THE EFFECTS OF HYPERBARIC OXYGEN THERAPY ON PAIN THRESHOLDS AND ANXIODEPRESSIVE BEHAVIORS IN A PRE-CLINICAL FIBROMYALGIA PAIN MODEL

by

CASSIE MAE ARGENBRIGHT

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Abstract

THE EFFECTS OF HYPERBARIC OXYGEN THERAPY ON PAIN THRESHOLDS AND ANXIODEPRESSIVE BEHAVIORS IN A PRE-CLINICAL FIBROMYALGIA PAIN MODEL

Cassie Mae Argenbright, BS

The University of Texas at Arlington, 2021

Supervising Professor: Perry Fuchs

Fibromyalgia (FM) is a chronic, widespread pain disorder generally of a non-inflammatory nature. FM has many known affective and cognitive comorbidities, including fatigue, sleep disturbances, cognitive deficits, and mood disturbances. As a result, FM treatment approaches are mixed in nature and lack a robust characterization that address symptoms on a sensory, affective, and cognitive level. However, there is promise in the implementation of hyperbaric oxygen therapy (HBOT) for alleviating fibromyalgia pain and comorbidities, despite no work investigating the efficacy of this treatment in prominent preclinical FM models. Thus, this project aims to investigate the affective components, specifically anhedonia and anxiety, associated with an acidic saline model of fibromyalgia in rats. Additionally, we seek to find evidence for the potential efficacy of HBOT in the treatment of a pre-clinical model of fibromyalgia and its associated comorbidities.

In this study, forty-eight female Sprague Dawley rats were randomized to a fibromyalgia pain condition or a saline control condition. After verification of the induction of the model using measures of mechanical thresholds, animals were then randomized to receive two 60-minute treatments of hyperbaric oxygen therapy at 2.0 atmospheric absolute (ATA, pressure equivalent

to a depth of 33 feet of sea water) or a control treatment with the absence of oxygen/pressure manipulations above 1 ATA (sea level). After the initial treatment session, mechanical thresholds were taken every 24 hours to investigate the effects of hyperbaric oxygen therapy after one session, two sessions, and the period following treatment. Additionally, animals were exposed to an in-cage sucrose preference test for 72 hours, immediately following their second treatment session, to measure between-group anhedonic changes in behavior. 24 hours following treatment, animals were tested for between-group differences using the open-field paradigm, which assessed anxiety-like behavior through changes in locomotion. Results revealed that the acidic saline model was efficacious in replicating pain thresholds indicative of fibromyalgia-like pain. However, data did not provide support for the presence of anxio-depressive comorbidities associated with FM. Additionally, HBOT did not effectively increase mechanical thresholds as expected. Future studies should seek to identify the experimental circumstances within which the negative affective comorbidities associated with FM are presented through the acidic saline model. Additionally, further investigation into the exposure-response relationship between HBOT and the acidic saline model could provide insights into the mechanisms under which this preclinical representation of FM operates.

Keywords: fibromyalgia, hyperbaric oxygen therapy, anhedonia, depression, anxiety, affective, sucrose preference test, open field

Table of Contents

List of Illustrations

Chapter 1

INTRODUCTION

1.1 The Fibromyalgia Pain Experience

Chronic widespread pain disorders, such as fibromyalgia, are highly represented in our population but well understudied. Upwards of 2% of the U.S. population (Centers for Disease Control and Prevention, 2020) and an estimated 3-6% of the world population (Wray, 2015) suffers from fibromyalgia. Age appears to be a risk factor, such that fibromyalgia diagnosis rates increase with age, with approximately 8% of the U.S. population qualifying to be diagnosed with fibromyalgia by the age of 80 (Wray, 2015), as well as gender, where women are 2 times more likely to be diagnosed (Centers for Disease Control and Prevention, 2020) and represent 75-90% of individuals diagnosed (Wray, 2015). A lack of robust treatment methods for fibromyalgia has resulted in a large fiscal and societal burden, with economic evidence implicating that our current treatment approaches and clinical understandings of the disorder are simply inadequate for treating fibromyalgia. In fact, the National Fibromyalgia and Chronic Pain Association has assessed this issue from a more macro perspective, suggesting fibromyalgia accounts for 1-2% of the nation's overall productivity loss, with U.S. national estimated costs being between $12-14 billion per year (Wray, 2016). As of 2016, the mean annual cost for adult fibromyalgia patient care was $3,804, with patient out of pocket expenses including consultations with health care professionals, over-the-counter medications, and employment of home health aides, to name a few (Lacasse et al., 2016). Working participants reported having lost an average of more than 5 work-days, with non-working patients having reported a loss of over 25 productive days, in just a 3 month period (Lacasse et al., 2016). These studies only illuminate the increasing gap of lost productivity due to fibromyalgia and the increasing cost for traditional medicinal treatment

routes. The National Fibromyalgia Association showed that as of 2012, nearly $1,500 a year was being spent a year out-of-pocket by diagnosed individuals on non-traditional therapeutic pain management methods (The National Fibromyalgia Association, 2020). This patient trend towards the search for alternative treatment modalities outside of a typical clinical management regimen implies a crucial problem for those suffering from this type of chronic pain: *Current clinical treatment approaches are not effectively treating fibromyalgia pain as a whole.* Understanding this clinical challenge provides the foundation for the investigation of a robust and efficacious treatment plan for fibromyalgia patients.

1.1.1 Fibromyalgia Diagnosis

As a result of a mixed acceptance from the medical community, initial efforts to understand the nature of fibromyalgia pain had employed verbal descriptors to aid in its definition. This came primarily from the understanding that general clinical descriptions involving “achiness” and “stiffness” do not fully elucidate the actual complexities of pain sensations associated with this disorder (Leavitt et al., 1986). Though fibromyalgia is characterized by a lack of joint or muscle inflammation, its symptom similarity to disorders that produce joint, muscle, and tissue damage has led the condition being commonly categorized with rheumatic disorders, primarily rheumatoid arthritis, and subsequently referred to treatment through rheumatologists (American College of Rheumatology, 2021a; American College of Rheumatology, 2021b). Further, the overlap in presentation of symptoms and concomitance between fibromyalgia and rheumatoid arthritis has led early studies to seek to discriminate fibromyalgia type pain from that of rheumatoid arthritis and other arthridites (RA). Results have implicated a unique nature of this chronic pain form, markedly described using words such as “radiating”, “steady”, “gnawing”, “unlocalized”, “crushing”, “vicious”, and “exhausting”, more

so than individuals diagnosed with RA or osteoarthritis (Leavitt et al., 1986; Marques et al., 2001).

Further investigation beyond verbal descriptors has revealed that fibromyalgia pain is more often described by patients to not be merely localized to joints, but largely present in areas such as their upper and lower back, hips, arms, thighs, and head (Leavitt et al., 1986; Burckhardt, Clark & Bennett, 1992), making for a unique characterization of fibromyalgia as a non-inflammatory chronic pain disorder in comparison to much more widely understood pain disorders. Because of this, the American College of Rheumatology (ACR) 1990 diagnostic criteria for fibromyalgia included the identification of mild or greater tenderness in 11 out 18 tender points, among the presence of widespread pain (Wolfe et al., 1990). Despite the advancement, these diagnostic criteria served to be too divisive in the identification of pain tender points, and too vague in the identification of symptom concomitance and quantification of widespread pain (Wolfe, 2010). In later years, the ACR has introduced revisions to these criteria to include measures of symptom severity and widespread pain, regardless of other clinical diagnoses (Wolfe et al., 2011; Wolfe et al., 2016). For measures of symptom severity, the Symptom Severity (SS) scale was introduced, within which patients are asked to identify the intensity of symptoms, such as restless sleep, fatigue, and difficulties thinking or remembering, on a 0-3 Likert-type scale (Wolfe et al., 2011). Patients are also asked to identify if they have experienced cramps in the lower abdomen, depression, or headaches in the past 6 months (Wolfe et al., 2011). Summed scores can range from a minimum of 0, to a maximum of 12, quantifying a measure of patient symptom severity (Wolfe et al., 2011). For quantification of widespread pain, the widespread pain index (WPI) was introduced, where patients are asked to identify pain presence on each side of the body within the last week, in areas such as their upper back, lower

back, upper arm, lower arm, etc. (Wolfe et al., 2011). Pain presence is rated as either a 0 ("absent") or 1 ("present"), with a maximum score of 19 (Wolfe et al., 2011). The current 2016 ACR diagnostic criteria for fibromyalgia in adults now includes: 1) generalized pain in at least 4 of 5 regions; 2) symptom presence for at least 3 months; 3) a WPI score ≥ 7 and a SS score ≥ 5, *or* a WPI score of 4 - 6 and a SS score ≥ 9; and 4) diagnosis that does not exclude the presence of other clinical disorders (Wolfe et al., 2016). These more current criteria have allowed for fibromyalgia to be assessed as a *continuum* of its unique symptoms, providing a more precise quantification of widespread pain, and reducing misclassification or discrimination based on the presence of coexisting disorders (Wolfe et al., 2016).

The unlocalized and unprecedented nature of fibromyalgia pain has yet to be completely accepted as a chronic pain disorder within the scientific community, largely because of the combination of difficulty in attaining a fibromyalgia diagnosis, due in part to the prevalence of various cognitive and/or affective deficits, and difficulty differentiating fibromyalgia from somatoform or psychosomatic disorders. Many members of the medical community express strong dissent towards the inclusion of fibromyalgia as an authentic pain disorder due to its lack of palpable, non-subjective symptoms, tissue damage, and perceived tendency to encourage self-victimization (Ehrlich, 2003a; Ehrlich 2003b; Hadler & Greenhalgh, 2005; Huibers & Wessely, 2006; Wolfe, 2009). Furthermore, despite the growing scientific literature surrounding etiological understanding of this disorder, patients often still encounter medical professionals who label them as "complainers" who primarily suffer from "psychological problems" (Cunningham & Jillings, 2006). However, systematic meta-analyses have highlighted the therapeutic importance of receiving a fibromyalgia diagnosis for patient overall well-being by

promoting feelings of relief, validation, credibility, clarity, and hope for the future (Sim & Madden, 2008).

1.1.2 Fibromyalgia Comorbidities

Fibromyalgia is often exacerbated, and occasionally defined, by its comorbidities, especially in the development of preclinical representations of the disorder. A fibromyalgia diagnosis is not discriminatory, and co-occurrence rates with various medical or sensory disorders have been reported as high as 36.7% with lupus, 12.8% with IBS, 23.8% with migraines, 16.8% with osteoarthritis, and 21.1% with inflammatory arthritides, such as rheumatoid arthritis (Cole et al., 2006; Marcus & Bhowmick, 2013; Wolfe et al., 2011; Haliloglu et al., 2014). However, many such comorbidities frequently associated with fibromyalgia pain, and those most often targeted by treatment clinical treatment regimens, are affective and cognitive in nature: depression and anxiety, difficulty sleeping, as well as certain cognitive deficits (Goldenberg et al., 2008; Ambrose, Gracely, & Glass, 2012; Aguglia et al., 2011). A large sample study investigating some of these affective fibromyalgia comorbidities reported through a health insurance database, indicated that 12.3% of patients had depression, 5.4% had anxiety, 7.7% had anxiety-like symptoms, and 5.7% suffered from sleep disorders (Berger et al., 2007). Another large sample study indicated that 44.7% of patients reported taking medication for depression, 43.6% reported medication use for anxiety, 67.5% reported issues with insomnia, and 43.6% had reported issues of memory loss (Walitt et al., 2015). These comorbidities and prevalence rates of fibromyalgia related disorders only solidifies our previous understanding of pain as a whole, as more than a mere sensory stimulus, but rather as a sensory, cognitive, and affective *experience*. The current use of the SS scale in fibromyalgia diagnostic criteria has only served to emphasize the relationship between fibromyalgia pain and its associated affective

comorbidities, with SS scale scores showing a positive correlation with anxiety and depression diagnoses among fibromyalgia patients, as well as higher rates of pain and sleep disturbances among fibromyalgia patients diagnosed with anxiety and depression (Singh & Kaul, 2018). The close relationship between fibromyalgia and its association with affective disturbances demonstrates the need for diagnostic methods to be inclusive of the negative emotionality associated with the disorder, in order to gain sufficient understanding aimed at improving patient quality of life.

1.1.3 Fibromyalgia Treatments

As a result of an incomplete etiological understanding, but increasing acceptance of an approach towards diagnosis as a unique continuum of sensory, cognitive, and affective components, fibromyalgia treatments currently focus on patient symptom management. While various over-the-counter NSAIDs, prescription opioids, and other pharmacological cocktails are often employed for disorder management, there are currently only three FDA approved drugs for the treatment of fibromyalgia: duloxetine, milnacipran, and pregabalin (Forte et al., 2015). Non-pharmacological fibromyalgia treatments have been identified and organized into four primary categories: physical therapies, exercise, cognitive-behavioral therapies (CBT), and alternative or complementary treatments (Cassisi et al., 2008). Treatment regimens inclusive of physical therapies have provided some evidence for beneficial outcomes among fibromyalgia patients, with cranial electrical stimulation (Taylor et al., 2013), acupuncture (Vas et al., 2016), and massage therapy (Kalichman, 2010) all generally reducing sensory discomfort for at least a short period of time. In studies investigating the use of aerobic exercise, meta-analyses have revealed strict exercise adherence for long periods of time can improve fibromyalgia's impact on daily life, regardless of whether land-based or pool-based exercise is utilized with any combination

with complementary exercise training (Häuser et al., 2010; Thomas & Boltman, 2010). However, these same studies have only shown significant efficacy for individuals who exercise 2-3 times per week, for at least 4-6 weeks (Häuser et al., 2010), posing significant challenges in regard to noncompliance and attrition rates (Thomas & Boltman, 2010). Meta-analyses of cognitive-behavioral interventions to employ stress reduction and mindfulness techniques among patients indicated that CBT has been beneficial for affective fibromyalgia related challenges, such as pain self-efficacy and depressed mood, but there is little to no evidence for reduction of actual pain reported, fatigue, sleep disturbances, or health-related quality of life (HRQoL) (Bernardy et al., 2010). Alternative or complementary treatment modalities for fibromyalgia, such as music therapy (Alparslan et al., 2016) and aromatherapy utilizing essential oils (Ko et al., 2007), have provided evidence for reduced sensory discomfort among participants, but either have failed to investigate or produce significant results for affective measures on quality of life. Results of the various approaches to fibromyalgia pain and fibromyalgia-related symptom management have shown continued failure to produce an encompassing methodology that addresses this form of musculoskeletal pain as a whole, inclusive of sensory, affective, and cognitive comorbidities.

1.1.4 Etiology of Fibromyalgia

Despite there being no one identified etiological cause of fibromyalgia, a growing literature base investigating some of the biological mechanisms associated with this type of pain has identified some important relational factors. A primary theory in etiological understandings of fibromyalgia involves central sensitization (Bellato et al., 2012; Sluka & Clauw, 2016), or an abnormal state of responsiveness as a result of increased membrane excitability and synaptic efficacy in nociceptive pathways (Latremoliere & Woolf, 2009), as displayed through fibromyalgia related hyperalgesia and allodynia (Staud et al., 2001). Fibromyalgia patients also

display an increase in temporal summation, or “wind up” — a phenomenon within which repeated stimuli of the same intensity generate an increased rate of perceived pain (Staud et al., 2001), supporting the theory of abnormal central sensitization.

Various central nervous system neurochemicals have also been implicated in fibromyalgia pathogenesis, including serotonin, norepinephrine, dopamine, glutamate, and substance P. Serotonin has long been known to modulate pain and pain-related behaviors along both central and peripheral nociceptive pathways (Neugebauer, 2020). Decreased rates of this pain modulating neurotransmitter have been seen in both cerebrospinal fluid (CSF) (Russell et al., 1992) and numerous studies investigating serum (Alnigenis & Barland, 2001). Decreased levels of norepinephrine have also been recorded in CSF of fibromyalgia patients (Russell et al., 1992), and the role of both serotonin and norepinephrine are further implicated in fibromyalgia etiology through the efficacy of serotonin-norepinephrine reuptake inhibitors (SNRIs) in treatment regimens for restoring their analgesic properties (Sluka & Clauw, 2016). Studies investigating the role of dopamine in fibromyalgia pathogenesis have implied the possibility of a disrupted endogenous dopamine release system, with those suffering from fibromyalgia showing a lack of dopaminergic response to experimental pain and an increase in dopamine response to typically non-painful stimuli (Wood et al., 2007b). Further studies have also implicated decreased presynaptic activity of dopaminergic neurons (Wood et al., 2007a), providing more evidence for disruption of a pain modulation system having a strong role in affective disorders as well (Jarcho et al., 2012). In terms of glutamate as a neurochemical contributor, increased glutamatergic activity has been identified in the insula of the brain in fibromyalgia patients (Harris et al., 2008; Harris et al., 2009), a vital area for pain processing. Holton and colleagues (2012) even identified decreased dietary consumption of glutamate significantly reduced

fibromyalgia related symptoms, such as IBS. The activation of glial cells provides further evidence for both glutamate and substance P-related fibromyalgia pathogenesis, due to their known role of releasing neuroactive chemicals, such as these, from primary afferent fibers in the spinal cord, which can amplify associated spinal cord excitability (Bellato et al., 2012; Watkins, Milligan, & Maier, 2001). In support of this theory, CSF samples collected from fibromyalgia patients has been recorded as having up to 3 times higher rates of substance P than that of controls (Russell et al., 1994).

While the previously discussed biological mechanisms serve as just a handful of etiological theories in the developing bodies of literature surrounding fibromyalgia pain, the scope of this thesis is not to serve as a comprehensive review but, rather, to highlight the complex relationship between fibromyalgia and many of the endogenous systems implicated in various types of pain and their affective comorbid disorders.

1.2 Preclinical Representations of Fibromyalgia

The study of various pain disorders in preclinical models as a precursor to clinical model studies is essential in gaining knowledge towards etiology, drug development, and grasping the greater impact of these conditions on patient quality of life. There are many models actively used in preclinical disciplines to study various types of chronic pain, including models for arthritis, neuropathy, spinal cord injury, and many more. However, without a definitive etiological understanding of fibromyalgia, it has been increasingly difficult to develop a singular preclinical fibromyalgia model to study greater life impacts associated with this pain disorder (DeSantana et al., 2013). The handful of models developed for preclinical fibromyalgia research primarily focused on replicating fibromyalgia symptomology and management challenges, and include the hyperalgesia priming model (Dina, Levine & Green, 2008), the fatigue-induced muscle pain

model (Yokoyama et al., 2007), the biogenic amine depletion model (Nagakura et al., 2009), the cold stress model (Nishiyori et al., 2011), the sound stress model (Khasar et al., 2009), the sub-chronic swim stress model (Quintero et al., 2000), and the acidic saline model (Sluka et al., 2001). For the scope of this thesis, we primarily focused on Sluka et al.'s acidic saline model (2001) due to long-lasting incorporation of both central and peripheral pain factors with the absence of peripheral tissue damage, replicable of fibromyalgia presentation in humans. The model utilizes repeated injections of 4.0 ± 0.1 pH saline into the left gastrocnemius muscle of the rodent to induce a bilateral hyperalgesia lasting up to 4 weeks (Sluka et al., 2001).

1.2.1 Pathophysiology of the Acidic Saline Model

The current understanding of the mechanisms of action associated with induction of hyperalgesia through repeated intramuscular injections is related to the activation of muscle afferent acid sensing ion channel (ASIC) 3, although these peripheral channels are not indicated in hypersensitivity maintenance of the model (Sluka et al., 2003; DeSantana et al., 2013). Following the second injection indicated by the model, increases in excitatory neurotransmitters and decreases in inhibitory neurotransmitters have been recorded in the rostral ventromedial medulla (RVM), implicating supraspinal mechanisms for the induction of associated hyperalgesia (Radhakrishnan & Sluka, 2009; DeSantana et al., 2013). In terms of maintenance of this hypersensitivity, studies using Sluka et al.'s model (2001) have provided evidence for an increase in neuronal receptive fields, as well as an increased sensitivity to both painful and non-painful stimuli, suggesting a central sensitization in animals (Sluka et al., 2003). There are strong implications for pain maintenance also being a result of increased spinal cord glutamate, as well as possibly glutamate mediated excitatory activity in the RVM (DeSantana et al., 2013). Overall, this model is best understood thus far to employ muscle afferent and supraspinal mechanisms for

induction of hyperalgesia, and both spinal and supraspinal central mechanisms for maintenance (DeSantana et al., 2013).

1.2.2 Affective Dimensions of the Acidic Saline Model

Previous research focused on the capacity of the acidic saline model to induce a comprehensive fibromyalgia-like experience, including the decreased pain thresholds and anxio-depressive comorbidities characteristic of the disorder, has produced contrasting results. Studies have indicated that the acidic saline model has been effective in mirroring both anxiety (Liu et al., 2014; Murasawa et al., 2020) and depression-like behaviors (Liu et al., 2014) across several preclinical measurements, while a more recent study by Argenbright et al. (unpublished) provided evidence that the acidic saline model may not be as efficacious in the replication of fibromyalgia related anxiety-like behaviors, as previously thought. While it is sufficiently understood that acid-induced hyperalgesia adequately models the sensory and possible etiological relationships associated with fibromyalgia, the conflicting results on the model's ability to induce different commonly comorbid negative emotionality calls for further investigation into the affective components induced by the model, as well as the mechanisms of action associated with.

1.3 Hyperbaric Oxygen Therapy (HBOT)

As with humans, there has been recent and emerging therapeutic approaches utilizing hyperbaric oxygen therapy (HBOT). HBOT is characterized by the intermittent inhalation of 100% oxygen in a sealed chamber that is pressurized to a level that is greater than that of sea level, or 1 atmospheric absolute (ATA) (Gill & Bell, 2004). At sea level, pound-force per square inch gauge (psig) is at zero, while atmospheric pressure is generally at 760 millimeters of mercury (mmHg), or 101.325 kilopascals (kPa). For every increasing atmospheric absolute, an

increase of approximately 15 psi is seen. Due to differential pressures between pressure within a container and the surrounding ambient barometric pressure, gauge pressure measurements are usually slightly altered in terms of atmospheres displayed and actual atmospheric absolutes (Davis & Hunt, 1977). This relationship between atmospheric absolutes and gauge pressure is illustrated in Table 1-1. Generally, early therapeutic approaches have utilized 2 to 3 times the atmospheric pressure found at sea level for the treatment of disorders such as decompression sickness, anemia, ischemia and hypoxia-related skin wounds, or lesions (Gill & Bell, 2004; Sahni, Singh, & John, 2003). As the research base and popularity of hyperbaric treatments has grown, there has been a marked increase in hyperoxia therapy for other disorders such as various types of pain (Sutherland et al., 2016), migraine (Bennett et al., 2015; Matera, Smith, & Lam, 2019), and neurological disorders (Sahni et al., 2003; Fischer & Barak, 2020). From a neurological approach, the benefits of hyperoxia have shown tremendous promise in both human and animal treatment models of autism spectrum disorder (Rossignol, 2007; Fischer & Barak, 2020), traumatic brain injury (Bennet, Trytko, & Jonker, 2012; Fischer & Barak, 2020), stroke (Sahni et al., 2003), cognitive impairment (Shwe et al., 2021), and Alzheimer's disease (Harch & Fogarty, 2018; Zhang & Le, 2010).

Table 1-1: Absolute pressure in comparison to gauge pressures at sea level and increasing atmospheric absolutes (Davis & Hunt, 1977).

Absolute Pressures		**Gauge Pressures**	
ATA	*mmHg (Torr)*	*psig*	*atm*
1	760	0	0
2	1520	14.7	1

3	2280	29.4	2
4	3040	44.0	3

Primary hyperbaric therapeutic effects are achieved through maximizing tissue oxygenation. While a portion of the oxygen found in the body is in solution, the majority of oxygen in the blood is bound to hemoglobin (Gill & Bell, 2004), and a normal measure of arterial hemoglobin oxygen saturation (SaO_2) is considered to be 97 ± 2% (Goldberg et al., 2012). Plasma oxygen concentrations found at sea level are typically 3 mL/L (Leach, Rees, & Wilmshurst, 1998). According to Henry's law, the amount of an ideal gas dissolved in solution is proportional to its partial pressure (Mechem, Manaker & Traub, 2014). Therefore, the amount of oxygen in plasma solution delivered to tissues increases in a pressurized state, as seen in HBOT (Gill & Bell, 2004). An increase in pressure from 101.325 kPa (1 ATA) to 303.975 kPa (3 ATA) increases plasma oxygen concentration to 60 mL/L, which maintains the oxygen requirement of tissues at rest without needing the other reservoir of oxygen typically bound to blood hemoglobin (Leach et al., 1998; Gill & Bell, 2004). This allows for maximized oxygenation of locations that may not be normally accessible to saturated hemoglobin, and promotes oxygenation in cases of impaired hemoglobin saturation (Gill & Bell, 2004).

1.3.1 The Implications of HBOT for Fibromyalgia

In the study of chronic pain, HBOT has already provided established evidence of anti-nociceptive and anti-inflammatory mechanisms (Sutherland et al., 2016), with efficacy of treatment having been shown in animal models of injury-related pain (Sümen, Çimşit, & Eroğlu, 2001), carrageenan induced paw edema (Wilson et al., 2006), arthritic pain (Wilson et al., 2007), and neuropathic pain (Thompson et al., 2010). Within the clinical literature, there have been

select studies that have focused on the possible therapeutic benefits of HBOT in individuals suffering from fibromyalgia pain. A randomized controlled trial conducted by Yildiz et al. (2004) produced results indicating that patients diagnosed with fibromyalgia experienced a significant decrease in fibromyalgia associated tender points and visual analog scale scores, alongside an increase in pain thresholds. Efrati et al. (2015) also investigated the potential role of HBOT in a clinical fibromyalgia population, and similarly found that treatment was effective in increasing physical pain thresholds and decreasing tender point count. However, they also found a significant increase in physical functionality as measured by the Fibromyalgia Impact Questionnaire (FIQ), psychological distress as measured by the Symptom Check List, and an overall increase in quality of life (QoL) assessments (Efrati et al., 2015). Another more recent randomized controlled trial by Hadanny et al. (2018) showed improvement in women's scores on various measures of fibromyalgia pain, such as the widespread pain index, alongside improvement in various measures of PTSD. This year, a study was conducted by Curtis et al. (2021) to validate the ability of HBOT to both improve and maintain improvement in physical and affective fibromyalgia symptoms. Results from this study also indicated the same significant results as seen in other RCTs, with functional impairments symptoms, anxiety and depression symptoms, and sleep disturbances all being alleviated for 3 months following treatment (Curtis et al., 2021). This evidence for the use of HBOT in the attenuation of both negative physicality and emotionality, such as depression and anxiety-like behaviors, among various types of pain models and central nervous system disorders has persisted through both clinical (Cao et al., 2013; Feng & Li, 2017; Sutherland et al., 2016; Yan et al., 2015) and preclinical studies (Lim et al., 2017, Sutherland et al., 2016). With fibromyalgia being a disorder widely emphasized by its negative affective components, there is promise in the potential therapeutic benefits of hyperbaric

treatment for both alleviation of fibromyalgia related hyperalgesia, as well as its related affective comorbidities.

1.4 Purpose

While there is growing knowledge in the field surrounding fibromyalgia and its treatment related to HBOT, there is still substantial research to be done. With much of our knowledge translating into clinical work being based on information obtained from the animal models, it is important to understand the underlying mechanisms of these pain models more fully and the disorder characteristics these same models are efficacious at replicating. More specifically, it is crucial to understand to what extent these animal models might fully mirror the disorders and comorbidities we are studying, and to what extent we can utilize them for further experimental treatment studies. In this study, we will examine this question in more depth in relation to the acidic saline model of fibromyalgia in rats, as well as the potential efficacy of HBOT as a therapeutic approach. We will do so by comparing results on behavioral tests of mechanical hypersensitivity along with those examining depression, or anhedonia, and anxiety, across several groups of animals. Animals will be randomized to a pain condition: a) repeated injections of acidic saline or b) a saline vehicle control. Animals will also be randomized to a treatment condition: a) two HBOT treatments or b) two control treatments. Analysis of anhedonia using the sucrose preference test, pain thresholds using mechanical paw withdrawal thresholds, and anxiety-like behavior using the open field paradigm will allow us to determine the efficacious features of this preclinical fibromyalgia model in replicating the fibromyalgia pain experience as a whole. Additionally, this will allow us to gauge to what degree the administration of HBOT treatment is adequate, not only in measures of pain reduction, but also in the attenuation of fibromyalgia- related negative emotionality.

1.4.1 Hypotheses

In regard to mechanical thresholds, we anticipated: (1) animals who receive repeated intramuscular acidic saline injections to have significantly lower thresholds post-model induction, compared to those who receive saline vehicle control injections and (2) an increase in mechanical thresholds after each therapy session for animals who receive subsequent hyperbaric treatment following acidic saline injections, such that their thresholds will be comparable to that of controls. For affective measures of anhedonia, we predicted: (3) low levels of anhedonia in animals that are randomized to the saline vehicle control group despite their subsequent treatment randomization, which would be exhibited by increased rates of sucrose consumption and (4) animals randomized to the acidic saline pain condition who receive a control treatment to have the lowest sucrose consumption rates, displaying the greatest depression-like behavior. However, we also expected (5) animals who receive repeated intramuscular injections of acidic saline followed by subsequent hyperbaric treatment to exhibit lowered anhedonic responses following treatment, with sucrose consumption rates similar to that of control animals following their second treatment session. In relation to anxiety-like behavior, we anticipated (6) animals randomized to receive repeated acidic saline injections with subsequent control treatment to display the greatest anxiety-like behavior, whereas (7) animals in the same pain condition who received subsequent hyperbaric oxygen therapy would show less anxiety-like behaviors such that they will be comparable to controls, with (8) controls being expected to show very little anxiety-like behavior.

Chapter 2

2.1 METHODS

2.1.1 Subjects

Forty-eight female Sprague Dawley rats were utilized for this study. Animals were purchased from Charles River and ordered by weight requirement (225-250 g). Animals were singly housed, with access to food and water ad libitum. All animals were maintained on a 12-hour light/dark cycle. All procedures for this study were approved by the University of Texas at Arlington Institutional Animal Care and Use Committee (IACUC).

2.1.2 Procedure

Baseline mechanical paw withdrawal threshold test (MPWT) measures were taken for all animals before being randomized to either the acidic saline (FM) pain condition (Sluka et al., 2001) or a saline vehicle control condition. For the induction of the acidic saline pain model of musculoskeletal pain, animals received a 4.0 ± 0.1 pH saline injection into the left gastrocnemius muscle while under anesthesia (isoflurane, 3% induction and 2% maintenance). Animals randomized to the saline control model received an injection of approximately 5.5 pH normal saline in the same location under identical anesthesia methods. After completion of the first half of the appropriate model randomization, animals underwent a post-injection one MPWT check to ensure there was no hypersensitivity in either hind paw. Any animals experiencing hypersensitivity post-injection one were excluded from further procedures. All animals were then be left for five days to allow the model to fully develop before undergoing the second half of the pain model or control model induction. On the fifth day, all animals underwent the exact same injection procedure they received on day one, indicating full assigned model induction. Animals

were allowed to habituate for 24 hours before undergoing another MPWT test to measure changes in post-induction withdrawal latencies.

Animals were then randomized to a hyperbaric oxygen therapy (HBOT) treatment condition or a control treatment condition. Animals randomly assigned to the HBOT treatment condition were subjected to 1-hour of 2.0 ATA. Animals assigned to the control treatment condition were placed in the chamber and experienced no manipulation of oxygen or pressure over the 1-hour period. 24 hours later, animals underwent a post-treatment one MPWT immediately before beginning their second hyperbaric or control treatment session. Subsequently, upon completion of the second treatment session, animals began an in-cage sucrose preference test, where measures of sucrose and water consumption were measured every 24 hours, for a total of 72 hours. 24 hours following the second treatment session, animals were subjected to a 5-minute open field measurement to analyze potential anxiety-like behaviors. Alongside daily measurements of sucrose consumption, animals underwent MPWT tests every 24 hours over the 72-hour period following the second treatment session. Taking measures of mechanical thresholds and anhedonic behaviors for 3 days following the second hyperbaric or control treatment session allowed us to adequately detect the presence of both acute and/or persistent therapeutic effects. Clarification for the experimental timeline is shown in Figure 2-1.

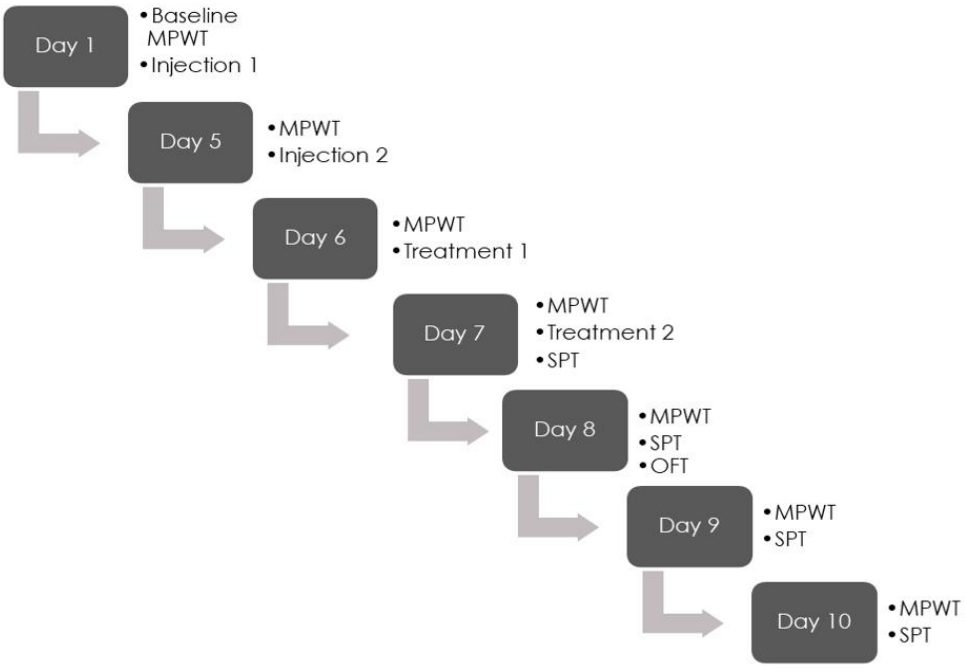

Figure 2-1: Experimental Timeline.

2.1.3 Mechanical paw withdrawal threshold (MPWT) testing

Animals were placed into a Plexiglass chamber with a mesh floor bottom that allows access to the plantar surface of the hind paws. Animals were allowed to habituate for 10 minutes prior to mechanical stimulation. Mechanical sensitivity was measured using von Frey monofilaments (3.85, 5.68, 9.74, 18.39, 39.42, 77.3, 135.3, and 251.34 mN) and quantified using the up-down method (Dixon, 1980). Each trial began with the 9.74 mN filament delivered to the left hind paw for approximately 1 second, then similarly to the right hind paw. If no withdrawal response was observed (i.e. licking or paw withdrawal), the next highest filament force was used. If a withdrawal response was observed, the next lowest force was used. This procedure was repeated until there was no response from the animal at the highest force (251.34 mN) or until a total of 5 stimuli were administered. 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. This test was conducted 3 times, with the scores from each trial being averaged to determine the mean withdrawal threshold for the left and right hind paw of the animal. A combined mechanical threshold (CMT) score was then averaged from the right and left paw values for each MPWT test for statistical analysis.

2.1.4 Sucrose preference test (SPT)

A two-bottle cage paradigm was used within which one drinking bottle contained a sweetened sucrose solution (2% wt/vol concentration) and the other will contained standard reverse osmosis water. A 2% wt/vol solution was achieved by dissolving 10 g of sucrose into a 500 mL cage bottle. Volume measures (mL) of consumption from each bottle left in the cage were taken every 24 hours following the second treatment session to determine preference for sweetened versus normal water, implying the presence or absence of hedonic behaviors. Location of the bottles were also switched every 24 hours to combat any place preference behavior. Sucrose preference percentage rates were calculated as follows: (% = [sucrose intake / total fluid intake] x 100).

2.1.5 Open field paradigm (OF/OFT)

24 hours following completion of the second treatment or control treatment session, animals were subjected to an open field test (OFT), within which changes in behavioral and motor activity were recorded by a computer software to investigate implications of anxiety-like behavior. For this paradigm, animals were placed in the center of a circular open field chamber

with a wooden base and aluminum sheet metal walls. Activity was recorded and quantified using Ethovision, a video tracking program that recorded total distance travelled and mean velocity in both the center and the along the perimeter of the chamber. Ethovision recorded behavioral and motor activity for a span of 5 minutes without interfering with the animal's natural behavioral repertoire. Quantifications were made using Ethovision to determine the amount of distance the animal travelled along the perimeter of the chamber, implicating more anxious behavior, as well as in the center of the chamber, implicating less anxious behavior.

2.1.6 Hyperbaric oxygen therapy treatment (HBOT)

Animals randomized to the hyperbaric treatment condition underwent two 60-minute sessions of 100% oxygen inhalation at a pressure of 2.0 ATA. The first treatment session occurred 24 hours after animals received their second injection of either 4.0 pH saline or a saline vehicle control. After their first treatment, animals were returned to their cage for 24 hours, before undergoing mechanical thresholds, and following to the second 60-minute treatment session. Animals randomized to the control treatment condition underwent the same procedure schedule as experimental animals but were instead placed in the HBOT chamber and experienced no manipulations of oxygenation or pressurization for 60 minutes during each control treatment session.

Chapter 3

RESULTS

3.1 Mechanical Thresholds by Condition over Time

To analyze changes in mechanical thresholds of animals over time, a 4 (condition) X 7 (time) mixed-model analysis of variance (ANOVA) was used, with condition as the between-subjects variable and time as the within-subjects variable. Significant main effects of condition, $F(3, 44) = 17.517$, $p < .001$, $\eta_p^2 = .544$, and time, $F(5, 220) = 24.894$, $p < .001$, $\eta_p^2 = .361$, were found. A significant time by condition interaction was also identified, $F(15, 220) = 4.061$, $p < .001$, $\eta_p^2 = .217$. Post hoc analyses were conducted using Fisher's Least Significant Difference (LSD), revealing that overall, animals in the acidic saline condition ($M = 293.70$, $SE = 16.199$; $M = 307.405$, $SE = 16.199$) had significantly lower thresholds than their control counterparts ($M = 394.706$, $SE = 16.199$; $M = 433.821$, $SE = 16.199$), regardless of time. In terms of time, baseline mechanical thresholds ($M = 449.469$, $SE = 2.662$) were significantly higher than thresholds at all other time points. Following full induction of the model, animals that received repeated insults of acidic saline ($M = 233.772$, $SE = 26.634$; $M = 237.939$, $SE = 26.634$) had significantly lower thresholds than their saline control counterparts ($M = 353.102$, $SE = 26.634$; $M = 420.942$, $SE = 26.634$). Analysis of mechanical thresholds following the first treatment revealed that animals in the FM condition that underwent HBOT ($M = 259.32$, $SE = 28.495$) showed no difference in thresholds compared to animals in the FM condition that received a control treatment ($M = 265.272$, $SE = 28.495$). However, all animals in the FM condition, despite treatment condition, showed significantly lower thresholds than all animals in the control condition that received either hyperbaric treatment ($M = 381.655$, $SE = 28.495$) or a control treatment ($M = 420.942$, $SE = 28.495$). The exact same unexpected mechanical threshold pattern emerged following the second treatment as well, with animals in the FM condition having underwent HBOT ($M = 259.851$, $SE = 25.029$) showing no significant difference in thresholds compared to FM animals

who received a control treatment (*M* = 268.891, *SE* = 25.029). There was no difference in thresholds seen in control animals, regardless of whether they received HBOT (*M* = 383.733, *SE* = 25.029) or a control treatment (*M* = 441.741, *SE* = 25.029). However, all FM animals had significantly lower thresholds than control animals, despite treatment conditions for all groups. This was contrary to our expectations, as we hypothesized that mechanical thresholds for animals in the FM condition that received HBOT would increase significantly after each treatment session. This same pattern was observed for analysis of mechanical thresholds collected 48 hours following the second treatment. FM animals showed no difference in thresholds between those that received HBOT (*M* = 313.455, *SE* = 24.781) or a control treatment (*M* = 301.647, *SE* = 24.781). There was also no significant difference among control animals, despite having received HBOT (*M* = 396.010, *SE* = 24.781) or a control treatment (*M* = 425.077, *SE* = 24.781). All FM animals showed significantly lower thresholds than control animals, despite differences in treatment conditions between groups. However, 72 hours following the second treatment, all groups showed significantly different thresholds from each other. FM animals that received HBOT (*M* = 239.175, *SE* = 25.768) exhibited the lowest withdrawal thresholds, followed by FM animals that received a control treatment (*M* = 323.318, *SE* = 25.768), and control animals who received HBOT (*M* = 407.357, *SE* = 25.768). Control animals that received a control treatment exhibited the highest withdrawal thresholds 72 hours following the second treatment session (*M* = 450.677, *SE* = 25.768) (Figure 3-1).

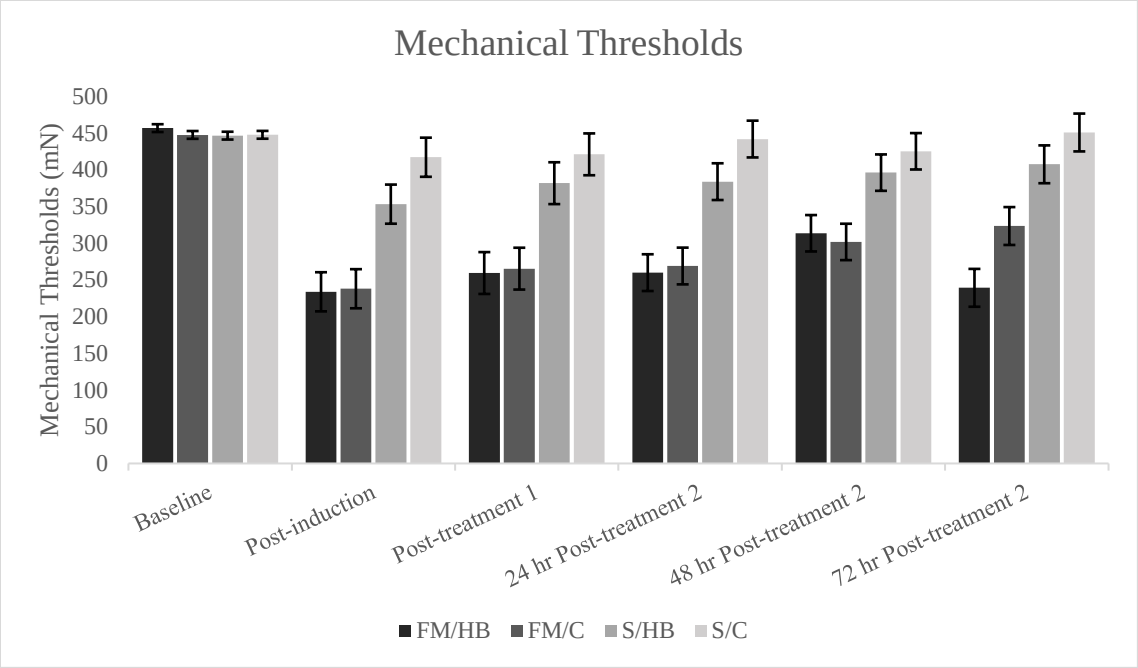

Figure 3-1: Mechanical Thresholds. Withdrawal thresholds (mN) displayed across the six experimental time points, with the maximum possible threshold at 456.63 mN. Groups are labeled by shorthand forms for pain condition (fibromyalgia = FM; saline = S) and treatment condition (hyperbaric = HB; control = *C).*

3.2 Sucrose Preference by Condition over Time

To analyze between-group differences of sucrose preference over time, we used a 4 (condition) X 3 (time) mixed-model analysis of variance (ANOVA), with condition as the between-subjects variable and time as the within-subject variable. There were no significant effects found for time, $F(2, 86) = 0.853$, $p = .43$, $\eta_p^2 = .019$, condition, $F(1, 43) = 0.611$, $p = .611$, $\eta_p^2 = .041$, or the time by condition interaction, $F(6, 86) = 0.255$, $p = .287$, $\eta_p^2 = .081$ (Figure 3-2).

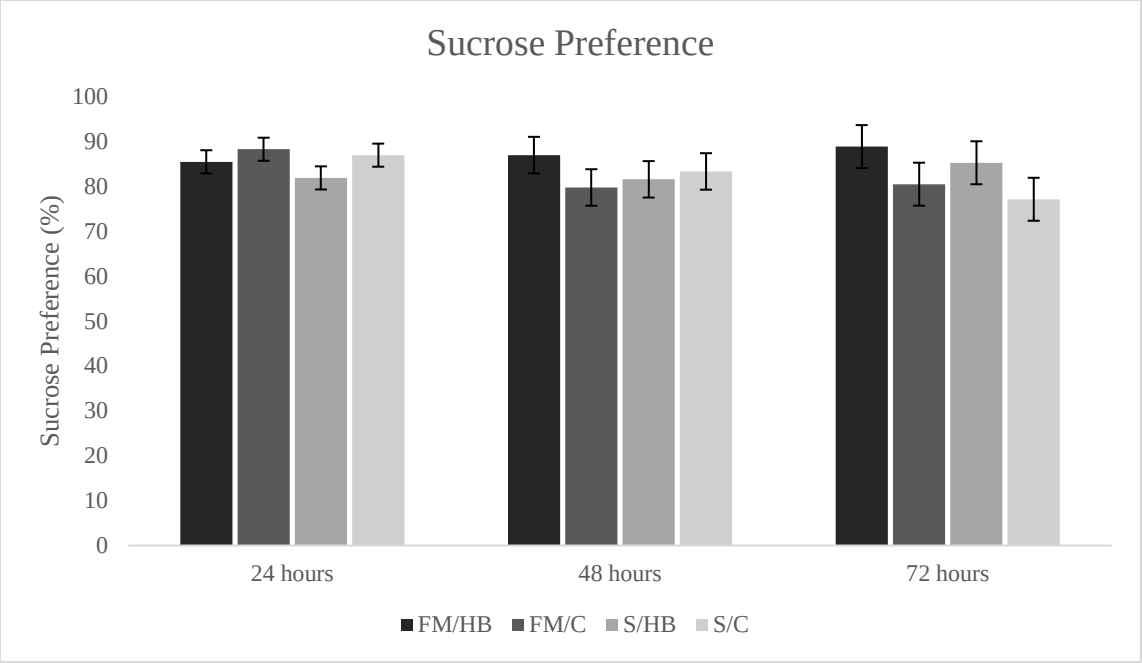

Figure 3-2: Sucrose Preference Rates. Values expressed in percentages (% = [sucrose intake (mL) / total fluid intake (mL)] x 100), across 24, 48, and 72 hours following the second treatment. Groups are labeled by shorthand forms for pain condition (fibromyalgia = FM; saline = S) and treatment condition (hyperbaric = HB; control = C).

3.3 Open Field Distance Between-Groups

3.3.1 Open Field Center Distance Between-Groups

To analyze between-group differences in distance traveled in the center of the open field apparatus, a one-way analysis of variance (ANOVA) was utilized. There was no significant main effect of condition found, $F(3, 44) = 2.435$, $p = .077$, $\eta_p^2 = .142$, although the relationship was trending towards significance. An important trending relationship to note includes animals in the FM condition that received a control treatment ($M = 510.2999$, $SE = 59.546$) having travelled the most distance in the center of the open field apparatus (Figure 3-3).

3.3.2 Open-Field Perimeter Distance Between-Groups

To analyze between-group differences in distance traveled along the perimeter of the open field apparatus, a one-way analysis of variance (ANOVA) was also utilized. No significant effects were observed, $F(3, 44) = .037$, $p = .991$, $\eta_p^2 = .002$ (Figure 3-3).

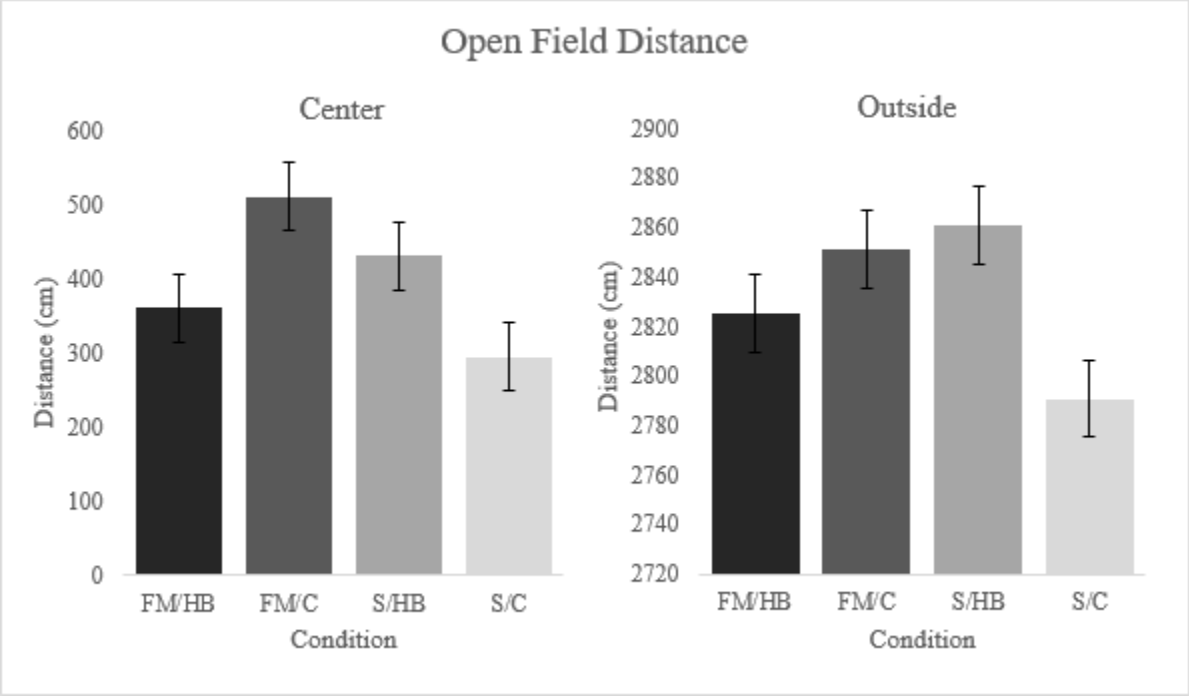

Figure 3-3: Open Field Distance. Measures of distance travelled (cm) for both the center and outside areas of the apparatus, 24 hours following the second treatment. Groups are labeled by shorthand forms for pain condition (fibromyalgia = FM; saline = S) and treatment condition (hyperbaric = HB; control = C).

Chapter 4

DISCUSSION

The purpose of this study was to provide further investigation into the efficacy of the acidic saline model of fibromyalgia in replicating both physical and negative affective

comorbidities. Further, we sought to utilize a back translational approach to gauge the potential efficacy of HBOT as a therapeutic mechanism for the alleviation of acidic saline induced pain and its associated comorbidities. Specifically, animals were randomized to a FM condition or a saline control condition, as well as to a HBOT treatment condition or a control treatment condition. Within the experimental timeline, animals underwent two treatment sessions, and mechanical thresholds were measured regularly to quantify the development of the model and to characterize its lasting effects in relation to the treatment. Animals were also subjected to a between-subjects open field test that served to quantify potential anxiety-like behaviors as a result of the pain model. Measures of anhedonia were obtained by conducting a sucrose preference test, within which animals' potential preferential differences in sucrose to water consumption sought to identify variations in depression-like behavior.

It was hypothesized that animals in the FM condition who received repeated insults of acidic saline would show significantly lower thresholds post-induction of the fibromyalgia model. Mechanical threshold data showed a significant difference between animals in the FM condition and the saline control condition following the second injection. This outcome was expected, as this model has shown continued efficacy in physical replication of the reduced mechanical thresholds associated with other preclinical models of pain. Thus, this study further contributes to the existing validity of the acidic saline model in replicating reduced physical thresholds indicative of fibromyalgia-like pain. It was also anticipated that after each treatment session, specifically among FM animals randomized to the HBOT condition, there would be an increase in paw withdrawal thresholds following each treatment session. Mechanical threshold data indicated no significant differences in thresholds between FM animals that received HBOT compared to those who received a control treatment, until 72 hours following the second

treatment. At this point, animals in the FM condition who received HBOT showed significantly lower thresholds than FM animals that received a control treatment. Though this study is the first to investigate the potential effect HBOT on the acidic saline model of fibromyalgia, these results were not in line with results of the few clinical studies that exhibited HBOT's efficacy in fibromyalgia pain management (Yildiz et al., 2004; Efrati et al., 2015; Hadanny et al., 2018; Curtis et al., 2021). In fact, the results of these studies provided strong evidence within clinical populations for the use of HBOT as a potential fibromyalgia treatment method. Additionally, applying a back-translational approach within the current study indicated that the efficacy of HBOT seen in clinical populations did not render increased thresholds among rats induced into a prominent animal model of fibromyalgia pain. However, it is plausible that the treatment regimen investigated by this study did not reach the therapeutic threshold needed to produce pain alleviation. Therefore, these results from novel exploration of the back-translational application of HBOT within Sluka et al.'s acidic saline model of fibromyalgia suggests future studies should seek to analyze variations in oxygenation and pressurization dosages, as well as the impact of more treatment schedules in regard to the ability to alleviate fibromyalgia-like pain.

For affective measures investigated in this study, we anticipated the animals randomized to the saline control condition to exhibit the lowest levels of anhedonic behavior, displayed by the greatest rates of sucrose preference. We also anticipated that animals in the FM condition who received a control treatment would exhibit the greatest levels of anhedonia, showing the lowest sucrose preference, compared to their HBOT treatment counterparts that we anticipated would show sucrose preference rates similar to that of controls. Analysis of the sucrose preference data did not support any of these hypotheses, as no significant differences were found between groups at 24 hours, 48 hours, or 72 hours following the second treatment. These results

were not expected and countered Liu et al.'s (2014) study that found animals within an acidic saline model displayed significant differences in anhedonic behaviors. It should be noted, however, that the current study utilized a different, and more continuous, methodological approach compared to that of Liu and colleagues. As a result, we believe these unique results have two potential implications: 1) the acidic saline model was simply not effective in inducing anhedonia, or 2) the pleasure associated with sucrose consumption among rodents is more salient than the pain associated with repeated insults of acidic saline. The consistent and voluminous intake of sucrose by rats has been well-documented (Colantuoni et al., 2001; Galic & Persinger, 2002; Bonacchi, Ackroff, & Sclafani, 2008), and has even served to contribute to the development of rodent models of binge eating disorders and the display of addiction-like behaviors (Colantuoni et al., 2001; Corwin, Avena, & Boggiano, 2011; Avena & Hoebel, 2012). Therefore, the natural behavioral repertoire of rats displays strong reward processing associated with sweetened solutions. While it might be possible that the acidic saline model was not efficacious in replicating anhedonia, we find the complete absence of anhedonia associated with the model to be unlikely, as anhedonia has been documented among clinical fibromyalgia patients (Boehme et al., 2020; Ross et al., 2010; Conte, Walco, & Kimura, 2003; Thieme, Turk & Flor, 2004), and among acidic saline preclinical representations (Liu et al., 2014). However, it is more probable that the type of pain produced by the model in this study was not aversive enough to override the reward processing associated with sucrose consumption and, thus, presented as lack of anhedonic behaviors.

It was further hypothesized that animals in the FM condition that received HBOT would show significantly less anxiety-like behavior within the open field paradigm by spending less time along the perimeter of the apparatus. Comparably, we anticipated that animals in the FM

condition that received a control treatment would show significantly more anxiety-like behavior, compared to saline control animals expected to show very little anxiety-like behavior. Analysis of the distance travelled in both the center and the perimeter of the open field apparatus did not support these hypotheses, as there were no significant between-groups differences. These results were not expected as previous studies investigating affective comorbidities associated with the acidic saline model produced results similar to that of our hypotheses, such that animals in the FM condition showed significantly more anxiety-like behavior in the open field paradigm compared to their saline control counterparts (Liu et al., 2014; Murasawa et al., 2020). However, these results do support the findings of a previous study conducted by our lab, within which no expected between-group differences were observed in anxiety-like behavior at baseline, post-induction of the model, or post-treatment with pregabalin (Argenbright et al., unpublished). For future studies, this calls into question the complexity of the relationship between the acidic saline model and the circumstances under which the model is able to replicate negative emotionality associated with fibromyalgia.

Sex-related differences may potentially have been a factor that contributed to variations in the obtained results between the current study and other preclinical investigations into affective dimensions within the acidic saline model. The current study, as well as Argenbright et al. (unpublished), utilized female rats, while Liu et al. (2014) and Murasawa et al. (2020) utilized male rats. Previous studies into the use of acidic saline have noted that sex-differences exist between men and women (Christidis et al., 2008; Law et al., 2008), as well as between male and female animals (Gregory et al., 2013; Nasir et al., 2016), primarily related to the amount of pain recorded and the manner in which the pain manifests. While the mechanisms of these sex-related differences remain unclear, more research is warranted to identify if these differences may

account for the variation in obtained results and whether they contribute to the development of negative affect on a preclinical level.

4.1 Conclusion

The information obtained from preclinical investigations of various pathologies and their affective dimensions serves as a crucial foundation for approaching clinical disease manifestations. In order to successfully utilize these preclinical models, understanding the full translational efficacy, including replication of pathological characteristics and comorbidities, is vital. In the study of fibromyalgia, a disorder with no single identified etiology or treatment, it is essential we have a complete understanding of the preclinical models used to investigate disease state variability and potential therapeutic approaches. The findings from the current study have provided controversial evidence for the ability of the acidic saline model to fully replicate the complex experience of fibromyalgia as a whole, by encompassing its sensory, cognitive, and affective elements.

The absence of anxiety-like behavior observed in the current study, as well as one previous study (Argenbright et al., unpublished), has resulted in contradiction to the antecedent studies that also utilized the open field paradigm to investigate the negative emotionality associated with the acidic saline model (Liu et al., 2014; Murasawa et al., 2020). While previous studies in our lab producing contradictory results were believed to be attributed to errors associated with repeated measures (Argenbright et al., unpublished), adjustments in methodology to incorporate a single between-subjects measure of open field produced a similar, unexpected absence of anxiety-like behavior. Further, lack of anticipated anhedonic behaviors contributed to the experimental condition of the current study being characterized solely by the negative sensory component associated with fibromyalgia. This absence of a collective negative affect

and sensory experience characteristic of fibromyalgia likely produced a much less aversive experience and, thus, failed to produce changes in the behavioral repertoire of the animals that might have been observed in measures of sucrose preference or open field. Further investigations are necessary to identify the underlying variables that may be contributing to the variation in development of negative affect previously observed.

The expected back-translational efficacy of HBOT as a therapeutic agent for treatment of fibromyalgia pain was derived from clinical manifestations of improved symptomology and pain thresholds associated with the treatment modality (Yildiz et al., 2004; Efrati et al., 2015; Hadanny et al., 2018; Curtis et al., 2021). Additionally, evidence for the use of HBOT in treatment of various other presentations of preclinical pain has provided even further promise (Sutherland et al., 2016; Sümen et al. 2001; Wilson et al., 2006; Wilson et al., 2007; Thompson et al., 2010). However, the inability of this study to bridge the gap between preclinical pain research and clinical fibromyalgia research might conceivably be attributed to the treatment regimen employed by this study. This study utilized two treatments of 100% oxygen at 2.0 ATA which, compared to other preclinical studies, was a rather low pressurization and number of treatment sessions (Sutherland et al., 2016; Sümen et al., 2001; Wilson et al., 2006; Wilson et al., 2007; Thompson et al., 2010). However, this treatment dosage of 2.0 ATA did prove to be beneficial for previous clinical fibromyalgia samples (Efrati et al., 2015; Hadanny et al., 2018; Curtis et al., 2021). Overall, there is potential that variations in treatment dosages, as well as variations in the roles that pressurization and oxygenation play as independent factors in HBOT, may elicit more beneficial therapeutic outcomes than observed in the current study.

The degree to which we understand the relationship between commonly observed affective comorbidities and preclinical models directly influences further research into

underlying biological and psychological mechanisms associated with less-understood pain states, such as fibromyalgia. Exploring the ability of potential therapeutic modalities to alleviate negative affect associated with chronic pain serves to steer clinical treatment approaches towards a focus not just on the sensory alleviation of pain, but rather treatment of pain as a sensory, affective, and cognitive experience. While the current study provided conflicting data on the ability of a prominent fibromyalgia model to replicate its characteristic pain experience as a whole, as well as alleviate the pain through HBOT treatment, future investigations should focus on experimental factors that contribute to development of the previously observed negative affect within the acidic saline model. Additionally, further research should appraise potential alterations in HBOT regimens to probe the effects on reduced thresholds produced by the acidic saline model. Going forward, studies should incorporate female animals as a more representative population of those who suffer from fibromyalgia.

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