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INVESTIGATION OF AFFECTIVE ANXIETY USING AN ACIDIC SALINE-INDUCED MODEL OF FIBROMYALGIA IN RATS

Michelle Bland

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by

MICHELLE K. BLAND

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ABSTRACT

INVESTIGATION OF AFFECTIVE ANXIETY USING
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Michelle K. Bland, B.S. Biology

The University of Texas at Arlington, 2021

Faculty Mentor: Dr. Perry N. Fuchs

Fibromyalgia is a complex, yet prevalent pain disorder characterized by noninflammatory, musculoskeletal chronic widespread pain and extensive comorbidity with negative emotional affect. Preclinical studies have successfully modeled the long-lasting, bilateral mechanical hyperalgesia characteristic of fibromyalgia in rodents using repeated, intramuscular injections of acidic saline, though few have examined whether this technique might also induce similar affective comorbidities to those seen in human patients. Thus, the present study utilized the open field test to assess whether acid-induced hyperalgesia produces measurable changes to locomotor activity, exploratory drive, or hind paw sensitivity. The model was also extended to evaluate pregabalin's efficacy as a subsequent anxiolytic treatment. No significant difference was found for distance or mean velocity traveled between pain conditions regardless of the area of the chamber measured.

On average, animals reared more at baseline, though there were no significant differences across pain conditions. Pregabalin produced a global increase in locomotor behavior without ataxic symptoms, though this effect did not differ regardless of whether the acidic saline model was present or not. Mixed findings warrant further investigation of affective dimensions within the acidic saline model; wherein future replications should examine whether the effects of acid-induced pain extend beyond sensory and affective components.

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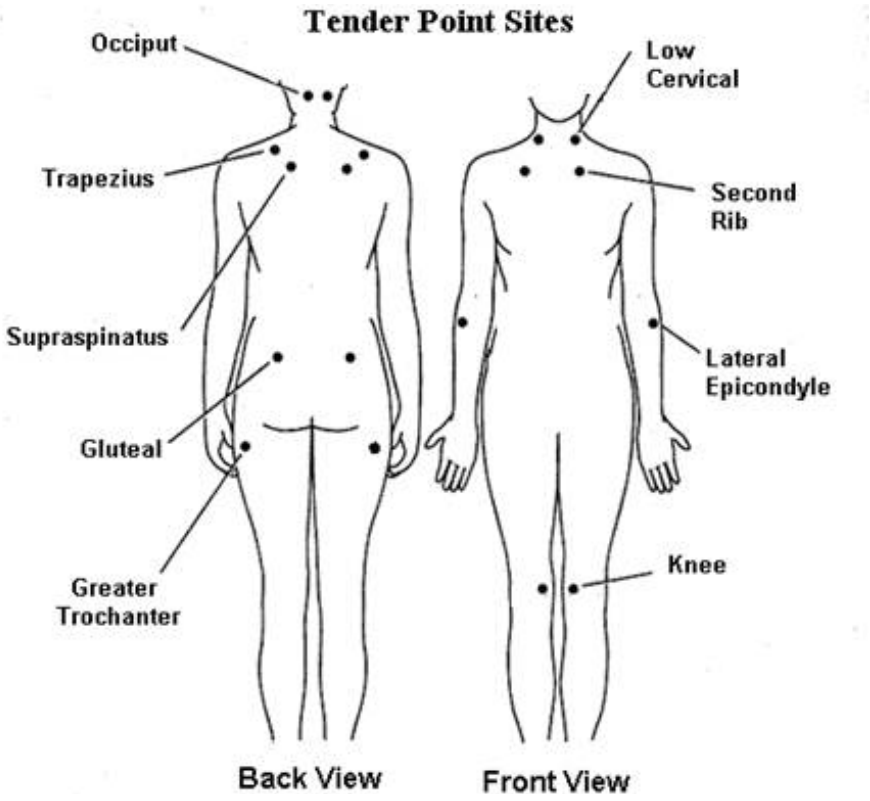
CHAPTER 1

INTRODUCTION

1.1 Fibromyalgia

Fibromyalgia (FM) is a pain disorder characterized by chronic widespread pain (CWP) lasting for more than 3 months, without detectable inflammation or structural abnormalities and for which no alternative cause can be identified (Cimmino, Ferrone, & Cutolo, 2011; Williams & Clauw, 2009). Patients with fibromyalgia present with heightened and painful responses to pressure, which is described in the tenth edition of the International Classification of Diseases (ICD) as the presence of ≥ 11 out of 18 localized tender point sites on either side of the body (see Figure 1.1; Wolfe et al., 1990; World Health Organization, 2004). This phenomenon is known as *abnormal pain perception processing*, in which individuals experience higher sensitivity to pain (e.g., a lowered threshold to normally painful stimuli, also known as *hyperalgesia*) than those without the disorder (Centers for Disease Control and Prevention, 2020; International Association for the Study of Pain, 2017). In some cases, patients may also present with *allodynia*, an abnormal pain response to a stimulus that does not normally provoke pain (IASP, 2017). Other symptoms include excessive fatigue, *paresthesia* (a numbness, tingling, or pricking sensation of the skin), joint stiffness, memory problems, sleep disturbances, and emotional and mental distress (Hsu, 2011; Yunus, 2001), often leading to cumulative dysfunction and disability in various domains of the person's daily life (Verbunt, Pernot, & Smeets, 2008).

Figure 1.1: Diagnostic location of 18 tender points.



Note: Fibromyalgia was previously diagnosed by assessing the presence of mechanical sensitivity at 9 pairs of pressure points distributed symmetrically across the median plane (United States Social Security Administration, 2012).

Astonishingly, fibromyalgia is estimated to affect around 4 million adults in the United States alone (CDC, 2020), though some researchers believe up to 75% of cases may go undiagnosed in a population (Clauw, Arnold, & McCarberg, 2011). Importantly, women appear to be disproportionally affected by fibromyalgia; the gender ratio of diagnosed cases is reported as anywhere from 3:1 to 6:1 in females to males, though survey research indicates the disparity might be as high as 9:1 (Queiroz, 2013; Hsu, 2011; Yunus, 2001). Prevalence is highest in middle-aged women, who comprise around 75% to 90% of those diagnosed (Bennett et al., 2007; Robinson et al., 2013; Wolfe et al., 2010). However, this overwhelming gender imbalance may actually arise from diagnostic protocols and the

tender point standard itself. In practice, females tend to report a greater number of tender points and more intense pain at these sites than their male counterparts, leading to an overrepresentation among diagnoses (Arout et al., 2018). Modern standards have progressively opted to eliminate the presence of tender points as a determining diagnostic criterion, and thus, gender differences in fibromyalgia appear to be far smaller than previously estimated, approximately 3.4% in women and 0.5% in men (Wolfe et al., 1990; Lindell et al., 2000).

Regardless of demographic distribution, fibromyalgia represents a major burden to all those who suffer from it and, despite changes to diagnostic protocols, the overall prevalence of this condition is still staggeringly high – though the exact figure is somewhat disputed. Early reports suggested fibromyalgia to affect nearly 2% of the general population (Wolfe et al., 1995); however, estimates from the 2000s onward tend to be higher – between 6% to 13% in some samples (Barsante Santos et al., 2010; Vincent et al., 2015). Although prevalence rates differ, it is still clear fibromyalgia represents a significant portion of the chronic pain population, resulting in considerable and frequent dysfunction on a day-to-day basis. Fibromyalgia patients tend to experience significant functional impairment due to their chronic pain and comorbidities (Penrod et al., 2004; Cosci et al., 2011), culminating in reduced physical functioning, mental health, and quality of life for these individuals and their spouses (Hoffman & Dukes, 2007; Bigatti & Cronan, 2002). Many face additional economic burdens from lost job productivity, work disability, and unemployment related to their condition (Lacasse et al., 2016; Al-Allaf, 2007; Sanchez et al., 2011) and experience high costs when purchasing prescribed pain medications and paying for consultations from the multiple medical specialists required for effective

management of symptoms (Berger et al., 2007; Wolfe et al., 1997). Despite the dire needs of these individuals, research and awareness of fibromyalgia is limited but greatly warranted. Along with the obvious benefits toward the patient themselves, as well as to their support networks and local communities, improved understanding of this condition and chronic widespread pain is likely to provide new groundwork for prevention and treatment of other chronic pain syndromes as a whole.

1.1.1 Pathophysiology in Humans

Despite the condition's high overall prevalence in the population and its significant impairment of patients, fibromyalgia is still poorly understood by researchers, physicians, and the general public. Before the eighteenth century, the specific presentation of musculoskeletal pain now associated with fibromyalgia was thought to be a form of rheumatism, later termed *fibrositis*, or inflammation of the fibre, by neurologist Sir William Gowers in 1904. The term fibromyalgia was first introduced in the 1970s once it was understood that, characteristically, pain was not accompanied by any detectable inflammation or structural abnormalities (Inanici & Yunus, 2004). However, fibromyalgia was not recognized as a medical condition in the United States until nearly twenty years later in 1987, when the American Medical Association first identified it as a disease entity causing illness and disability (Mandal, 2019). Indeed, our understanding of fibromyalgia and its mechanisms has only expanded within the last 35 years since its characterization, though there remains no widely accepted causal factor or explanation for the condition.

Currently, fibromyalgia pain is understood to be a noninflammatory, muscular-based form of chronic widespread pain (CWP), though the condition itself may often be comorbid with other inflammatory components (Cimmino et al., 2011). This definition was

largely based on the 1990 criteria of chronic widespread pain defined by American College of Rheumatology as being (1) bilateral (on both sides of the body), (2) localized simultaneously above and below the waist, (3) in both the axial and peripheral skeleton, and (4) that lasts for at least three months (Wolfe et al., 1990). The pain is also typically described as being diffuse and migratory, such that an originating site of the pain cannot always be pinpointed on or in the body (Aaron & Buchwald, 2003), though pain in deep tissues, such as in ligaments and joints, is commonly reported (Staud & Rodriguez, 2006). Within the bodies of literature and in practice, patients with fibromyalgia most consistently exhibit increased sensitivity to pressure, which is termed *mechanical hyperalgesia* or *mechanical allodynia* (Granges & Littlejohn, 1993; Petzke et al., 2003), leading to the popularity and practice of using the nine paired tender points as a diagnostic criterion (see Figure 1.1).

Currently, modern research into the potential mechanisms of fibromyalgia focuses heavily on determining a neurobiological origin for the condition. Based on studies and imaging conducted on diagnosed case samples and animal models, it is generally understood that the abnormal pain processing characteristic of fibromyalgia is related to amplification of pain and sensory signals within a malfunctioning central nervous system (Hsu, 2011; DeSantana & Sluka, 2008; Staud & Rodriguez, 2006). This general hypothesis was greatly strengthened by data collected from functional magnetic resonance imaging (fMRI) revealing greater brain activity in areas related to the processing of *nociceptive* (aversive) stimuli as well as activation of these regions at lesser stimuli than healthy controls (Gracely et al., 2002; Cook et al., 2004; Nebel & Gracely, 2009). These amplified signals are suggested to arise from *central sensitization*, or increased response to the same

stimulus, leading to the exaggerated perception of painful stimuli and the perception of innocuous stimuli as painful (Staud & Smitherman, 2002; Staud & Rodriguez, 2006).

The causal mechanism of sensitization within the central nervous system is not fully understood but is thought to be triggered by interactions between genetic predispositions and environmental stressors (i.e., from infection, trauma, or stress), with key genetic polymorphisms implicated in serotoninergic, dopaminergic, and catecholaminergic pathways (Buskila, 2009). Current theories in this field of research attempt to explain both initiation and maintenance of abnormal pain perception, modulation, and interpretation leading to chronic widespread pain. Broadly, these include deficits in endogenous pain inhibition systems (Julien et al., 2005; Jensen et al., 2009; Nielsen & Henriksson, 2007), neuroplastic changes to the brain and spinal cord (Petersel, Dror, & Cheung, 2010; Staud & Spaeth, 2008), alterations to the hypothalamus-pituitary-adrenocortical (HPA) axis and the autonomic nervous system itself (Williams & Clauw, 2009; Crofford, 2002), imbalance between excitatory and inhibitory descending controls (Clauw, Arnold, & McCarberg, 2011; DeSantana & Sluka, 2008), and increased neuronal excitability and enhanced temporal summation during firing (Price & Staud, 2005; Price et al., 2002). Actions of certain receptors, such as N-methyl-D-aspartate (NMDA) receptors, are believed to contribute to abnormal neuronal firing, along with specific neurotransmitters (e.g., glutamate) and neuropeptide enhancer molecules (e.g., substance P, nerve growth factor, and neurotrophins) which diffuse in the spinal cord and lead to widespread pain throughout the body (Hsu, 2011; Meeus & Nijs, 2006; Nielsen & Henriksson, 2007).

Until the late 2000s, central factors, like those previously discussed, were thought to be the only neurogenic mechanisms involved in fibromyalgia, as no specific muscle pathology or consistent muscle changes had been observed in diagnosed patients. However, increasing evidence has found measurable changes to peripheral tissues of fibromyalgia patients, such as increased levels of substance P and interleukin-1 (IL-1) (Sprott et al., 1998; Salemi et al., 2003), ragged red fibers and "moth-eaten" fibers (Bengtsson, Henriksson, & Larsson, 1986), as well as muscle tension, perfusion abnormalities (passage of fluid through the muscle tissue), and *ischemia* (inadequate blood flow to the muscle tissue) (Lund, Bengtsson, & Thorborg, 1986; Elvin et al., 2006). These discoveries in peripheral tissues are now believed to sensitize muscle nociceptors to sensory input, indirectly contributing to and maintaining central mechanisms of pain amplification seen in these patients (Staud & Rodriguez, 2006; Staud, 2006). In updating our previous understanding of abnormal pain processing, fibromyalgia is currently considered to result from the combined contributions of central sensitization and sensory augmentation, arising from interactions between biopsychosocial factors in the individual's genetics and environment.

1.1.2 Affective Components and Comorbidity

Despite rapid breakthroughs in fibromyalgia research within the last half century, there still remains significant gaps in our understanding of this pain condition. To this point, research has focused heavily on the physical, sensory dimension of pain to explain chronic widespread pain and abnormal pain processing. In practice, however, each individual's experience and presentation of pain is unique, distinct, and isolated. This extensive interpersonal variation between cases, including among those who share the

same diagnosis, which cannot fully be explained by a physical component alone. Instead, researchers believe there is also an emotional, *affective* dimension that interacts with aversive, nociceptive sensation to form the individual's subjective pain experience (Rainville, 2002). This idea of a multidimensional pain experience is the foundation for the current definition of pain used in research, which is defined as "an unpleasant sensory and emotional experience associated with actual or potential damage or described in terms of such damage" (IASP, 1999). Within this description, an affective-motivational dimension of pain, or *pain affect*, is recognized, having previously been defined as a person's cognitive appraisal of the unpleasantness of the pain sensation (Price & Harkins, 1992).

Pain affect has huge implications for research and clinical management of pain, especially in patients who suffer from chronic pain. It is now understood that all nociception undergoes modulation in the central nervous system by conscious and unconscious mental activity (Loeser, 1991), allowing sociocultural influences and personal biases to shape how pain is experienced by the individual in a bidirectional relationship (Price, 1999; Rainville, Bao, & Chrétien, 2005). In particular, cognitions related to cause, control, duration, outcome, and blame are frequently linked to negative emotions, such as anger, fear, and depression (Staud & Rodriguez, 2006; Staud, 2006). These negative appraisals are powerful and greatly influence the unified pain experience, such as by augmenting one's perceptions of the pain's unpleasantness (Wade et al., 1990; Taenzer, Melzack, & Jeans, 1986) and intensity (Staud et al., 2003; Staud et al., 2004).

Recent research suggests negative emotionality to play a dynamic role in the origin and maintenance of fibromyalgia pain, in that the chronic widespread pain associated with the condition is especially prone to the effects of negative mood (Davis, Zautra, & Reich,

2001; Staud et al., 2003). When comparing two groups of patients diagnosed with either fibromyalgia or general chronic musculoskeletal pain, Montoya and colleagues (2005) reported that tactile pain ratings among fibromyalgia patients were differentially modulated by emotional context compared to controls, as patients showed significantly higher vulnerabilities to pain in a negative affective state compared to a positive affective state. Even within pure fibromyalgia samples, studies show that a significant portion of variance in individual pain ratings are accounted for by negative affect (Staud et al., 2003; Staud et al., 2004; Staud et al., 2006). In the opposite direction, positive cognitions, such as pain acceptance and greater *self-efficacy* (belief in one's ability to influence events that occur in one's life; Bandura, 2010), are known to buffer the severity of pain associated with fibromyalgia and improve functional outcomes among patients (Kratz, Davis, & Zautra, 2007; Buckelew et al., 1996).

It has been suggested, however, the relationship between negative emotionality and fibromyalgia extends beyond a general vulnerability or dispositional association with pain. Even among other chronic pain disorders, fibromyalgia shows significant co-occurrence with mood and anxiety disorders (Bradley, 2005; Buskila & Cohen, 2007). 74% of fibromyalgia patients experience a lifetime prevalence of depression, while 60% exhibit extended comorbidity with certain anxiety disorders (Cole et al., 2006; Arnold et al., 2006; Thieme, Turk, & Flor, 2004), both of which share multiple familial risk factors with fibromyalgia (Raphael et al., 2004; Arnold et al., 2013). Furthermore, individuals who suffer from fibromyalgia are also more likely to be diagnosed with conditions known to be related to stress, such as irritable bowel syndrome (IBS) and frequent migraine headaches, as well as with sleep disorders and chronic fatigue syndrome (Hudson et al., 1993). Indeed,

a cross-sectional survey of fibromyalgia patients found that over 50% had seven or more comorbidities, including those aforementioned (Vincent et al., 2015).

While it is clear that strong relationships exist between fibromyalgia pain, negative affect and psychosocial beliefs, and comorbidity with certain psychiatric and chronic conditions, comprehensive research conducted on these connections is limited at this point. Due to this, there exists great controversy and criticisms over whether fibromyalgia should be classified as a somatoform disorder of psychogenic origin (Häuser & Henningsen, 2014). However, the general consensus among pain researchers and treating clinicians is that its manifestation of chronic widespread pain firmly categorizes fibromyalgia as a pain disorder, which itself interacts with biopsychosocial factors (Häuser & Fitzcharles, 2018). Currently, researchers do not yet know how, or if, the addition of an affective dimension to the prevailing model could further explain these connections. Concerted research in this domain is key to clarifying why emotional and psychosocial factors appear to be so strongly related to fibromyalgia and chronic widespread pain.

1.1.3 Treatments

Treatment options for fibromyalgia are as numerous and varied as its presentations in patients and aim to alleviate chronic widespread pain, increase restorative sleep, and improve physical functioning and quality of life in patients (Bellato et al., 2012). In most cases, an integrated approach is preferred for symptom management which combines the use of drug therapies with complementary interventions, such as exercise, physical therapy, acupuncture, and cognitive-behavioral therapy (Mease, 2005; Rooks, 2007). The most commonly prescribed pharmacotherapies for fibromyalgia are antidepressants, followed by opioids, nonsteroidal anti-inflammatory drugs (NSAIDs), muscle relaxants, and

sedatives (Skaer, 2014; Mease, Dundon, & Sarzi-Puttini, 2011; Kim, Landon, & Solomon, 2013). Furthermore, the United States Food and Drug Administration (FDA) currently approves three different medications specifically for treatment of fibromyalgia pain: duloxetine and milnacipran, both of which are dual serotonin-norepinephrine reuptake inhibitors (SNRIs), as well as pregabalin, a gabapentinoid alpha2-delta ($\alpha_2\delta$) ligand (Hsu, 2011; Mease et al., 2011). In this study, we specifically focused on the mechanism of action of pregabalin; however, drug therapies are typically prescribed in varying combinations to achieve highest efficacy and are highly tailored to the patient's symptom presentation and comorbidities (Mease et al., 2011; Kim et al., 2013).

Pregabalin, marketed under the brand name Lyrica, was the first drug approved by the FDA for management of fibromyalgia in 2007, having been originally marketed as an antiepileptic (Boomershine, 2010; Ben-Menachem, 2004). A structural analog of the inhibitory neurotransmitter GABA (gamma(γ)-aminobutyric acid), pregabalin is a potent ligand of the $\alpha_2\delta$ subunits located on voltage-gated calcium channels in the central nervous system (Ben-Menachem, 2004). Ligand binding acts to inhibit these channels and reduce calcium influx at nerve terminals, decreasing release of several excitatory neurotransmitters, such as glutamate, norepinephrine, and substance P (Dooley et al., 2007; Taylor, Angelotti, & Fauman, 2007). This decrease in neurotransmitter release is presumed to account for the *analgesic* (pain-relieving), anticonvulsant, and *anxiolytic*-like (anxiety-reducing) actions of this drug (Lyseng-Williamson & Siddiqui, 2008; Ben-Menachem, 2004). In fibromyalgia patients, pregabalin is found to significantly improve pain ratings and sleep quality compared to placebos (Boomershine, 2010). Further, in 8-, 12-, and 28-week randomized, double-blinded, placebo-controlled clinical trials, patients who received

pregabalin treatment reported significant improvement in fibromyalgia-specific symptoms and functionality, physical and emotional functioning, and overall perception of symptom improvement (Crofford et al., 2005; Arnold et al., 2008; Crofford et al., 2008). Due to its high efficacy and relatively low potential for drug-to-drug interactions, pregabalin is considered to be well-tolerated among patients and of high clinical utility in the treatment of fibromyalgia (Ben-Menachem, 2004; Arnold et al., 2018).

Despite FDA and clinician approval of the pharmacological therapies discussed, there is no known cure for fibromyalgia or the chronic widespread pain it presents with, and, furthermore, some patients do not respond fully to medication alone (Prabhakar et al., 2019; Arnold, 2006). Current treatment guidelines also call for concurrent evaluation and treatment of comorbidity, emphasizing the need for a multimodal approach individualized to each patient (Goldenberg, Burckhardt, & Crofford, 2004; Arnold, 2008). Due to these considerations, and in order to manage long-term symptoms, many patients seek out alternative or complementary interventions in combination with prescribed medications (Pioro-Boisset, Esdaile, & Fitzcharles, 1996; Goldenberg, Burckhardt, & Crofford, 2004; Crofford & Appleton, 2001). These nonpharmacological adjuvants broadly include exercise and dietary modifications, physical therapy, chiropractic or massage therapy, meditation, biofeedback, acupuncture, and cognitive-behavioral therapy (Leventhal, 1999; Arnold, 2006; Prabhakar et al., 2019). Among these, exercise is associated with greatest reductions in fibromyalgia-related symptoms and improvements in daily functioning, overall well-being, and reported self-efficacy when used alongside medication regimens (Busch et al., 2007; Rooks, 2007). This huge variation in modalities used for clinical management of fibromyalgia points toward its complexity as a chronic pain disorder,

requiring a comprehensive and multidisciplinary approach. While further research is warranted on patient outcomes and treatment efficacy, this thesis focuses on the use of pregabalin pharmacotherapy to treat bilateral mechanical hyperalgesia associated with fibromyalgia.

1.2 Preclinical Models of Fibromyalgia in Rodents

To this point, discussion of fibromyalgia research has primarily focused on clinical findings from patients who have previously been diagnosed with the condition. While human studies can examine within- and between-group differences among fibromyalgia patients and healthy controls, measurements primarily consist of self-report, medical imaging, and quasi-experimental conditions. To investigate the underlying mechanisms of fibromyalgia from a physiological perspective, researchers have developed techniques which model symptomatology and chronic widespread pain in animals, most commonly in rodents (e.g., rats and mice). In these domains of preclinical research, animal models are frequently used to resolve the functional pathways and systems implicated in complex disease states, which are otherwise difficult or impractical to test experimentally in human subjects due to high expense, invasiveness, and risk. To circumvent these challenges in humans, researchers and medical scientists turn instead to preclinical studies which have quickly become tremendous assets in the advancement and refinement of the current theoretical neurobiology of fibromyalgia (DeSantana, da Cruz, & Sluka, 2013; Sluka & Clauw, 2016).

Animal models of fibromyalgia are relatively new and few in number, the earliest ones having been developed at the turn of the 21st century. Because the condition's etiology is still unknown, researchers aim to mimic its symptomatology and pathology instead,

finding greatest success in rodents. Notably, Sluka, Kalra, and Moore (2001) successfully replicated long-lasting, bilateral hyperalgesia (exaggerated sensitivity to painful stimuli) in rats and mice using repeated, intramuscular injections of acidic saline at a pH of 4.0. Importantly, this paradigm induces prolonged mechanical hyperalgesia (increased pressure sensitivity) *without* observable tissue damage or inflammation. According to the model, a total of two injections of acidic saline are delivered unilaterally to the gastrocnemius muscle over an ideal window of time, reported as 2 to 5 days between injections. After the second injection, rodents display increased bilateral sensitivity to mechanical stimulation of the hind paws and back leg muscles, measured by mechanical paw withdrawal thresholds, for up to 4 weeks (Radhakrishnan et al., 2004; Sluka et al., 2001).

Use of this procedure in both rats and mice accurately mimics the clinical presentation of fibromyalgia in humans with its characteristic widespread hyperalgesia, minimal muscle tissue damage, and wider alterations in central nociceptive processing (DeSantana, da Cruz, & Sluka, 2013). Importantly, acid-induced pain and hyperalgesia are also able to be reversed by analgesic strategies used commonly in clinical management of fibromyalgia (Nielsen, Mathiesen, & Blackburn-Munro, 2004), e.g., with administration of SNRI antidepressants (Kim et al., 2009), $\alpha_2\delta$-ligands (Yokoyama et al., 2007; Saeki et al., 2019), and opioids (Sluka et al., 2002), as well as with exercise (Bement & Sluka, 2005; Sharma et al., 2010). For example, pregabalin treatment is shown to decrease both cutaneous and deep tissue hyperalgesia in acid-induced rat models (Yokoyama et al., 2007). The exact reason why or how Sluka et al.'s model (2001) induces fibromyalgia-like symptoms in rodents is unknown, but researchers propose physiological stress associated with multiple low-intensity insults to the muscle enacts molecular and cellular changes to

the nociceptors and in central neurons that result in sensitization and maintenance of pain (DeSantana, da Cruz, & Sluka, 2013; Sluka & Clauw, 2016).

Despite the model's high replicability and success in producing long-lasting hyperalgesia (Kim et al., 2009; Yokoyama et al., 2007; Sluka et al., 2002; Sharma et al., 2009), there is minimal evidence as to whether acidic saline injections are also able to induce affective comorbidities (e.g., depression or anxiety), though these symptoms have been documented in rodent models utilizing other pain induction techniques (Nagakura et al., 2009; Nishiyori & Ueda, 2008; Nasu et al., 2019; Khasar et al., 2009; Nazeri et al., 2018). Aside from this study, the only other research to examine affective symptomatology in association with acid-induced mechanical hyperalgesia was conducted by Liu et al. (2014), who found the model to be associated with anxiety-like and depression-like behaviors in 50% to 60% of rats after induction of experimental pain. To measure negative affect, the researchers used a battery of behavioral tests: open field, elevated plus maze, forced swimming, sucrose consumption, and sucrose preference. Rats in the chronic pain condition showed remarkable aversion to open areas in elevated plus maze and open field (anxiety-like behavior), longer immobility in forced swimming (despair mood), and lower sucrose preference and consumption (anhedonic response). From these findings, it was concluded that anxio-depressive behaviors did indeed co-occur with the acid-induced pain model described by Sluka et al. (2001).

1.2.1 Open Field Paradigm

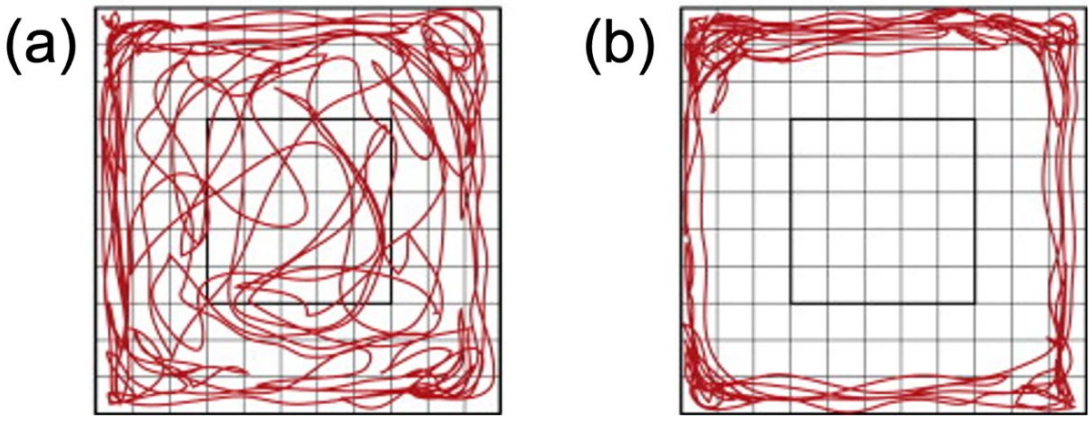
Expanding upon Liu et al.'s novel findings (2014) using the acidic saline paradigm, the current study specifically utilizes the open field paradigm (OF) to measure anxiety-like behavior in rat model. To preface, it is notoriously difficult to model and measure affective

behaviors in animals, which are incapable of giving a verbal report or conscious assessment of their current emotional state. Instead, negative emotionality must ultimately be inferred based on measurable deviations from an animal's normal behavioral repertoire (Paul, Harding, & Mendl, 2005); as such, researchers deem aberrant behaviors as "anxiety-like" or "depression-like" in context, since they are interpreted as being representative, but not definitive, of anxiety or depression, respectively. To measure anxiety-like behavior in rodents, behavioral tests, such as open field and elevated plus maze, exploit the animal's natural aversion to exposed fields (Asano, 1986; Lister, 1987). According to the theory, when a rat or mouse is first introduced to an exposed yet inescapable area, the subsequent quality and quantity of its exploratory drive and locomotor activity may be measured as an indicator of fear or anxiety (Gould, Dao, & Kovacsics, 2009).

As a behavioral paradigm, the open field test subjects a rodent (e.g., rat or mouse) to an unknown environment consisting of a bare apparatus whose top is uncovered and where escape is prevented by enclosing walls (Gogas et al., 2007; Carter & Shieh, 2015). To avoid influencing the animal's natural behavior in the open field, passive computer tracking software uses infrared beams and video cameras to analyze the animal's movements over a set time, e.g., 5 minutes, and quantifies horizontal activity, time spent in various regions of the open field (e.g., near the walls versus in the center), and total distance traveled (Valvassori, Varela, & Quevedo, 2013). In addition, a researcher may separately record and assess other rodent behaviors, such as rearing (a form of exploratory behavior; Seibenhener & Wooten, 2015), as supplementary parameters for analysis (Gogas et al., 2007; Carter & Shieh, 2015).

Thus, when a rat or mouse is placed in a novel environment consisting of a bare chamber, the animal tends to show an initial behavioral preference to stay near the walls (*thigmotaxis*) while avoiding the center of the field. This is because, as prey animals, rodents are averse to being physically exposed in a new environment and, presumably, find the open center to be a stressful location. Normal animals typically acclimate to the chamber and eventually explore the center area over time (see Figure 1.2, left; Carter & Shieh, 2015), while more anxious animals spend significantly less time in the open area and more time closer to the walls (see Figure 1.2, right; Carter & Shieh, 2015). Importantly, treatment with *anxiolytic* (anxiety-reducing) drugs before administration of the open field test increases time spent in the center area and decreases the latency to enter the center area after being placed into the apparatus, further supporting the test's ability to accurately measure anxiety-like behaviors (Gogas et al., 2007).

Figure 1.2: The open field test as a measure of rodent anxiety.



Note: In the open field test, (a) normal exploratory behavior following acclimation versus (b) a rodent model of anxiety (Carter & Shieh, 2015).

Of the behavioral paradigms used to evaluate emotionality in animals, the open field paradigm is considered a gold standard to assess anxiety-like behavior in rats and mice. Along with the elevated plus maze, open field is consistently shown to be both reliable and sensitive (Hegmann & DeFries, 1968; Ivinskis, 1968; Carola et al., 2002; Ho, Eichendorff, & Schwarting, 2002; Seibenhener & Wooten, 2015), leading to its long-standing popularity as an assessment tool within affective pain literature. Despite this, rodent models of fibromyalgia using acidic saline induction generally lack inclusion of affective measurements, including that of open field, and therefore cannot examine whether animals see an increase in negative emotionality following experimental induction of chronic widespread pain (Liu et al., 2014). Further research in these domains is greatly warranted considering the strong bidirectional associations between fibromyalgia and its high comorbidities with negative emotional affect (Price, 1999; Rainville, Bao, & Chrétien, 2005).

1.3 Hypotheses

While the bodies of research regarding the neurobiological contribution to fibromyalgia pain have grown substantially through the use of preclinical rodent models, there still remains large gaps in our understanding of the affective comorbidities accompanying fibromyalgia, especially in Sluka et al.'s acidic saline model (2001). While one preliminary study has suggested anxio-depressive behaviors to accompany acid-induced pain in rats (Liu et al., 2014), there have been no further studies examining whether negative emotionality co-exists in this acidic saline model, such as that which is exhibited by human fibromyalgia patients.

Therefore, the present study sought to replicate the work done by Sluka et al. (2001) and Liu et al. (2014) by using the open field test to measure differences in anxiety-like behavior before and after pain induction with repeated acid injections. To further expand the scope of the acidic saline model, we also explored the efficacy of pregabalin as a post-induction anxiolytic treatment by analyzing a third trial of open field following administration of the drug. Thus, patterns of exploratory drive and locomotor activity in the open field test were compared and interpreted across three time points (baseline, post-induction, post-treatment) and across pain and treatment conditions (acidic saline/saline, acidic saline/pregabalin, saline/saline, saline/pregabalin).

For the first aim of this study, the acidic saline models were expected to exhibit greater anxiety-like behavior than controls after pain induction, which could be indicated by differences in total, central, or peripheral locomotion based on pain condition at post-induction. It was hypothesized that acidic saline models would exhibit greater anxiety-like behavior and hind paw sensitivity than controls after pain induction and would also travel less distance, travel at a slower velocity, and would rear less often than vehicle controls.

The second aim of this thesis was to evaluate pregabalin's efficacy as an anxiolytic treatment in the acidic saline model; specifically, animals treated with pregabalin were expected to exhibit decreased anxiety-like behavior, e.g., increased locomotion, and decreased hind paw sensitivity, e.g., increased rearing, after the drug treatment had been administered. It was hypothesized that while the acidic saline/saline condition would travel the least distance and at the slowest velocity of all four groups at post-treatment, the acidic saline/pregabalin condition would travel at a distance and velocity comparable to vehicle controls. Additionally, the acidic saline/pregabalin condition was expected to rear

comparably to controls during post-treatment testing, whereas the acidic saline/saline animals were expected to rear the least out of all the groups.

CHAPTER 2

METHODOLOGY

2.1 Subjects

Forty-eight female Sprague-Dawley rats (225-250 g; The University of Texas at Arlington vivarium) from Charles River (Hollister, CA) were used in this study. All animals were housed in pairs and maintained on a controlled 12-hour light-dark cycle with constant temperature and humidity. Water and food were provided *ad libitum* before and throughout the study. Animals were cared for in accordance with the ethical guidelines outlined by the International Association for the Study of Pain for the use of laboratory animals, and the experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas at Arlington (protocol number: A20.004). After being allowed to acclimate for one week from their transfer, animals were randomly assigned to a pain condition (4.0 pH acidic saline vs. 0.9% normal saline) and then to a treatment condition (pregabalin vs. 0.9% normal saline). The four groups used for analysis were as follows: acidic saline/normal saline, acidic saline/pregabalin, normal saline/normal saline, and normal saline/pregabalin.

2.2 Materials

2.2.1 Mechanical Paw Withdrawal Threshold

Prior to mechanical stimulation, animals were allowed to acclimate for 10 minutes in a Plexiglass chamber with mesh flooring, which allowed access to the plantar surface of the hind paws. Using the up-down method (Dixon, 1980), von Frey monofilaments (3.85,

5.68, 9.74, 18.39, 39.42, 77.3, 135.3, and 251.34 mN) were used to apply a one-second stimulus to each hind paw, sufficient to bend the filament. Trials were begun with the 9.74 mN filament and, if no withdrawal response was observed (i.e., paw withdrawal, raising, or licking), the next highest filament was administered. If withdrawal was observed, the next lowest filament was administered instead. The procedure was repeated until there was no response at the highest filament force (251.34 mN), or until five stimuli were administered in total, constituting a single trial.

The 50% paw withdrawal threshold for each trial was calculated using the following formula: [Xth]log = [vFr]log + ky, where [vFr] is the force of the last von Frey used, k = 0.2593 is the average interval (in log units) between the von Frey monofilaments, and y is a value that depends upon the pattern of withdrawal responses. If an animal did not respond to the highest von Frey monofilament (251.34 mN), then y = 1.00 and the 50% mechanical paw withdrawal response for that paw was calculated to be 456.63 mN.

The test was conducted using 3 separate trials per hind paw, and the scores from each trial were averaged to determine the mean withdrawal threshold for the left and right paw of the animal. A final combined mechanical threshold (CMT) score was then averaged from the right and left paw values for each MPWT test. Broadly, animals were assessed to be in pain if they withdrew their paw at lower filament forces compared to baseline measurements.

2.2.2 Open Field Test

Animals were placed in the center of an uncovered, circular chamber with a wooden base of 100 cm diameter and an aluminum sheet metal wall of 45 cm height. Once the animal was placed in the testing chamber, Ethovision applied video tracking software

immediately began recording the animal's free activity within the open field over a five-minute interval, allowing the researcher to observe from a monitor located outside the testing room without interrupting or altering the animal's normal repertoire. This software also quantified the distance (cm) traveled by the animal, as well as its mean velocity (cm/s) of travel, in total and in the center of the field, specifically. In addition to these measures, the number of times an animal reared during each trial was manually assessed and counted by a researcher, where rearing was operationally defined as lifting of the front paws for any reason other than grooming. Thus, distance traveled, mean velocity of travel, and rearing behavior exhibited in the open field test could quantify locomotor activity (as an indicator of anxiety-like behavior), exploratory drive, and hind paw sensitivity.

2.2.3 Acidic Saline Model

In the study, experimental pain was induced according to the model by Sluka, Kalra, and Moore (2001), which employs a 2-injection paradigm, administered five days apart, that induces a bilateral hyperalgesia. Prior to injections, all animals were anesthetized using isoflurane gas (3% induction, 2% maintenance). Experimental animals were injected with 1 microgram of acidic saline (pH 4.0) in the left gastrocnemius muscle following baseline tests and then again five days later to allow habituation. Animals treated with the vehicle control received an injection of 0.9% normal saline to the left gastrocnemius muscle during the same time schedule. Per the Sluka et al. model (2001), a manipulation check (consisting only of MWPT measurements) was performed before administration of the second injection to ensure long-lasting bilateral hyperalgesia was properly induced.

To make acidic saline, 0.01 N sterile hydrochloric acid (HCl) was added dropwise to 0.9% sterile saline until pH meter read 4.0 ± 0.1. Adjustments were made by adding 0.01

N sterile sodium hydroxide (NaOH) dropwise until proper pH was achieved. Acidic saline was prepared on the day of injection to ensure consistent pH throughout the study. The vehicle control for repeated injections of acidic saline was 0.9% normal saline at physiological pH (pH 5.5 to 7.4), delivered during the same five-day schedule.

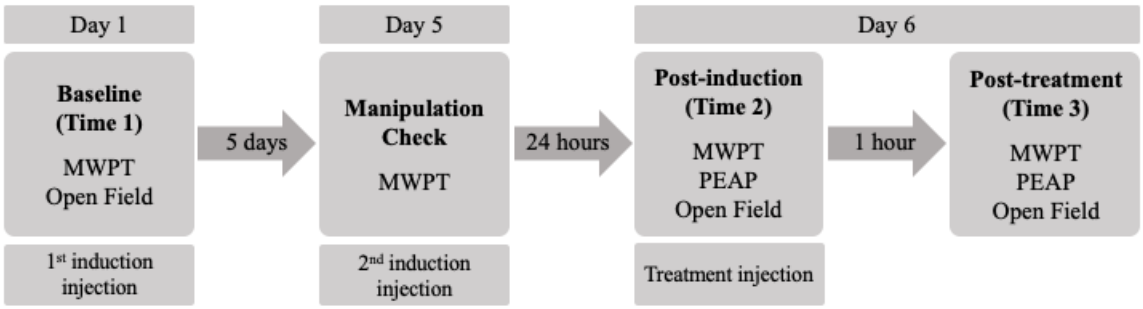
2.2.4 Pregabalin Treatment

After completion of post-induction open field tests, animals in both pain conditions were randomized to receive an intraperitoneal (i.p.) injection of pregabalin or a vehicle control (0.9% normal saline). Since pregabalin is sold as a solid, the drug was suspended in sterile injection water for delivery and administered based on weight. Thus, all animals were weighed prior to injections to ensure a dosage of 30 mg/kg. After injection, animals were allowed to habituate for an hour, followed by a third and final test day.

2.3 Procedure

This thesis was written as part of a larger preclinical model examining noninflammatory musculoskeletal pain associated with fibromyalgia, which was conducted between February 2020 and September 2020. Injections and testing took place over a six-day period, in which the open field data collected at three time points (baseline, post-induction, post-treatment) were used for analysis (Figure 2.1).

Figure 2.1: Injection and testing timeline over a six-day period.



Prior to experimental manipulation, baseline MWPTs and open fields were collected on the first day of the study, followed by a first injection with acidic saline (or 0.9% normal saline) to promote gradual induction of experimental pain. Injection protocols were performed according to the model originally described by Sluka et al. (2001), where experimental animals receive two unilateral injections of pH 4.0 saline to the left gastrocnemius muscle over a five-day period. Based on the model, bilateral mechanical hyperalgesia should not be induced until after administration of a second acidic saline injection, which is scheduled on the fifth day following the first injection; thus, on day 5, MWPT measurements were repeated for each animal prior to administering the second injection and were compared to baseline measurements. Any animals shown to exhibit mechanical hypersensitivity during this manipulation check, e.g., a CMT score significantly lower from baseline, were to be excluded from further manipulation. However, no animal's CMT scores significantly differed between these two time points and, therefore, all animals were carried forward to receive a second injection.

Once it was verified that no animals were exhibiting mechanical hypersensitivity after the manipulation check on day 5, experimental animals received a second injection of acidic saline, again to the left gastrocnemius muscle, on the same day. Control animals were subjected to identical hypersensitivity testing and injection procedures over the same five-day time frame but were injected with the 0.9% normal saline vehicle control instead. Following the second injection, both groups were allowed to habituate for 24 hours post-induction before a second round of MWPTs and open field tests were collected on day 6. In addition to these tests, place escape/avoidance paradigm (PEAP) measurements were

collected as part of the larger study but were not included in this thesis. Thus, on day 6, the sequence of testing at post-induction was (1) MWPT, (2) PEAP, and (3) open field.

Immediately following the second round of testing on day 6, animals in both pain conditions were randomly assigned to receive a treatment injection of pregabalin or 0.9% normal saline on the same day, after which the animals were given one hour to habituate. Following this rest period, animals were subjected to a third and final round of post-treatment tests in the following order: (1) MWPT, (2) PEAP, and (3) open field. Once measurements were complete, animals were humanely euthanized using CO_2 gas and a secondary measure of euthanasia (i.e., bilateral thoracotomy or decapitation) as required by the veterinarian.

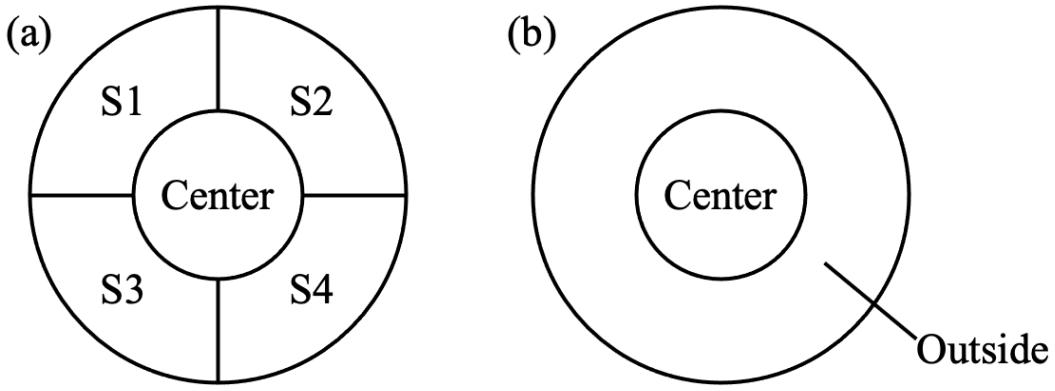
2.4 Analyses Plan

While MWPT and PEAP were collected as part of a larger study, these measurements were not assessed in this thesis, which specifically analyzed data collected from open field over three time points (Figure 2.1). Descriptions of these other measures were included to give a thorough timeline of all manipulations performed during the study.

Thus, for this thesis, temporal changes in open field activity and behavior in all groups were analyzed using a 3 (time: pre-induction, post-induction, post-treatment) x 4 (condition: acidic saline/saline, acidic saline/pregabalin, saline/saline, saline/pregabalin) mixed model Analysis of Variance (ANOVA), followed by post-hoc comparisons (Fisher's least-significant difference and simple effects) if appropriate. Mixed model ANOVAs were run for the following open field measures: distance traveled (total, center, and outside), mean velocity of travel (total, center, and outside), and total rears exhibited during each 5-minute testing interval.

Using the total and center field components automatically defined by the Ethovision software, an outside dimension was manually calculated after data collection had concluded. The distance traveled in the outer component was calculated as the difference between total and center distance, *Outside distance = Total distance – Center distance*. The mean velocity of outside travel was calculated by averaging the velocities measured in each quadrant, *Outside velocity* = (*S1 velocity + S2 velocity + S3 velocity + S4 velocity*) /4.

Figure 2.2: Sectioning of the open field.



Note: (a) The circular open field split into a center and four quadrants (S1, S2, S3, S4). (b) An outside perimeter can be defined using total, center, and quadrant calculations.

CHAPTER 3

RESULTS

3.1 Analysis of Total Distance

A 3 (time: baseline, post-induction, post-treatment) x 4 (condition: acidic saline/saline, acidic saline/pregabalin, saline/saline, saline/pregabalin) mixed model Analysis of Variance (ANOVA; Figures 3.1 and 3.2) failed to reveal a significant main effect of condition, $F(3, 44) = .67$, $p = .573$, $\eta^2_p = .04$, or time, $F(2, 88) = 2.17$, $p = .121$, $\eta^2_p = .05$, on the total distance that animals traveled. However, there was a significant interaction of condition and time, $F(6, 88) = 4.21$, $p = .001$, $\eta^2_p = .22$.

Analysis of simple effects of condition on time (Figure 3.1) revealed that animals traveled differently depending on their condition. Animals in the acidic saline/saline condition traveled significantly more at baseline ($M = 3635.98$, $SD = 136.02$) than at post-induction ($M = 3215.01$, $SD = 182.25$), whereas animals in the saline/saline condition traveled significantly more at baseline ($M = 3398.50$, $SD = 136.02$) than at post-treatment ($M = 2904.74$, $SD = 252.90$). Finally, animals in the saline/pregabalin condition traveled significantly more at post-treatment ($M = 3885.87$, $SD = 252.90$) than at post-induction ($M = 3120.09$, $SD = 182.25$).

Further analysis of simple effects of time on condition (Figure 3.2) revealed a significant effect pregabalin on the total distance traveled in the open field. At post-treatment specifically, the acidic saline/pregabalin condition ($M = 3663.12$, $SD = 252.90$) and the saline/pregabalin condition ($M = 3885.87$, $SD = 252.90$) each traveled significantly

more than the saline/saline condition (M = 2904.74, SD = 252.90).

Figure 3.1: Estimated marginal means of total distance between condition and time.

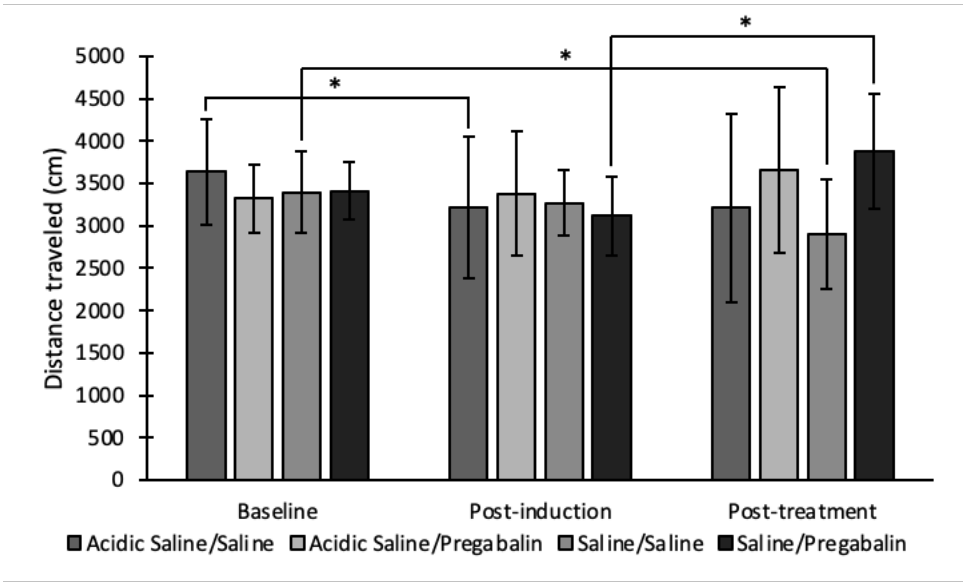
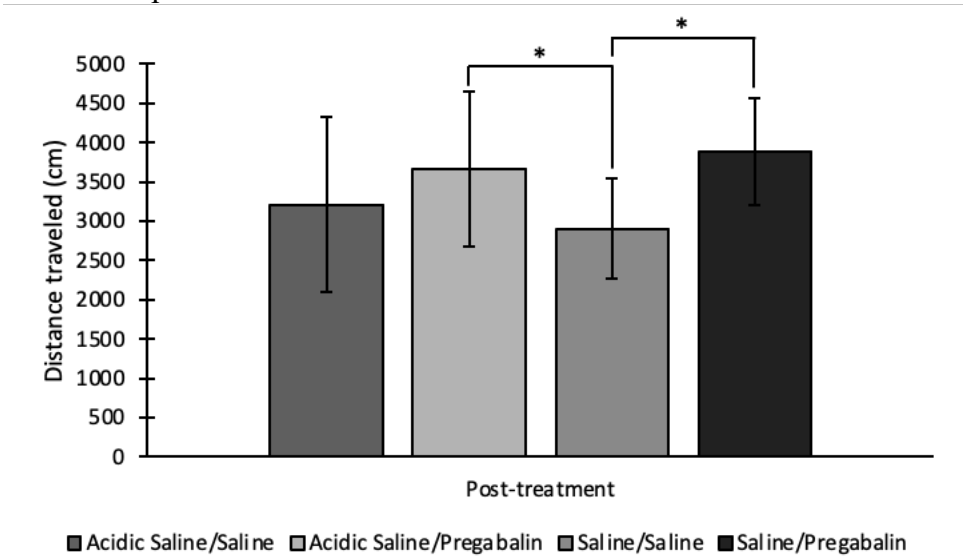


Figure 3.2: Estimated marginal means of total distance between conditions at post-treatment.



3.2 Analysis of Total Mean Velocity

A 3 (time: baseline, post-induction, post-treatment) x 4 (condition: acidic saline/saline, acidic saline/pregabalin, saline/saline, saline/pregabalin) mixed model ANOVA (Figures 3.3 and 3.4) did not reveal a significant main effect of condition on

average velocity of travel, $F(3, 44) = 1.22$, $p = .313$, $\eta^2_p = .08$. However, the main effect of time was significant, $F(2, 88) = 3.75$, $p = .027$, $\eta^2_p = .08$; Fisher's least significant difference post hoc revealed that, on average, animals traveled significantly slower at post-induction ($M = 11.01$, $SD = .31$) compared to baseline ($M = 11.60$, $SD = .23$) and post-treatment ($M = 11.93$, $SD = .40$) measurements. A significant interaction between condition and time was also found, $F(6, 88) = 5.15$, $p < .001$, $\eta^2_p = .26$.

Analysis of simple effects of condition on time (Figure 3.3) revealed that, on average, animals in the acidic saline/saline condition traveled faster at baseline ($M = 12.04$, $SD = .46$) than at post-induction ($M = 10.92$, $SD = .62$). On the other hand, saline/saline animals faster at baseline ($M = 11.50$, $SD = .46$) than at post-treatment ($M = 9.85$, $SD = .79$). Interestingly, both pregabalin condition showed similar trends of velocity over time; that is, the acidic saline/pregabalin animals and the saline/pregabalin animals each traveled fastest at post-treatment (Ms = 13.52, 13.23, SDs = .79, .79) than compared to baseline (Ms = 11.28, 11.57, SDs = .46, .46) or post-induction (Ms = 11.44, 10.63, SDs = .62, .62).

Further analysis of simple effects of time on condition (Figure 3.4) also revealed pregabalin had a significant effect on travel velocity. After treatment, the acidic saline/pregabalin condition ($M = 13.52$, $SD = .79$) and the saline/pregabalin condition ($M = 13.23$, $SD = .79$) each traveled significantly faster than the saline/saline condition ($M = 9.85$, $SD = .79$). Within the acidic saline model, animals in the acidic saline/pregabalin traveled significantly faster at post-treatment ($M = 13.52$, $SD = .79$) than the acidic saline/saline animals ($M = 11.11$, $SD = .79$).

Figure 3.3: Estimated marginal means of total mean velocity between condition and time.

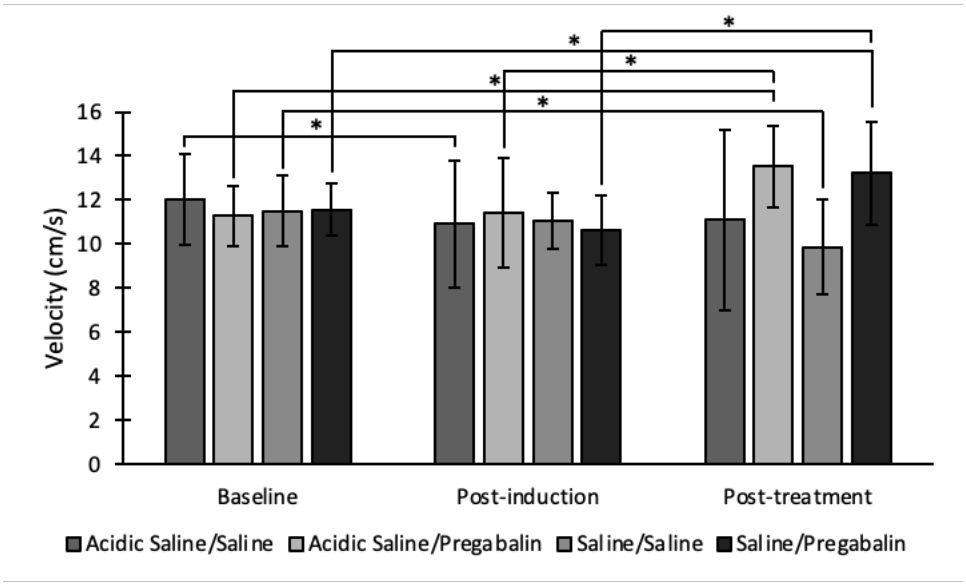
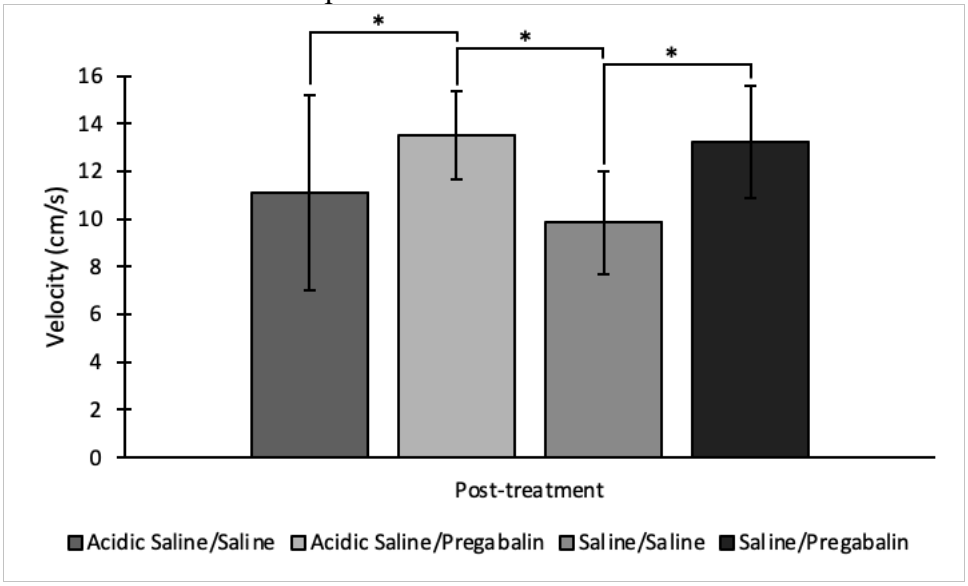


Figure 3.4: Estimated marginal means of total mean velocity between conditions at post-treatment.



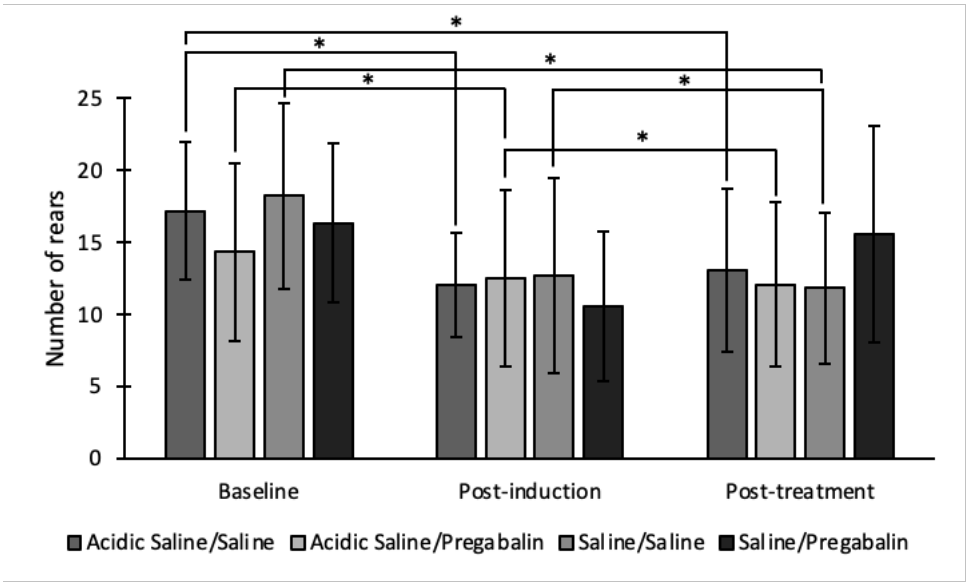
3.3 Analysis of Total Rears

A 3 (time: baseline, post-induction, post-treatment) x 4 (condition: acidic saline/saline, acidic saline/pregabalin, saline/saline, saline/pregabalin) mixed model ANOVA (Figure 3.5) did not reveal a significant main effect of condition on rears, $F(3, 44) = .22$, $p = .883$, $\eta^2_p = .02$, but did find a significant main effect of time, $F(2, 88) =$

13.23, $p < .001$, $\eta^2_p = .23$. Fisher's least significant difference post hoc revealed that animals reared most at baseline ($M = 16.52$, $SD = .83$) than during tests repeated at post-induction ($M = 11.96$, $SD = .80$) and post-treatment ($M = 13.15$, $SD = .88$). However, the interaction between condition and time was not significant for rears, $F(6, 88) = 1.62$, $p = .152$, $\eta^2_p = .10$.

However, some simple effect comparisons were significant (Figure 3.5) despite the overall interaction effect not reaching significance. Specifically, animals in the acidic saline/saline condition and the saline/saline condition reared most at baseline (Ms = 17.17, 18.25, SDs = 1.66, 1.66), with rears in each group decreasing at post-induction (Ms = 12.08, 12.67, SDs = 1.61, 1.61) and at post-treatment (Ms = 13.08, 11.83, SDs = 1.75, 1.75). On the other hand, animals in the acidic saline/pregabalin condition reared most at baseline (M = 16.33, SD = 1.66) and at post-treatment (M = 15.58, SD = 1.75), and reared least at post-induction (M = 10.58, SD = 1.61).

Figure 3.5: Estimated marginal means of total rears between condition and time.



3.4 Analysis of Center Distance

A 3 (time: baseline, post-induction, post-treatment) x 4 (condition: acidic saline/saline, acidic saline/pregabalin, saline/saline, saline/pregabalin) mixed model ANOVA (Figures 3.6 and 3.7) did not reveal a significant main effect of condition on the distance animals traveled in the field's center, $F(3, 41) = .67$, $p = .573$, $\eta^2_p = .05$. However, a significant main effect of time was found, $F(2, 82) = 14.53$, $p < .001$, $\eta^2_p = .26$; Fisher's least significant difference post hoc revealed that animals traveled least in the center at post-induction ($M = 197.04$, $SD = 27.29$) than at baseline ($M = 303.04$, $SD = 21.42$) or at post-treatment ($M = 363.54$, $SD = 36.24$). There was also a significant interaction between condition and time for this measure, $F(6, 82) = 4.34$, $p = .001$, $\eta^2_p = .24$.

Analysis of simple effects of condition on time (Figure 3.6) revealed that animals in the acidic saline/saline condition traveled more in the center at baseline ($M = 325.91$, $SD = 45.30$) than at post-induction ($M = 184.57$, $SD = 57.72$). Animals in the acidic saline/pregabalin condition traveled most in the center at post-treatment ($M = 512.71$, $SD = 73.10$) compared to baseline ($M = 291.96$, $SD = 43.20$) or post-induction ($M = 213.64$, $SD = 55.03$). Finally, the extent to which the saline/pregabalin animals traveled in the field's center differed across all three time points; hierarchically, these animals traveled most in the center at post-treatment ($M = 448.40$, $SD = 69.98$), followed by baseline ($M = 278.88$, $SD = 41.36$), and post-induction ($M = 147.41$, $SD = 52.69$).

Further analysis of simple effects of time on condition (Figure 3.7) also revealed pregabalin treatment significantly affected how much animals traveled in the center area. After treatment, the acidic saline/pregabalin condition ($M = 512.71$, $SD = 73.10$) and the saline/pregabalin condition ($M = 448.40$, $SD = 69.98$) each traveled significantly more in

the center than saline/saline condition (M = 241.66, SD = 69.98). Within the acidic saline model, the acidic saline/pregabalin animals (M = 512.71, SD = 73.10) traveled significantly more in the center at post-treatment than the acidic saline/saline animals (M = 251.41, SD = 76.66).

Figure 3.6: Estimated marginal means of center distance between condition and time.

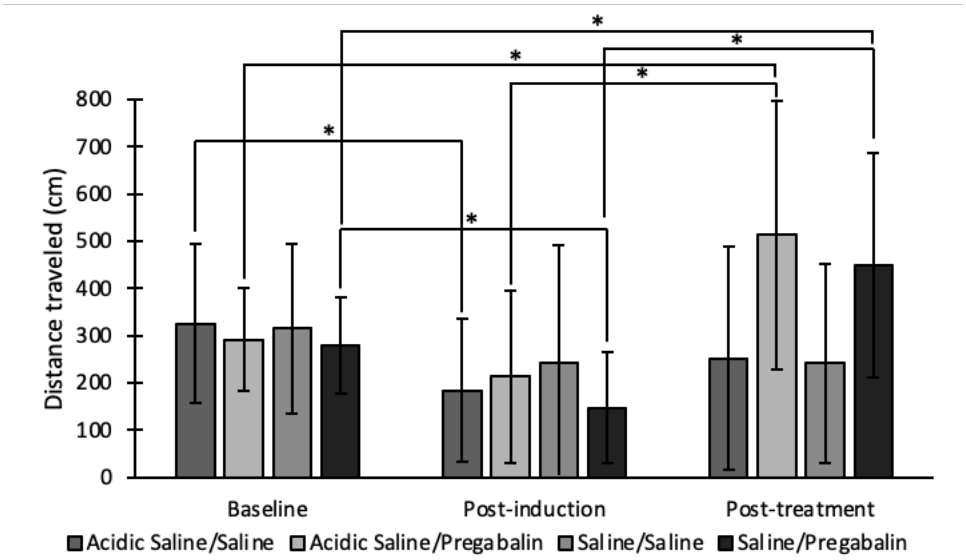
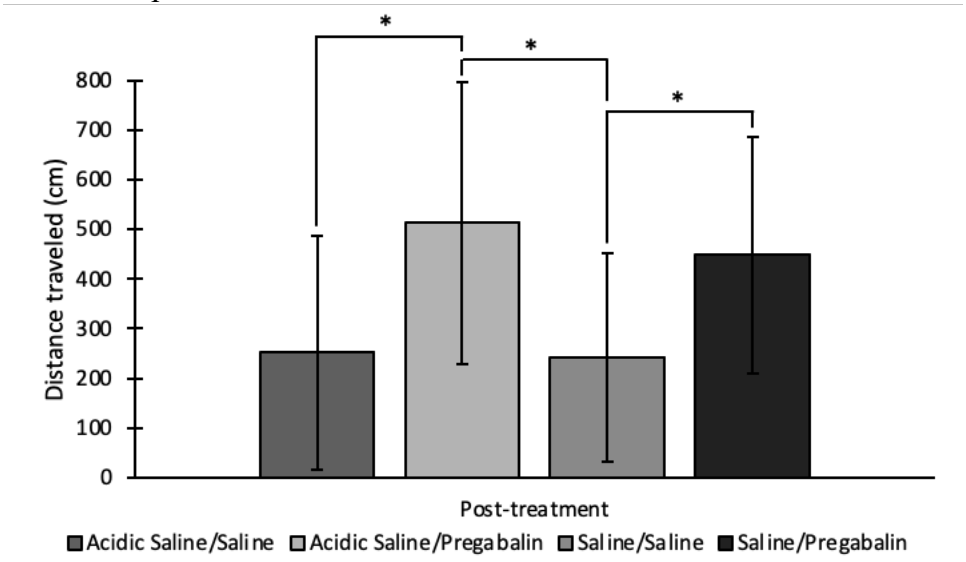


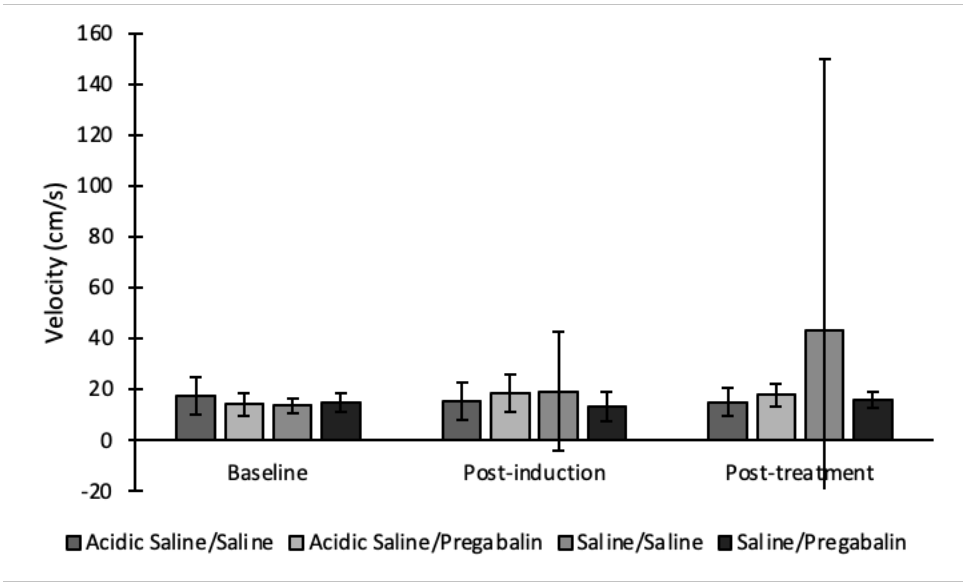
Figure 3.7: Estimated marginal means of center distance between conditions at post-treatment.



3.5 Analysis of Center Mean Velocity

A 3 (time: baseline, post-induction, post-treatment) x 4 (condition: acidic saline/saline, acidic saline/pregabalin, saline/saline, saline/pregabalin) mixed model ANOVA (Figure 3.8) did not find significant main effects of condition, $F(3, 41) = .77$, $p = .516$, $\eta^2_p = .05$, or time, $F(2, 82) = .73$, $p = .486$, $\eta^2_p = .02$, on the velocity at which animals traveled in the center of the open field, nor was there a significant interaction effect between condition and time for this variable, $F(6, 82) = .65$, $p = .689$, $\eta^2_p = .05$.

Figure 3.8: Estimated marginal means of center mean velocity between condition and time.



3.6 Analysis of Outside Distance

A 3 (time: baseline, post-induction, post-treatment) x 4 (condition: acidic saline/saline, acidic saline/pregabalin, saline/saline, saline/pregabalin) mixed model ANOVA (Figures 3.9 and 3.10) did not reveal a main effect of condition, $F(3, 41) = 1.10$, $p = .359$, $\eta^2_p = .08$, or time, $F(2, 82) = .38$, $p = .687$, $\eta^2_p = .01$, on the distance animals traveled in the field's outer perimeter, $F(3, 44) = .93$, $p = .435$, $\eta^2_p = .06$. However, the interaction between condition and time was significant, $F(6, 82) = 3.53$, $p = .004$, $\eta^2_p = .21$.

Analysis of simple effects of condition on time (Figure 3.9) revealed that the saline/pregabalin animals traveled more in the perimeter at post-treatment (M = 3437.47, SD = 190.81) than at post-induction (M = 2972.68, SD = 171.74). On the other hand, animals in the saline/saline condition traveled more in the perimeter at baseline (M = 3083.10, SD = 135.16) and at post-induction (M = 3029.00, SD = 171.74), and traveled least in this area at post-treatment (M = 2663.08, SD = 190.81).

Further analysis of simple effects of time on condition (Figure 3.10) revealed pregabalin treatment had a significant effect on how much animals traveled in the outer perimeter of the field. After administration of treatment, the acidic saline/pregabalin condition (M = 3396.74, SD = 199.30) and the saline/pregabalin condition (M = 3437.47, SD = 190.81) each traveled significantly more in this outer area compared to the saline/saline condition (M = 2663.08, SD = 190.81).

Figure 3.9: Estimated marginal means of outside distance between condition and time.

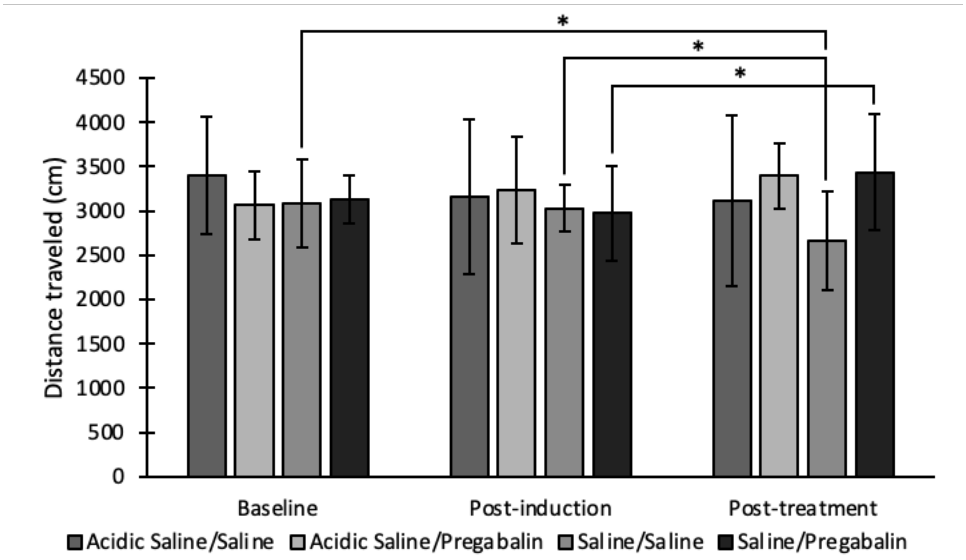
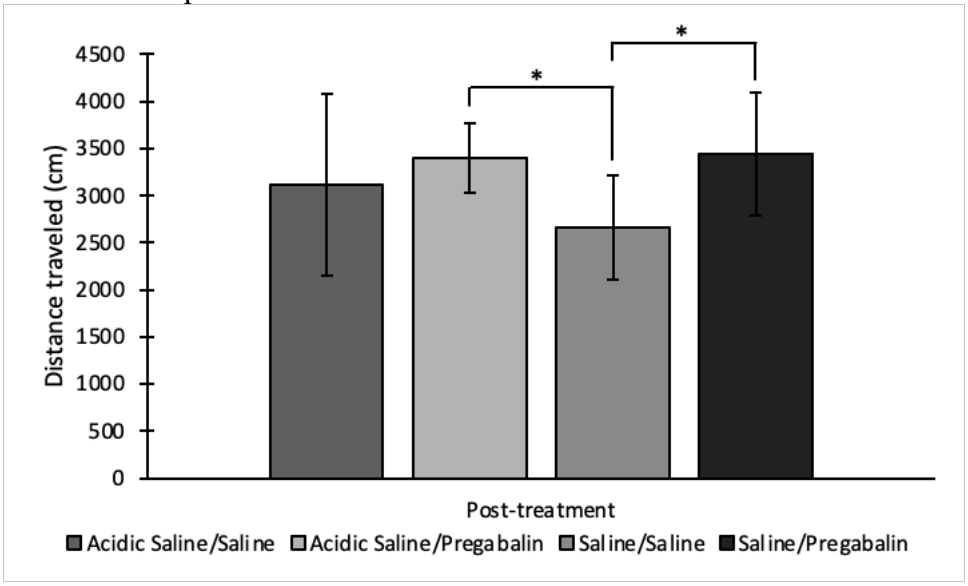


Figure 3.10: Estimated marginal means of outside distance between conditions at post-treatment.



3.7 Analysis of Outside Mean Velocity

A 3 (time: baseline, post-induction, post-treatment) x 4 (condition: acidic saline/saline, acidic saline/pregabalin, saline/saline, saline/pregabalin) mixed model ANOVA (Figures 3.11 and 3.12) did not reveal a significant main effect of condition on how fast animals traveled in the outside perimeter of the field, $F(3, 44) = .83$, $p = .483$, $\eta^2_p = .05$. However, a main effect of time was significant, $F(2, 88) = 4.54$, $p = .013$, $\eta^2_p = .09$; Fisher's least significant difference post hoc revealed that, on average, animals traveled faster in the perimeter during post-treatment testing ($M = 12.58$, $SD = .38$) than during post-induction testing ($M = 11.66$, $SD = .30$). The interaction effect between condition and time was also significant, $F(6, 88) = 4.70$, $p < .001$, $\eta^2_p = .24$.

Analysis of simple effects of condition on time (Figure 3.11) revealed that the acidic saline/pregabalin condition and the saline/pregabalin condition each traveled fastest in the perimeter at post-treatment (Ms = 13.91, 13.76, SDs = .76, .76) than at baseline (Ms = 11.55, 11.88, SDs = .48, .48) or at post-induction (Ms = 12.01, 11.19, SDs = .60, .60).

Additionally, analysis of simple effects of time on condition (Figure 3.12) revealed pregabalin treatment significantly affected how fast animals traveled on average in the outer perimeter of the field. At post-treatment, the acidic saline/pregabalin condition (M = 13.91, SD = .76) and the saline/pregabalin condition (M = 13.76, SD = .76) traveled significantly faster in the perimeter than the saline/saline condition (M = 10.81, SD = .76).

Figure 3.11: Estimated marginal means of outside mean velocity between condition and time.

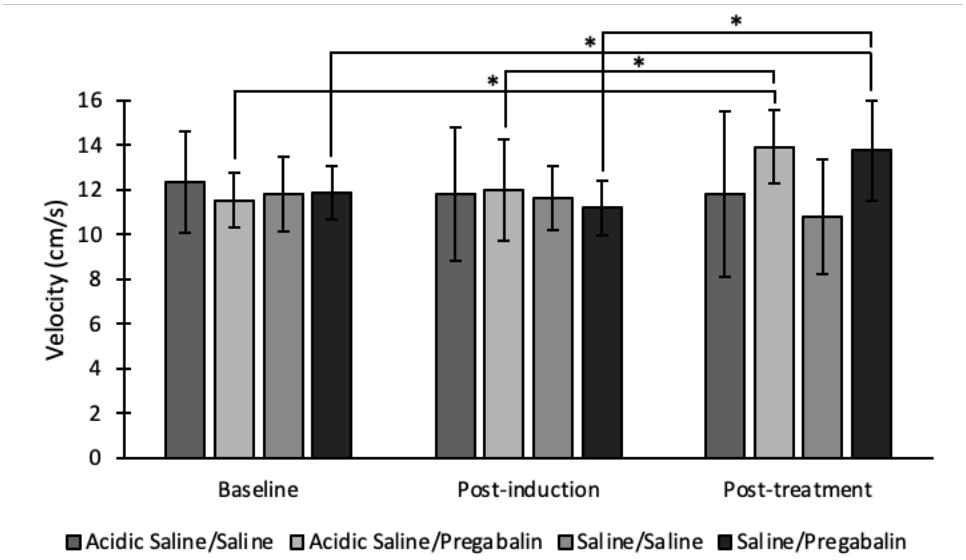
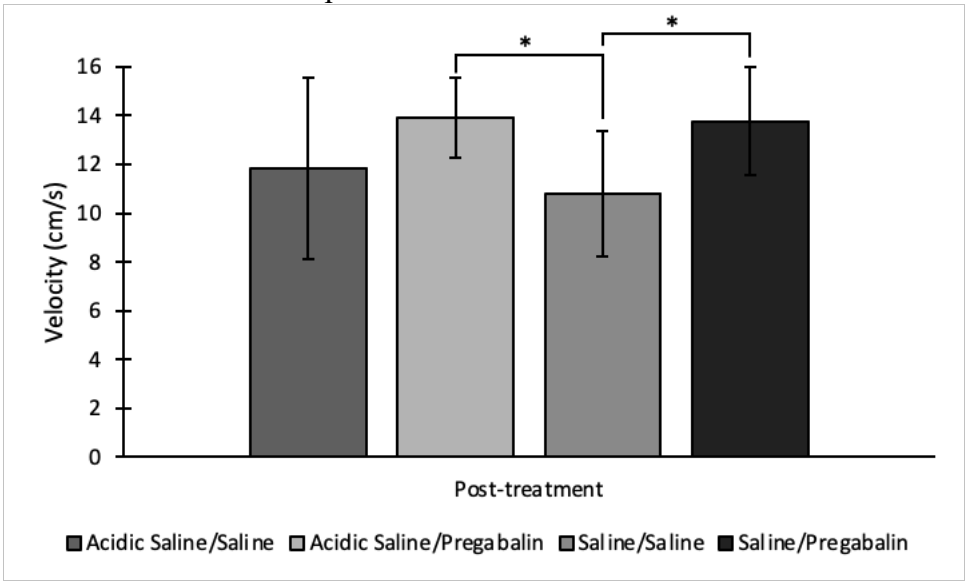


Figure 3.12: Estimated marginal means of outside mean velocity between conditions at post-treatment.



CHAPTER 4

DISCUSSION

Within the last half century, rapid breakthroughs in fibromyalgia research have led to a more nuanced understanding of this disorder and chronic widespread pain as a whole. However, despite significant progress, there are still many components of fibromyalgia that are much less understood, particularly regarding its complex affective comorbidities. Given the difficulty of parsing out this relationship in clinical patient samples, recently developed preclinical animal models might offer new utility to study the co-occurrence of affective behavior in more stringent and controlled systems. Notably, the acidic saline model developed by Sluka et al. (2001) has been consistently validated as inducing a long-lasting bilateral mechanical hyperalgesia in rodents, similar to the presentation of chronic widespread pain in fibromyalgia patients. However, there is limited research examining whether this model also produces anxiety as a comorbidity alongside acid-induced hyperalgesia. Successful co-induction of anxiety-like behavior in the acidic saline model would increase its validity as being analogous to the nuanced presentation of fibromyalgia syndrome in humans, increasing the model's utility and desirability for future preclinical research.

Thus, the present study replicated Sluka et al.'s acidic saline model (2001) in a group of rats using repeated, intramuscular injections of saline at pH 4.0. The model was also extended to assess pregabalin's efficacy as a post-induction, anxiolytic treatment. To do so, animals were randomly assigned to a pain condition (4.0 pH acidic saline vs. 0.9%

normal saline) and then to a treatment condition (pregabalin vs. 0.9% normal saline), resulting in four final groups: acidic saline/saline, acidic saline/pregabalin, saline/saline, and saline/pregabalin. To measure anxiety as a dimension of negative affect in rats, the open field test was used to measure distance traveled, mean velocity of travel, and total number of rears, which were then statistically compared across the four conditions and across three different time points (baseline, post-induction, post-treatment). Significant between-group differences in locomotion or exploration could then be used to interpret anxiety-like behavior, hind paw sensitivity, and exploratory drive in our sample.

While analysis of mechanical paw withdrawal thresholds was outside the scope of this thesis, a brief summary of the findings was included to guide interpretation of open field data. Based on post-induction and post-treatment assessments, significant differences were found between conditions, which were consistent with the acidic saline model and with pregabalin's efficacy as an analgesic. Specifically, at post-induction, the mechanical thresholds of the acidic saline groups were significantly reduced compared to vehicle controls, indicating bilateral mechanical hyperalgesia had been successfully induced according to Sluka et al. (2001). As expected, at post-treatment, the acidic saline/saline condition exhibited lowest paw withdrawal thresholds relative to the other groups, whereas the acidic saline/pregabalin condition showed thresholds similar to that of controls. Thus, pregabalin treatment is believed to have significantly decreased the mechanical hyperalgesia induced by repeated acid injections. As a whole, findings from mechanical thresholds indicate induction and treatment of experimental pain was successful and in line with expectations.

4.1 Does the Acidic Saline Model Increase Anxiety-Like Behavior?

In the present study, the acidic saline models were expected to exhibit greater anxiety-like behavior than controls after pain induction, which could be indicated by differences in total, central, or peripheral locomotion based on pain condition at post-induction. Specifically, we hypothesized that animals in the acidic saline conditions would travel less distance and at a slower velocity at post-induction than vehicle controls. However, the main effects of condition on distance and mean velocity of travel were not significant regardless of the area of the chamber measured and, more importantly, were not significantly different across pain conditions at time 2. Additionally, while two main effects of time were found for center distance and total mean velocity, these effects represented global changes in locomotion rather than differences based on pain condition. Based on these findings, it cannot be said that the acidic saline model altered open field locomotion in a manner significant enough to indicate a corresponding increase in anxiety-like behavior.

In addition, rearing was assessed as a third behavioral measure during open field testing, where post-induction differences in rearing between pain conditions could indicate differences in hind paw sensitivity or changes in exploratory drive. It was hypothesized that the acidic saline conditions would rear less than vehicle controls after pain induction. However, comparison of total rears across time and condition did not reveal any significant differences across pain conditions, and, furthermore, there was no effect of pain condition on rearing behavior exhibited post-induction. This may imply that exploratory behavior is unaffected by the acidic saline model, as rearing did not change regardless of whether the model was present or not. A global main effect of time was also significant, such that

animals reared more often at baseline than after pain induction or after treatment. However, this finding was generally expected because initial exposure to novel paradigms usually elicits an increase in exploratory drive.

In general, these findings were in contrast to those reported by Liu and colleagues (2014), the only other study to evaluate negative affect in conjunction with the acidic saline paradigm (2001). In addition to open field, Liu et al. (2014) utilized four other behavioral tests (elevated plus maze, sucrose consumption, sucrose preference, and forced swimming) to measure anxio-depressive comorbidity in rats repeatedly injected with acid. In the open field test specifically, animals in the acidic saline condition traveled significantly shorter durations in the center than did the vehicle controls. However, the present study failed to find a similar effect of pain condition on differences in locomotor behavior or exploratory behavior measured by the open field test, and, ultimately, anxiety-like behavior in the acidic saline condition did not surpass that of saline controls. Based on these results, Sluka et al.'s acidic saline model (2001) did not induce strong enough changes in anxiety-like behavior measured by the open field test in this study.

4.2 Is Pregabalin Effective as Anxiolytic Treatment?

The second aim of this thesis was to evaluate pregabalin's efficacy as an anxiolytic treatment in the acidic saline model, which could be indicated by condition-based differences during post-treatment testing; specifically, animals treated with pregabalin were expected to exhibit decreased anxiety-like behavior, e.g., increased locomotion, and decreased hind paw sensitivity, e.g., increased rearing, after the drug treatment had been administered. It was hypothesized that while the acidic saline/saline condition would travel the least distance and at the slowest velocity of all four groups at post-treatment, the acidic

saline/pregabalin condition would travel at a distance and velocity comparable to vehicle controls. Additionally, the acidic saline/pregabalin condition was expected to rear comparably to controls during post-treatment testing, whereas the acidic saline/saline animals were expected to rear the least out of all the groups.

Further analysis of simple effects for distance and velocity measured at post-treatment indicated that pregabalin significantly increased locomotion in the open field but had no effect on exploratory rearing. Overall, animals who received pregabalin not only traveled at a faster overall velocity but also traveled more distance in the field's center and in total after administration of the drug treatment compared to controls, regardless of whether the acidic saline model was present or not. Within in the acidic saline model specifically, animals that received pregabalin traveled significantly farther in the center and faster overall than those that did not. While increases in locomotion were only seen after pregabalin was administered, it cannot be determined whether this global trend represents an anxiolytic effect as the acidic saline model did not induce strong enough changes in anxiety-like behavior to be measured by post-induction open field testing.

These findings are somewhat in contrast to previous research that found pregabalin to have both locomotor-reducing and ataxic (coordination-reducing) effects in rats (Vartanian et al., 2006). This may be due to pregabalin's anticonvulsant features (French et al., 2003; Miller et al., 2003; Arroyo et al., 2004), for which it was originally marketed as a treatment for epilepsy before becoming approved to treat fibromyalgia due to its unanticipated analgesic and anxiolytic effects in patients (Boomershine, 2010; Ben-Menachem, 2004; Zimcikova et al., 2017). Vartanian and colleagues (2006) found that high dosages of pregabalin between 30-300 mg/kg produced ataxic symptoms in rats and also

reduced spontaneous locomotion (measured by travel distance). However, in the present study, pregabalin was not found to inhibit animal activity when administered at 30 mg/kg, the same dosage as that which obtunded rat activity in Vartanian et al. (2006). Instead, this dosage of pregabalin was found to increase both distance and velocity of locomotion measured in open field after administration of the drug treatment, regardless of the presence of the acidic saline model. However, because anxiety-like behavior did not significantly differ across conditions when measured at post-induction, we cannot say whether this subsequent increase in locomotion after pregabalin treatment represents the drug having an anxiolytic effect on behavior.

4.3 Limitations and Future Directions

Due to the limited scope of preclinical research surrounding affective comorbidity in preclinical models of fibromyalgia, it was especially important to determine whether the acidic saline model developed by Sluka et al. (2001) could also replicate anxious comorbidity in rats, and, subsequently, whether pregabalin treatment might induce an opposing anxiolytic effect. While Liu et al. (2014) found acid-induced pain to exacerbate anxio-depressive behaviors in rats, we did not find a similar effect of the model when assessing anxiety-like behavior in the open field test, as there was no condition-based difference between locomotion or exploration measured after pain induction. Broadly, pregabalin was found to increase locomotor behavior measured after administration of the drug treatment, though this effect was global and did not differ regardless of whether the acidic saline model was present or not.

Due to the design of the study, it is possible that repeated exposure to the open field paradigm across multiple time points may have resulted in a test-retest effect as animals

exhibited a decline in exploratory behavior over the study period. According to the theory, the novelty of the open field is central to the test's ability to accurately measure anxiety-like behavior in rats (Gould, Dao, & Kovacsics, 2009); however, the use of repeated measurements of open field is possible to have diminished its novelty over each subsequent test, which could very well change how the animals behave within the paradigm over time. If this were the case, the presence of any anxious comorbidity induced by the acidic saline model would be unlikely to be picked up by repeated testing of the open field paradigm.

Furthermore, because pregabalin was administered at a dosage of 30 mg/kg, animals were specifically selected to ensure a consistent weight range between 225-250 g. If there were considerable differences within the sample that were unrelated to weight, there could be a conceivable effect of these outside variables on our measurements. For example, it is possible that age could influence differences in locomotor and exploratory behavior if the sample contained animals of significantly varying ages. However, while we were aware of this potential confound, the test-retest effect was suspected to be primarily responsible for the variance seen in open field locomotion and exploration.

Overall, further investigation of affective dimensions within Sluka et al.'s model is greatly warranted based on conflicting findings between Liu et al. (2014) and the current study. At this point, we cannot determine what is contributing to the aspect of anxiety seen in acidic saline model, and, thus, future studies ought to assess whether other behavioral paradigms, such as the elevated plus maze, are more effective than the open field test at teasing apart this component. Additional replications of the acidic saline model should also examine whether the effects of acid-induced pain extend beyond sensory and affective components. Importantly, fibromyalgia also shows significant comorbidity with excessive

fatigue and memory impairment in human patients, however, no study has assessed whether cognition-based impairment exists in the acidic saline model. Various behavioral paradigms could be used to assess cognition in a rodent sample, such as the rat gambling task, Morris water task, or radial arm maze, among others.

Ultimately, preclinical models have been indispensable in expanding our understanding of fibromyalgia and chronic widespread pain, as these techniques allow researchers to more carefully isolate and scrutinize the condition's complex components in animals. Sluka et al.'s acidic saline model, in particular, shows great utility as a validated method with which to study fibromyalgia in rodents, calling for a more comprehensive assessment of its potential comorbidities.

CHAPTER 5

CONCLUSION

The present study utilized the open field paradigm to test the following two objectives: firstly, we examined whether Sluka et al.'s (2001) acidic saline model of fibromyalgia induces anxious comorbidity in rats; secondly, we evaluated whether pregabalin is efficacious as a subsequent anxiolytic treatment. Thus, the acidic saline model was hypothesized to exhibit greater anxiety-like behavior and hind paw sensitivity than controls after pain induction and were expected to travel less distance, travel at a slower velocity, and to rear less often than vehicle controls. It was also hypothesized that pregabalin treatment would decrease anxiety-like behavior and hind paw sensitivity indicated as an increase in locomotion and rearing during subsequent open field tests.

Overall, results revealed that (1) there was no significant difference for the distance or mean velocity traveled across pain conditions after pain induction, regardless of the area of the chamber measured, (2) on average, animals reared more at baseline, though, again, there was no significant difference between conditions, and (3) pregabalin produced a global increase in locomotor behavior, though this effect did not differ regardless of whether the acidic saline model was present or not. Based on these findings, further investigation of affective dimensions within Sluka et al.'s model is greatly warranted, wherein future replications of the acidic saline model should examine whether the effects of acid-induced pain extend beyond sensory and affective components.

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BIOGRAPHICAL INFORMATION

Michelle Bland is a senior undergraduate student at the University of Texas at Arlington who is pursuing an Honors Bachelor of Science in Biology and an Honors Bachelor of Science in Psychology with two minors in Neuroscience and Biochemistry. In 2018, Michelle began participating in university research and has since made major contributions to four different labs in the Biology, Psychology, and Chemistry departments. As an undergraduate research assistant, she has prior experience with health and aging research under Dr. Robert Gatchel, for which she has published two articles on the epidemiology and treatment of chronic pain in *Pain Management* and the *Journal of Applied Biobehavioral Research*. As of 2021, Michelle's ongoing research focuses on social determinants of physical and mental health under Dr. Lauri Jensen-Campbell, preclinical pain models of anxiety and cognition under Dr. Perry Fuchs, and target-directed total synthesis of organic oxazolone molecules under Dr. Carl Lovely, respectively. Alongside her studies and research interests, Michelle is the current president of the Psi Chi International Honor Society in Psychology chapter at the University of Texas at Arlington and has held the position since 2019. Following graduation in spring 2021, she intends to pursue a dual doctoral program to obtain an M.D. and Ph.D.