexpressing BDNF or even injecting a single dose of BDNF resulted in an increase in neurogenesis in the dentate gyrus within the hippocampus. Along with BDNF, the transcriptional regulars of neurogenesis in the DG and/or SVZ include Sox2, NeuroD1, Pax6, Gsx2, Sp8, Prox1, Ascl1, TLX (also known as NR2E1). Histone modification by MBD1 or by small non-coding RNAs such as mir-124 also plays a role in regulating neurogenesis. Morphogens, neurotransmitters, growth factors, and cytokines also control NSPC activity (S. M. G. Braun & Jessberger, 2014).. These include GABA, glutamate,

BDNF, EGF, FGF2, Wnt ligands, Shh, BMP, IL6, and TNF alpha. Positive factors regulating neurogenesis include physical activity, environmental enrichment, and olfactory or hippocampus-dependent learning. These factors either enhance NSPC proliferation and/or the survival of new neurons. Negative factors resulting in the loss of newborn neurons include stress, inflammation, alcohol abuse, and age. Targeting neurogenesis may be beneficial for cognition and can also have positive effects on stress response in patients suffering from major affective disorders. Thoughts have been suggested that brain lesions followed by neurogenesis targeting can repair damaged brain tissue.

Hippocampus Anatomy

The hippocampus is found within the medial temporal lobe found deep within the brain. This region is composed of three sections: the CA1, CA2, CA3, and the dentate gyrus (DG) (Figure 2).

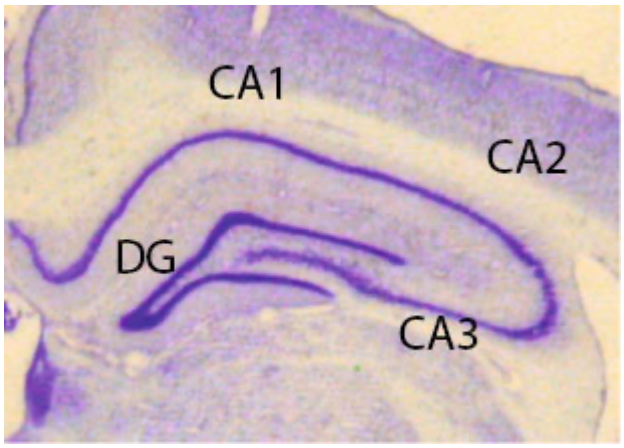


Figure 2. Hippocampus Coronal Slice.

Visualization of the hippocampus and each of the subregions

The Schaffer Collateral pathway indicates that the major input into the hippocampus (perforant path) is directed into the DG (synapse 1) and then projected into the CA3 region (synapse 2). When CA1 projects back to the entorhinal cortex (synapse 3), the Schaffer Collateral pathway completes the loop.

Despite the Schaffer Collateral pathway hypothesis which claims a unidirectional pathway, recent studies have shown that the pathway is multitudinous. The entorhinal cortex not only projects into the DG but also the CA3 and CA1. The CA3 then feedbacks into the DG through excitatory cells from the dentate hilus (Knierim, 2015).

Norepinephrine and Epinephrine

The catecholamines norepinephrine (NE) and epinephrine (EP) are neurotransmitters and hormones that are released from the locus coeruleus during stressful events. This is better yet known as the body's “flight or fight” response. The synthesis of NE and EP begins with Phenylalanine which was once referred to as an unimportant step in the synthesis (Kirshner, n.d.). Phenylalanine converts into the amino acid Tyrosine through the enzymatic reaction involving Phenylalanine hydroxylase. Tyrosine to L-DOPA (enzyme Tyrosine Oxidase) and L-DOPA to Dopamine through yet another enzymatic reaction. Within the pathway, the discovery

of the rate-limiting step has been identified to be the conversion of L-DOPA to Dopamine by utilizing the enzymatic reaction of Aromatic L-amino acid Decarboxylase (*TIlE J0URN.TI. Oo Put RMAC LOGY*, n.d.). Finally, Dopamine is synthesized to NE and NE to EP through first a Dopamine B-hydroxylase and a NE N-methyl transferase (Kirshner, n.d.). The synthesis of NE and EP can be seen in Figure 3.

Despite the synthesis of EP following the synthesis pathway of NE, NE is found to have many therapeutic applications in medicine. Rather than merely utilizing it only for serotonin, NE has become a novel therapeutic agent in the treatments of anxiety and depression (Jakubovski et al., 2019).

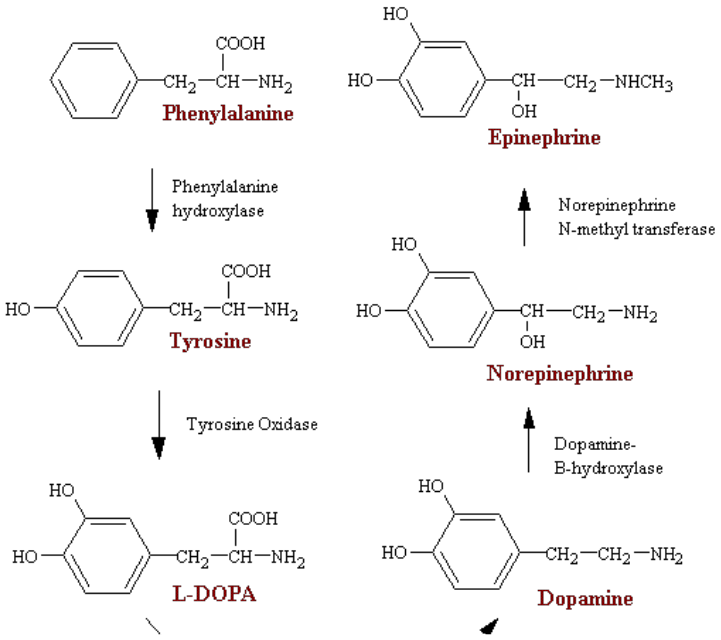


Figure 3.

Norepinephrine and Epinephrine Synthesis Pathway

Adrenergic Receptors

Adrenergic receptors (AR) are G-Coupled Protein Receptors (GPCRs) which consist of 400-500 amino acids in lengths that weave through membranes of cellular components. GPCRs are targeted by NE and EP for pathway activation. Each of these receptors contains 7 hydrophobic regions with 3 regions being intramembrane and extramembrane. Each chain has an N and C terminal, the C terminal differs in length depending on the receptor subtype. For

example, alpha2 GPCRs have a shorter C terminal than beta but the beta has a longer C terminal than alpha1 (Strosberg, 1993). For each GPCR, 3 distinct types of ARs exist such as alpha1, alpha2, and beta. Along with the types, 9 subtypes also exist which are listed in Table 2 (Piascik & Perez, 2001).

Table 2. Adrenergic Receptor Subtypes. The AR subtypes consist of 9 total with 3 coming from each of the 3 types of GPCRs.

Adrenergic Receptor Subtypes		
α1	α2	β
α1A	α2A	β1
α1B	α2B	β2
α1D	α2C	β3

There had been a common misconception about alpha1C and alpha1D prior to the current findings (Strosberg, 1993). The alpha1C was thought to be a novel receptor and was added to the alpha1A list. It wasn't until later that the alpha1C was cloned and was discovered to be the alpha1A rather than a novel alpha1C. For the alpha1D, it was determined to be the alpha1A but was found to have similarities to alpha1B, which made the receptor slightly different than the alpha1A. As such, alpha1D was discovered. The alpha1D's purpose is yet to

be analyzed. What is known about alpha1D is that it is involved in the contraction of large caliber type arteries (Piascik & Perez, 2001).

Despite the presence of adrenergic receptors on the cell membrane, alpha2C-ARs were found to have intra-and extracellular GPCRs through investigation of transfected fibroblasts whereas alpha2A receptors are only found on the extracellular surface of fibroblasts like the locations of the alpha1B (Piascik & Perez, 2001).

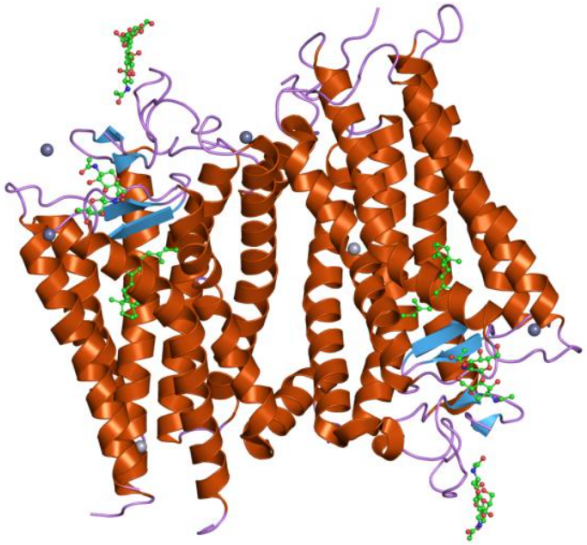


Figure 4. The Rhodopsin Family Protein Structure.

While determining the similarities of the alpha1-AR subtypes genomic code, it was found that an intron that followed the TM6 domain resembled to be a part of the rhodopsin family which has a variety of 80% GPCRs but with which the beta-ARs do not resemble. Figure 4 denotes an image of the rhodopsin family.

Norepinephrine and Neurogenesis

The effects that NE has on neurogenesis are questionable, but prior findings have indicated that NE plays a role in neurogenesis. NE acts as a neurotransmitter in the central

nervous system and the sympathetic nervous system which is responsible for the body's stress responses. Studies have shown that when the body is under stress, the amount of cortisol and NE increases, thus hindering neurogenesis with a decline in neural density (Bremner, 2006). Areas of the brain that are involved in the stress response include the amygdala, hippocampus, and prefrontal cortex. Because of the increase in NE during stress, the body's neurotransmitters become imbalanced. The dysregulation of NE becomes balanced when antidepressants are used in the treatment of traumatic stress disorders (such as PTSD).

Antidepressants are the first line of treatment used to treat disorders of depression, anxiety, and PTSD. SNRIs stimulate the regulation of NE which is found to stimulate neurogenesis at a faster rate than that of SSRIs due to the modulation of the earlier stages of neurogenesis (Park, 2019).

The Effects of Cirazoline in the Treatment of Psychiatric Disorders

Cirazoline, an alpha1A agonist is commonly used in research to study the effects of the adrenergic system in numerous studies (Datta et al., 2019; Shen & Shi, 2021). Unlike other ways to study the adrenergic system, Cirazoline's ability to cross the blood-brain barrier makes it a popular choice in adrenergic system studies. Prior studies have indicated that Cirazoline poses an increase in learning and memory and an improvement in psychiatric symptoms when is used in treatment for 3 months (Doze et al., n.d.), however, antidepressants that induce neurogenesis begin to show effects in as little as one month. The effects of neurogenesis and improvements in cognition and mood are correlated which, in 14 to 28 days, a study found that

chronic treatment with antidepressants increases BrdU-positive cells rather than acute treatments (1-5 days) (Malberg et al., 2000). Within this study, we aimed to investigate the effects of a one-month treatment of Cirazoline (an alpha1A agonist), and its effects on learning, memory, and mood. We hypothesized that mice treated with Cirazoline would improve their performance in cognitive tasks as well as in their mood when faced with the behavioral examination.

Materials and Methods

Animals

Male and female C57BL/6 mice were purchased through The Jackson Laboratory at 16 weeks old and were received by the University of North Dakota's (UND) Center for Behavioral Research (CBR) core. All animals were provided standard veterinary care and received food and water ad lib. Standard acidified water was provided by the UND CBR staff. Mice were housed socially and with nestling.

Cirazoline

Mice were transferred from CBR to UND's behavioral core holding room where they were allowed to habituate for 1 week on a 12-hour light/dark cycle (06:00-06:00). Mice were randomly assigned to a treatment group that received either 40 µM (10mg/L) of Cirazoline (Fisher Scientific) or the standard water in their water bottles. Cirazoline was dissolved into acidic mouse water and administered ad lib. through the mice's water bottles for both 2 and 4

weeks. Cirazoline treatment continued until the final behavioral experiment was concluded to avoid adverse withdrawal symptoms.

Behavioral Testing

Following both a 2-week and 4-week treatment, behavioral testing was done which included the Elevated Zero (EZM) and Elevated Plus maze (EPM), Tail Suspension (TST), Forced Swim (FST), the Marble Burying Test (MBT), and Morris Maze (MWM). Each of the previous tests was used on select treatment groups. Mice who underwent 2-weeks of treatment were subjected to the EZM, EPM, FST, TST, and MBT. After 4-weeks of treatment, an additional cohort was run through the EZM, EPM, TST, FST, and MBT. Additionally, mice underwent the MWM in the 4-week treatment group.

Elevated Zero Maze.

Utilized to test for anxiety, the EZM is a modified version of the EPM that is elevated off the ground (Kulkarni et al., 2007). The apparatus is 61 cm in diameter, with a height of 50 cm. Each mouse was placed in the maze facing the open arms. Mice were allowed to explore the maze for 5 minutes and AnyMaze was utilized to track the mice's position. Front paws in the open area were counted as entries and time in the open.

Elevated Plus Maze.

The EPM apparatus used for this test has the shape of a cross (see figure 5) and has two open arms (25 x 5 x 0.5 cm) across each other and perpendicular to this are two closed arms (25

x 5 x 16 cm) (A. A. Braun et al., 2010; Walf & Frye, 2007). There is a central platform in the middle of these arms which is half exposed to the open area and the closed area. Mice were allowed to move around the maze for 5 minutes. The distance traveled, number of entries into each arm, the time spent in each arm, and the percentage of entries into the open arms was calculated.

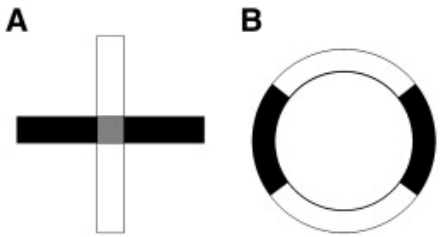


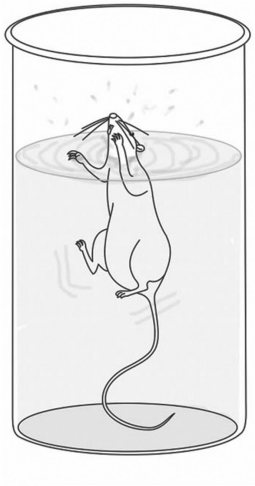
Figure 5. **Anxiety Test Apparatuses.** 5A) The elevated plus maze apparatus. 5B) The elevated zero maze.

The apparatus for the EPM can be found in figure 5A whereas the EZM can be found in figure 5B. Each of the black areas is known as the "enclosed zone" and the white areas are the "open areas." In EPM, the grey zone indicates the area which is partially enclosed and open.

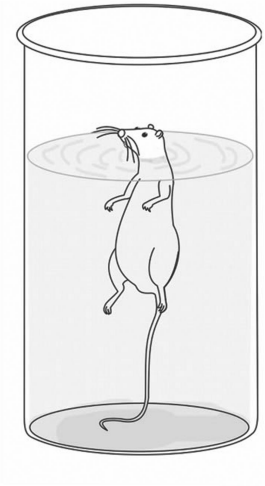
Tail Suspension Test.

Utilizing Bioseb's TST apparatus, mice are allowed to acclimate in their testing room for 1 hour before the TST was performed. A camera was used to monitor any movement, while the Bioseb apparatus measured time immobile as well, energy, and the quantification of power exertion. The apparatus contained 3 compartments (figure 6) which each contain a hook.

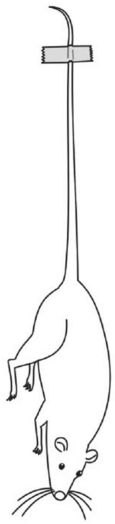
Scotch tape was wrapped around the mice's tail (2-3 mm short of the tail base) and each mouse was hung by the hook. Time began once mice were suspended for a total of 6 minutes (Can et al., n.d.).



Mobility



Immobility



Immobility

Mobility

Figure 6. **Forced Swim and Tail Suspension.** Displayed are the FST (above) and the TST (below).

Mice were counted as either mobile, or immobile depending on their responses to the stimuli presented (water vs hanging). Each test consisted of 6-minute testing.

Forced Swim Test.

Mice were placed in an inescapable transparent tank (30 cm height x 20 cm diameter) that was filled with room temperature water (15 cm). Like figure 6 each mouse was placed within the cylindrical tank with the mice's mobility being measured for 6 minutes (Can et al., n.d.).

Marble Burying Test.

A modified version of the Marble Burying Test (MBT), utilized to measure symptoms of OCD, was used which did not include nestlet shredding investigation (Angoa-Pérez et al., 2013). In the marble burying test, 20 marbles were placed on the surface of clean bedding for 30 minutes. The number of marbles is scored blind with marbles being counted when buried at a minimum of ½ the size of the marble's diameter.

Morris Water Maze.

The MWM is a trial dependent test for learning and memory which took place over 5 days (Vorhees & Williams, 2006). 4 visual cues of a diamond, star, circle, and square were placed along the north, east, south, and west areas of the 122 cm in diameter pool of water. A submerged (1-2cm submersion) platform was placed in a specific location which was not moved

throughout the trials. Mice were required to utilize spatial cues to assess their location within the pool and find their way to the platform in order to escape the maze. Each trial consisted of placing the mice at one location of pool with their face facing the inner walls of the pool. The mice were allowed for 2 minutes to find the platform before being guided to the platform to end the trial. Powdered milk was utilized in the pool water as to allow AnyMaze to track and record the time the mice took to reach the platform. Powdered milk allowed for a decrease in reflectivity of the water and allowed for better mice tracking.

Results

2-Week Cirazoline Treatment

Mice treated for 2 weeks were subjected to the TST. Utilizing a value of N = 10/group with 4 total groups (Table 3).

Table 3. Treatment Groups. Indication of each treatment group utilized in the 2-week, tail suspension study.

Treatment	N
Male Drug	10
Male No Drug	10
Female Drug	10
Female No Drug	10

An ANOVA was utilized to analyze for any variance in the time spent immobile, energy spent, and the amount of power used over time. Immobility amongst each group was found to not be significant in finding any differences amongst the 4 treatment groups (P<0.309). Results found in figure 7 and 8.

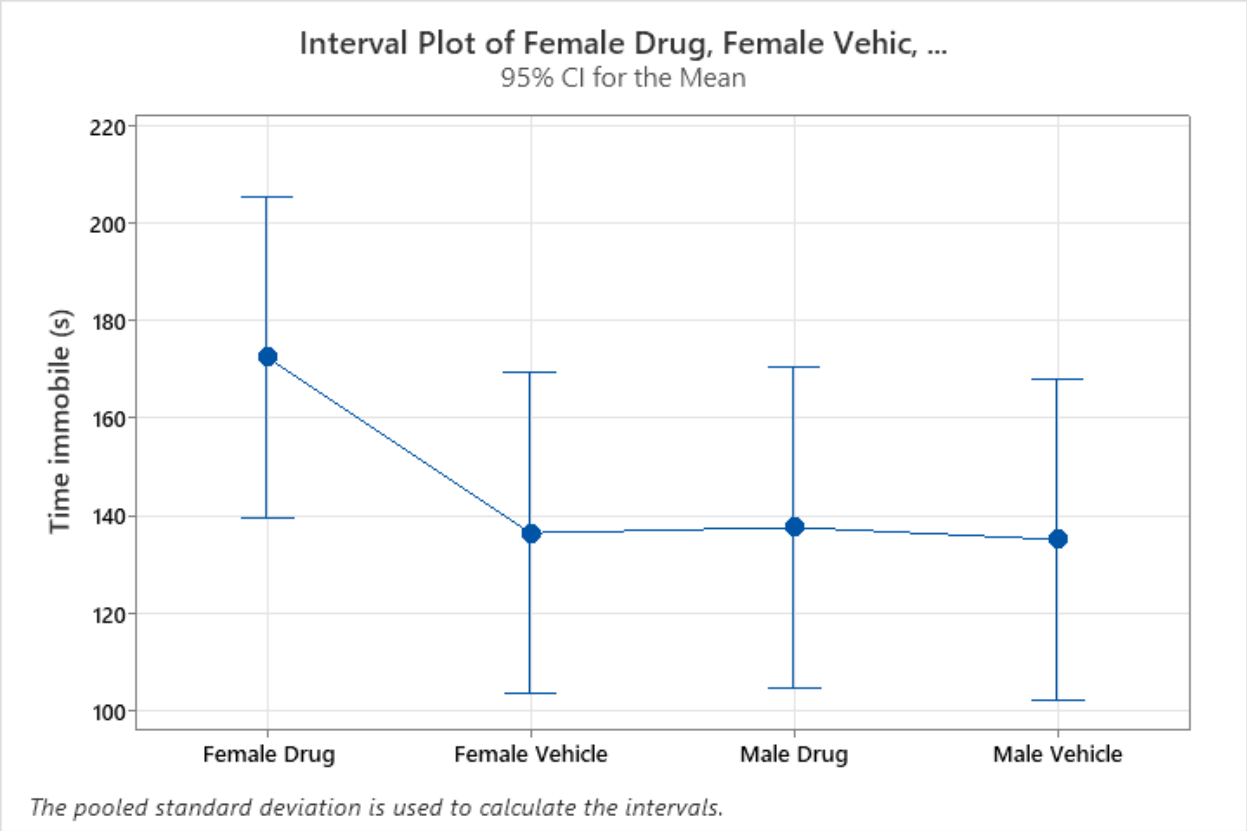


Figure 7. Tail Suspension Test Interval Plot. A 95% confidence interval plot indicating the data recorded from each treatment group during the TST test.

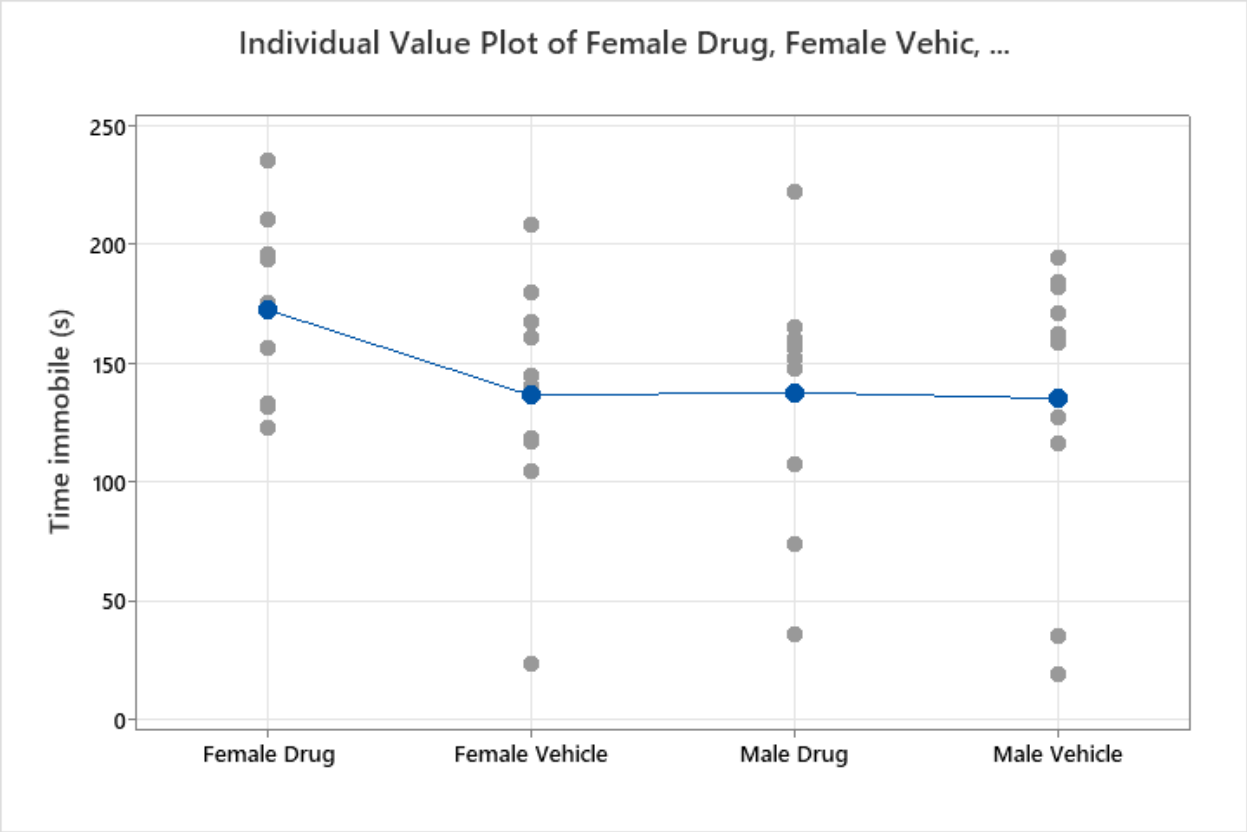


Figure 8. Tail Suspension Individual Value Plot. An individual value plot indicating the points of data recorded from each treatment group during the TST test.

Energy spent during the TST was then examined. With a $P > 0.818$, ANOVA was run to determine the significance and correlations of independent factors (treatment groups) to the

dependent variable of energy spent. The interval plots can be found in figures 7 and 8. A Games-Howell comparisons of dependent variables can be found in figure 9.

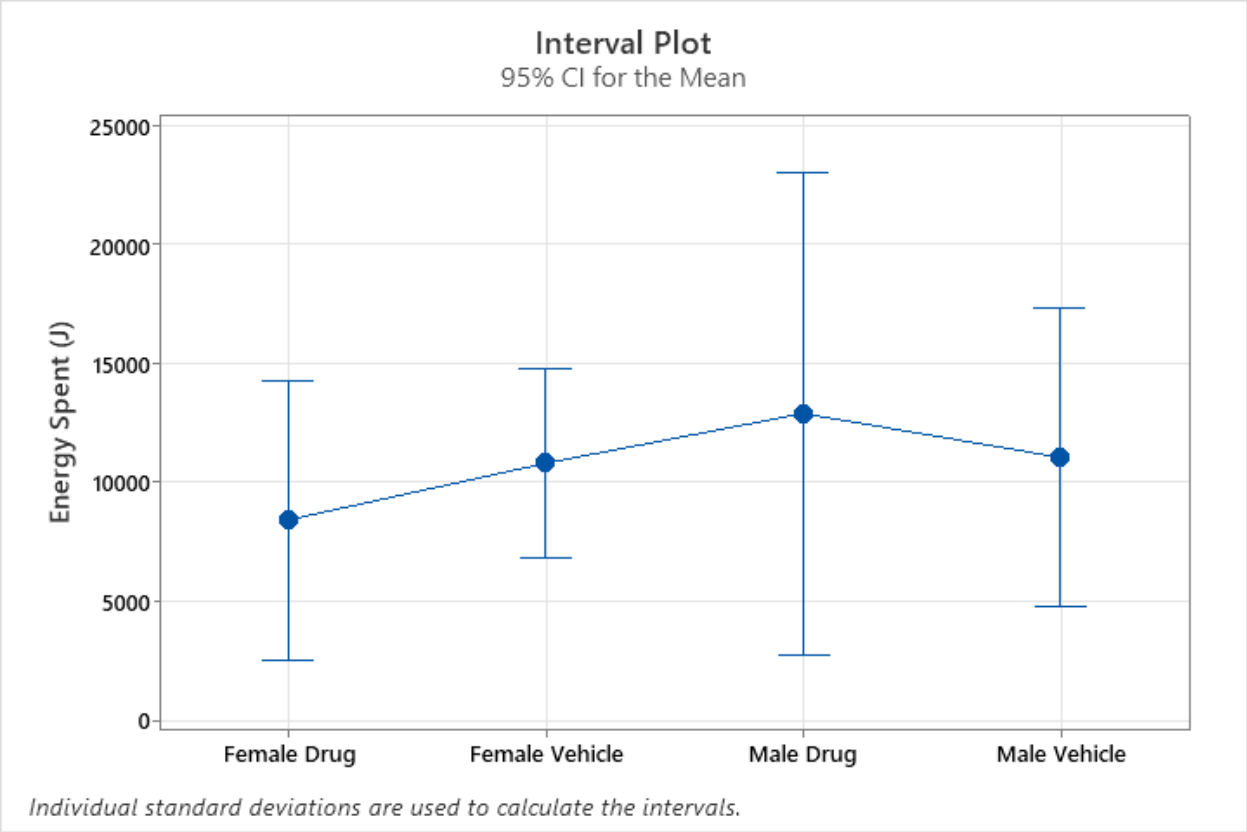


Figure 9. Energy Spent in Tail Suspension. Interval plots, including their 95% confidence intervals, are shown for energy spent within the TST apparatus.

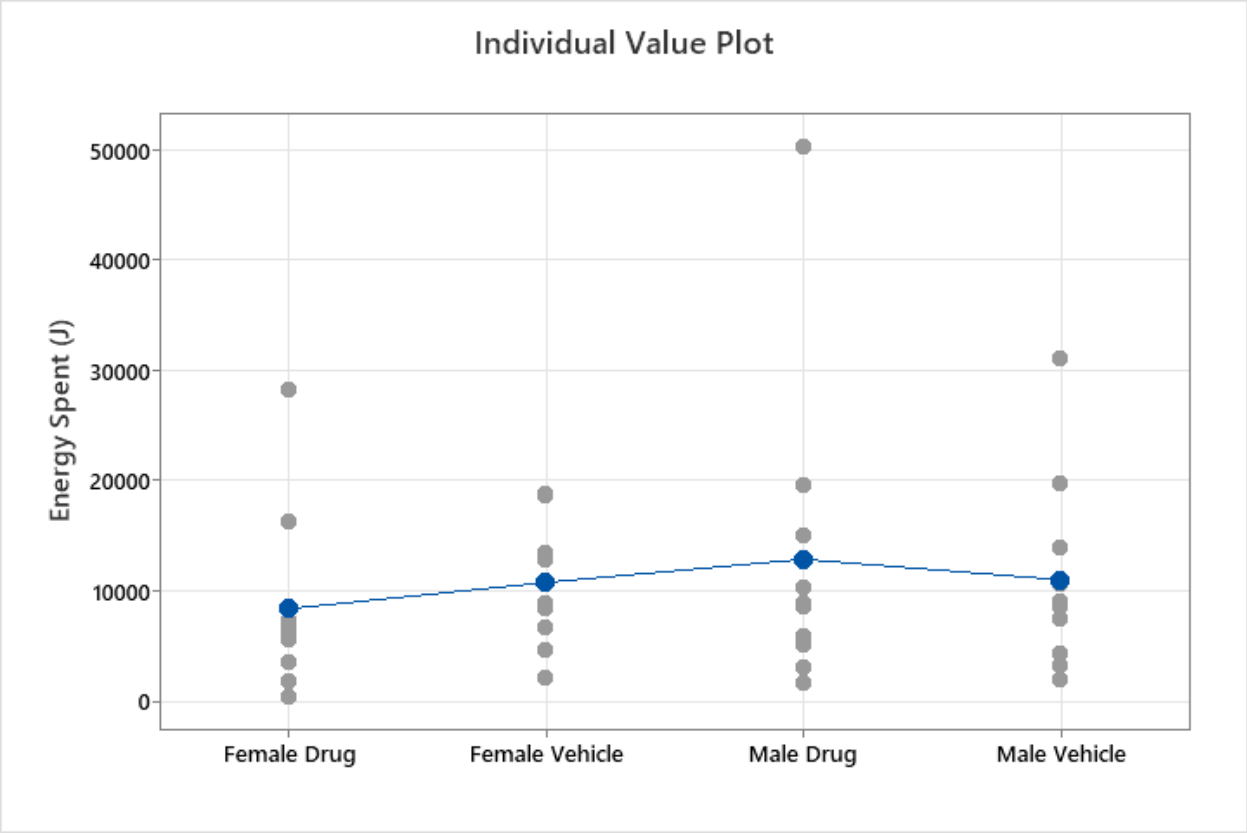


Figure 10. **Individual Value Plot of Energy Spent**. The individual interval plot displays the individual value points for energy exerted throughout the test in Joules.

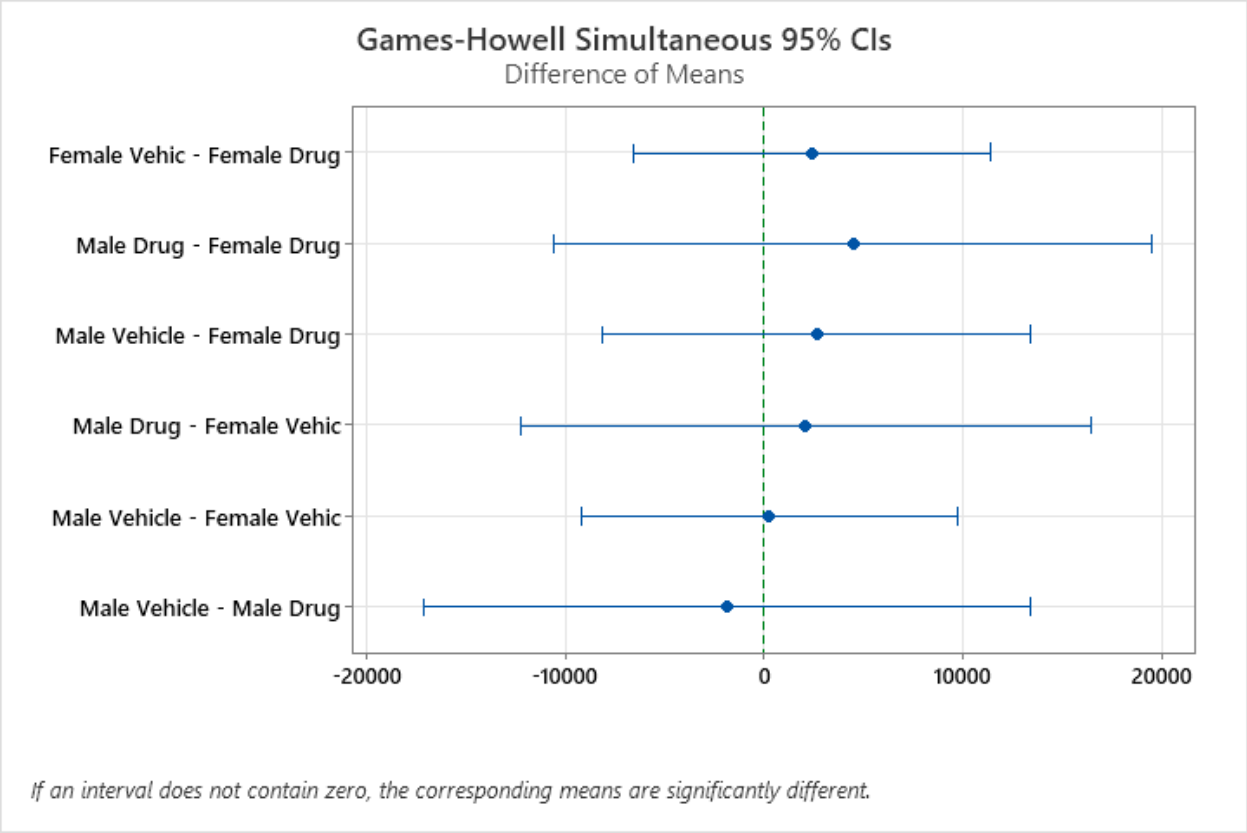


Figure 11. Games-Howell Test. The results of the Games-Howell Simultaneous test which compared the means between each of the individual factors and their associated dependent factor values.

The power was then evaluated (J/s). With a P-value of 0.916, the following interval, value, and Games-Howell test can be displayed in figures 11, 12, and 13 respectively.

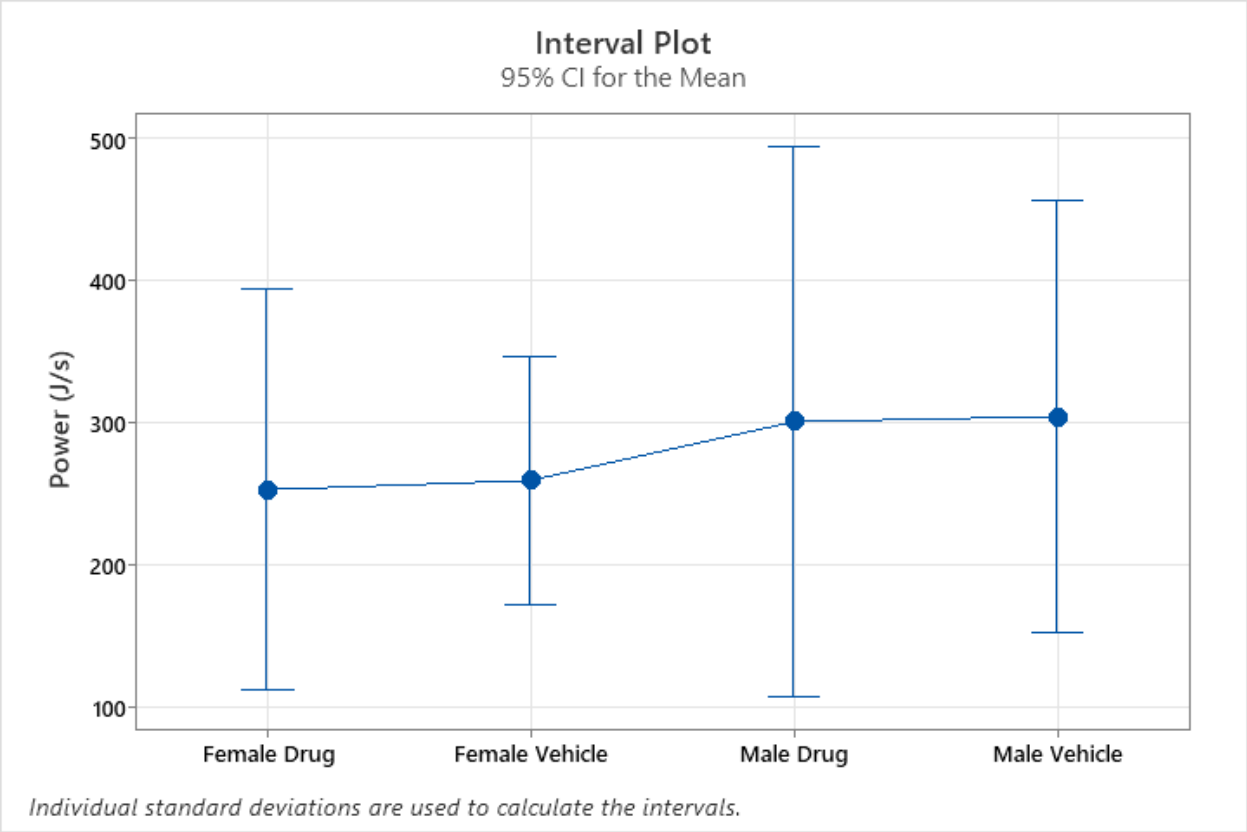


Figure 12. The Interval Plot of 2-week Cirazoline. The 2-week Cirazoline Treatment during the TST.

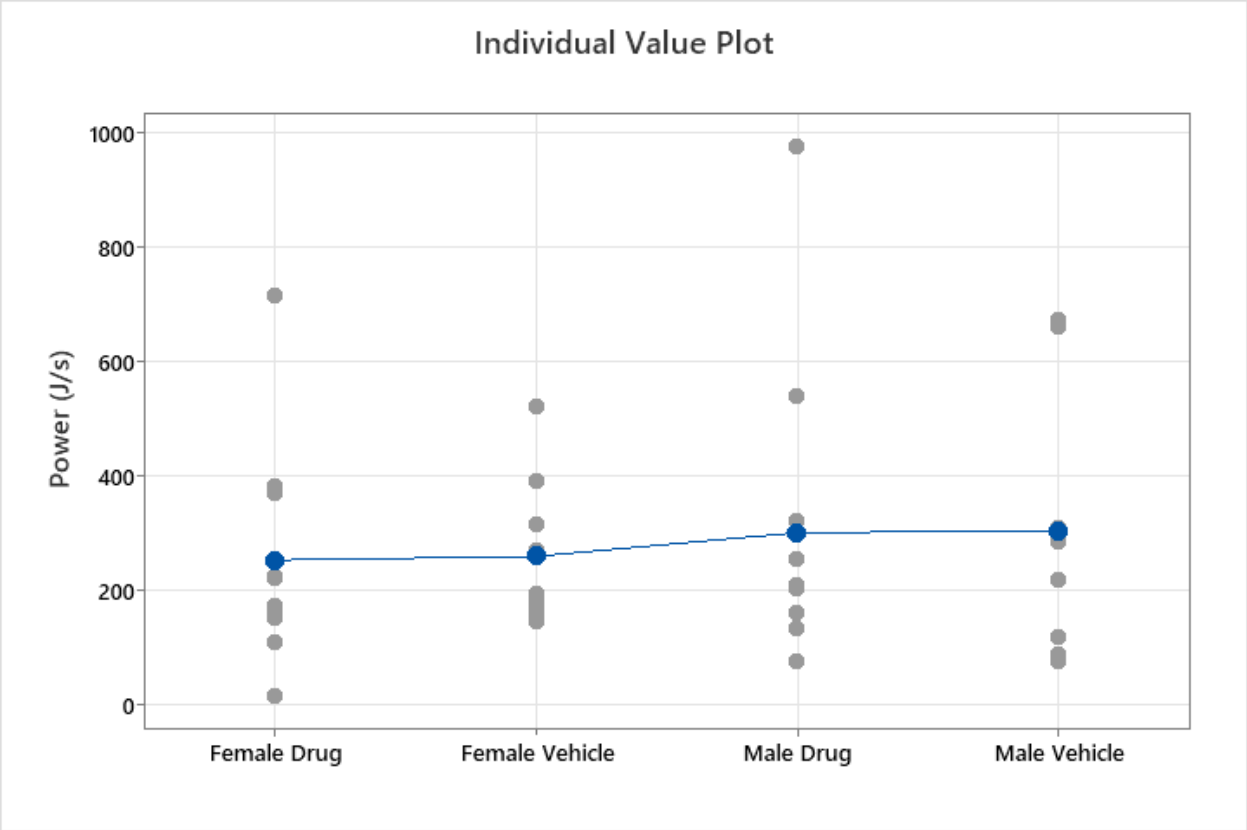


Figure 13. Value Plot of 2-week Cirazoline. Individual value plot which is associated with the 2-week Cirazoline TST test.

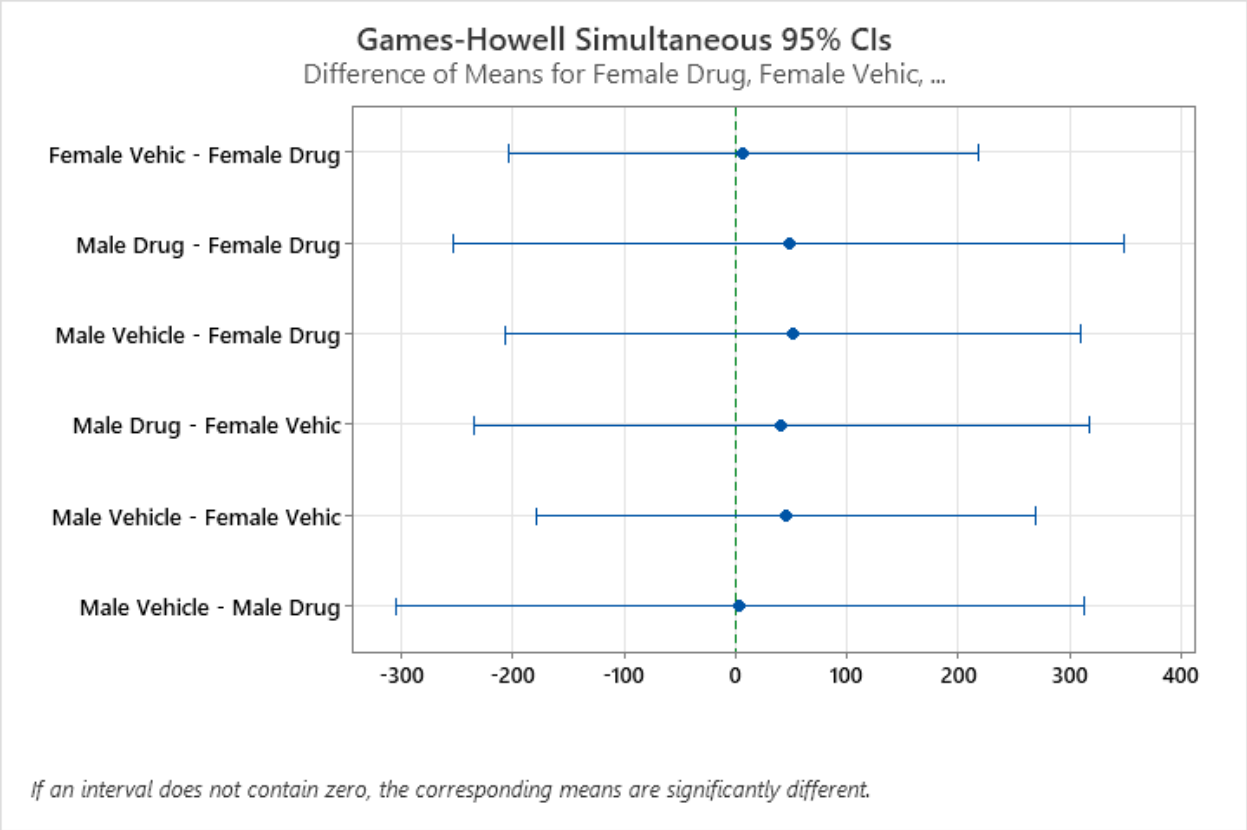


Figure 14. Games-Howell Simultaneous For 2-week Cirazoline. The Games-Howell Simultaneous comparison of means for the 2-week Cirazoline TST test.

4-Week Cirazoline Treatment

To further the 2-week Cirazoline treatment, the same treatment groups found in Figure 14 (above) were assigned from a new cohort of mice, but were treated with a 4-week treatment of cirazoline. Mice underwent the treatments of EZM, EPM, FST, MBT, TST, and the MWM. An ANOVA test was done and analyzed utilizing the software Minitab to look for any significant variance of the means in each of the mentioned tests.

The EZM was examined which, with a P-value < 0.010 through a Tukey test, individual factor for variance were examined. The following results were recorded from the EZM (Figures 15, 16, and 17).

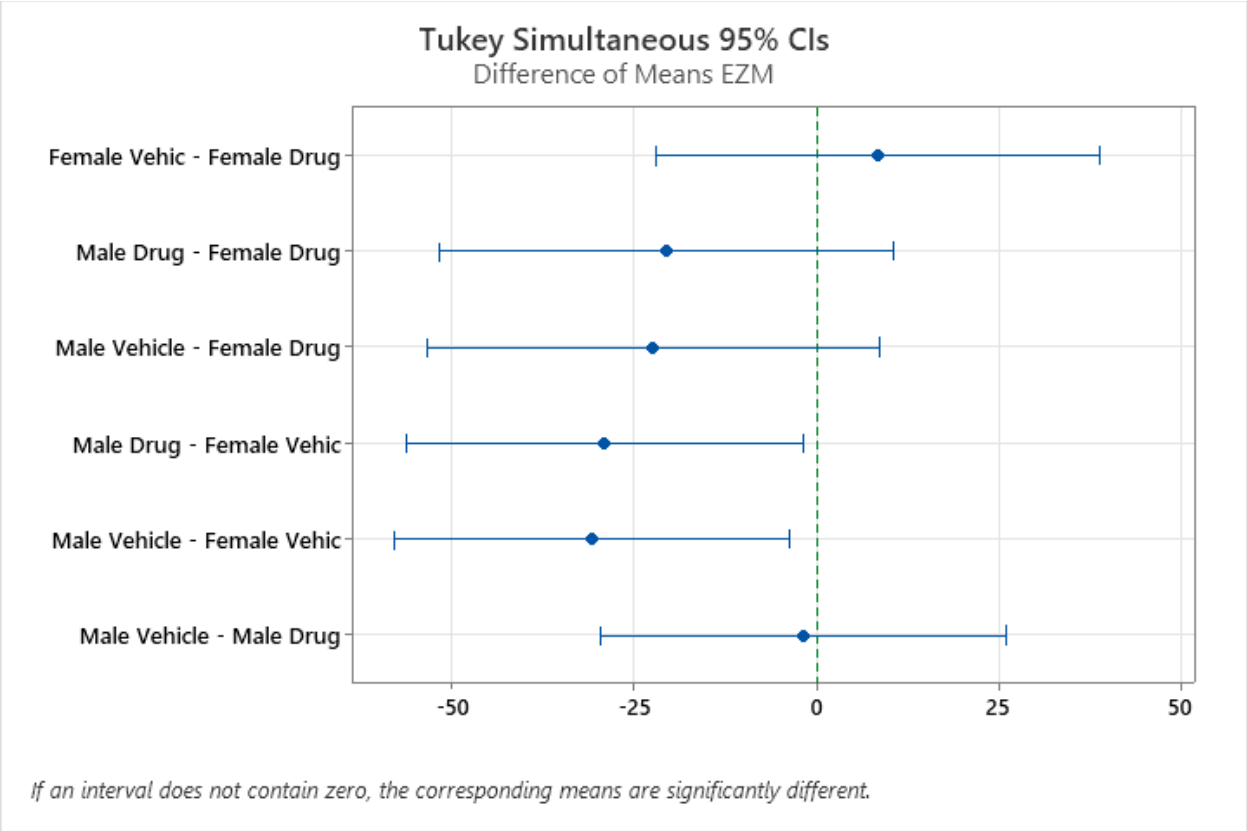


Figure 15. Tukey Simultaneous for 4-week Cirazoline in EZM. Tukey Simultaneous with a P-value < 0.05, the Tukey analysis looked for the specific significant differences amongst individual variables. Vehicle indicates no treatment.

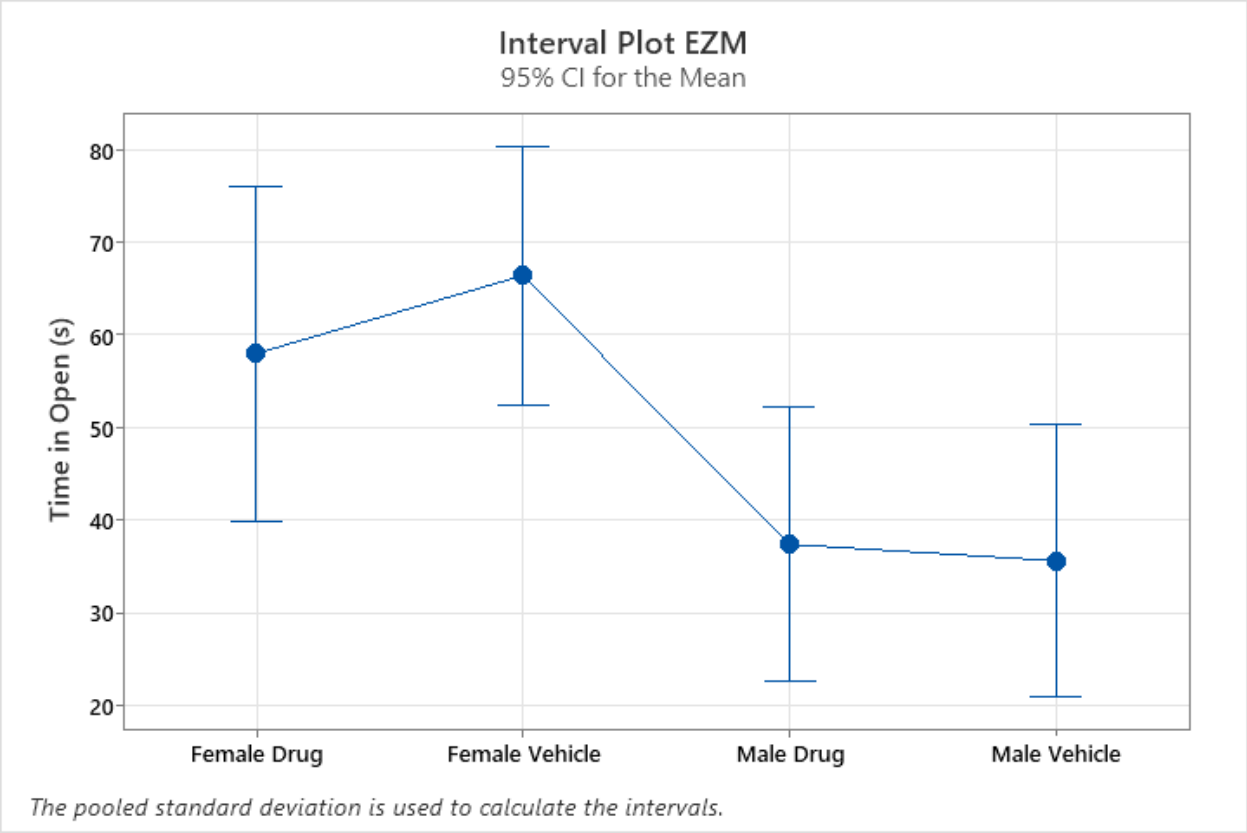


Figure 16. Interval Plot EZM Associated With the 4-week Cirazoline treatment. Vehicle indicates no treatment.

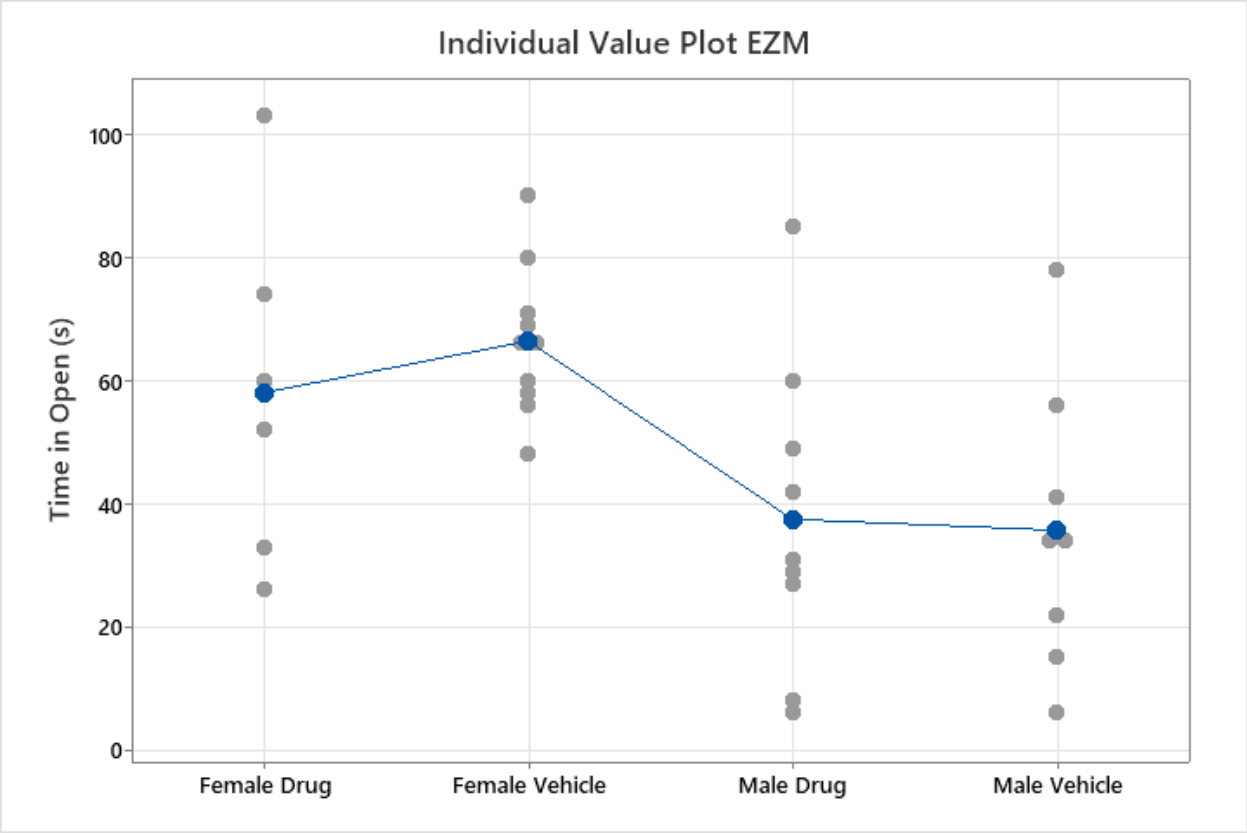


Figure 17. Individual Value Plot EZM – 4 Week Treatment. Individual Plot of EZM which are associated with the 4-week Cirazoline treatment in the EPM.

Next, the EPM was examined. An ANOVA test utilizing the statistical software, Minitab, was utilized. With P = 0.084, a Tukey and Fisher Pairwise Comparison was done to investigate the variances. Figures 18 and 19 indicate the respective analyses.

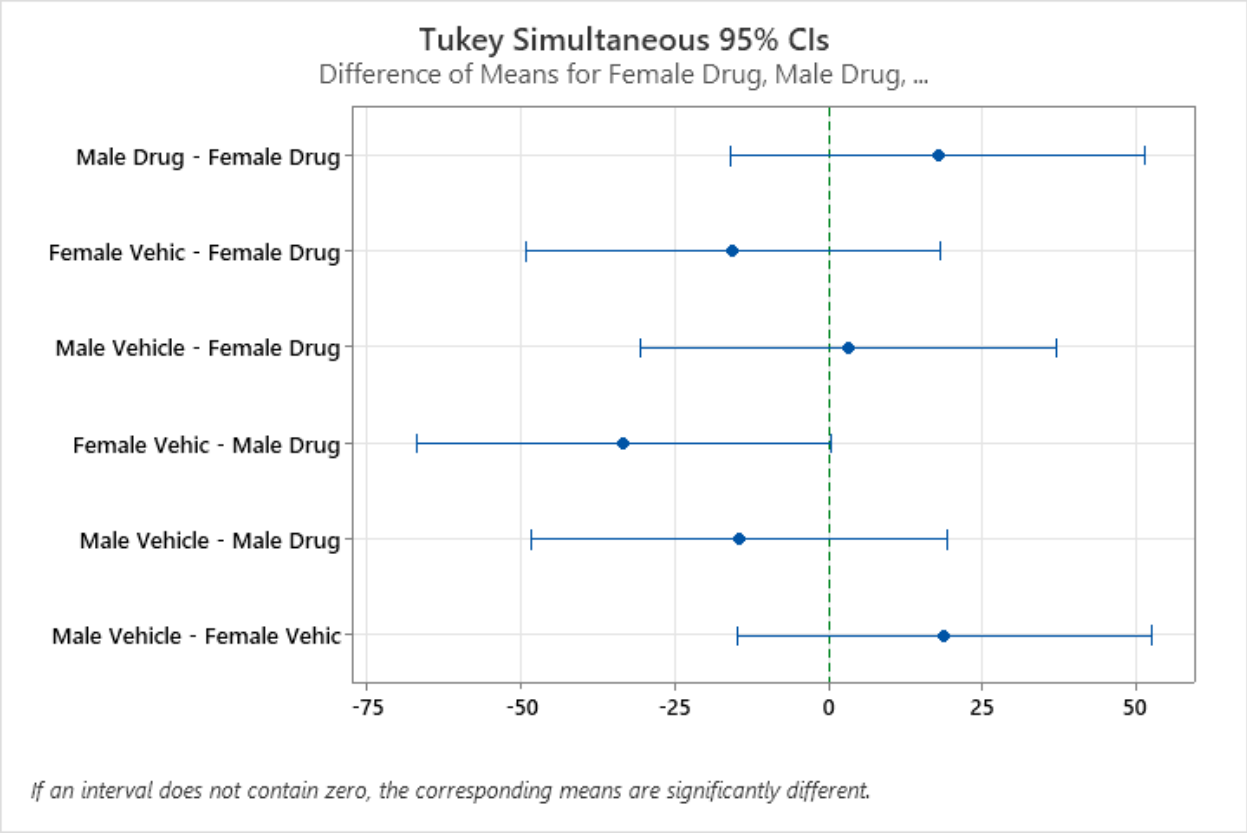


Figure 18. Tukey Simultaneous of the EPM. Done to compare the extent of differences amongst the means of the time-spent in the open.

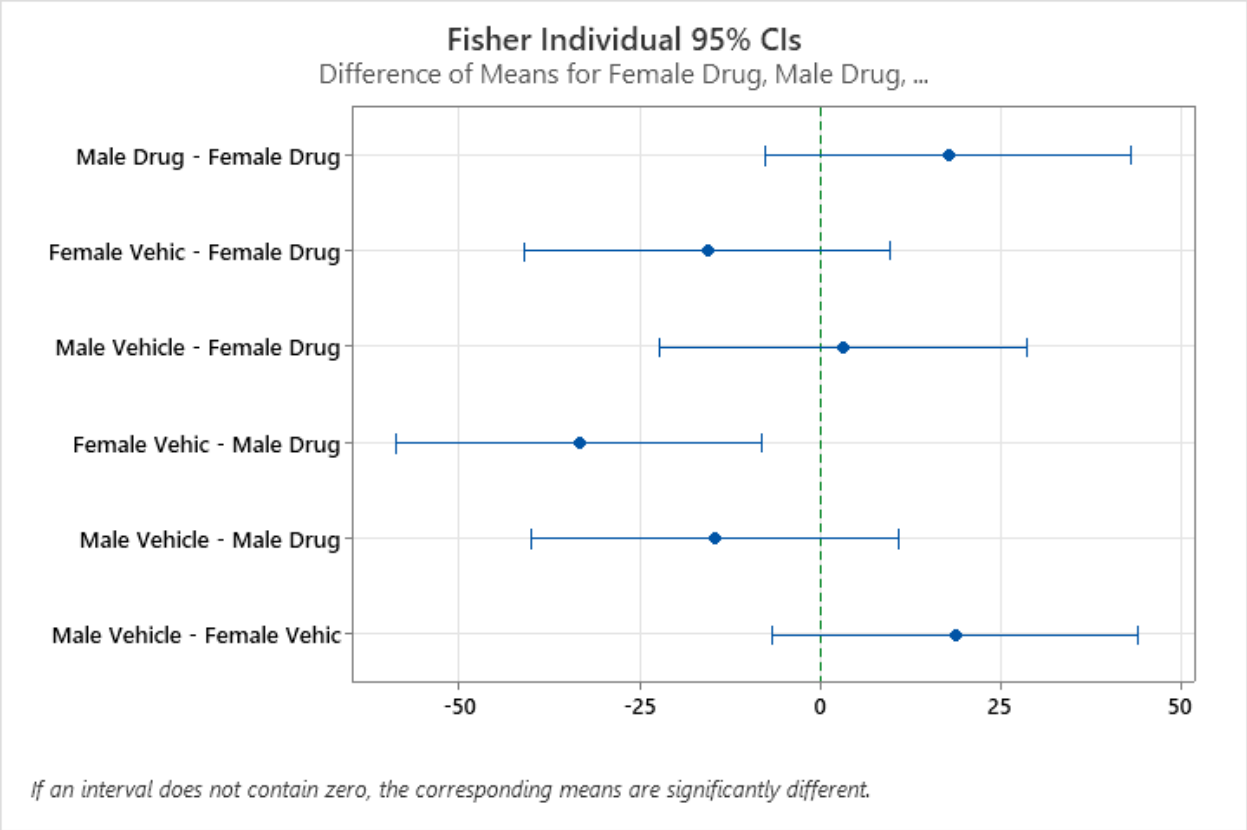


Figure 19. A Fisher Individual Analysis in 4-week EPM Cirazoline. Utilized to indicate any differences amongst the means in the EPM during the 4-week Cirazoline treatment.

The means were then examined for trends with the P-value being below 0.10 (P = 0.084). Figures of the interval plots can be found in figures 20 and 21.

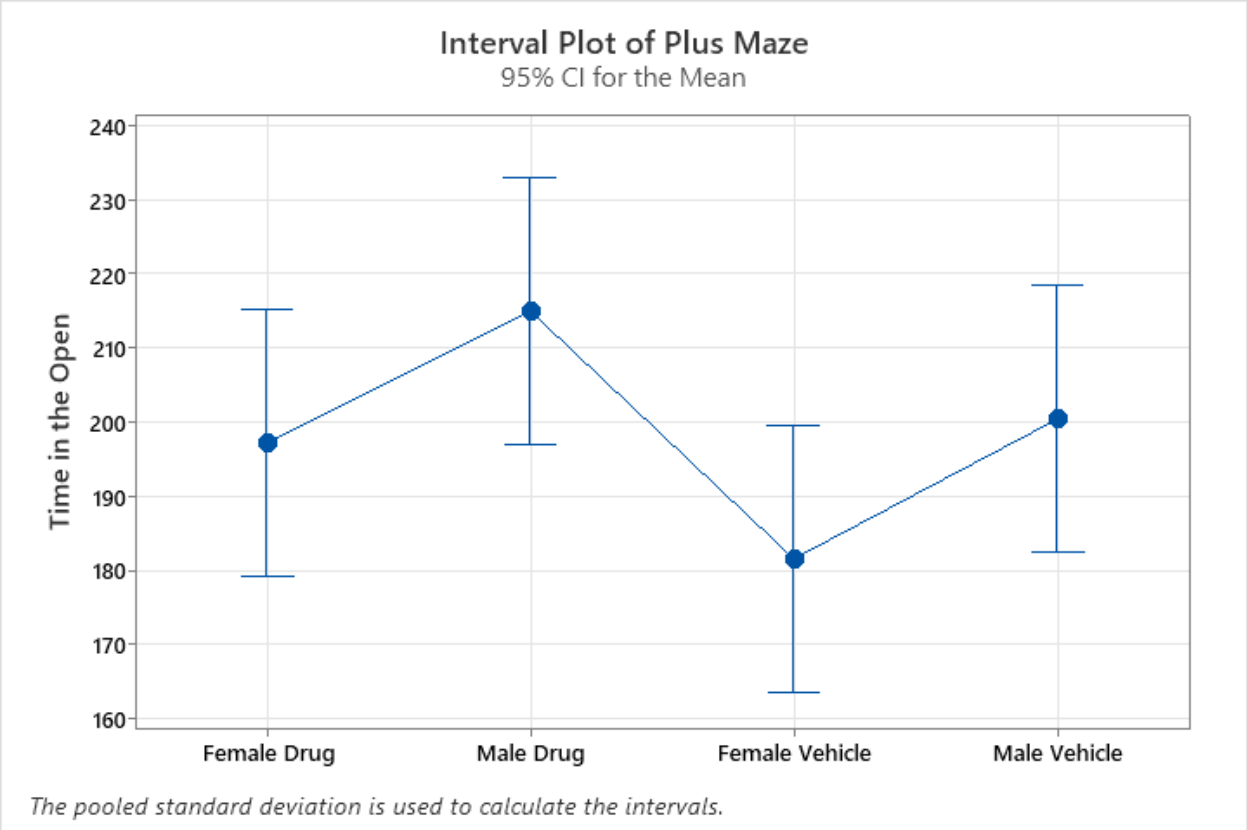


Figure 20. Visual interval plot of the EPM. Results indicate the time in the open (+/- 95% confidence interval).

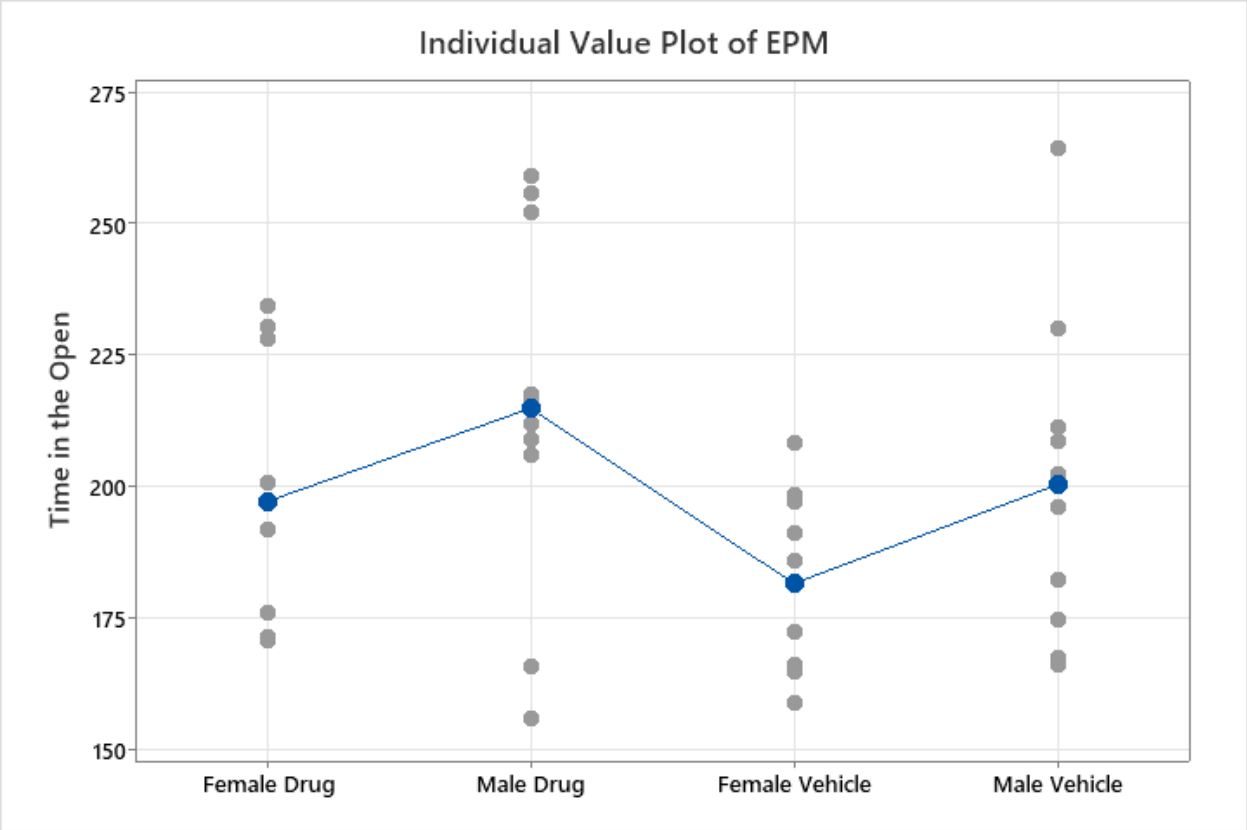


Figure 21. Individual Values of the EPM – 4 Week Cirazoline.

An individual investigation was done, clustering the treatment groups together and making the only independent factor being sex was investigated (figure 22).

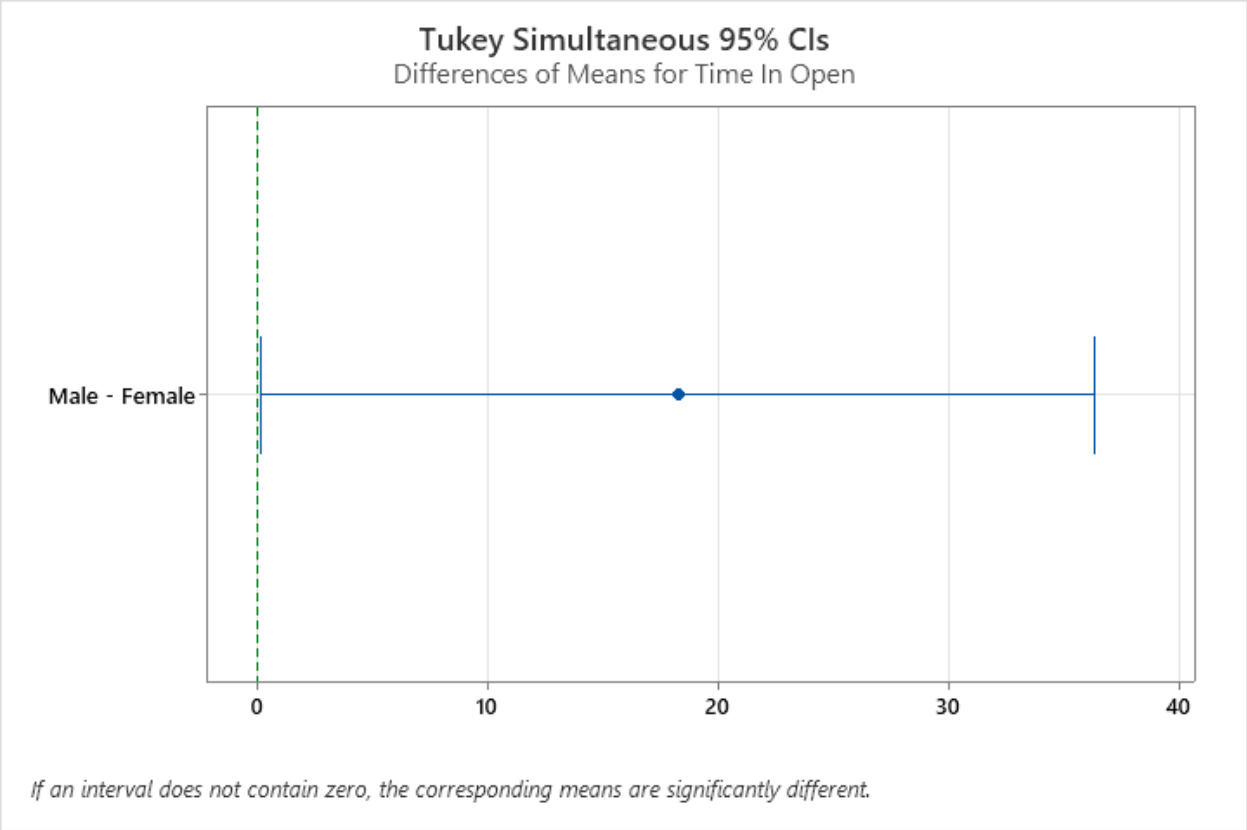


Figure 22. Tukey Analysis for Sex Differences. A Tukey Analysis for the difference between the means in the time spent in the open in the EPM. Treatment groups were neglected.

The marble burying test was then analyzed to investigate significant differences amongst the means of number of marbles buried amongst the different treatment groups. ANOVA was run (P = 0.236). Additionally, running the same analysis on the FST resulted in a P = 0.685.

Next, ANOVA through Minitab software tested for analysis of variance amongst the means of the immobility, energy and power values received during the TST. Results from

looking at the P value for variance in immobility was 0.806. The energy spent was then analyzed. A P-value of 0.004 in energy resulted in furthering a Tukey analysis to determine where the differences existed (figure 23). The Interval plots can be found in figures 24 and 25.

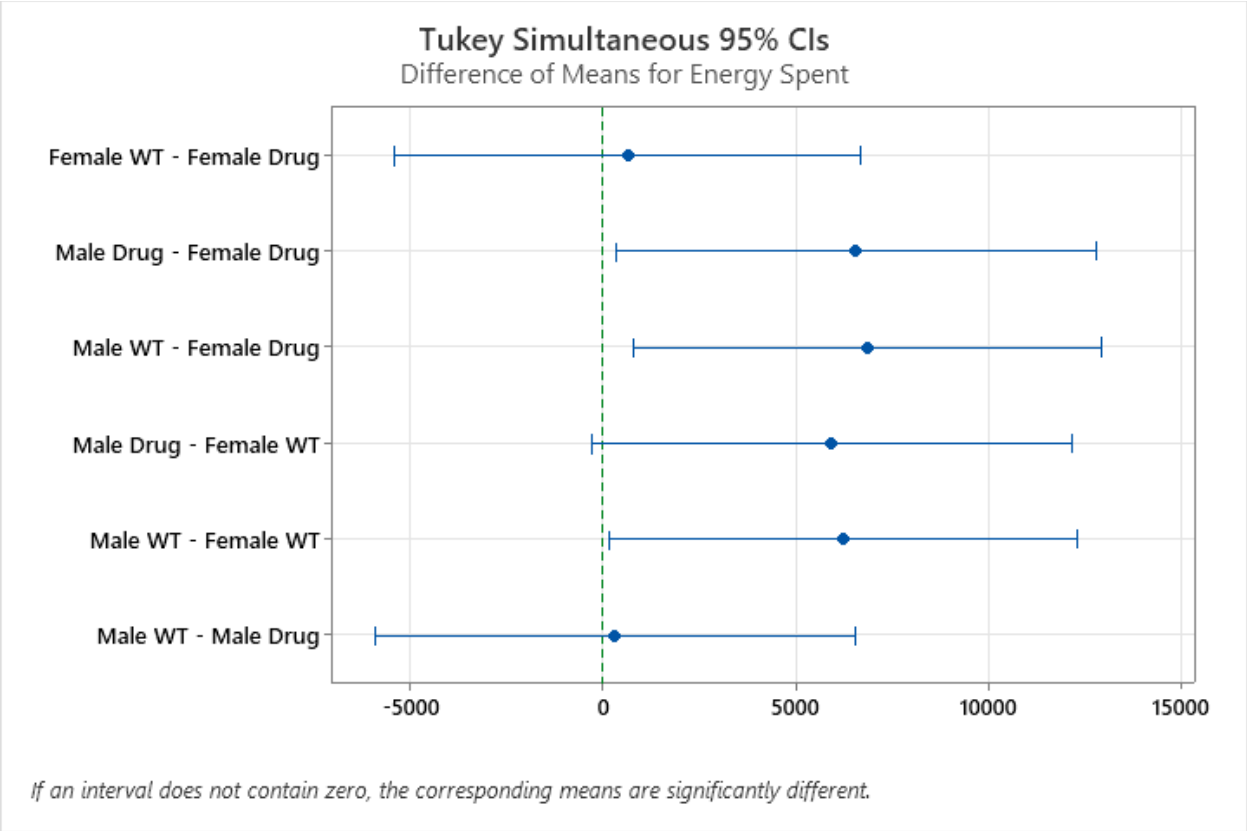


Figure 23. Tukey Simultaneous Analysis Among Treatment Groups. Utilized to investigate where the differences between the means exist amongst treatment groups.

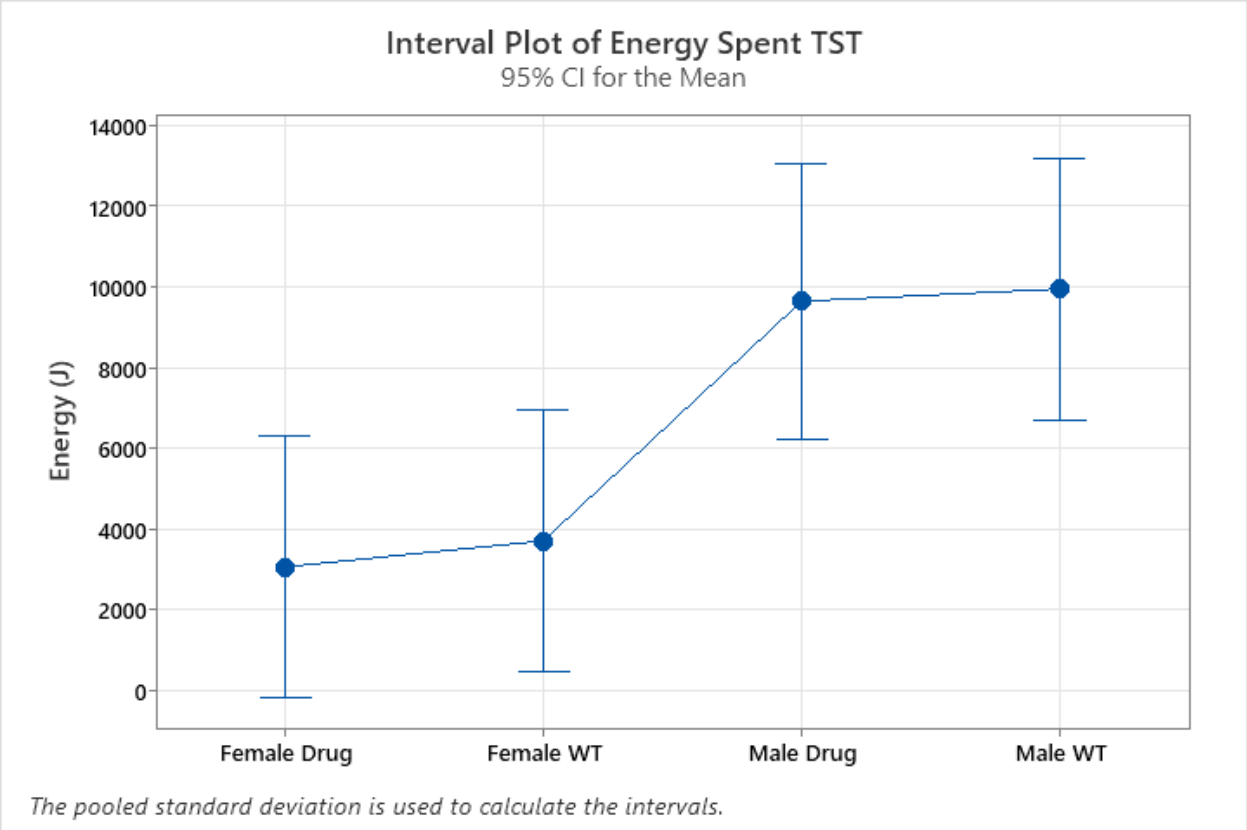


Figure 24. **The Interval Plot of Energy Spent in the TST.**

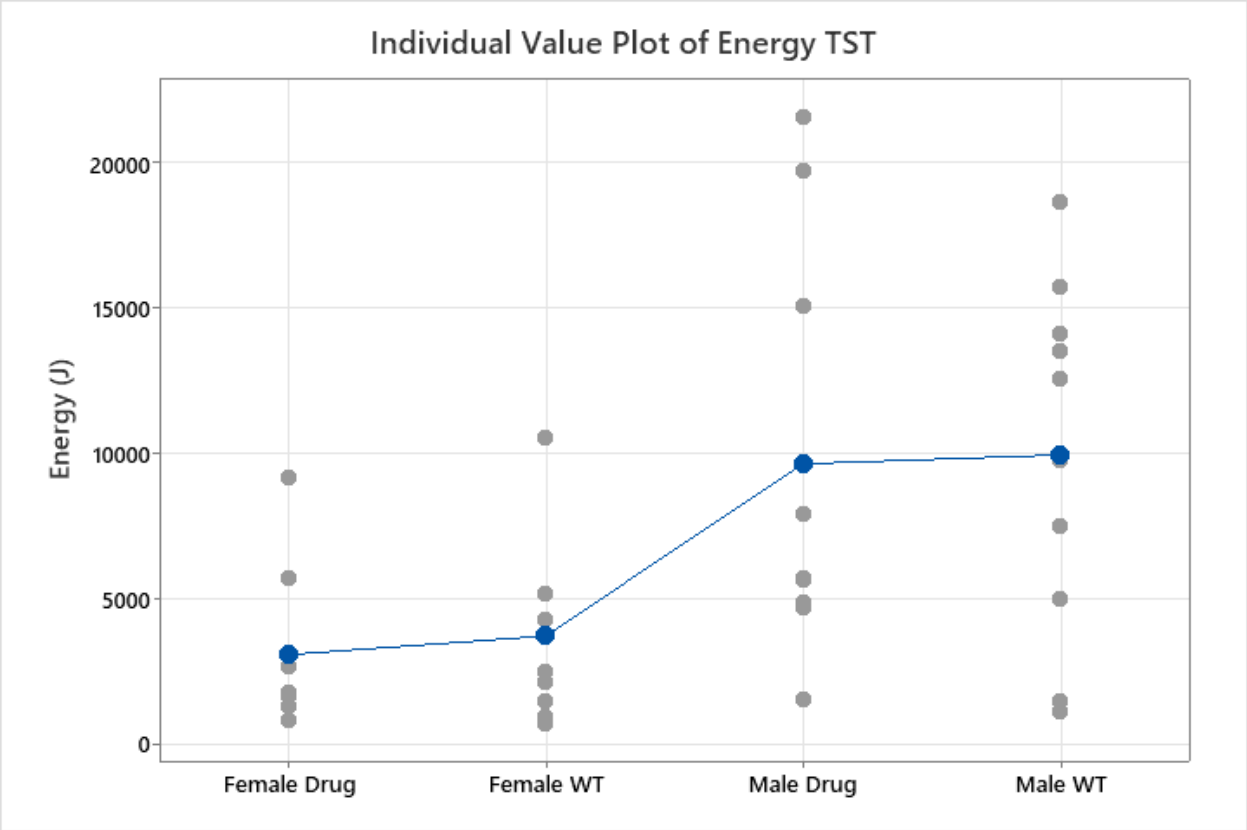


Figure 25. **Individual Value Plot for Energy Spent in the TST**.

Analysis of the amount of power spent amongst the treatment groups indicated a P value of 0.031 when ran through ANOVA. The Tukey, interval plot, and individual value plots can be found in figures 26, 27, and 28.

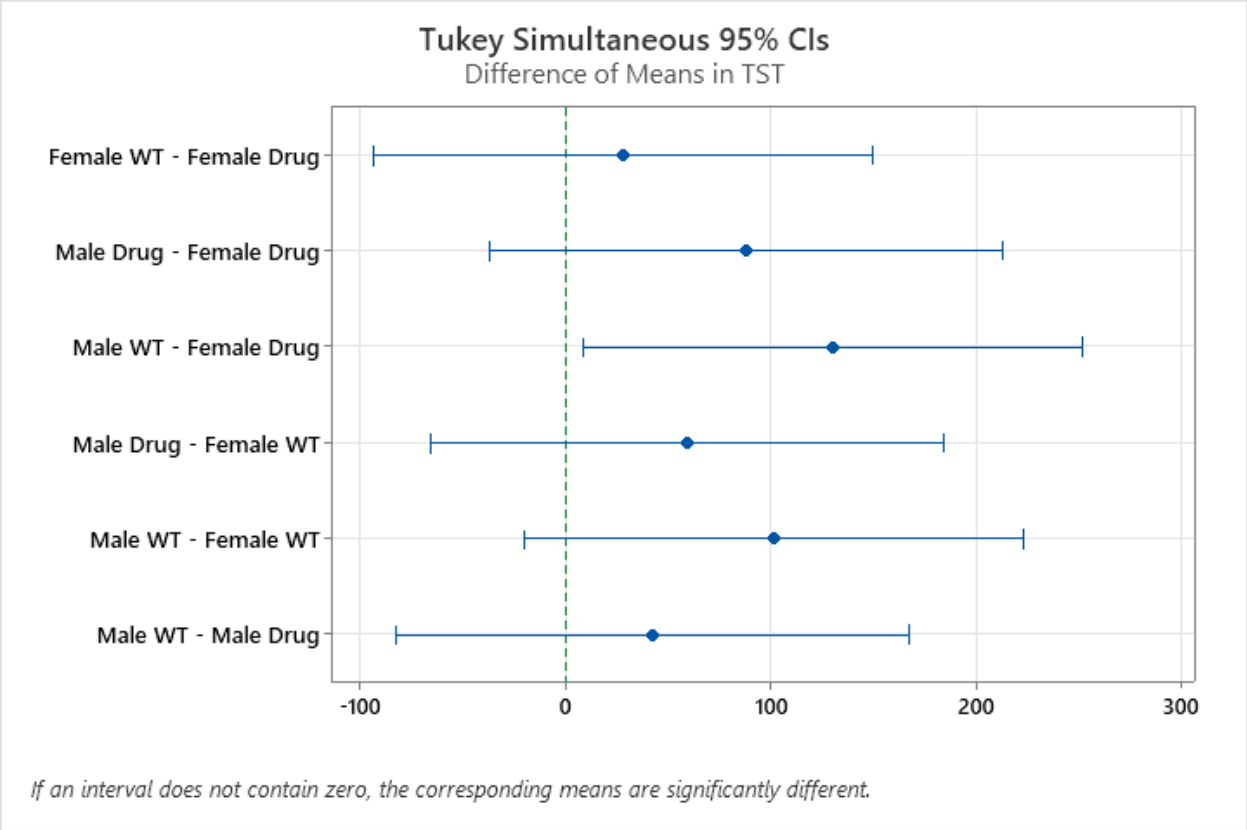


Figure 26. Tukey Simultaneous to Locate Significant Differences in TST.

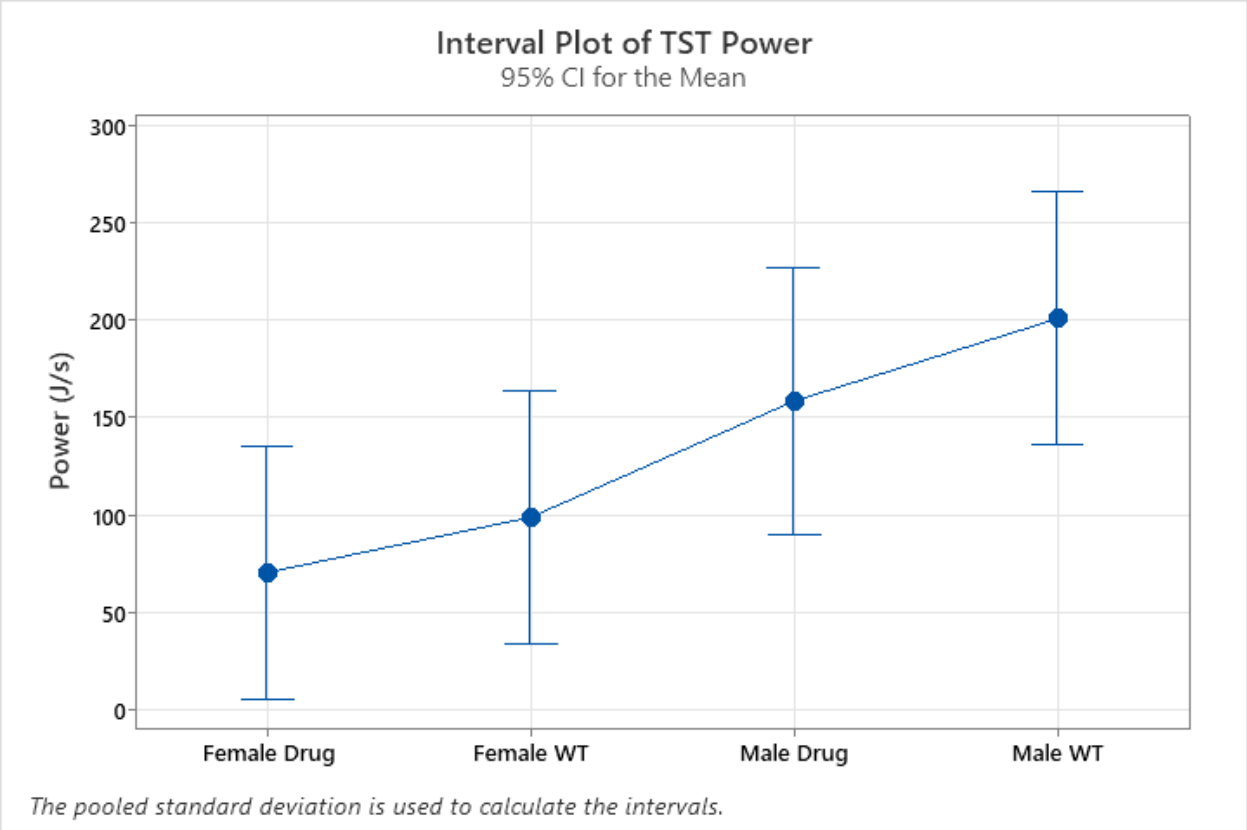


Figure 27. The interval plot of the TST. Displays a 95% confidence interval for the means of the TST measured in joules per second.

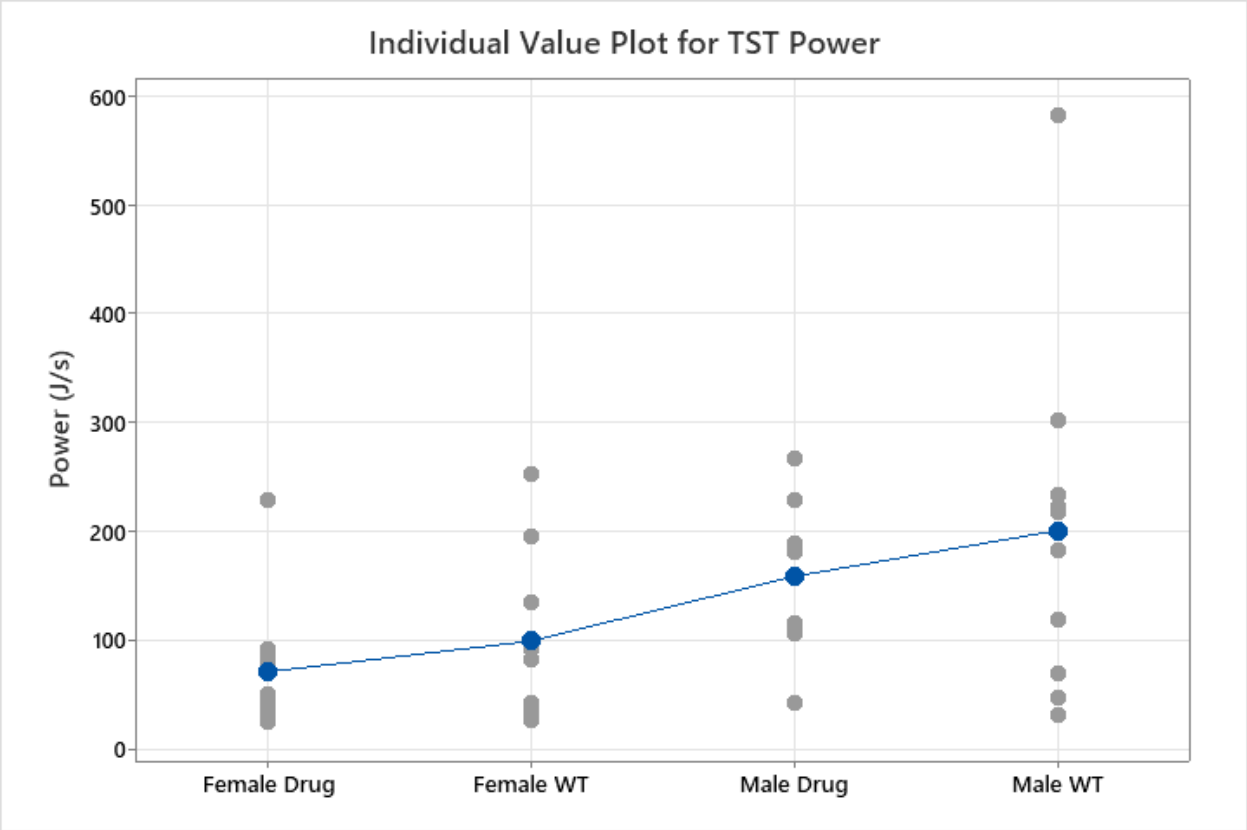


Figure 28. Individual Value Plot of Power in 4-week Cirazoline TST.

An ANOVA test was run on the additional cohort that had undergone the MWM utilizing the statistical software, Minitab. Analysis of each individual factor towards the distance traveled a P-value of 0.067 was found amongst the treated group in trial 3.

Discussion

Results from the 2-week Cirazoline Treatment did not indicate any significant differences in sex, treatment, or any improvements in psychiatric-based symptoms. This was expected, as current treatments for depression and anxiety take 2-4 weeks to work despite

being either due to 5-HT1A desensitization, or neurogenic effects (Commons & Linnros, 2019). As a result of neurogenesis within the hippocampus, being an underlying link between the pathophysiology of psychiatric disorders, a month analysis was hypothesized to induce either cognitive improvement or symptomatic improvement in vivo.

Analysis of the 4-week Cirazoline treatment indicated that sex-based differences were highly apparent. Through the Tukey analysis of the difference of the means of the TST, Male wildtype (WT) had a significant difference amongst their means in the amount of power exerted as compared to that of females on the drug. Treated female mice exerted less energy over time than male WT. Additionally, the total amount of energy exerted over the course of the experiment was also significantly different between the treated female and drug, male WT and treated females, and male WT and female WT during the TST test. This was unexpected, as current research focuses majorly on male mice whereas female mice are not tested as much due to their hormonal variability. Some studies have not found variability due to the estrous cycle (Fulenwider et al., 2019), whereas the current results indicate a difference between sex and performance. The present study did not consider the effects of the estrous cycle.

In the EZM, another significant difference in the means was evident when analyzing the difference between males and females. The results of the Tukey Test, thus ($P < 0.01$) there were significant differences amongst the means with the time spent in the open. Male WT and female WT (known as a vehicle on the graph) showed differences as well in the treated males

and the non-treated female mice. Females spent less time in the open than their male counterparts. This insinuates that female mice have a higher anxiety level than males. There is no doubt that sex plays a huge role in behavior (Bangasser & Cuarenta, 2021). Analysis that removed the treatment group indicated a sex difference amongst males (treated and none) when compared to females (treated and non). Studies have been done which correlate the function of testosterone in anxiety and depression. It is believed that testosterone has an antidepressant/anxiety effect (McHenry et al., 2014). Despite investigating the role that the adrenergic system had in attenuating psychiatric symptoms, treatment was not found to be significantly beneficial in our study. Prior findings have found that longer Cirazoline treatments and constitutively active mutants (CAM) alpha1A-Adrenergic Receptor (-AR) had lower levels of depression, anxiety, and improvements in cognition when ran through similar behavioral tasks (Doze et al., 2009).

No other differences were found other than sex differences among the tasks. Thus, indicating that females and males should be treated differently when investigated.

Induction of Depressive/Anxious Behavior in Mice Through 24-Hour Restraint

Within the lab, prior studies and data obtained through studies done on the effect of alpha1A-AR on psychiatric symptoms within the laboratory were gathered without inducing a depressive or anxious phenotype prior to treatment. Alpha1A-CAM and Cirazoline treated mice were never exposed to represent the disorder in which were being treated. Literature searches

(Pubmed 2010-2022) which include behavioral research on depression and anxiety do not utilize psychiatric models before treatment. Rather, treatment is done prior to the behavioral testing.

With stress being an important factor in psychiatric disorders, animal models were investigated to mimic depressive/anxious behavior (Campos et al., 2013; Chu et al., 2016; Hao et al., n.d.; Harro, n.d.). By choosing the 24-hour restraint model, a one-day restraint was chosen to induce a long-term depressive/anxious phenotype with minimal time required. Treatment should begin following the restraint for the ease of experimentation.

Materials and Methods

Animals

Male and female C57BL/6 mice were purchased through The Jackson Laboratory at 16 weeks old and were received by the University of North Dakota's (UND) Center for Behavioral Research (CBR) core. All animals were provided standard veterinary care and received food and water ad lib. Standard acidified water was provided by the UND CBR staff. Mice were housed socially and with nestling.

24-Hour Restraint

Mice were transferred from CBR to UND's Behavioral Core where mice were allowed to acclimate for 1 hour prior to either being restrained or not (control). From 10:00 am-10:00 am, mice were restrained in darkness in a cylindrical vehicle with adjustable restraint

measurements suitable for each mouse's size. Mice were unable to move their head and forelimbs and were deprived of food and water for 24-hours. Mice were able to slightly move their hindlimbs but were unable to turn around during the experiment. Figure 29 implicates the apparatus used in the referenced protocol (Chu et al., 2016). Mice were returned to their home cages following the restraint.

Figure 29. Referenced 24-hour Restraint Protocol's Apparatus for mice Restraint.

Behavioral Tests

Following the restraint, mice were allowed to rehabilitate in their home cages for 3 days. Following the 3rd day, both non-restrained and restrained mice underwent the TST and FST to investigate the acute depressive phenotype.

Forced Swim Test.

Restrained and unrestrained mice were placed in a transparent tank (30 cm height x 20 cm diameter) that was filled with water (15 cm) at room temperature. Each mouse was placed within the cylindrical tank with the mice's mobility being measured for 6 minutes (Can, Dao, Arad, et al., n.d.).

Restrained and unrestrained mice were placed in a transparent tank (30 cm height x 20 cm diameter) that was filled with water (15 cm) at room temperature. Each mouse was placed within the cylindrical tank with the mice's mobility being measured for 6 minutes (Can et al., n.d.).

Tail Suspension Test.

Bioseb's TST apparatus was used. All mice (control and restrained) were allowed to acclimate to their testing room for 1 hour before the TST was performed. A camera was used to monitor any movement, while the Bioseb apparatus measured the time they were immobile along with energy, and the quantification of power exertion. The apparatus contained 3 compartments where each containing a hook. Scotch tape was wrapped around the mice's tail (2-3 mm short of the tail base) and each mouse was hung by the hook. The clock was started once mice were suspended for a total of 6 minutes.

Results

The TST and FST were done to measure the amount of time the mice were immobile. An ANOVA was done utilizing Minitab software to test for significant differences between the means of the restrained and unrestrained mice. The FST revealed a P-value of 0.345. Analyzing the TST revealed a P-value of 0.013; the interval and individual value plots can be seen in figures 30 and 31, respectively.

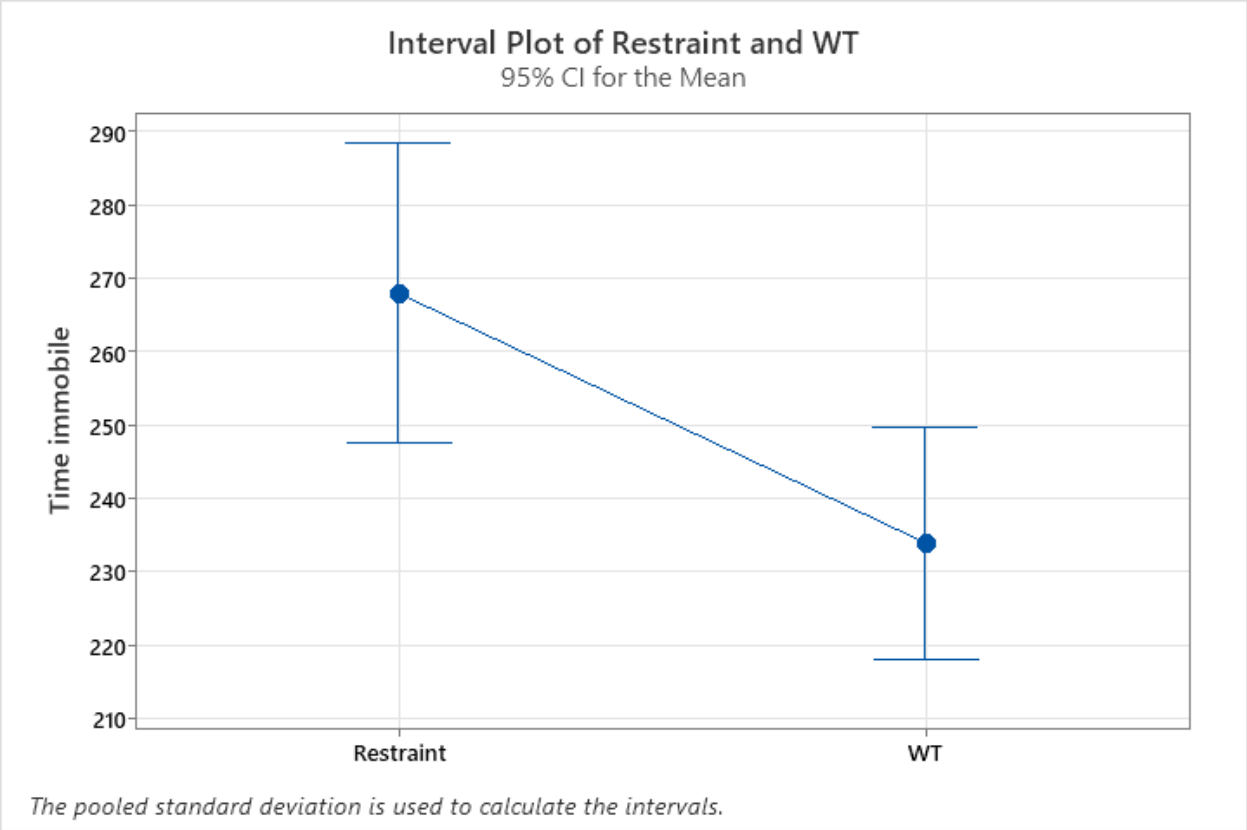


Figure 30. The Interval Plot of Restraint and WT.

The interval plot associated with the time spent immobile of the restrained mice when compared to the non-restrained (WT) mice (P = 0.013).

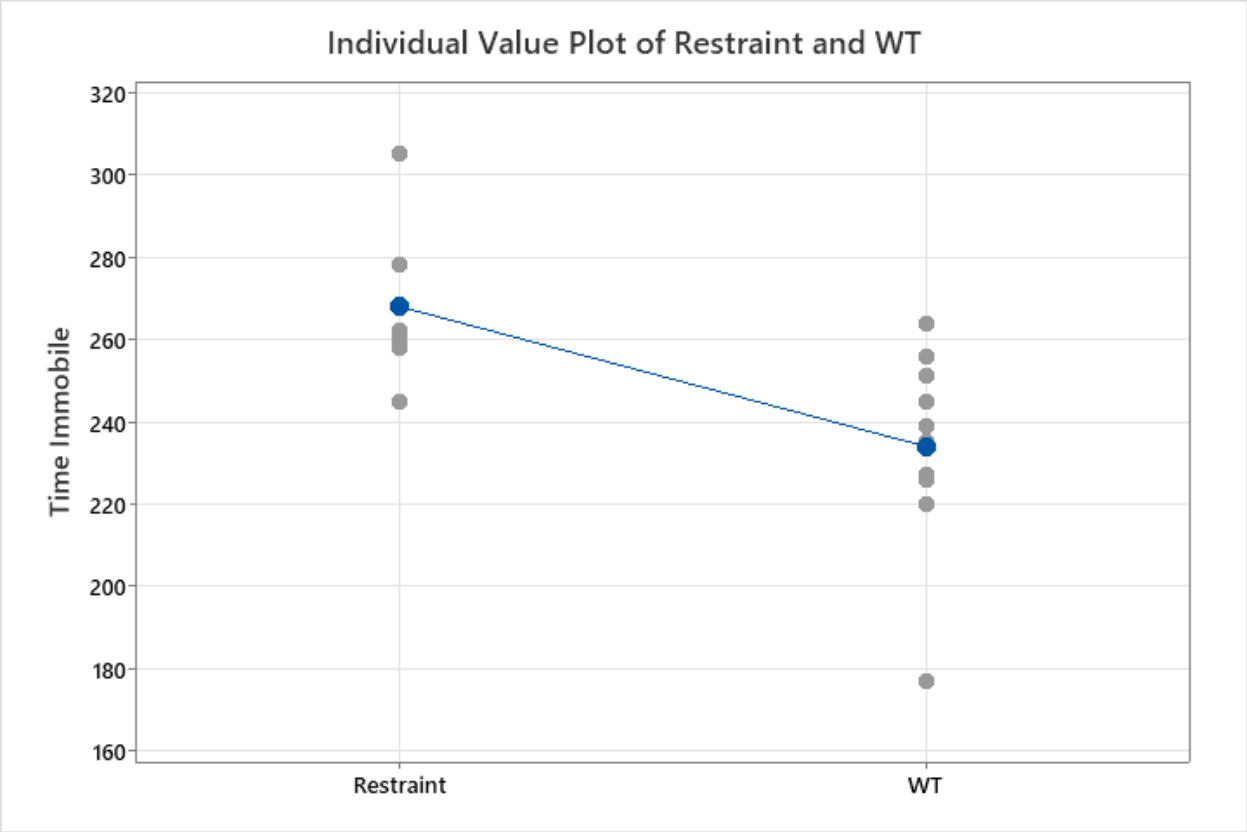


Figure 31. Individual Values of the Restrained Mice and the Non-restrained Mice.

Discussion

The 24-hour restraint model indicated a significant difference, and we can conclude with 99.99% confidence that the restraint induced depressive phenotypic symptoms of learned

helplessness (Vollmayr & Gass, n.d.). The learned helplessness model offers a key distinction in the symptoms of depression and anxiety. Those that are exposed to stress have a greater chance of developing psychiatric symptoms as increased stress increases the likelihood of illness emergence.

In this experiment, mice were exposed to a 24-hour restraint while being deprived of food and water. Their light-dark cycle was fixed to only night, and the mice were given 3 days to acclimate before testing. As expected, there was a lack of will to escape the TST apparatus as the mice became accustomed to being helpless. Although results were anticipated to be similar in the FST, results did not conclude significant differences in the time spent immobile during the FST.

Discussion

The adrenergic system plays a key role in the treatment and understanding of the etiology of mental illnesses. Despite the antidepressants' effect of increasing the number of neurotransmitters, such as norepinephrine (NE), the mystery behind their mechanism is poorly understood. It is apparent that neurogenesis does occur in the hippocampal region of the brain around the time of symptom improvement. With neurogenesis taking ~1 month to occur, it was hypothesized that treatment of an alpha1A-AR agonist would induce an antidepressant-like action as a potential phenotypical action of the adrenergic system. Although results utilizing the agonist, Cirazoline, did not find any significant difference in a 2-week acute treatment or a

month-long treatment, significance was found in the behavioral experiments. Males and females have long been divided into separate experiments due to their biological differences. Current research is working to disprove the need for separate experiments.

In the results from the Cirazoline experiment, sex differences were significantly found among the treated and untreated mice. As such, male and female mice cannot be compared in terms of their equal responses in behavioral experiments. Female mice were found to have more anxiety than males whether treated or untreated. Treatment, when neglected, indicated a significant difference when sex was compared independently.

In the 24-hour restraint, significance was found in the TST which concluded that after 24 hours of restraining, an acute depressive phenotype is formed. Although this was not found in the FST, not all tests are measured to be equal. The TST not only includes immobility but also does not have the bias of mice being able to float in the FST. By being able to float, mice are less likely to continue to be immobile during the FST when learned. However, the TST does not allow for this bias as mice are not able to learn that harm will not occur if they give up. This is a key indicator for the learned helplessness model.

Although not examined in the present study, cognitive impairments are a highlighted symptom of not only depression and anxiety but also schizophrenia. Schizophrenia is a debilitating mood disorder that affects 1% of the population and reduces life expectancy by 15 years (McCutcheon et al., 2020). Symptoms of schizophrenia are labeled as being either positive

or negative symptoms. Positive symptoms, such as delusions and hallucinations, are often the initial symptoms reported to clinicians. However, negative symptoms, such as amotivation, social withdrawal, cognitive defects in working memory, executive function, and processing speed are at the core of the symptomatic burden of the disorder.

The results of negative symptoms become apparent in early life, and it provides evidence for the linkage of the pathogenesis of schizophrenia to early neural development (McCutcheon et al., 2020). It was thought that disruption occurs during the "synaptic pruning" stage of development which leads to cognitive deficits. It's been found that there is an increase in gray matter loss and diverged neural networks at the onset of the illness. Cognitive decline is an early symptom of Schizophrenia; however, increased memory difficulties usually lead up to the first psychotic episode later in adulthood.

Despite the results from the MWM did not indicate a significant difference between the trials between the treatment group and the non-treatment group, a difference was observed in the 3rd trial of the MWM ($P = 0.067$). Those treated with the drug did adapt to the trial much quicker than those not treated despite not having a P value < 0.05. As such, with a 93.3% confidence, the treatment group was associated with an improvement in cognition (learning and memory).

Current treatments for schizophrenia include antipsychotics which target various systems in the brain (serotonergic, dopaminergic, glutamatergic, etc.) which have had the

benefits of aiding positive symptoms of the disorder (hallucinations, thought disorder) but antipsychotics have very minimal effect on negative symptoms (cognition, social withdrawal) (Stępnicki et al., 2018). In the MWM, the alpha1A-AR agonized which, unlike antipsychotics, is the opposite mechanism targeted by antipsychotics. Quetiapine, an antipsychotic drug used to treat both schizophrenia and other mood disorders, is an alpha1 antagonist and an alpha2A/B/C antagonist along with having multiple other mechanisms such as norepinephrine transporter inhibition (Maneeton et al., 2016).

Cirazoline, which was used in the MWM, is an alpha1A agonist with partial agonistic properties to alpha1B and alpha1D. Improvements in cognitive function as a result of cirazoline's mechanism may counteract the negative symptoms untreatable by quetiapine with a modification to the effects associated with the noradrenergic system. Despite this study not examining Schizophrenia, the symptomology of Schizophrenia is shared among different disorders. As such, Schizophrenia, and other mood disorders should not be discounted from the results of the study.

Future Studies

As previously mentioned, behavioral tests for depression and anxiety have been performed with and without proper mouse models to express somatic symptoms. Schizophrenia as well was not studied in the above-mentioned studies. Schizophrenia needs to also be evaluated along with depression and anxiety as a basis for negative symptom

improvement. The field of psychiatric research remains at a loss while models are continuously improving to provide accurate results for symptomatic improvement. In our lab, prior studies require a mouse model of depression and anxiety to produce more, accurate results prior to concluding the role of the alpha1A-AR in depression and anxiety. As such, the 24-hour restraint model has been verified for use along with alpha1A pathway investigation. Future studies can include the 24-hour restraint prior to Cirazoline treatment to investigate any improvements in somatic symptoms.

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