ANALYSIS OF THE ANTI-MIGRAINE EFFECTS OF THC USING HOME CAGE WHEEL RUNNING

By

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To the Faculty of Washington State University:

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I would like to acknowledge and thank many people who have helped me throughout my neuroscience career. First, I would like to thank my advisor and mentor, Mike Morgan. His guidance, tenacity, and wisdom have greatly contributed to my growth as a person and a scientist. If it were not for his patience, mentorship, and sense of humor, I would not be in the position I am today. Second, I would like to thank Rebecca Craft who, besides myself, has been there since my first day as a researcher. Dr. Craft’s mentorship inspired me to pursue a career in pain research, and the knowledge I have gained from her has stuck with me since I started working in her lab nearly seven years ago. Third, I would like to thank Barb Sorg and Ray Quock for all our insightful conversations and for their guidance and assistance throughout my graduate career. I would like to thank my current and past lab members. I especially owe a great amount of gratitude to the undergraduate students who have worked with us over the years. I have learned a great deal about life, teaching, and mentorship from them. Their curiosity, enthusiasm, and hard work significantly contributed to the work presented in this dissertation. I also want to acknowledge everyone on the third floor of the Classroom Building at WSU Vancouver. Their support and help over the years has been wonderful. Last, but not least, I would like to thank my family. Their encouragement, patience, and support has provided me with a tremendous amount of inspiration and motivation over the years.
Migraine is a common neurological disorder characterized by severe headache and depression of normal activity. Despite its marked prevalence, migraine is poorly treated and no new anti-migraine agents have emerged in over 25 years. Two reasons for this lack of progress are the absence of clinically relevant behavioral outcomes for assessing migraine pain in rodents and a limited understanding of the underlying mechanisms. The purpose of this dissertation was to develop a clinically relevant method to assess migraine pain to evaluate the anti-migraine efficacy of ∆9-tetrahydrocannabinol (THC) in female rats. Activation of primary afferents via dural administration of allyl isothiocyanate (AITC) depressed home cage wheel running for three hours. Administration of the anti-migraine medication sumatriptan reversed AITC-induced depression of home cage wheel running. These observations are consistent with the migraine-induced decreases in activity in humans and subsequent recovery by sumatriptan. Chapter 3 shows that administration of THC also prevented migraine-depressed wheel running. This effect occurred only when rats were treated with THC immediately following AITC administration and not when administered 90 minutes
before or after AITC. Administration of cannabinoid type-1 and type-2 receptor antagonists revealed that the anti-migraine effects of THC were mediated by activation of the cannabinoid type-1 receptor. Chapter 4 examined whether THC efficacy is maintained with repeated administration. THC prevented migraine-depressed wheel running in rats whether they were pretreated with THC or vehicle for 2.5 days. In contrast, morphine transiently prevented migraine-depressed wheel running in rats pretreated with saline, but not morphine for 2.5 days. Moreover, migraine-depressed wheel running was exacerbated in rats pretreated with morphine compared to rats pretreated with THC or vehicle. The present studies reveal three findings: 1) Depression of home cage wheel running is an objective and clinically relevant method to assess migraine pain in rats; 2) Administration of THC can prevent migraine-depressed wheel running via CB1 receptor activation; and 3) Repeated THC administration does not exacerbate migraine pain or produce antinociceptive tolerance. These novel findings suggest that THC may be an effective migraine treatment.
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ABBREVIATIONS

ALTC—allyl isothiocyanate
ANOVA—analysis of variance
CB₁—cannabinoid type-1
CB₂—cannabinoid type-2
CGRP—calcitonin gene-related peptide
MOH—medication overuse headache
NSAID—non-steroidal anti-inflammatory drug
PACAP—pituitary adenylate cyclase-activating polypeptide
PAG—periaqueductal gray
SEM—standard error of the mean
TCC—trigeminocervical complex
THC—Δ⁹-tetrahydrocannabinol
TRPA1—transient receptor potential ankyrin 1
DEDICATION

This dissertation is dedicated to my parents
who have supported me throughout this journey.
CHAPTER 1:
GENERAL INTRODUCTION

Migraine is a recurring primary headache disorder and is the most common neurological disorder in the world. Despite its marked prevalence, treatment options remain poor. The objective of this chapter is to describe migraine, its treatment, and its preclinical assessment. The clinical and preclinical data on the anatomy, physiology, and pharmacology of migraine will be presented. This chapter will also highlight the lack of clinically relevant behavioral measures for headache and hypothesize that cannabinoids may be a more effective treatment and home cage wheel running may be a more clinically relevant approach to assess migraine pain in rats.

Migraine

Migraine is the seventh leading cause of disability worldwide and affects approximately 11.5% of the world’s population (Lipton et al., 2001; Vos et al., 2012). Its prevalence is highest during the patient’s most productive years, between the ages of 25 and 55 (Lipton et al., 2001; Burch et al., 2015). This loss of productivity, accompanied by a decrease in overall well-being, imposes a significant impact on daily life with significant functional impairment that includes both physical and emotional consequences (Leonardi et al., 2005).

Migraine is characterized by attacks of unilateral throbbing head pain, which can last anywhere from 4-72 hours (Headache Classification Committee of the International Headache Society (IHS), 2013). Accompanying the headache is heightened sensitivity to light touch (alldynia), light (photophobia), and sound (phonophobia) (Headache Classification Committee of the International Headache Society (IHS), 2013). Before the onset of headache, patients typically experience autonomic, affective, and cognitive symptoms (e.g., nausea, yawning,
depression, reduced concentration), termed the premonitory phase (Giffin et al., 2003). In approximately one-third of migraine patients, attacks are associated with changes in cortical excitability, termed migraine aura, which occur just before the headache (Rasmussen and Olesen, 1992). After the headache subsides, patients report feeling weak and tired for hours to days, termed the postdrome phase (Giffin et al., 2016). This combination of seemingly unrelated symptoms reflects the complex nature of migraine. Migraine is not simply a bad headache, as it encompasses an entire neurological disorder that affects cortical, subcortical, and brainstem areas involved in the regulation of autonomic, affective, cognitive, and sensory functions (Goadsby et al., 2009).

**Sex differences in migraine**

Migraine is approximately 2-3 times more common in women than men (Victor et al., 2010). Although boys and girls have similar prevalence of migraine (Victor et al., 2010), prevalence rises in both sexes after puberty, but with a much greater rise in women than men (Victor et al., 2010). The higher prevalence of migraine in women, the fact that more women with migraine seek out medical advice, and the predominance of women in clinical trials compared to men only emphasizes the importance of including female subjects in preclinical studies of migraine (Vetvik and MacGregor, 2016). However, only 11 of approximately 150 preclinical migraine studies use female rodents (Stucky et al., 2011; Chanda et al., 2013; Vermeer et al., 2014; Pradhan et al., 2014a; 2014b; Bhandare et al., 2015; Tipton et al., 2015; Vermeer et al., 2015; Christensen et al., 2016; Huang et al., 2016; Christensen et al., 2017). Thus, the present studies will focus exclusively on female rats (Chapters 2-4).

**Migraine pathophysiology**

An understanding of the anatomy, physiology, and pharmacology underlying migraine has greatly increased over the last 25 years. However, a full understanding of the
Pathophysiology is not clear. The debilitating headache associated with migraine is due to activation of the trigeminovascular system, a broad term that comprises the neuronal, non-neuronal, and vascular architecture of the trigeminal system. Trigeminal nociceptors are pseudounipolar neurons that originate in the trigeminal ganglion. These neurons send peripheral projections that innervate pial, arachnoid, and dural blood vessels (e.g., superior sagittal sinus, middle meningeal artery) or simply remain as free nerve endings (Penfield and McNaughton, 1940; Ray and Wolff, 1940). These are non-myelinated (C-fibers) and thinly myelinated (Aδ-fibers) axons that project through the ophthalmic division of the trigeminal nerve. Although the pain pathway from the meninges to the cortex has been well characterized, the neurochemical mechanisms that trigger the activation of this pathway to produce migraine are not clear.

Primary afferent neurons in the meninges contain various vasoactive neuropeptides [e.g., calcitonin gene-related peptide (CGRP), substance P, neurokinin A, pituitary adenylate cyclase-activating polypeptide (PACAP)], all of which have been implicated in migraine pathophysiology (Uddman et al., 1985; Edvinsson et al., 1988; Uddman and Edvinsson, 1989; Uddman et al., 1993). Upon stimulation of the trigeminal nerve, these neuropeptides are released and cause vasodilation of dural and pial blood vessels (Williamson et al., 1997; Ebersberger et al., 1999; Petersen et al., 2004). Vasodilation releases more sensitizing factors in the meninges (e.g., PACAP, CGRP) which are thought to sensitize primary afferent neurons and initiate nociceptive signaling.

The central projections of these primary afferents travel through the caudal medulla of the brainstem and terminate in the superficial laminae of the trigeminal nucleus caudalis and upper cervical spinal cord (Kaubé et al., 1993a; 1993b). Further, these same neurons converge on other trigeminal neurons in the trigeminocervical complex (TCC) that receive inputs from
facial skin and muscle (Davis and Dostrovsky, 1988a; 1988b; Bartsch and Goadsby, 2003). Nociceptive information is then relayed to the thalamus through the quintothalamic tract (Goadsby et al., 2009). Nociceptive processing in the thalamus occurs in various thalamic nuclei (e.g., ventroposteromedial thalamus, medial nucleus of the posterior complex) (Zagami and Lambert, 1991), before the information is relayed to cortical regions. Various cortical regions (e.g., anterior cingulate cortex, frontal cortex, visual/auditory association cortices) participate in the final processing of pain information that originated in the meninges (Weiller et al., 1995; Bahra et al., 2001).

Significant progress in understanding the anatomy and physiology of migraine pain has been made over the years. However, a better understanding of the pharmacology and physiology underlying migraine would allow for the development of more effective treatments.

**Migraine treatments**

Approaches to treating migraine can be divided into nonpharmacological and pharmacological therapies. Non-pharmacological therapies [for review, see (Silberstein and Rosenberg, 2000)] typically include educating the patient about identifying migraine triggers so the patient can make lifestyle changes (Goadsby et al., 2002). Pharmacological treatments can either be prophylactic (i.e., taken daily to prevent the migraine from occurring) or abortive (i.e., taken immediately during the onset of the headache to terminate the attack). Current prophylactic therapies (e.g., topiramate, gabapentin) are nonspecific, have minimal efficacy, and are associated with substantial cognitive side effects (Welch, 1993). Current abortive therapies include nonsteroidal anti-inflammatory drugs (NSAIDs; e.g., aspirin, acetaminophen, naproxen, ibuprofen), ergot derivatives, and triptans.
NSAIDs are widely available as over-the-counter drugs. The rationale for their use in migraine is supported by the hypothesis that migraine is an inflammatory disorder due to the role of prostaglandins in migraine pathophysiology (Párdutz and Schoenen, 2010). Although many controlled studies have demonstrated their efficacy (i.e., pain-free for two hours after administration), their use is limited by slow absorption, gastrointestinal side effects, and increased headache recurrence (Párdutz and Schoenen, 2010).

An alternative to NSAIDs are derivatives of ergotamine. Ergot derivatives work to alleviate migraine by interacting with serotonin receptors to induce vasoconstriction (Baron and Tepper, 2010). The major advantage of ergot derivatives is their affordability (Goadsby et al., 2002). However, their use is not recommended due to their complex pharmacology and potent and long-lasting vasoconstricting effects (Goadsby et al., 2002). Further, repeated ergot use leads to increased headache recurrence (Bigal and Tepper, 2003).

A third treatment are the triptans. Triptans are serotonin 1B/1D receptor agonists that are currently the only drug prescribed specifically for migraine. The first triptan, sumatriptan (1991), was a significant advance in migraine therapy. Despite its long history, the mechanism underlying triptan actions remains unclear. There is evidence suggesting that triptans treat migraine by constricting blood vessels (Connor et al., 1997; Martin et al., 1997), inhibiting trigeminal primary afferent neurons (Williamson et al., 1997; Gupta et al., 2000), and inhibiting central trigeminal neurons (Goadsby and Hoskin, 1996; Cumberbatch et al., 1997; Goadsby and Knight, 1997). There is evidence that sumatriptan does not cross the blood-brain barrier, suggesting a peripheral site of action (Ahn and Basbaum, 2005). However, sumatriptan also dampens central trigeminovascular neurons, suggesting a central site of action (Levy et al., 2004). Thus, there are a number of possible mechanisms through which sumatriptan could modulate migraine.
Although triptans are efficacious in a subset of migraineurs, there are numerous disadvantages. Triptans often have low oral bioavailability and short half-lives (resulting in high headache recurrence) (Antonaci et al., 2016). Further, triptans have insufficient rates of tolerability, as patients do not adhere to the treatment due to a variety of side effects (e.g., dizziness, throat tightness) (Hepp et al., 2015). Triptans are also ineffective in patients with refractory chronic migraine; thus, there are no effective treatment options for these patients (Schuster and Rapoport, 2016). Finally, up to a third of migraineurs and 40% of all migraine attacks do not respond to triptans (Ferrari et al., 2001).

The most common adverse effect associated with regular NSAID, ergot, and triptan use is a condition known as medication overuse headache (MOH). MOH manifests as increased frequency and intensity of migraine headaches and enhanced sensitivity to normally innocuous sensory stimuli and migraine triggers (Diener et al., 2016; Westergaard et al., 2016). In some patients, triptan overuse can transform migraine from an episodic to a chronic condition (Dodick and Freitag, 2006). Further, although MOH is not as common as other primary headache disorders such as migraine, people with MOH are more likely to report adverse effects of headache on education, career, and overall quality of life (Diener et al., 2016; Westergaard et al., 2016).

Depending on the frequency of the use of available anti-migraine treatments (e.g., triptans, ergots, opioids, NSAIDs), some drugs are more likely than others to elicit MOH (Thorlund et al., 2016); however, the risk for novel treatments to cause the development of MOH is rarely assessed in preclinical studies. Emerging drug therapies for migraine (e.g., CGRP monoclonal antibodies, PACAP receptor antagonists) have entered clinical trials before being evaluated for their potential to elicit MOH in animal models. As such, it is critical to identify and
validate novel therapies that will remain efficacious in migraine patients with repeated use. This will be addressed further in Chapter 4.

**Cannabinoids and pain**

Cannabinoids may present a novel therapeutic avenue for migraine. Cannabinoids are lipophilic compounds that are present in cannabis. The major psychoactive cannabinoid, ∆9-tetrahydrocannabinol (THC), was first purified in 1964 and has many behavioral effects (Gaoni and Mechoulam, 1964; Bruijnzeel et al., 2016).

Cannabinoids bind to cannabinoid type-1 (CB1) and type-2 (CB2) receptors, along with other targets (e.g., TRPV1). Cannabinoid receptors are among the most abundant G-protein coupled receptors in the nervous system. CB1 and CB2 receptors are located presynaptically and modulate neurotransmitter release (Iversen, 2003). CB1 receptors are present in brain regions implicated in migraine (e.g., periaqueductal gray) and in areas relevant to pain modulation (e.g., rostral ventromedial medulla, substantia gelatinosa, and spinal interneurons) (Aggarwal, 2013). In contrast, CB2 receptors are primarily concentrated in the peripheral tissues, especially cells of the immune system (Mackie, 2008).

Multiple experiments have provided firm preclinical evidence of cannabinoid analgesia. Regardless of the route of administration (e.g., oral, intraperitoneal, intraplantar, subcutaneous, etc.), cannabinoids are effective against chronic inflammatory pain (Craft et al., 2013), neuropathic pain (Herzberg et al., 1997), chemotherapy-induced peripheral neuropathy (Harris et al., 2016), diabetic neuropathy (Ulugol et al., 2004), visceral pain (Jaggar et al., 1998; Kwilasz and Negus, 2012), and acute pain (Tseng and Craft, 2001; Wakley and Craft, 2011).

Given the pharmacology and reported therapeutic benefits of cannabis in pain medicine (Noyes and Baram, 1974; Milstein et al., 1975; Noyes et al., 1975; Karst et al., 2010; Kraft, 2012; Chiou et al., 2013; Maione et al., 2013), it should not be surprising that this benefit
appears to extend to migraine. The use of cannabinoids to treat migraine headache dates back over 2000 years when Greek physicians Galen and Dioscorides described medical indications for cannabis (Brunner, 1973). In 1887, Dr. Stephen Mackenzie advocated for the use of marijuana twice daily for “chronic daily headache”, likely chronic migraine by description (Mackenzie, 1887). In 1915, Sir William Osler, the father of modern medicine, advocated for cannabis use in migraine by stating “Cannabis indica is probably the most satisfactory remedy” (Baron, 2015).

Although there are no controlled experiments examining the anti-migraine effects of marijuana, survey data indicates surprisingly widespread use. A survey of 54 patients in a drug treatment center reported that marijuana was commonly used as a self-medication treatment for migraine (el-Mallakh, 1989). Analysis of the reasons for cannabis use in Germany, Austria, and Switzerland revealed that 10.2% of patients used it for migraine and headache (Schnelle et al., 1999). And finally, a survey of 2480 patients in the Oakland Cannabis Buyer’s Club (Oakland, CA, USA) revealed that 5% used it for its anti-migraine properties (Baron, 2015). These data demonstrate that the need for controlled studies of cannabis efficacy for migraine is significant.

A number of possible mechanisms of action underlying the anti-migraine effects of cannabinoids have been postulated. Intravenous administration of CB₁ agonists inhibits dural vessel dilation and neuronal firing in the TCC, suggesting that CB₁ receptors are present on trigeminovascular neuronal projections (Akerman et al., 2007). Cannabinoids are thought to directly and indirectly inhibit the release of glutamate from primary afferents present in laminae I and II (Farquhar-Smith et al., 2000; Jennings et al., 2001; Morisset et al., 2001). It is also known that CB₁ receptors are present on the terminals of GABAergic neurons in the periaqueductal gray (PAG) (Tsou et al., 1998; Maione et al., 2006). Descending projections from the ventrolateral PAG, a brain region implicated in migraine, are known to modulate neurons of the
TCC (Akerman et al., 2007; 2013). Finally, THC alone has also been shown to dose-dependently suppress the propagation velocity, amplitude, and duration of cortical spreading depression, a key component of migraine pathophysiology (Kazemi et al., 2012). Given that migraine is a complex disorder involving neurons, non-neuronal cells, and vasculature, the contribution of cannabinoid receptors underlying the anti-migraine effects of cannabinoids such as THC needs to be examined in carefully controlled studies.

Despite plenty of evidence of the anti-migraine effects of cannabinoids in vitro and in anesthetized animals using electrophysiological recordings from the TCC, few studies have examined the anti-migraine effects of cannabinoids in vivo. Animal studies have shown that a systemic injection of nitroglycerin, a common migraine trigger, decreases levels of endogenous cannabinoids and increases CB$_1$ receptor levels in the trigeminal nucleus caudalis (Greco et al., 2010), suggesting that increasing levels of the endogenous cannabinoid anandamide may protect against migraine pain. Other studies have demonstrated that administration of a CB$_2$ receptor agonist significantly reduces nitroglycerin-induced hyperalgesia (Greco et al., 2014) and inhibition of fatty acid amide hydrolase, an enzyme that degrades anandamide, increases anandamide levels and decreases nitroglycerin-induced hyperalgesia in rats (Greco et al., 2015).

Although a lot of circumstantial evidence indicates that marijuana is an effective treatment for migraine, no preclinical behavioral studies have been conducted. The experiments in Chapter 3 will fill this knowledge gap by examining the behavioral effects of THC and its pharmacology in a rat model of migraine.

**Preclinical assessment of pain and migraine**

*Preclinical assessment of migraine*
Novel therapies for migraine have been slow to develop. In fact, the last drug specifically designed for migraine was sumatriptan in 1991. The severe lack of adequate behavioral assays for spontaneous ongoing pain, such as headache, contributes to the failure of drug development (Strassman and Burstein, 2013). This problem is compounded for migraine drug development, as the headache field has suffered from having no clinically relevant behavioral assay (Strassman and Burstein, 2013).

There are numerous ways to induce migraine pain in rodents. The best animal model of migraine should have a similar etiology and phenotype to the human condition. However, given that migraine is a complex disorder with variable phenotypes, no animal model replicates all components of migraine. As such, the best animal models of migraine are based on activation of dural afferents. The most common stimulus to activate dural afferents is “inflammatory soup”, a mixture of serotonin, prostaglandin E2, histamine, and bradykinin (Strassman et al., 1996); however, the primary argument for this approach is based on the fact that the initial trigger for migraine is an inflammatory response in the dura (Moskowitz, 1990). Further, the use of inflammatory soup is a better model of meningitis, than migraine (Storer et al., 2015).

Rather than activating numerous targets with inflammatory soup, a simpler approach is to activate transient receptor potential ankyrin 1 (TRPA1) channels in the dura using the selective TRPA1 agonist allyl isothiocyanate (AITC). Activation of TRPA1 degranulates mast cells to release histamine and serotonin (Fischer et al., 2016) – key components of migraine pathophysiology (Akerman et al., 2016). TRPA1 receptors have also been shown to contribute to migraine in both humans and rodents (Edelmayer et al., 2012; Nassini et al., 2012). Thus, activation of TRPA1 on dural afferents provides a simple, direct, and consistent approach to generate migraine pain in rats. Dural administration of AITC will be used to induce migraine-like pain in Chapters 2-4.
A seminal behavioral headache study demonstrated that chemical activation of dural afferents using inflammatory soup induced facial allodynia in awake male rats (Oshinsky and Gomonchareonsiri, 2007) - the first behavioral correlate of a “headache” in awake animals. However, recent studies examining spontaneous migraine pain (De Felice et al., 2013; Melo-Carrillo and Lopez-Avila, 2013; Christensen et al., 2016; Sufka et al., 2016) have called this approach into question. Although migraine causes allodynia in a subset of migraineurs, allodynia is not a diagnostic criterion (Headache Classification Committee of the International Headache Society (IHS), 2013). Moreover, allodynia is considered a marker of migraine progression (Burstein et al., 2004; Louter et al., 2013), rarely assessed clinically (Mathew et al., 2004), and may outlast the headache and be present during interictal periods (Aguggia, 2012). Thus, although allodynia may be a marker of central sensitization processes underlying the progression of a migraine attack (Burstein et al., 2000), measuring spontaneous headache pain, as opposed to allodynia, remains a challenge.

**Pain-depressed behaviors**

One approach to solve the migraine assessment problem in animals has been to measure depression of behavior. Pain-depressed behaviors are defined as behaviors that decrease in frequency, rate, duration or intensity in response to a noxious stimulus or pain state (Negus et al., 2010). A number of pain-depressed behavioral models have been developed and characterized in male rodents. The most common of these focus on measuring decreases in rewarding behaviors such as feeding or operant responding for intracranial self-stimulation. There are many advantages to examining pain-depressed feeding: little experimental infrastructure is required and minimal training is needed (Stevenson et al., 2006; Negus et al., 2010). For example, chemical activation of dural afferents decreased feeding for six hours (Malick et al., 2001). However, this assay was not used in future studies due to the difficulty of
the paradigm (Strassman and Burstein, 2013). Assessing pain-depressed feeding requires depriving the rodents of food or water prior to evaluation, removing the animal from its home cage, limiting assessment to a session of 60 minutes or less, and possibly capturing indirect effects of treatments on appetite or thirst (Stevenson et al., 2006; Miller et al., 2011). Further, these conditions can be stressful for animals and do not mimic the conditions under which pain is assessed in chronic pain patients.

A major problem with assessment of pain-evoked behaviors (e.g., flinching, mechanical allodynia, thermal hyperalgesia) is distinguishing whether a treatment has antinociceptive effects as opposed to merely disrupting behavior because of side effects such as sedation. In contrast, administration of analgesic compounds will increase pain-depressed behaviors, thereby avoiding confounds from motor impairment or sedation. Second, as mentioned in the clinical criteria for migraine, pain states in humans that require clinical intervention are often associated with a depression of behavior (Negus et al., 2010). Third, the use of pain-depressed behaviors allows for preclinical research on the mechanisms and determinants of the affective, non-reflexive component of pain (Negus et al., 2010). Fourth, many pain-depressed behaviors can be assessed using automated equipment resulting in no experimenter bias (Negus et al., 2006). And fifth, pain-related behavioral depression plays a prominent role in the diagnosis of pain conditions in both humans and in veterinary medicine (Negus et al., 2010), lending validity to the use of these measures in preclinical pain research.

**Pain-depressed wheel running**

Pain-induced reductions in overall locomotion have also been used to assess pain and analgesia (Matson et al., 2007). Pain-depressed locomotion closely mimics the decreases in activity that typically occurs in chronic pain patients. The advantage of measuring locomotion is the unevoked nature of the primary endpoint (Matson et al., 2007). Wheel running in rats is an
especially good model of human locomotion because it is a voluntary behavior that shows clear diurnal rhythms (Lockard, 1966). Inflammatory pain has been shown to reduce wheel running in male mice tested one hour each day (Cobos et al., 2012). Assessing nociception during a specific hour allows for a close association between the treatment and wheel running, in addition to limiting the amount of data to analyze. However, a one-hour test does not mimic the human condition in that chronic pain disrupts behavior throughout the day. Although a number of studies have examined pain-induced decreases in wheel running with mixed results (Table 1.1), depressed wheel running due to migraine has never been demonstrated.

We have previously shown that home cage wheel running is an objective, sensitive, and clinically relevant method to assess inflammatory pain and opioid analgesia in male and female rats (Kandasamy et al., 2016; 2017). There are several advantages to assessing pain and analgesia using home cage wheel running. First, in contrast to most preclinical pain research, pain is assessed during the animal’s active period (i.e., dark phase of the light cycle). Second, testing occurs in a stress-free environment, which removes confounds due to handling or the stress of a novel environment. Third, continuous monitoring of home cage wheel running allows for the entire time course of pain and analgesia to be evaluated on an hour-by-hour or day-by-day scale. Fourth, wheel running is a natural behavior that requires minimal training. These advantages indicate that home cage wheel running improves on existing methodologies by providing an objective, sensitive, clinically relevant measure of the entire duration and magnitude of pain and analgesia in the rat. This approach can be applied to virtually any pain condition and may be especially useful for migraine pain, as it is characterized by spontaneous headache that results in a depression of activity. This hypothesis will be tested in Chapter 2.

Concluding remarks
Migraine is a complex neurological disorder that involves a combination of neuronal, non-neuronal, and vascular contributors. Current migraine treatments (e.g., triptans) are only efficacious in a subset of patients and most treatments are accompanied by a variety of side effects (e.g., medication overuse headache, cognitive deficits), some of which are often worse than the headache itself. Thus, there is a significant clinical need for novel anti-migraine treatments with limited side effects. Cannabinoids provide a novel treatment avenue for migraine as there is anecdotal and *in vitro* data supporting its efficacy. However, there are a lack of controlled studies examining cannabinoid analgesia against migraine. Therapies for migraine have been slow to develop. This delay is due, in part, to the lack of clinically relevant behavioral assays that capture the entire duration and magnitude of migraine pain. Current methods to assess migraine pain such as measuring allodynia in laboratory rodents cannot dissociate between the antinociceptive and adverse effects of a drug. In contrast, home cage wheel running provides an objective, sensitive, and clinically relevant measure of migraine pain and anti-migraine efficacy of a drug. Further, a large majority of preclinical migraine studies have used male rats, even though migraine is 2-3 times more common in females than males.

The following studies will use female rats to examine: 1) the extent to which home cage wheel running can be used to assess the duration and magnitude of migraine pain (Chapter 2); 2) the anti-migraine effects of THC and its pharmacology (Chapter 3); and 3) whether repeated THC administration leads to MOH (Chapter 4).
Table 1.1: Summary of studies demonstrating depressed wheel running following pain, and the effects of analgesics

<table>
<thead>
<tr>
<th>Pain Model</th>
<th>Species</th>
<th>Sex</th>
<th>Acquisition Period Prior to Pain</th>
<th>Wheel in home cage?</th>
<th>Duration of assessment</th>
<th>Analgesic</th>
<th>Restored Function?</th>
<th>Reference</th>
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<tr>
<td>CFA (1 paw)</td>
<td>Rat</td>
<td>M/F</td>
<td>3 or 8 days</td>
<td>Yes</td>
<td>23 h/day</td>
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<td>8 days</td>
<td>Yes</td>
<td>23 h/day</td>
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<td>No</td>
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<td>-</td>
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<td>24 h/day</td>
<td>-</td>
<td>-</td>
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<td>1 hour</td>
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<td></td>
<td></td>
<td></td>
<td>Caffeine</td>
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<td>3 days</td>
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<td>-</td>
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<td>-</td>
<td>No</td>
<td>17 hours</td>
<td>Botulinum Toxin Type A</td>
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<td>Gender</td>
<td>Duration</td>
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<td>17 hours</td>
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<td>30 min</td>
<td>Morphine Yes</td>
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<td>24 hours/day</td>
<td>Liposome-encapsulated oxymorphone Yes</td>
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Ferrari MD, Roon KI, Lipton RB, Goadsby PJ (2001) Oral triptans (serotonin 5-HT(1B/1D))


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Pitzer C, Kuner R, Tappe-Theodor A (2016a) Voluntary and evoked behavioral correlates in inflammatory pain conditions under different social housing conditions. PAIN Reports 1:e564.


Wakley AA, Craft RM (2011) Antinociception and sedation following intracerebroventricular
CHAPTER 2:

DEPRESSION OF HOME CAGE WHEEL RUNNING IS A RELIABLE AND CLINICALLY RELEVANT METHOD TO ASSESS MIGRAINE PAIN IN RATS

Abstract

The development of new anti-migraine treatments is limited by the difficulty in assessing migraine pain in laboratory animals. Depression of activity is one of the few diagnostic criteria for migraine that can be mimicked in rats. The goal of the present study was to test the hypothesis that depression of home cage wheel running is a reliable and clinically relevant method to assess migraine pain in rats. Adult female rats were implanted with a cannula to inject allyl isothiocyanate (AITC) onto the dura to induce migraine pain, as has been shown before. Rats recovered from implantation surgery for 8 days in cages containing a running wheel. Home cage wheel running was recorded 23 hours a day. AITC and the migraine medication sumatriptan were administered in the hour prior to onset of the dark phase. Administration of AITC caused a concentration-dependent decrease in wheel running that lasted 3 hours. The duration and magnitude of AITC-induced depression of wheel running was consistent following three repeated injections spaced 48 hours apart. Administration of sumatriptan attenuated AITC-induced depression of wheel running when a large dose (1 mg/kg) was administered immediately following AITC administration. Wheel running patterns did not change when sumatriptan was given to naïve rats. These data indicate that home cage wheel running is a sensitive, reliable, and clinically relevant method to assess migraine pain in the rat.
**Introduction**

Migraine is characterized by severe pain and heightened sensitivity to sensory stimuli that results in depression of normal daily activities. Migraine is difficult to study in laboratory animals because pain occurs in the absence of tissue injury and because of the limitations of existing behavioral assays (Strassman and Burstein, 2013). Most preclinical studies of migraine assess periorbital and/or hindpaw allodynia as the dependent measure for migraine pain. Although migraine causes allodynia in a subset of migraineurs, allodynia is not a diagnostic criterion (Headache Classification Committee of the International Headache Society (IHS), 2013). Moreover, allodynia is considered a marker of migraine progression (Burstein et al., 2004; Louter et al., 2013), rarely assessed clinically (Mathew et al., 2004), and may outlast the headache and be present during interictal periods (Aguggia, 2012). Thus, the development of better ways to assess migraine pain in laboratory animals would significantly advance migraine research.

In contrast to allodynia, the reduction in routine physical activity caused by migraine is a diagnostic criterion that is easy to replicate in laboratory animals. A number of studies have used depression of activity (e.g., locomotor activity, feeding, rearing) to assess pain resulting from headache in humans (Mannix et al., 2016) and rodents (Malick et al., 2001; Edelmayer et al., 2012; Melo-Carrillo and Lopez-Avila, 2013; Christensen et al., 2016; Sufka et al., 2016). The problem with these studies is that assessment was limited to 60 min or less making it impossible to quantify the full duration and magnitude of the migraine. In humans, the time course for the headache phase of migraine can last from 4 to 72 hours.

We have recently shown that home cage wheel running is a sensitive and objective method to assess the magnitude and duration of chronic inflammatory pain (Kandasamy et al., 2016). Home cage wheel running is an especially good model of human activity because it is a
voluntary behavior that shows clear diurnal rhythms that can be continuously and objectively quantified in the rat in a stress-free environment (Kandasamy et al., 2016). The primary goal of the present study was to test the hypothesis that home cage wheel running is a sensitive, reliable, and clinically relevant method to assess the duration and magnitude of migraine pain in laboratory rats.

Although a number of different animal models of migraine have been developed (e.g., dural inflammatory soup, systemic nitroglycerin), we used microinjection of the TRPA1 agonist allyl isothiocyanate (AITC) onto the dura to generate migraine pain. Similar to inflammatory soup, AITC is a simple and direct method to activate the dural afferents responsible for headache pain. Moreover, activation of TRPA1 degranulates mast cells to release histamine and serotonin (Fischer et al., 2016) – all of which are key components of migraine pathophysiology (Akerman et al., 2016). TRPA1 receptors have also been shown to contribute to migraine in both humans and rodents (Edelmayer et al., 2012; Nassini et al., 2012). The clinical relevance of home cage wheel running as a method to assess migraine will be evaluated by determining whether AITC produces a concentration-dependent depression of wheel running that can be reversed by the migraine treatment sumatriptan. Reliability will be assessed by measuring the consistency of AITC to depress home cage wheel running following injections on different days. All of the experiments were conducted in female rats to be consistent with the much higher rates of migraine in women than men (Stang and Osterhaus, 1993; Smitherman et al., 2013).

**Materials and Methods**

*Subjects*
Data were collected from 64 adult female Sprague-Dawley rats bred at Washington State University Vancouver (Vancouver, WA, USA). All rats were 50-70 days old at the start of the study and randomly assigned to treatment groups. A within-subjects design was used when possible to reduce the number of animals needed. All procedures were approved by the Washington State University Animal Care and Use Committee and conducted in accordance with the International Association for the Study of Pain’s Policies on the Use of Animals in Research.

**Surgery**

Prior to surgery, rats were housed in pairs in a 22-24 ℃ colony room on a 12/12-hour light/dark cycle (lights off at 1800 h). Animals were anesthetized with pentobarbital (50 mg/kg, i.p.) and implanted with a guide cannula (18 gauge; 4 mm long) aimed above the dura mater (AP: +1.0 mm; ML: +1.0 mm; DV: 0.8 mm). Loctite® super glue (Henkel AG & Company, KGaA, Düsseldorf, Germany) was used to form a tight seal around the guide cannula and skull, and then dental cement was used to anchor the guide cannula to two screws in the skull. Rats were maintained under a heat lamp until awake. Following surgery, each rat was housed individually in an extra tall cage (36 x 24 x 40 cm) with a running wheel. The rat was allowed to recover for 8 days following surgery in a sound-attenuating booth (2.1 x 2.2 m; Industrial Acoustics Company, Inc., Bronx, NY, USA). Food and water were available ad libitum.

**Running wheel**

A Kaytee Run-Around Giant Exercise Wheel (Kaytee Products, Inc., Chilton, WI) with a diameter of 27.9 cm was suspended from the top of the rat’s home cage. The floor of the cage was covered with cellulose bedding (BioFresh™, Ferndale, WA, USA). A thin aluminum plate (0.8 mm x 5.08 cm x 3.81 cm; K&S Precision Metals, Chicago, IL, USA) was attached to one spoke of the running wheel to interrupt a photobeam projecting across the cage with each
rotation. The beam was set 18 cm above the floor of the cage so that only the rotation of the wheel, not the normal activity of the rat, would interrupt the beam. The number of wheel revolutions was summed over 5 min bins for 23 hours each day using Multi-Varimex software (Columbus Instruments, Columbus, OH, USA) beginning at 1700 h, the onset of the dark phase of the light cycle when rats are most active. A full description of the running wheel with video is available in our previous publication (Kandasamy et al., 2016).

**Drugs**

Allyl isothiocyanate (AITC; Sigma-Aldrich, Inc., St. Louis, MO, USA) was mixed in mineral oil at concentrations of 1% and 10% and injected into the periosteal space in a volume of 10 µL. Sumatriptan succinate (Sigma-Aldrich, Inc., St. Louis, MO, USA) was dissolved in saline (Hospira Inc, Lake Forest, IL, USA) at doses of 0.1 mg/kg and 1 mg/kg and injected subcutaneously at a volume of 1 mL/kg. All drugs were made fresh on the day they were injected.

**Baseline acquisition**

Rats were allowed unrestricted access to the wheel for 23 hours/day for 8 days following surgery and prior to induction of migraine pain. The number of wheel revolutions that occurred during the 23 hours prior to the first dural injection was used as the baseline activity level. Rats that ran less than 400 revolutions on the baseline day (n = 7 out of 71) were not included in further testing (Kandasamy et al., 2016).

**Experiment 1: AITC concentration-response**

If wheel running is a clinically relevant measure of AITC-induced migraine pain, then the duration and magnitude of depressed wheel running should depend on the intensity of the headache. This hypothesis was tested by measuring depression of wheel running after different concentrations of AITC. Following baseline testing on Day 8, the rat was injected with 10 µL of
1% AITC, 10% AITC, saline, or mineral oil onto the dura mater using an injection cannula inserted into the guide cannula. All injections were complete by 1650 h. The rats were returned to their home cage and wheel running activity recorded for the next 23 hours beginning at 1700 h. This procedure was repeated every other day with the drugs administered in a counterbalanced manner, although no rat was treated in more than three conditions. Rats were euthanized 48 hours after the last injection.

**Experiment 2: Repeated 10% AITC injections**

If depression of wheel running is a reliable measure of migraine pain, then the magnitude and duration of AITC-induced depression of wheel running should be consistent with repeated administration. This hypothesis was tested by measuring wheel running following injection of 10% AITC onto the dura every other day until the rat had received three injections. Surgical implantation of the cannula, baseline testing, and timing of the AITC injection was identical to Experiment 1.

**Experiment 3: Sumatriptan efficacy against AITC-induced pain**

If depression of wheel running is a clinically relevant measure of migraine pain, then the anti-migraine medication sumatriptan should attenuate AITC-induced depression of wheel running. This hypothesis was tested by injecting sumatriptan (0.1 mg/kg and 1 mg/kg, s.c.) or saline either 1 or 90 min post-AITC injection. Human data demonstrates that sumatriptan is only effective if administered soon after migraine onset (Diener et al., 2008). In order to administer sumatriptan 90 min after AITC administration, animals were removed from their home cages at 1500 h, injected with AITC, and returned to their home cage. Rats were removed again 90 min later, injected with sumatriptan (1 mg/kg, s.c.) or saline, and returned to their home cage at approximately 1650 h. Wheel running was measured for 23 hours beginning at 1700 h. A within-subjects, counterbalanced design was used so that each rat was tested with AITC three times.
Saline was administered on one occasion and sumatriptan on the other two. Some rats were tested with different doses of sumatriptan while other rats were tested at different times (1 or 90 min). Two days separated each dural injection.

**Experiment 4: Sumatriptan effects on wheel running**

In the absence of migraine pain, sumatriptan should have no effect on wheel running. This hypothesis was tested by injecting sumatriptan into naïve rats following 8 days of habituation to wheel running. These rats had no surgery and were not treated with AITC. Rats were removed from their home cages and injected with sumatriptan (1 mg/kg, s.c.) or saline and returned to their home cages at approximately 1650 h. Wheel running was measured for 23 hours beginning at 1700 h. A within-subjects, counterbalanced design was used so that each rat received both saline and sumatriptan injections. Two days separated these injections.

**Data analysis**

Baseline activity was defined as the total number of wheel revolutions during the 23 hours preceding the first injection. An average hourly nighttime running rate was used as the baseline for hour-by-hour analysis. Given individual differences in wheel running, subsequent wheel running data are presented as a percent change from each rat’s baseline value. All data are expressed as mean ± SEM. Nearly all running occurs during the dark phase of the light cycle (Kandasamy et al., 2016), so only data collected during the dark phase were analyzed. Percentage of baseline running was averaged over the 3-hour period following injection of AITC in order to compare the magnitude of migraine-pain induced depression of wheel running. Data were analyzed with an independent samples t-test or one-way ANOVA. Because animals whose guide cannulas were defective (n = 6) and whose wheels malfunctioned (n = 6) were not available for all of the within-subjects conditions, groups were treated as independent samples. Statistical significance was defined as a probability of < 0.05.
Results

Experiment 1: AITC concentration-response

Microinjection of AITC onto the dura caused a concentration-dependent reduction in wheel running. The highest concentration of AITC (10%) caused a pronounced depression of wheel running that lasted for 3 hours (Fig. 2.1A). Administration of a lower concentration of AITC caused a more modest and shorter lasting depression of wheel running (Fig. 2.1A). Analysis of the magnitude of AITC-induced depression of wheel running during this 3-hour period (Fig. 2.1B) revealed a significant difference in wheel running between AITC conditions ($F(3,173) = 7.459, p < 0.001$). Post-hoc analysis revealed that wheel running was significantly lower following administration of 10% AITC compared to saline-, mineral oil-, or 1% AITC-treated rats (Tukey test: Sal vs. 10% AITC, $p < 0.001$; Mineral oil vs. 10% AITC, $p = 0.016$; 1% vs. 10% AITC, $p = 0.04$). The depression in wheel running following administration of 1% AITC was not significantly different than saline- (Tukey test, $p = 0.153$) or mineral oil-treated (Tukey test, $p = 0.710$) controls.

Experiment 2: Repeated 10% AITC injections

Rats were given three dural injections of 10% AITC to test the reliability of repeated injections to depress wheel running. Administration of 10% AITC caused a consistent depression of wheel running that lasted approximately 3 hours following each injection (Fig. 2.2A). The magnitude of the depression seemed to increase with each subsequent injection, especially during the second hour, but a one-way ANOVA on total wheel running during the 3 hours following AITC administration revealed no significant differences between the three injections ($F(2,56) = 1.716, p = 0.189$; Fig. 2.2B).

Experiment 3: Sumatriptan efficacy against AITC-induced pain
Administration of the anti-migraine medication sumatriptan immediately after injection of AITC attenuated the depression of wheel running. Reversal of AITC-induced depression of wheel running was first evident 2 hours after sumatriptan administration and only following administration of the highest dose, 1.0 mg/kg (Fig. 2.3A). There was a significant difference in wheel running during the first three hours following AITC administration (Fig. 2.3B) in rats injected with 1 mg/kg of sumatriptan compared to rats treated with saline or a low dose of sumatriptan (0.1 mg/kg) \( F(2,71) = 4.041, p = 0.022 \).

A separate group of rats was injected with sumatriptan (1 mg/kg) or saline 90 min after AITC administration to determine whether sumatriptan could reverse migraine pain once it had been established. Running patterns are nearly identical between sumatriptan- and saline-treated rats when injected 90 min after AITC (Fig. 2.4). Comparison of total wheel running in these two groups during the 3 hours following sumatriptan administration revealed no differences between groups \( t(43) = 0.002, p = 0.969 \).

**Experiment 4: Sumatriptan effects on wheel running**

Naïve rats were injected with either sumatriptan (1 mg/kg) or saline to determine the effects of sumatriptan alone on wheel running. Sumatriptan had no consistent effect on wheel running in naïve animals (Fig. 2.5). Comparison of these two groups during the first 3 hours after sumatriptan administration revealed no significant difference \( t(94) = 0.860, p = 0.392 \).

**Discussion**

The present data indicate that depression of home cage wheel running is a reliable and clinically relevant method to assess migraine pain in laboratory rats. AITC-induced activation of dural afferents produced a concentration-dependent reduction in wheel running that persisted for at least 3 hours. Repeated injections of 10% AITC onto the dura caused a consistent
reduction in wheel running following each injection. Finally, administration of the anti-migraine medication sumatriptan produced dose-dependently reverend AITC-induced depression of wheel running when administered immediately after the AITC injection, but did not affect wheel running in pain-free animals.

Our finding that dural administration of AITC produces a 3 hour reduction in wheel running confirms previous studies indicating that activation of TRPA1 receptors produces migraine-like pain. TRPA1 receptors have been shown to contribute to migraine in both human and rat. Activation of trigeminal TRPA1 receptors causes meningeal vasodilation and calcitonin gene-related peptide release (Nassi et al., 2012) as well as periorbital and hindpaw allodynia (Edelmayer et al., 2012) in rodents. Case studies have reported that inhalation of TRPA1 agonists can trigger headache in people (Benemei et al., 2010; Nassini et al., 2012).

Although the depression of wheel running following AITC administration onto the dura lasted for 3 hours, the diagnostic criterion for migraine in humans requires a duration of a minimum of 4 hours (Headache Classification Committee of the International Headache Society (IHS), 2013). It is unlikely that AITC administration produces a syndrome that fully replicates a human migraine. Dural administration of an “inflammatory soup” has also been used to induce migraine-like pain, but neither of these models truly captures a human migraine. Given that migraine is a complicated process that involves the brain, local blood vessels, and trigeminal afferents (Jacobs and Dussor, 2016), simple chemical activation of dural afferents via AITC or inflammatory soup is unlikely to engage all of these systems. However, the 3-hour depression of wheel running reported in this manuscript and the widespread allodynia reported by others (Edelmayer et al., 2012) suggests that administration of AITC mimics migraine-like pain.

Previous studies have shown that administration of AITC to the dura produces allodynia that persists for up to 5 hours post-injection (Edelmayer et al., 2012) - significantly longer than
depression of wheel running. Given that allodynia may be present during interictal phases (Aguggia, 2012), it is likely that assessing allodynia as a measure of migraine in rodents may be confounded by allodynia being present in either the postdrome phase of a migraine attack or the interictal period. Furthermore, given that dural AITC-induced hindpaw allodynia persists for 5 hours, it is unlikely that depression of wheel running is caused by hindpaw allodynia.

We also show that repeated AITC administration did not cause a significant increase in the magnitude or duration of depression of wheel running. A trend towards greater depression of wheel running was evident with repeated injections, particularly in the second hour post-AITC (Fig. 2.2A), but this difference did not reach statistical significance. A floor effect may have prevented the expression of more intense pain. Sensitization of the trigeminovascular system has been demonstrated using multiple dural infusions of inflammatory soup (Oshinsky and Gomonchareonsiri, 2007; Melo-Carrillo and Lopez-Avila, 2013), so a similar enhancement would be expected with repeated AITC injections. These findings suggest that it may be possible to induce chronic migraine with repeated dural injections of AITC. Continuous home cage wheel running would provide an objective and simple method to detect potential spontaneous migraine episodes, especially if the incidence of chronic migraine arises in only a subset of animals as is the case with humans.

Sumatriptan is a prototypical anti-migraine agent and has been used to demonstrate the predictive validity of animal models of migraine. Our data showing that sumatriptan is only effective in reversing AITC-induced depression of wheel running when given at a sufficient dose (1 mg/kg) and latency following induction of headache further validates home cage wheel running as a method to assess migraine in rats. Sumatriptan had no efficacy when administered 90 min post-AITC injection (Fig. 2.4) as has been reported in humans where the efficacy of triptans is attenuated if patients wait to take the drug (Diener et al., 2008). Furthermore, similar
to the clinical situation (Winner et al., 2003), our data suggest that sumatriptan relieves pain 2 hours after its administration (Fig. 2.3A). Although pretreatment with sumatriptan may prevent AITC-induced depression of wheel running as has been reported previously with other behaviors (Edelmayer et al., 2012; Melo-Carrillo and Lopez-Avila, 2013), triptans are generally considered abortive anti-migraine agents, and thus, are rarely taken before the onset of migraine symptoms.

Depression of wheel running is also a clinically relevant method to assess migraine pain in rats in that it mimics the reduction in normal physical activity characteristic of migraine in humans (Headache Classification Committee of the International Headache Society (IHS), 2013). Migraine has been reported to reduce activity for 57.3 million days per year for women (Stang and Osterhaus, 1993), and is often so severe as to require bed rest (Brandes, 2002). Our data showing depression of home cage wheel running is consistent with these clinical observations.

Assessment of home cage wheel running provides a number of advantages over other tests to assess migraine pain in animals. In contrast to pain-evoked methods to assess migraine such as assessing periorbital and/or hindpaw allodynia, which may induce stress by testing the animal in a novel environment, home cage wheel running reveals the effect of spontaneous migraine pain in a stress-free environment. Moreover, data collection is objective, independent of the researcher, and captures both the magnitude and duration of migraine pain.

Given that home cage wheel running is also sensitive to the disruptive side effects of analgesics (Kandasamy et al., 2017), this test allows existing and experimental treatments to be simultaneously evaluated for both anti-migraine efficacy and side effect profile. This key advantage separates home cage wheel running from traditional tests of migraine pain in rats such as assessment of allodynia, which can be blocked by drugs with either antinociceptive or
sedative effects. A key goal of drug treatment should be to restore function as opposed to replacing one problem (pain) with another (sedation).

Although depression of wheel running provides an objective and clinically relevant measure of migraine pain, exercise itself has been shown to reduce pain (Grace et al., 2016). However, this type of exercise-induced antinociception typically requires multiple weeks of daily wheel running (Sluka et al., 2013). Our previous studies (Kandasamy et al., 2016; Kandasamy et al., 2017) and those of others (Whitehead et al., 2016) show that 5-8 days of baseline wheel running has no antinociceptive effect. These findings are consistent with human behavior in that consistent prolonged exercise can be used to maintain health, whereas pain or illness will disrupt a person’s exercise regimen.

The present data build a firm foundation for the use of home cage wheel running to assess migraine pain in rats. The obvious problem with preclinical migraine research is that it does not mimic the spontaneous onset of migraine. Both pharmacological (Oshinsky et al., 2012) and genetic (Chanda et al., 2013) approaches have been used to attempt to overcome this disconnect between preclinical models and the human situation, but these procedures are limited by their inability to continuously monitor migraine pain-related decreases in behavior. Assessment of home cage wheel running provides a simple method to model the seemingly random occurrences of migraine that occur in humans. The validity of home cage wheel running as a preclinical method to assess migraine will be tested in future studies by examining the effects of prophylactic and other abortive anti-migraine therapies.

Female rats were used in the present study to enhance the clinical relevance of the research given that migraine is more common in women than men (Stang and Osterhaus, 1993; Smitherman et al., 2013). Given that the higher incidence of migraine in women has been linked to female sex hormones (Vetvik and MacGregor, 2016), one would predict that the magnitude
and duration of migraine-induced depression of wheel running would be greater in female than male rats, and vary depending on the phase of the estrous cycle. However, the occurrence of sex differences may require the use of a more natural migraine model (e.g., systemic nitric oxide donor) as opposed to that induced by an irritant such as AITC or inflammatory soup.

In sum, the results of the present study demonstrate that home cage wheel running objectively captures AITC-induced depression of activity that closely resembles migraine pain in patients. It is an easy-to-use test that allows precise quantification of the magnitude and duration of migraine pain. The use of home cage wheel running should help to unveil the pathophysiology underlying migraine headache and provide a clinically relevant method to assess the anti-migraine efficacy of novel therapeutics.
Figure 2.1. *Dural injection of AITC depresses home cage wheel running in a concentration-dependent manner.* (A) Administration of 10% AITC depressed wheel running for 3 hours. The magnitude and duration of AITC-induced depression of wheel running was greatly reduced when the concentration was reduced to 1%. (B) Total wheel revolutions during the 3 hours following administration of 10% AITC (n = 17) was significantly lower compared to animals receiving 1% AITC (n = 20), mineral oil (n = 7), or saline (n = 14). * indicates \( p < 0.001 \) vs. saline, \( p = 0.016 \) vs. mineral oil, \( p = 0.04 \) vs. 1% AITC.
Figure 2.2. Consistent depression of wheel running following repeated injections of 10% AITC onto the dura. (A) Administration of 10% AITC depressed wheel running for approximately 3 hours on all three trials. The magnitude of depressed wheel running was greatest following the third AITC injection, especially during the second hour, although this difference did not reach statistical significance when total wheel revolutions during the 3 hours following AITC administration were analyzed (see panel B). n = 6-7/condition
Figure 2.3. Administration of sumatriptan reverses AITC-induced depression of wheel running.

(A) Administration of the high (1 mg/kg), but not the low dose (0.1 mg/kg) of sumatriptan immediately after administration of AITC shortened the duration of AITC-induced depression of wheel running. (B) Recovery of wheel running in the 3 hours following 1.0 mg/kg sumatriptan (n = 11) administration was significantly greater than following administration of 1 mg/kg of sumatriptan compared to rats treated with 0.1 mg/kg (n = 7) or saline (n = 7). * indicates p < 0.05 vs. saline.
Figure 2.4. *Sumatriptan injected ninety minutes after induction of headache did not reverse AITC-induced depression of wheel running.* Sumatriptan (1 mg/kg) or saline (1 mL/kg) was injected 90 min after dural injection of 10% AITC. Administration of sumatriptan (n = 8) at this time point had no effect on depressed wheel running compared to saline-treated animals (n = 7).
Figure 2.5. *Sumatriptan had no effect on wheel running in rats not treated with AITC.* (A) Wheel running was variable following administration of sumatriptan (1 mg/kg) or saline (1 mL/kg) in the absence of headache. Neither sumatriptan (n = 14) nor saline (n = 18) depressed wheel running. (B) Wheel running was unaffected in the 3 hours following sumatriptan or saline.
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CHAPTER 3:

ANTI-MIGRAINE EFFECT OF Δ⁹-ΤΕΤΡΑΗΔΡΟΚΑΝΝΑΒΙΝΟΛ IN THE FEMALE RAT

Abstract

Current anti-migraine treatments have limited efficacy and many side effects. Although anecdotal evidence suggests marijuana is useful for migraine, this hypothesis has not been tested in a controlled experiment. Thus, the present study tested whether administration of Δ⁹-tetrahydrocannabinol (THC) produces anti-migraine effects in the female rat. Microinjection of the TRPA1 agonist allyl isothiocyanate (AITC) onto the dura mater produced migraine-like pain for 3 hours as measured by depression of home cage wheel running. Concurrent systemic administration of 0.32, but not 0.1 or 1.0 mg/kg of THC prevented AITC-induced depression of wheel running. This same dose was ineffective when administered 90 min before or after AITC. Administration of the highest dose of THC (1.0 mg/kg) produced disruptive side effects that depressed wheel running independent of any anti-migraine effects. Administration of the CB₁ but not the CB₂ receptor antagonist attenuated the anti-migraine effect of 0.32 mg/kg THC. These data reveal that: 1) THC reduces migraine-like pain when administered at a specific dose (0.32 mg/kg) and time (immediately after AITC); 2) THC’s anti-migraine effect is mediated by CB₁ receptors; and 3) Wheel running is an effective method to assess migraine treatments because only treatments producing antinociception without disruptive side effects will restore normal activity.
Introduction

Migraine is characterized by severe headache and heightened sensitivity to sensory stimuli that results in depression of normal daily activities. Despite the prevalence and severity of primary headache disorders, treatments for migraine are surprisingly limited. Currently available prophylactic and abortive therapies manage less than 50% of migraine cases due to lack of efficacy or adverse side effects (e.g., nausea, dizziness, drowsiness) (Stovner et al., 2009; Diener et al., 2015). Furthermore, efficacious drugs which are used repeatedly (e.g., triptans, ergots, NSAIDs) can lead to medication overuse headache, a condition in which headaches transform from an episodic to a chronic and more intense condition (Dodick and Freitag, 2006). Thus, there is a critical need to identify and employ novel anti-migraine agents.

Given the reported therapeutic benefits of cannabis and cannabinoids such as ∆9-tetrahydrocannabinol (THC) for a wide range of pain conditions (Noyes and Baram, 1974; Milstein et al., 1975; Noyes et al., 1975; Karst et al., 2010; Kraft, 2012; Chiou et al., 2013; Maione et al., 2013), it is not surprising that some people use marijuana as a treatment for migraine (el-Mallakh, 1989). A survey investigating reasons for self-medication with cannabis in Germany, Austria, and Switzerland revealed that 10.2% used it for migraine and headache (Schnelle et al., 1999). Medical marijuana has also been reported to reduce the frequency of migraines (Rhyne et al., 2016; Cuttler et al., 2016; Sexton et al., 2016). Preclinical research suggests that cannabinoids can modulate migraine pain by inhibiting the activity of A- and C-fiber inputs from the dura mater via activation of cannabinoid type-1 (CB1) receptors (Akerman et al., 2007). Although these data are promising, we are not aware of any study that systematically examines the antinociceptive efficacy of THC in an animal model of migraine.

Migraine is difficult to study in laboratory animals because pain occurs in the absence of tissue injury, leaving scientists without reliable or valid behavioral assays (Strassman and
Burstein, 2013). Mechanical allodynia has served as the primary dependent measure for headache in laboratory animals, but allodynia is a marker of migraine progression (Burstein et al., 2004; Louter et al., 2013) and is rarely assessed clinically (Mathew et al., 2004). Moreover, mechanical allodynia may outlast the headache and be present during interictal periods (Aguggia, 2012). In contrast to allodynia, the reduction in routine physical activity caused by migraine is a diagnostic criterion that is easy to assess in laboratory animals. We have previously shown that activation of dural afferents using the TRPA1 agonist allyl isothiocyanate (AITC) depresses home cage wheel running and this depression is restored by the anti-migraine treatment sumatriptan (Chapter 2). Thus, home cage wheel running provides an objective, sensitive, and clinically relevant measure of migraine pain in rats. The present study will test the hypothesis that THC will prevent migraine-depressed wheel running in a CB1-dependent manner.

Materials and Methods

Subjects

Data were collected from 53 adult female Sprague-Dawley rats bred at Washington State University Vancouver (Vancouver, WA, USA). Female rats were selected because migraine is much more common in women than men. All rats were 50-70 days old at the start of the study and randomly assigned to treatment groups. A within-subjects design was used to reduce the number of animals needed. All procedures were approved by the Washington State University Animal Care and Use Committee and conducted in accordance with the International Association for the Study of Pain’s Policies on the Use of Animals in Research.

Surgery
Prior to surgery, rats were housed in pairs in a 22-24 °C colony room on a 12/12-hour light/dark cycle (lights off at 1800 h). Animals were anesthetized with pentobarbital (50 mg/kg, i.p.) and implanted with a guide cannula (18 gauge; 4 mm long) aimed above the dura mater (AP: +1.0 mm; ML: +1.0 mm; DV: 0.8 mm, from lambda). Loctite® super glue was used to form a tight seal around the guide cannula, and dental cement anchored the guide cannula to two screws in the skull. Rats were maintained under a heat lamp until awake. Following surgery, each rat was housed individually in an extra tall cage (36 x 24 x 40 cm) with a running wheel. The cage was located in a sound-attenuating booth (2.1 x 2.2 m; Industrial Acoustics Company, Inc., Bronx, NY, USA) for the remainder of the experiment to limit the influence of outside stimuli. Food and water were available ad libitum.

**Running wheel**

A Kaytee Run-Around Giant Exercise Wheel (Kaytee Products, Inc., Chilton, WI, USA) with a diameter of 27.9 cm was suspended from the top of the rat’s home cage. The floor of the cage was covered with cellulose bedding (BioFresh™, Ferndale, WA, USA). A thin aluminum plate (0.8 mm x 5.08 cm x 3.81 cm; K&S Precision Metals, Chicago, IL, USA) was attached to one spoke of the running wheel to interrupt a photobeam projecting across the cage with each rotation. The beam was set 18 cm above the floor of the cage so that only the rotation of the wheel, not the normal activity of the rat, would interrupt the beam. The number of wheel revolutions was summed over 5-min bins for 23 hours each day using Multi-Varimex software (Columbus Instruments, Columbus, OH, USA). Recording began at 1700 h, the onset of the dark phase of the light cycle when rats are most active. A full description of the running wheel with video is available in our previous publication (Kandasamy et al., 2016). Rats were allowed unrestricted access to the wheel for 23 hours/day for 8 days following surgery. The number of wheel revolutions that occurred during the 23 hours prior to the first dural injection of AITC was
used as the baseline activity. Rats that ran less than 400 revolutions on the baseline day (Kandasamy et al., 2016) were not included in further testing (n = 5/58).

**Drugs**

Allyl isothiocyanate (AITC; Sigma-Aldrich, Inc., St. Louis, MO, USA) was mixed in mineral oil at a concentration of 10% and injected into the periosteal space in a volume of 10 \( \mu \text{L} \). \( \Delta^9 \)-tetrahydrocannabinol (Sigma-Aldrich, Inc., St. Louis, MO, USA) was dissolved in vehicle (1:1:18; ethanol:cremophor:saline) and injected intraperitoneally at doses of 0.1, 0.32, and 1.0 mg/kg in a volume of 1 mL/kg. The CB\(_1\) receptor antagonist SR141716A (1.0 mg/kg) and the CB\(_2\) receptor antagonist SR144528 (3.2 mg/kg) (Tocris Bioscience, Minneapolis, MN, USA) were dissolved in the same vehicle as THC and injected intraperitoneally in a volume of 1 mL/kg. Antagonist doses were consistent with doses in female rats from a previous publication (Craft et al., 2012).

**Experiment 1: THC administration during onset of headache**

The objective of this experiment was to determine whether THC administration prevents AITC-induced depression of wheel running. Following baseline testing on Day 8, the rat was injected with 10 \( \mu \text{L} \) of 10% AITC or mineral oil onto the dura mater using an injection cannula inserted into the guide cannula. The rat was injected with either vehicle or THC (0.1, 0.32, 1.0 mg/kg) immediately following AITC administration. All injections were completed by 1650 h so the rats could be returned to their home cages prior to the start of the 23 hours of recording beginning at 1700 h. This procedure was repeated every other day with the THC doses administered in a counterbalanced order. No rat was injected with AITC more than three times (Chapter 2). Rats were euthanized 48 hours after the last injection.

**Experiment 2: THC administration ninety minutes before onset of headache**
The objective of this experiment was to test whether pretreatment of THC 90 min before AITC injection prevents AITC-induced depression of wheel running. Surgical implantation of the cannula and baseline testing were identical to Experiment 1. In this experiment, the rat was removed from its cage at approximately 1500 h and injected with either vehicle or THC (0.1, 0.32 mg/kg, i.p.). Ninety minutes later, the rat was injected with 10 µL of 10% AITC or mineral oil onto the dura mater. All injections were complete by 1650 h. The rat was returned to its home cage and wheel running was recorded for the next 23 hours beginning at 1700 h. This procedure was repeated every other day with the THC doses administered in a counterbalanced order.

**Experiment 3: THC administration ninety minutes after onset of headache**

The objective of this experiment was to determine whether THC administration ninety minutes after AITC injection reverses AITC-induced depression of wheel running. Surgical implantation of the cannula and baseline testing were identical to Experiment 1. In this experiment, the rat was removed from its cage at approximately 1500 h and injected with 10 µL of 10% AITC or mineral oil onto the dura mater. Ninety minutes later, the rat was injected with either vehicle or THC (0.1, 0.32 mg/kg, i.p.). All injections were completed by 1650 h. The rat was returned to its home cage and wheel running was recorded for the next 23 hours beginning at 1700 h. This procedure was repeated every other day with the THC doses administered in a counterbalanced order.

**Experiment 4: Role of cannabinoid receptors in the anti-migraine effects of THC**

The goal of this experiment was to determine whether CB₁ or CB₂ receptors mediate the anti-migraine effects of THC. Surgical implantation of the cannula, baseline testing, and drug injections were identical to Experiment 1. Rats were injected with either vehicle, the CB₁ receptor antagonist SR141716A (1.0 mg/kg, i.p.), or the CB₂ receptor antagonist SR144528 (3.2
mg/kg, i.p.) 30 min prior to administration of AITC or mineral oil and then THC (0.32 mg/kg) or vehicle. All injections were completed by 1650 h. The rats were returned to their home cages and wheel running was recorded for the next 23 hours beginning at 1700 h. This procedure was repeated every other day with the different cannabinoid receptor antagonists or vehicle administered in a counterbalanced order.

Data analysis

An average hourly nighttime running rate was used as the baseline for hour-by-hour analyses. Given individual differences in wheel running, all wheel running data are presented as a percent change from each rat's baseline value. All data are expressed as mean ± SEM. Nearly all running occurs during the dark phase of the light cycle, so only data collected during the dark phase when drugs were administered are presented. Data were analyzed with two-way ANOVA (dose x hour) followed by LSD post-hoc analysis over the 3-hour period following injection of AITC or THC to get a precise measure of the magnitude of migraine pain-induced depression of wheel running. A Mann-Whitney U or Kruskal-Wallis test was used to compare two or three groups using medians. Because animals whose guide cannula was defective (n = 4) were not available for all of the within-subjects conditions, groups were treated as independent samples. Statistical significance was defined as a probability <0.05.

Results

Experiment 1: THC administration during onset of headache

Microinjection of AITC onto the dura caused a reduction in wheel running that lasted for 3 hours (Fig. 3.1, top). Concurrent administration of 0.32 mg/kg of THC prevented AITC-induced depression of wheel running compared to lower (0.1 mg/kg) or higher (1.0 mg/kg) doses or administration of vehicle (Fig. 3.1, top). Analysis of the magnitude of wheel running during this
3-hour period revealed a significant difference between THC doses ($F(3,31) = 4.793, p = 0.007$). Post-hoc analysis revealed that wheel running was significantly higher following administration of 0.32 mg/kg THC compared to all other doses (LSD test: Vehicle vs. 0.32 mg/kg, $p = 0.002$; 0.1 mg/kg vs. 0.32 mg/kg, $p = 0.021$; 1.0 mg/kg vs. 0.32 mg/kg, $p = 0.005$). The depression of wheel running following administration of 1.0 mg/kg of THC was similar to that produced by AITC administration alone (i.e., the vehicle group; LSD test, $p = 0.784$) or 0.1 mg/kg of THC (LSD test, $p = 0.496$). Although administration of 0.32 mg/kg of THC produced the greatest recovery of wheel running, analysis of individual rats indicates that this dose was ineffective in two rats and lower and higher doses (0.1 and 1.0 mg/kg) provided recovery in some rats (Fig. 3.1, bottom).

Microinjection of mineral oil onto the dura had no effect on wheel running (Fig. 3.2, top). Likewise, wheel running was relatively stable following administration of 0.1 and 0.32 mg/kg of THC ($F(3,21) = 1.443, p = 0.259$). Although mean wheel running was 50% of baseline levels following administration of 1.0 mg/kg of THC, the response in individual rats was mixed. Five of the seven rats showed a dramatic reduction in activity, but administration of 1.0 mg/kg of THC had no effect on wheel running in two rats (Fig. 3.2, bottom). Despite this variability, analysis of medians revealed a strong trend for administration of 1.0 mg/kg THC to significantly depress wheel running compared to vehicle-treated rats (Mann-Whitney U test: $p = 0.063$).

**Experiment 2: THC administration ninety minutes before onset of headache**

Rats were injected with vehicle or THC (0.1 and 0.32 mg/kg) 90 min before AITC administration to determine whether THC pretreatment prevents AITC-induced depression of wheel running. Neither dose of THC prevented AITC-induced depression of wheel running. Microinjection of AITC caused a 3-hour decrease in wheel running in all groups (Fig. 3.3, top). Analysis of the magnitude of wheel running during this 3-hour period revealed no significant
differences in wheel running between groups ($F(2,25) = 2.735, p = 0.084$). Analysis of medians revealed no significant differences between groups (Fig. 3.3, bottom; Kruskal-Wallis test: $p = 0.07$).

**Experiment 3: THC administration ninety minutes after onset of headache**

Rats were injected with vehicle or THC (0.1 and 0.32 mg/kg) 90 min after AITC microinjection to determine whether THC reverses AITC-induced depression of wheel running. Neither dose of THC reversed AITC-induced depression of wheel running (Fig. 3.4, top). Analysis of the magnitude of wheel running during the 3 hours following THC administration revealed no significant differences in wheel running between groups ($F(2,18) = 0.220, p = 0.805$). Although analysis of medians revealed no significant differences between groups (Fig 3.4, bottom; Kruskal-Wallis test: $p = 0.891$), analysis of individual rats suggests that two rats benefited from administration of 0.32 mg/kg THC.

**Experiment 4: Role of cannabinoid receptors in the anti-migraine effects of THC**

To determine which cannabinoid receptor contributes to the anti-migraine effect of 0.32 mg/kg THC, rats were treated with vehicle, a CB$_1$, or a CB$_2$ receptor antagonist 30 minutes prior to the AITC and THC injections. The anti-migraine effect of THC was attenuated in animals treated with the CB$_1$ receptor antagonist compared to animals treated with vehicle or the CB$_2$ receptor antagonist (Fig. 3.5, top). Analysis of the magnitude of wheel running during this 3-hour period revealed a trend towards a significant difference in wheel running between groups ($F(2,18) = 3.026, p = 0.074$). Post-hoc analysis revealed that this trend was driven by significantly less wheel running in rats treated with the CB$_1$ receptor antagonist compared to vehicle-treated rats given THC (LSD test, $p = 0.025$). Animals treated with the CB$_2$ receptor antagonist did not differ from rats treated with vehicle (LSD test, $p = 0.176$). Analysis of medians revealed that animals treated with the CB$_1$ antagonist ran significantly less than animals treated
with THC alone (Fig. 3.5, bottom; Mann Whitney U test: \( p = 0.048 \)). Administration of the cannabinoid receptor antagonists had no effect in rats not treated with THC or not given AITC to induce a headache. Running patterns did not differ in animals treated with the CB\(_1\) and CB\(_2\) receptor antagonists 30 min prior to AITC injections in rats given vehicle instead of THC [Fig. 3.6A; \( F(1,11) = 0.919, p = 0.358 \)]. Similarly, administration of the cannabinoid receptor antagonists had no effect on wheel running in animals treated with mineral oil onto the dura mater [Fig. 3.6B; \( F(2,15) = 0.602, p = 0.561 \)].

**Discussion**

The present data show that administration of THC prevents depression of home cage wheel running caused by migraine-like pain in a time- and dose-dependent manner. AITC-induced activation of dural afferents reduced wheel running that persisted for approximately three hours, as we have shown before (Chapter 2). Administration of 0.32 mg/kg THC immediately after the onset of headache prevented AITC-induced depression of wheel running. This anti-migraine effect was absent if THC was administered either 90 min before or after AITC microinjection, or if lower or higher doses of THC were administered. Administration of the CB\(_1\), but not the CB\(_2\), receptor antagonist blocked the anti-migraine effect of THC.

Preclinical studies show that THC is effective in reducing multiple types of pain including pain caused by acute stimuli (Tseng and Craft, 2001), chronic inflammation (Craft et al., 2013), lactic acid (Kwilasz and Negus, 2012), and neuropathy (Harris et al., 2016). THC also suppresses the propagation velocity, amplitude, and duration of cortical spreading depression, a key component of migraine pathophysiology (Kazemi et al., 2012). This is the first preclinical study to show that administration of THC also reduces migraine-like pain in an awake animal.
Our data indicate that THC reduces migraine pain if administered at a specific dose (0.32 mg/kg) and time (immediately after AITC).

Anecdotal evidence indicates that medical marijuana may be effective in aborting migraine attacks after they have started (Rhyne et al., 2016). Our data does not provide evidence for an abortive effect of THC on migraine in 5 of the 7 rats tested, at least when THC is administered 90 min after the AITC injection. Similarly, administration of the anti-migraine medication sumatriptan had no effect on AITC-induced depression of wheel running when administered 90 min after AITC (Chapter 2). These findings are consistent with the well-known limitations of sumatriptan to abort migraine in humans if administered after migraine onset (Diener et al., 2008). However, a major difference between our data and anecdotal reports from migraine patients is that we focused on THC specifically, whereas marijuana contains over 100 different cannabinoids. Thus, it is possible that some of these other cannabinoids can reverse migraine pain that has progressed to a stage that is unaffected by THC alone. It is also possible that abortive effects of THC are mediated by mechanisms that precede the direct activation of dural afferents by AITC used in the present study.

Our finding that the CB₁ receptor mediates the anti-migraine effects of THC confirms previous studies indicating a role for the CB₁ receptor in migraine. Activation of CB₁ receptors in the ventrolateral periaqueductal gray attenuates activation of trigeminovascular afferents evoked by noxious stimulation of the dura mater (Knight and Goadsby, 2001; Akerman et al., 2013). Human data indicate that genetic mutations that limit the expression of the CB₁ receptor increase the risk of migraine (Juhasz et al., 2009). Our results are consistent with these findings and suggest that the CB₁ receptor may be a useful therapeutic target for the treatment of migraine.
CB₁ receptors may inhibit migraine via a central mechanism or by direct inhibition of dural afferents. CB₁ receptors are present on fibers in the trigeminal tract and trigeminal nucleus caudalis (Tsou et al., 1998). Activation of these receptors via THC likely inhibits the release of neuropeptides associated with migraine such as calcitonin gene-related peptide (CGRP). However, activation of CB₁ receptors also inhibits dural blood vessel dilation induced by electrical stimulation or administration of CGRP, capsaicin, or nitric oxide (Akerman et al., 2004). Cannabinoids may also interact with serotonin, a neurotransmitter implicated in migraine, to modulate migraine pain (Voth and Schwartz, 1997; Bartsch et al., 2004; Haj-Dahmane and Shen, 2009; Akerman et al., 2013). Given the complex mechanisms of action underlying the effects of cannabinoids on nociception, blood flow, and behavior (see (Greco and Tassorelli, 2015) for review), THC may alleviate migraine pain through multiple mechanisms.

Migraine is three times more common in women than men (Vetvik and MacGregor, 2016); however, the majority of preclinical studies of migraine use male subjects. Thus, finding effective anti-migraine therapies for women and using female subjects in preclinical studies remains a priority. Previous studies have demonstrated that female rats are more sensitive to the antinociceptive effects of THC than male rats against acute (Tseng and Craft, 2001) and chronic inflammatory pain (Craft et al., 2013). Given the high prevalence of migraine in females, cannabinoids may be an especially effective therapy for women.

Although migraine pain is difficult to assess in laboratory animals (Strassman and Burstein, 2013), depression of home cage wheel running provides an objective method to assess the duration and magnitude of migraine-like pain (Chapter 2). Assessment of home cage wheel running is especially useful in evaluating drug treatments because the goal is restoration of function, which requires that an effective drug reduces pain without inducing disruptive side effects. For example, high doses of morphine block mechanical allodynia induced by
inflammatory pain, but do not restore depressed wheel running because of disruptive side effects (Kandasamy et al., 2017). Likewise, the present data show that the highest dose of THC (1.0 mg/kg) does not restore migraine-depressed wheel running, and in fact, depresses wheel running in pain-free rats. Other tests of pain-depressed behavior, such as intracranial self-stimulation, show a similar depression of behavior following administration of high doses of THC (i.e., 1.0 mg/kg) (Leitl and Negus, 2015).

There is a significant amount of individual variability in pain (Fillingim, 2005) and analgesia (Galer et al., 1992) in humans; however, individual differences are rarely assessed in preclinical studies. A genetic basis for individual variability of pain in rats and humans has been reported (Diatchenko et al., 2005; Bali et al., 2014), but there is little behavioral evidence examining this phenomenon. Our data show that home cage wheel running provides a unique opportunity to assess individual differences in responses to THC. For example, 1.0 mg/kg of THC depressed wheel running in 5 of 7 animals, but appeared to have no effect in the other two (Fig. 3.2, bottom). A better understanding of the neural basis for individual differences in the behavioral and analgesic effects of THC and migraine is needed.

In conclusion, we demonstrate that THC, when given at a specific dose and time, prevents migraine-like pain as measured by home cage wheel running. An important finding is that although higher doses of THC probably reduce migraine-like pain, disruptive side effects prevent the restoration of normal activity. Further, we demonstrate that the anti-migraine effects of THC are mediated by the CB1 receptor. The present study builds a firm foundation for the behavioral analysis of cannabinoids such as THC as a treatment for migraine in humans. Additional controlled studies in both humans and animals are needed to more fully characterize the anti-migraine effects of THC and other cannabinoids.
Figure 3.1. THC dose dependently prevents AITC-induced depression of wheel running. Top: Microinjection of AITC onto the dura mater produced migraine-like pain indicated by depression of wheel running that lasted 3 hours. Administration of 0.32 mg/kg of THC immediately after AITC administration restored wheel running to near baseline levels. Administration of lower and higher doses of THC (0.1 and 1.0 mg/kg) did not reverse AITC-induced depression of wheel running. Bottom: Analysis of median (lines) wheel running activity for the 3-hour duration of the migraine shows relatively consistent within-group responses (symbols) to administration of AITC and THC. However, the reversal of migraine-like pain produced by administration of 0.32 mg/kg of THC did not occur in all of the rats: two rats that did not run at all during this period (n = 8-9/group).
Figure 3.2. The highest dose of THC depresses running in pain-free animals. Top:

Microinjection of mineral oil onto the dura as a control for AITC had no effect on wheel running. Administration of low (0.1 mg/kg) and medium (0.32 mg/kg) doses of THC had no effect on wheel running, whereas administration of 1.0 mg/kg of THC had mixed effects (Bottom). A dramatic depression in wheel running occurred in 5 of the 7 rats injected with 1.0 mg/kg of THC. Medians are represented by lines. Individual responses are represented by symbols. These data were averaged over the 3-hour period following AITC administration. (n = 6-7/group).
Figure 3.3. Ninety-minute THC pre-treatment does not prevent migraine-depressed running.

Top: Administration of THC (0.1 and 0.32 mg/kg) ninety minutes before microinjection of AITC onto the dura mater did not prevent AITC-induced depression of wheel running. Bottom: Medians (lines) and individual responses (symbols) to AITC are relatively consistent regardless of prior THC administration. These data were analyzed over the 3 hours following AITC administration (n = 9-10/group).
Figure 3.4. Ninety-minute THC post-treatment does not restore migraine-depressed running.

Top: Administration of THC (0.1 and 0.32 mg/kg) ninety minutes after microinjection of AITC onto the dura mater did not restore AITC-induced depression of wheel running. Bottom: Although the median response (lines) to AITC was relatively consistent regardless of THC administration, examination of individual effects (symbols) suggests that THC was beneficial in at least 2 of the 7 rats treated with 0.32 mg/kg. Medians are analyzed over the 3 hours following THC administration (n = 7/group).
Figure 3.5. Administration of the CB₁ receptor antagonist blocks the anti-migraine effects of THC. **Top:** Administration of the CB₁, but not CB₂, receptor antagonist 30 min before AITC and THC (0.32 mg/kg) injections blocked the anti-migraine effect of THC. **Bottom:** A significant decrease in wheel running is evident in rats injected with the CB₁ receptor antagonist compared to vehicle-treated rats when medians (lines) are analyzed over the first 3 hours following THC administration (n = 7/group; each symbol represents data from one rat).
Figure 3.6. Administration of cannabinoid receptor antagonists have no effect of wheel running in the absence of AITC and THC administration. A) Administration of the CB₁, but not CB₂, receptor antagonist 30 min before AITC injection had no impact on the duration or magnitude of AITC-induced depression of wheel running (n = 6-7/group). B) Administration of vehicle or the CB₁, or CB₂ receptor antagonist 30 min before a control injection of mineral oil onto the dura mater had no impact on wheel running (n = 6/group).
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CHAPTER 4:
LACK OF TOLERANCE AND MEDICATION OVERUSE HEADACHE TO REPEATED
THC ADMINISTRATION IN A RAT MODEL OF MIGRAINE

Abstract

Repeated use of anti-migraine agents such as triptans and opioids results in medication
overuse headache (MOH), a condition characterized by more intense and more frequent
migraine attacks. Thus, there is a great need to develop treatments that do not lose efficacy or
exacerbate headache with repeated administration. We have previously shown that ∆9-
tetrahydrocannabinol (THC) prevents migraine-induced depression of home cage wheel running
in female rats. However, the efficacy of THC as an anti-migraine therapy depends on the ability
of repeated THC administration to alleviate migraine without producing MOH. Anecdotal
evidence in humans suggests that repeated administration of THC will provide consistent
prevention of migraine-induced depression of home cage wheel running in rats. This hypothesis
was tested by examining whether repeated THC administration alters migraine-like pain
produced by administration of the TRPA1 agonist allyl isothiocyanate (AITC) onto the dura
mater. As reported previously, AITC depressed home cage wheel running for 3 hours.
Concurrent systemic administration of 0.32 mg/kg THC prevented AITC-induced depression of
wheel running whether rats were pretreated with vehicle or THC. In contrast, repeated
administration of morphine resulted in antinociceptive tolerance and exacerbated migraine pain.
These data reveal that unlike morphine, THC retains its anti-migraine properties after repeated
administration, and that THC is resistant to MOH in a rat model of migraine.
Introduction

Medication overuse headache (MOH) is caused by frequent and prolonged use of anti-migraine therapies (e.g., triptans, opioids, etc.), and is a debilitating headache disorder characterized by more intense and more frequent migraine attacks (Westergaard et al., 2016). Although MOH is not as common as other headache disorders such as migraine, people with MOH are more likely to report adverse effects of headache on education, career, and overall quality of life (Diener et al., 2016; Westergaard et al., 2016). Due to varying mechanisms of action underlying anti-migraine drugs, some classes of drugs may be less likely than others to elicit MOH (Thorlund et al., 2016). Preclinical studies provide a unique opportunity to screen drugs for MOH.

Sustained administration of analgesics (e.g., opioids, NSAIDs) and anti-migraine treatments (e.g., sumatriptan, rizatriptan) sensitizes the trigeminal nociceptive system (De Felice et al., 2010a; 2010b; Hitomi et al., 2016; Su et al., 2016). Overuse of anti-migraine medications results in neural plasticity that renders animals more susceptible to common migraine triggers (e.g., stress, nitric oxide donor) (De Felice et al., 2010b; Kopruszinski et al., 2016). Several anti-migraine agents (e.g., opioids, triptans, NSAIDs) elicit MOH in animal models of migraine; however, the purpose of these studies was to elucidate the mechanisms underlying MOH (Srikiatkhachorn et al., 2000; De Felice and Porreca, 2009; De Felice et al., 2010b). Unfortunately, animal studies demonstrating the potential for novel anti-migraine agents to elicit MOH are rare. These studies are vital for the discovery and development of new migraine therapies.

The previous chapter (Chapter 3) showed that administration of ∆9-tetrahydrocannabinol (THC) relieves migraine pain in female rats via CB1 receptor activation. This indicates that THC may be an effective anti-migraine therapy, but that will be true only if its efficacy is maintained...
with repeated administrations. The present experiment tested this hypothesis by evaluating the anti-migraine efficacy of THC after repeated administration using home cage wheel running. The anti-migraine efficacy of repeated morphine administration was used as a positive control.

**Materials and Methods**

**Subjects**

Data were collected from 31 adult female Sprague-Dawley rats bred at Washington State University Vancouver (Vancouver, WA, USA). Female rats were selected because MOH is much more common in women than men (Westergaard et al., 2016). All rats were 50-70 days old at the start of the study and randomly assigned to treatment groups. A within-subjects design was used to reduce the number of animals needed. All procedures were approved by the Washington State University Animal Care and Use Committee and conducted in accordance with the International Association for the Study of Pain’s Policies on the Use of Animals in Research.

**Surgery**

Prior to surgery, rats were housed in pairs in a 22-24 °C colony room on a 12/12-hour light/dark cycle (lights off at 1800 h). Animals were anesthetized with pentobarbital (50 mg/kg, i.p.) and implanted with a guide cannula (18 gauge; 4 mm long) aimed above the dura mater (AP: +1.0 mm; ML: +1.0 mm; DV: 0.8 mm, from lambda). Loctite® super glue was used to form a tight seal around the guide cannula, and dental cement anchored the guide cannula to two screws in the skull. Following surgery, rats were maintained under a heat lamp until awake. The rat was then housed individually in an extra tall cage (36 x 24 x 40 cm) with a running wheel. The cage was located in a sound-attenuating booth (2.1 x 2.2 m; Industrial Acoustics Company,
Inc., Bronx, NY, USA) for the remainder of the experiment to limit the influence of outside stimuli. Food and water were available ad libitum.

**Running wheel**

A Kaytee Run-Around Giant Exercise Wheel (Kaytee Products, Inc., Chilton, WI, USA) with a diameter of 27.9 cm was suspended from the top of the rat’s home cage. The floor of the cage was covered with cellulose bedding (BioFresh™, Ferndale, WA, USA). A thin aluminum plate (0.8 mm x 5.08 cm x 3.81 cm; K&S Precision Metals, Chicago, IL, USA) was attached to one spoke of the running wheel to interrupt a photobeam projecting across the cage with each rotation. The beam was set 18 cm above the floor of the cage so that only the rotation of the wheel, not the normal activity of the rat, would interrupt the beam. The number of wheel revolutions were summed over 5 min bins for 23 hours each day using Multi-Varimex software (Columbus Instruments, Columbus, OH, USA). Recording began at 1700 h, the onset of the dark phase of the light cycle when rats are most active. A full description of the running wheel with video is available in our previous publication (Kandasamy et al., 2016). Rats were allowed unrestricted access to the wheel for 23 hours/day for 8 days following surgery. The number of wheel revolutions that occurred during the 23 hours prior to the first pretreatment injection was used as the baseline activity.

**Drugs**

Allyl isothiocyanate (AITC; Sigma-Aldrich, Inc., St. Louis, MO, USA) was mixed in mineral oil at a concentration of 10% and injected into the periosteal space in a volume of 10 µL. ∆9-Tetrahydrocannabinol (Sigma-Aldrich, Inc., St. Louis, MO, USA) was dissolved in vehicle (1:1:18; ethanol:cremophor:saline) and injected intraperitoneally at doses of 0.1, 0.32, and 1.0 mg/kg in a volume of 1 mL/kg. Morphine hydrochloride (NIDA Supply Program, Bethesda, MD, USA) was dissolved in saline and injected intraperitoneally at doses of 0.32, 1.0, and 3.2 mg/kg.
in a volume of 1 mL/kg. THC doses were consistent with effective doses in female rats tested in Chapter 3 studies and morphine doses were chosen based on results from a previous publication (Kandasamy et al., 2017).

**Experiment 1: Repeated THC administration**

The objective of this experiment was to determine whether THC retains its anti-migraine efficacy after repeated administration. Following baseline testing on Day 8, the rat was injected with vehicle or THC (1.0 mg/kg, i.p.) at 1645 h on Day 9 and returned to its home cage. The rat also received twice-daily vehicle or THC injections (1045 h and 1645 h) on Days 10 and 11 and immediately returned to its home cage.

On Day 12, the rat was injected with 10 µL of 10% AITC or mineral oil onto the dura mater using an injection cannula inserted into the guide cannula. The rat was injected with either vehicle or THC (0.1 or 0.32 mg/kg, i.p.) immediately following AITC administration. All injections were completed by 1650 h so the rats could be returned to their home cages prior to the start of the 23 hours of recording beginning at 1700 h. This procedure was repeated every other day with the THC doses administered in a counterbalanced order (Days 14 and 16). The rat received two vehicle or THC injections (1.0 mg/kg, i.p.) at 1045 h and 1645 h on days not receiving AITC (Days 13 and 15). Rats were euthanized 48 hours after the last injection.

**Experiment 2: Repeated morphine administration**

The objective of this experiment was to test the hypothesis that morphine will cause MOH with repeated administration. Following baseline testing on Day 8, the rat was injected with saline or morphine (3.2 mg/kg, i.p.) at 1645 h on Day 9 and returned to its home cage to run. The rat also received twice-daily saline or morphine injections (1045 h and 1645 h) on Days 10 and 11 and returned to its home cage to run.
On Day 12, the rat was injected with 10 μL of 10% AITC or mineral oil onto the dura mater using an injection cannula inserted into the guide cannula. The rat was injected with either vehicle or morphine (0.32 or 1.0 mg/kg, i.p.) immediately following AITC administration. All injections were completed by 1650 h so the rats could be returned to their home cages prior to the start of the 23 hours of recording beginning at 1700 h. This procedure was repeated every other day with the morphine doses administered in a counterbalanced order (Days 14 and 16). The rat received two saline or morphine injections (3.2 mg/kg, i.p.) at 1045 h and 1645 h on days not receiving AITC (Days 13 and 15). Rats were euthanized 48 hours after the last injection.

**Data analysis**

An average hourly nighttime running rate was used as the baseline for hour-by-hour analyses. Given individual differences in wheel running, all wheel running data are presented as a percent change from each rat’s baseline value. All data are expressed as mean ± SEM unless stated otherwise. Nearly all running occurs during the dark phase of the light cycle, so only data collected during the dark phase when drugs were administered are presented. Data were analyzed with two-way ANOVA (dose x hour) followed by LSD post-hoc analysis over the 3-hour period following injection of AITC or THC/morphine to get a precise measure of the magnitude of migraine pain-induced depression of wheel running. Medians were analyzed using the Kruskal-Wallis test. Because animals whose guide cannula was defective (n = 4) or whose data were lost due to computer malfunction (n = 7) were not available for all of the within-subjects conditions, groups were treated as independent samples. Statistical significance was defined as a probability (p) < 0.05.

**Results**

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**Experiment 1: Repeated THC administration**

In rats pretreated with vehicle for 2.5 days, microinjection of AITC onto the dura caused a reduction in wheel running that lasted for 3 hours (Fig. 4.1A). Concurrent administration of 0.32 mg/kg THC prevented AITC-induced depression of wheel running compared to a lower dose (0.1 mg/kg) or vehicle (Fig. 4.1A). Analysis of the magnitude of wheel running during this 3-hour period revealed a significant difference between THC doses ($F(2,14) = 4.533, p = 0.03$). Post-hoc analysis revealed that wheel running was significantly higher following administration of 0.32 mg/kg THC compared to all other doses (LSD test: Vehicle vs. 0.32 mg/kg, $p = 0.017$; 0.1 mg/kg vs. 0.32 mg/kg, $p = 0.022$).

In rats pretreated with THC for 2.5 days, microinjection of AITC onto the dura caused a reduction in wheel running that lasted for 3 hours (Fig. 4.1B). Similar to vehicle-treated rats, concurrent administration of 0.32 mg/kg of THC prevented AITC-induced depression of wheel running compared to a lower dose (0.1 mg/kg) or vehicle (Fig. 4.1B). Analysis of the magnitude of wheel running during this 3-hour period revealed a significant difference between THC doses ($F(2,19) = 4.983, p = 0.018$). Post-hoc analysis revealed that wheel running was significantly higher following administration of 0.32 mg/kg THC compared to all other doses (LSD test: Vehicle vs. 0.32 mg/kg, $p = 0.009$; 0.1 mg/kg vs. 0.32 mg/kg, $p = 0.015$). Analysis of the mean magnitude of wheel running during the first three hours following THC administration revealed no significant differences between vehicle- and THC-pretreated animals ($F(1,33) = 2.686, p = 0.111$); Fig. 4.1C].

**Experiment 2: Repeated morphine administration**

In rats pretreated with saline for 2.5 days, microinjection of AITC onto the dura caused a reduction in wheel running that lasted for 3 hours (Fig. 4.2A). Concurrent administration of 1.0 mg/kg morphine produced a transient reduction in AITC-induced depression of wheel running
compared to a lower dose (0.32 mg/kg) or saline (Fig. 4.2A). Analysis of the magnitude of wheel running during this 3-hour period revealed no significant differences between morphine doses ($F(2,20) = 2.195, p = 0.137$). However, given the short duration of action of morphine in the rat (60-90 min), mean wheel running in the first hour following AITC injection was analyzed. This analysis revealed significantly higher running in animals treated with 1.0 mg/kg morphine compared to a lower dose (0.32 mg/kg) or saline ($F(2,39) = 8.795, p = 0.001$; LSD test: Vehicle vs. 1.0 mg/kg, $p = 0.001$; 0.32 mg/kg vs. 1.0 mg/kg, $p = 0.001$).

In rats pretreated with morphine for 2.5 days, microinjection of AITC onto the dura caused a reduction in wheel running that lasted for at least 3 hours (Fig. 4.2B). Concurrent administration of morphine or saline did not prevent AITC-induced depression of wheel running (Fig. 4.2B). Analysis of the magnitude of wheel running during this 3-hour period revealed no significant differences between morphine doses ($F(2,18) = 1.841, p = 0.187$). Analysis of the mean magnitude of wheel running during the first three hours following morphine administration revealed no significant differences between vehicle- and morphine-pretreated animals ($F(1,38) = 1.618, p = 0.211$; Fig. 4.2C).

The duration of AITC-induced depression of wheel running appears to be enhanced in animals pretreated with morphine compared to those pretreated with THC or vehicle/saline (Fig. 4.3A). Although analysis of means at Hour 4 revealed a trend towards a significant difference between groups ($F(2,26) = 2.989, p = 0.068$), this difference between groups is particularly evident upon analysis of individual data. Only one of the morphine-pretreated rats had returned to baseline levels of running by Hour 4, whereas the median for vehicle/saline- and THC-pretreated rats was at baseline levels by Hour 4. This difference in median wheel running between groups was statistically significant (Kruskal-Wallis test: $p = 0.038$; Fig. 4.3B).
Discussion

The present data show that THC, but not morphine, prevents migraine-depressed home cage wheel running after repeated administration. Activation of dural afferents via AITC produced a three-hour reduction in wheel running, as was shown in Chapters 2 and 3. Administration of 0.32 mg/kg THC prevented AITC-induced depression of home cage wheel running in rats pretreated with 1.0 mg/kg THC or vehicle for 2.5 days. In contrast, administration of 1.0 mg/kg morphine transiently prevented migraine-induced depression of wheel running in saline-pretreated animals, but not in animals pretreated with 3.2 mg/kg morphine for 2.5 days. Further, analysis of all three groups revealed that AITC-induced depression of wheel running is exacerbated in morphine-pretreated rats compared to THC- and vehicle/saline-pretreated rats.

These data indicate that tolerance to the anti-migraine effect of THC does not develop after repeated injections. This finding is consistent with anecdotal evidence in humans that marijuana may be an effective therapy for long term migraine treatment (Rhyne et al., 2016). Importantly, these results also demonstrate that while prophylactic THC use may not protect against migraine pain (see Chapter 3), the abortive effects of THC remain intact after repeated use. The lack of tolerance to THC contrasts with other studies examining the antinociceptive effects of THC to acute pain (Wakley et al., 2014). In addition to different pain models, much larger doses and more injections were used to induce tolerance to the antinociceptive effects of THC for acute pain (Wakley et al., 2014; 2015). It is possible that larger doses and/or more sustained administration of THC may be needed to induce tolerance to the anti-migraine effects of THC. Our finding that tolerance does not occur to administration of therapeutic doses of THC for 2.5 days is consistent with clinical observations showing a lack of cannabis tolerance with regular social uses in humans, although it develop after heavy use (González et al., 2005).
Our finding that morphine transiently blocked migraine-induced depression of wheel running is consistent with other rodent (Williamson et al., 2001; Martino and Perkins, 2008; Dong et al., 2011; Chanda et al., 2013) and human (Gallagher, 1986; Stiell et al., 1991) data. In fact, morphine and other opioids are used to treat severe cases of migraine, particularly when other agents are ineffective or contraindicated (e.g., during pregnancy or in patients with heart disease) (Silberstein and Lipton, 1994).

However, our data show that repeated administration of therapeutic doses of morphine for 2.5 days prevents morphine from preventing migraine-induced depression of wheel running. Tolerance to the antinociceptive effects of morphine has been shown in a wide range of pain conditions. In addition, repeated morphine administration extended the duration of AITC-induced depression of wheel running by one hour. This result is consistent with clinical observations of MOH. Opioids are not recommended for migraine as they are pro-nociceptive and interfere with triptan efficacy (Tepper, 2012). Opioid use in humans also exacerbates migraine and transforms migraine from an episodic to a chronic condition (Bigal and Lipton, 2009).

In contrast to opioids, repeated THC administration does not appear to be pro-nociceptive. This is consistent with clinical observations in patients who manage migraine pain using marijuana. There is no evidence for exacerbated headaches following repeated marijuana use. Data from previous studies also indicate that the antinociceptive effects of THC after repeated administration differ in pathological pain states. In neuropathic pain states, morphine antinociception was reduced after chronic treatment as indicated by a significant rightward shift of the morphine dose-response curve (Mao et al., 2000). In contrast, the antinociceptive effect of THC in rats with neuropathic pain remained comparable to that of sham rats after chronic
treatment (Mao et al., 2000). Our data demonstrating a lack of tolerance in THC-pretreated animals compared to morphine-treated animals is consistent with these observations.

In conclusion, we demonstrate that THC, but not morphine, retains its anti-migraine properties after repeated administration. Unlike morphine, repeated THC administration does not exacerbate AITC-induced depression of wheel running. These findings suggest that THC may be an effective anti-migraine therapy for migraine patients, and that this efficacy can be maintained over time.
**Figure 4.1.** THC retains its anti-migraine properties despite repeated administration. A) Animals were pretreated with vehicle for 2.5 days. B) Animals were pretreated with 1.0 mg/kg THC for 2.5 days. Microinjection of AITC onto the dura mater produced migraine-like pain indicated by depression of wheel running that lasted 3 hours. Administration of 0.32 mg/kg of THC immediately after AITC administration prevented AITC-induced depression of wheel running. Administration of a lower dose of THC (0.1 mg/kg) or vehicle did not prevent AITC-induced depression of wheel running. C) Analysis of wheel running over the first three hours revealed no differences in wheel running between vehicle- and THC-pretreated animals regardless of acute administration of vehicle or THC (0.1 or 0.32 mg/kg). n = 5-7/group
Figure 4.2. Morphine does not retain its anti-migraine property after repeated administration. A) Animals were pretreated with saline for 2.5 days. B) Animals were pretreated with 3.2 mg/kg morphine for 2.5 days. Microinjection of AITC onto the dura mater produced migraine-like pain indicated by depression of wheel running that lasted 3 hours. In saline-pretreated animals, administration of 1.0 mg/kg of morphine immediately after AITC administration prevented AITC-induced depression of wheel running (A); however, this effect was absent in morphine-pretreated animals (B). Administration of a lower dose of morphine (0.32 mg/kg) or saline did not prevent AITC-induced depression of wheel running. C) Analysis of wheel running over the first three hours revealed no differences between saline- and morphine-pretreated animals. n = 6-8/group
Figure 4.3. AITC-induced depression of wheel running is exacerbated by repeated morphine administration. A) AITC-induced depression of wheel running lasts for 3 hours in THC- and control (vehicle/saline)-treated rats. Depression of wheel running lasts four hours in morphine-treated rats. B) Analysis of medians (lines) in the fourth hour after AITC revealed consistent decreased wheel running in rats treated with morphine compared to THC or vehicle. n = 7-14/group; each symbol represents and individual response of one rat.
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CHAPTER 5:
GENERAL DISCUSSION

The present studies reveal three main findings: 1) Depression of home cage wheel running is an objective and clinically relevant method to assess migraine pain in rats (Chapter 2); 2) Administration of THC can prevent AITC-induced depression of wheel running via CB₁ receptor activation (Chapter 3); and 3) Neither tolerance to the anti-migraine properties nor MOH are evident following repeated THC administration (Chapter 4). These studies are the first set of controlled studies to demonstrate the anti-migraine efficacy of THC and suggest that THC may be an especially effective treatment for migraine.

Contribution 1: Home cage wheel running to assess migraine pain

The first major contribution of the present studies is the development of home cage wheel running as a clinically relevant behavioral endpoint for migraine pain in rodents. Although most preclinical migraine studies use evoked hypersensitivity (i.e., mechanical allodynia) to assess migraine pain, its clinical relevance is questionable. Allodynia is a marker of migraine progression (Burstein et al., 2004; Louter et al., 2013), and is only an indirect measure of the headache itself. In contrast, depression of wheel running mimics the reduction in normal physical activity that accompanies migraine in humans (Headache Classification Committee of the International Headache Society (IHS), 2013). Migraine pain decreases all forms of daily functioning (e.g., going to school, completing errands, etc.) in migraine patients (Mannix et al., 2016). The data presented in Chapter 2 show that depression of home cage wheel running is consistent with these clinical observations.
Assessment of home cage wheel running provides several other advantages over traditional tests (e.g., measuring periorbital and/or hindpaw allodynia) to assess migraine pain in animals. Pain-evoked measures often induce stress by testing the animal in a novel environment, restricting movement to a small container for extended periods of time, and assessing nociception during the rat's inactive period. Home cage wheel running significantly improves on these approaches by measuring migraine pain in a stress-free environment during the rat's active period. Importantly, data collection is objective, independent of the experimenter, and simultaneously captures both the magnitude and duration of migraine pain.

We have previously shown that home cage wheel running is sensitive to the disruptive side effects of morphine and buprenorphine (Kandasamy et al., 2017). Thus, this procedure can be used to simultaneously assess the sedative and analgesic properties of a drug. This is especially evident in Chapter 3 where the high dose of THC (1.0 mg/kg) depressed running whether rats had migraine-like pain or not. If traditional methods were used, drugs could appear to prevent allodynia because of sedative or other disruptive effects.

Home cage wheel running can also be used to study individual variability associated with pain and/or analgesia. Individual variability in pain-depressed behaviors have been described by others (Stevenson et al., 2011; Muralidharan et al., 2016; Pitcher et al., 2017). There is also a significant amount of individual variability in pain (Fillingim, 2005) and analgesia (Galer et al., 1992) in humans; however, individual differences are rarely assessed in preclinical studies. Our data in Chapter 3 and 4 show that home cage wheel running provides a unique opportunity to assess individual differences in behavioral responses to THC. For example, 1.0 mg/kg of THC depressed wheel running in 5 of 7 animals, but had no effect in the other two (Chapter 3). Similarly, 0.32 mg/kg THC was only efficacious in 5 of 6 rats repeatedly treated with THC (Chapter 4). A better understanding of the neural basis for individual differences in the
behavioral and analgesic effects of THC and migraine will reveal unique opportunities for the development and use of cannabinoid-based migraine therapies in migraineurs.

Home cage wheel running provides the best measure of translating the human migraine phenotype from the bedside to the bench, as it mimics the effects of pain on physical functioning. Given that the goal of analgesia should be to restore function in the absence of disruptive side effects, home cage wheel running clearly complements traditional reflexive behavioral endpoints and provides important information regarding pain targets and candidate analgesics. The use of home cage wheel running will surely improve the translation of new and effective anti-migraine therapies back to the bedside from the bench.

**Contribution 2: THC possesses anti-migraine properties in female rats**

The second major contribution of the present studies is the demonstration of the anti-migraine properties of THC. Although there is plenty of anecdotal evidence suggesting that marijuana possesses anti-migraine properties (Baron, 2015; Rhyne et al., 2016), controlled preclinical experiments examining the anti-migraine effects of THC have not been published. Our data suggest that when administered at a particular dose and time (0.32 mg/kg immediately after AITC), THC can prevent migraine-depressed wheel running (Chapter 3). These anti-migraine effects of THC were reversed by rimonabant indicating mediation by CB₁ receptors (Chapter 3).

Our data showing a role for the CB₁ receptor in migraine is consistent with data from humans. The human gene encoding the CB₁ receptor (cnr1) has been mapped to the same chromosome that has been linked with migraine (chr6) (Nyholt et al., 2005), and carriers of cnr1 alterations are more vulnerable to migraine (Juhasz et al., 2009). CB₁ receptors have been detected in the rostral ventromedial medulla, PAG, and TCC, which are migraine generators.
and pain modulators (Mailleux and Vanderhaeghen, 1992; Moldrich and Wenger, 2000). Targeting the CB₁ receptor provides a novel approach for the design and development of new cannabinoid-based anti-migraine therapies.

Anecdotal evidence suggests that marijuana can both prevent and abort migraine attacks in people (Rhyne et al., 2016); however, an interesting finding in Chapter 3 is that THC administration either 90 min before or after AITC microinjection does not prevent or restore migraine-depressed wheel running. Previous studies have shown that THC decreases cortical spreading depression, the neurological process preceding migraine pain (Kazemi et al., 2012). One difference between our study and anecdotal reports is that patients often use marijuana multiple times as a prophylactic treatment whereas our animals only received one systemic injection of THC. Thus, there may be chronic cannabinoid-induced adaptations in vasculature that prevents the generation of migraine pain in humans (Sidney, 2002). As such, the prophylactic properties of marijuana are likely mediated by mechanisms that precede the direct activation of dural afferents by AITC as used in the present study. Further, another major difference between our data and anecdotal reports from migraine patients is that we focused on THC specifically whereas marijuana contains over 100 different cannabinoids that likely act together to produce an effect. Thus, other cannabinoids or compounds present in marijuana may reverse migraine pain that has progressed to a stage that is not sensitive to THC alone.

Although the mechanism by which CB₁ receptors modulate migraine is not known, several unique hypotheses for the anti-migraine effects of THC have been proposed. THC inhibits the release of serotonin from the blood platelets of migraineurs during an attack (Volfe et al., 1985) presumably via inhibition of serotonin type-3 receptors (Fan, 1995). Serotonin is implicated in migraine pathophysiology and blockade of serotonin receptors underlies the anti-migraine effects of triptans and ergots (Goadsby et al., 2017). Given the close interaction of
cannabinoids and serotonin on various levels of the nervous system (Svízenská et al., 2008), THC may exert its anti-migraine effects through direct interaction with serotonin and/or its receptors.

Another hypothesis is that the anti-inflammatory properties of THC may contribute to its anti-migraine effects. A long-standing hypothesis for the mechanism underlying migraine is that activation of the trigeminovascular system results in neurogenic inflammation of the dura mater (Moskowitz, 1993). THC inhibits prostaglandin E$_2$ synthesis (Burstein et al., 1973), decreases platelet aggregation (Schaefer et al., 1979), and stimulates lipoxygenase (Fimiani et al., 1999) – all of which contribute to its anti-inflammatory properties. Lastly, endogenous cannabinoids are rapidly generated in response to pro-inflammatory stimulation of immune cells and dampen the pro-inflammatory response (Berdyshev et al., 2001). Thus, THC and other cannabinoids may exert anti-migraine effects by decreasing inflammation at the level of the dura mater.

The present data provide a strong rationale for the investigation and development of cannabinoid-based therapies for migraine. These data are consistent with anecdotal reports and support the use of THC/marijuana as a therapy for migraine patients.

**Contribution 3: THC retains anti-migraine efficacy after repeated administration**

The third major contribution resulting from the present studies is that repeated THC administration does not diminish its anti-migraine efficacy. Despite the multitude of novel anti-migraine agents being developed (e.g., CGRP monoclonal antibody, PACAP receptor antagonist) (Akerman et al., 2016), there have been no preclinical studies examining whether these drugs retain their efficacy after repeated treatment. Finding drugs that retain their efficacy and do not exacerbate migraine pain is of utmost importance and has clear clinical implications for the treatment of migraine. We tested whether repeated administration of THC or morphine...
produced tolerance or medication overuse headache (MOH) in female rats. Our findings suggest that 2.5 days of THC administration does not affect the anti-migraine efficacy of THC or produce MOH, whereas 2.5 days of morphine administration results in the development of tolerance and MOH in a rat model of migraine (Chapter 4).

Most of the central and peripheral effects of cannabinoids are susceptible to tolerance when these drugs are administered for several days in rodents (Dewey, 1986; Abood and Martin, 1992; Maldonado and Rodríguez de Fonseca, 2002). This tolerance mimics the clinical scenario in that tolerance has been reported in marijuana users (Jones et al., 1981; Hollister, 1986). However, the usual pattern of social cannabis use might not lead to tolerance, except in the case of extremely heavy social abusers (Haney et al., 1999; Hart et al., 2002; González et al., 2005). The difference between moderate and heavy exposure may also account for the variable effects of THC to produce tolerance in animal studies. Our data showing that tolerance does not develop after 2.5 days of 1.0 mg/kg THC pre-treatment (Chapter 4) and other data showing that tolerance does develop after 9 days of 5.4 mg/kg THC pre-treatment (Wakley et al., 2014) is consistent with these clinical observations. Another difference between these two paradigms is the presence of a pain state. Tolerance to THC’s anti-migraine and antinociceptive effects may develop differently depending on the pain model used. Our data suggest that repeated administration of a low dose of THC is resistant to tolerance development in a rat model of migraine.

In contrast to THC, repeated injections of morphine produced tolerance (Chapter 4). Administration of morphine transiently prevented AITC-induced depression of wheel running in saline-pretreated animals, but was ineffective in morphine-pretreated animals. This observation is consistent with clinical observations of morphine efficacy against migraine in humans, as
morphine is typically used as a “rescue drug” to terminate migraine pain in emergency settings. However, repeated use of opioids results in the development of tolerance in humans.

The problem with using morphine as a treatment for migraine is that repeated use of morphine leads to more frequent and more intense bouts of migraine attacks (Bigal and Lipton, 2009). Human data also indicates that opioids elicit abnormal pain that differ in quality from the original complaint (Ali, 1986; Arnér and Meyerson, 1988). The data presented in Chapter 4 is consistent with these observations. Although morphine pretreatment did not enhance the magnitude of AITC-induced depression of wheel running, it did increase the duration by one hour. In contrast, THC pretreatment did not exacerbate AITC-induced depression of wheel running in that the magnitude and duration of migraine-depressed wheel running was comparable between THC- and vehicle/saline-pretreated animals. Thus, our results suggest that THC may be an especially effective treatment for migraine that does not elicit MOH.

The negative effects of sustained administration of anti-migraine agents is well known (Bongsebandhu-phubhakdi and Srikiatkhachorn, 2012). Repeated systemic administration of sumatriptan to rats results in increased susceptibility to cortical spreading depression (Green et al., 2014), the development of periorbital/hindpaw allodynia, and increased expression of CGRP in trigeminal dural afferents (De Felice et al., 2010). Thus, repeated triptan exposure renders rats susceptible to a variety of neural changes at the cortical and primary afferent levels. Similarly, chronic daily acetaminophen administration increases platelet serotonin levels in rats (Srikiatkhachorn et al., 2000) thus providing a basis for the transformation of migraines from an episodic to chronic condition. Previous studies have used large doses of morphine (two 75 mg subcutaneous pellets) to demonstrate its ability to elicit MOH (Okada-Ogawa et al., 2009). The experiments presented in Chapter 4 extend this approach by intermittently administering morphine and THC via repeated daily injections and demonstrate that five pretreatments of 3.2
mg/kg of morphine is sufficient to exacerbate migraine pain in morphine-pretreated animals, whereas there was no evidence of MOH in rats pretreated with THC.

Medication overuse headache is a chronic pain condition that in principle is preventable with the right treatment. Our data suggest that THC can be an effective anti-migraine therapy that maintains its efficacy over a sustained period of time. THC also provides a novel treatment option for chronic migraine patients, whose treatment options are limited due to fears of the adverse effects associated with repeated use of anti-migraine therapies.

**Migraine in females**

A unique aspect to the present studies is the exclusive use of female rats. Despite the greater prevalence of migraine in women, preclinical studies of migraine focus almost exclusively on male animals (Bolay et al., 2011). This is problematic because migraine is far more common in females and data obtained from male subjects may not generalize to female subjects. Migraine is approximately three times more common in women than men, yet only 11 out of approximately 150 preclinical studies have used female rodents to examine migraine pain behaviors (Stucky et al., 2011; Chanda et al., 2013; Vermeer et al., 2014; Pradhan et al., 2014a; 2014b; Bhandare et al., 2015; Tipton et al., 2015; Vermeer et al., 2015; Christensen et al., 2016; Huang et al., 2016; Christensen et al., 2017). Six of these studies solely examine mechanical allodynia. Four studies demonstrated that migraine pain depresses open field locomotion (Stucky et al., 2011; Vermeer et al., 2014; 2015; Christensen et al., 2017). Lastly, only one study examined migraine-depressed wheel running; however, these results were not significant due to the lack of a painful stimulus (they examined intravenous low dose nitroglycerin) and limited observation time (30 minutes) (Christensen et al., 2016). Thus, the studies presented in Chapters 2-4 are the first to use migraine-depressed home cage wheel running in female rats to
report the entire duration and magnitude of AITC-induced migraine pain and THC antinociception.

As a result of the sex bias in preclinical migraine studies, the mechanisms underlying increased migraine in female patients is unclear. Sex hormones appear to play a role (Vetvik and MacGregor, 2016); however, the goal of the present studies was not to characterize the effect of estrous stage or sex hormones on migraine pain or THC antinociception. Rather, the goal was to assess migraine in a clinically relevant manner and that includes focusing on female rats.

Current clinical guidelines for migraine do not address the differential management of migraine in men and women, except for menstrual migraine (Silberstein et al., 2012). In fact, it is recommended that acute treatment of migraine should be the same in men and women, regardless of menstruation (Silberstein et al., 2012). However, specific perimenstrual prophylaxis (i.e., using a prophylactic anti-migraine agent before menstruation) or hormone manipulation may be used to limit the frequency of menstrual-related migraine in women (Silberstein et al., 2012). The increased prevalence of migraine in women likely warrants the need for differential treatments between men and women. Given the increased antinociceptive efficacy of THC in female compared to male rats under acute and chronic pain conditions (Tseng and Craft, 2001; Craft et al., 2012; 2013), THC may be an especially effective treatment for migraine in females.

**Future Directions**

Although the present data provide evidence for the use of THC as a treatment for migraine, this must be systematically tested in humans. No clinical trial has examined the anti-migraine efficacy of cannabinoids. The ideal study design to investigate the effects of
cannabinoids on migraine would be a randomized, placebo-controlled clinical trial with a cannabinoid washout period prior to the start of the trial (Rhyne et al., 2016). This trial should provide participants with standardized doses of drug and track a variety of dependent measures such as patient adherence, number of headache days, intensity of headaches, beneficial/adverse effects of the drug on overall functioning, and beneficial/adverse effects of the drug on the properties of the headache in a systematic fashion.

Our exclusive focus on THC revealed that THC is sufficient to prevent migraine pain in female rats. Cannabinoids appear to modulate many pathways specific to migraine, including interactions with serotonergic and opioid mechanisms of action. These interactions indicate that the anti-migraine effects of THC are complex, and regulation of several physiological mechanisms may be needed to provide complete migraine relief. Careful dissection of the interactions between cannabinoids, vasculature, neurons, and non-neuronal cells is greatly needed to fully understand the mechanisms and determinants underlying the anti-migraine effects of THC.

Concluding remarks

The studies described in this dissertation have greatly contributed to our knowledge of preclinical migraine assessment and the anti-migraine properties of THC in the rat. The work presented in Chapter 2 is expected to transform migraine pain assessment, as it provides a reliable and clinically relevant measure that is sensitive to existing (sumatriptan) and novel (THC) anti-migraine therapies. The work presented in Chapter 3 provides the first behavioral evidence of the anti-migraine efficacy of THC in the female rat. These data also suggest that adjusting THC doses can provide antinociception in the absence of disruptive side effects. Further, we show that activation of the CB₁ receptor provides an important therapeutic target for
new anti-migraine therapies. The work presented in Chapter 4 reveals that unlike morphine, THC retains its anti-migraine efficacy and does not exacerbate headache after repeated administration. In sum, the work presented in this dissertation builds a firm foundation for the preclinical assessment of migraine and the clinical assessment of cannabinoid-based therapies in migraine patients.
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