2017

Modeling Migraine Chronification and its Relief; the Effects of THC on Recurrent NTG-Induced Migraine Endpoints in Rats

Blake Sowers
University of Mississippi. Sally McDonnell Barksdale Honors College

MODELING MIGRAINE CHRONIFICATION AND ITS RELIEF; THE EFFECTS OF THC ON RECURRENT NTG-INDUCED MIGRAINE ENDPOINTS IN RATS

By:
Blake Andrew Sowers

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford
May 2017

Approved by:

_____
Advisor: Dr. Kenneth Sufka

_____
Reader: Dr. Todd Smitherman

_____
Reader: Dr. Lainy Day

ABSTRACT

This study investigated the analgesic effects of a THC pro-drug in a rodent model of recurrent migraine. Rats received 4 nitroglycerin-induced (NTG: 10mg/kg/2ml) migraine episodes every third day for 12 days; saline, cremaphor-vehicle, propranolol (10mg/kg/ml), or THC-VAL-HS (0.5, 1.0, and 2.0 mg/kg/ml) were given IP 30 minutes before NTG. Behavioral endpoints of photosensitivity, activity, orbital tightening, and tactile allodynia were assessed 110 m after NTG. Migraine severity increased over the course of the four episodes, pointing toward chronification and an important step in model validation. However, neither propranolol nor THC-VAL-HS significantly attenuated any of the migraine-related endpoints. These data are in contrast with clinical reports that marijuana mitigates migraine severity. These findings suggest that higher doses of THC-VAL-HS and/or other cannabinoid constituents in marijuana may be responsible for such anecdotal anti-migraine activity of cannabis.

ACKNOWLEDGMENTS

I would like to extend the sincerest gratitude to those who have helped make this thesis project possible. First, to Dr. Sufka, for being an incredible advisor and mentor, not only during this project, but during my entire tenure at the University of Mississippi. Second, to all of the graduate students and research assistants in the Psychopharmacology Research lab, whose time and effort down in the lab helped make this project a success. Third, to my readers, Dr. Todd Smitherman and Dr. Lainy Day, for their time and insight on this project. A tremendous thanks goes to the Sally McDonnell Barksdale Honors College, for giving me the resources and support to pursue many of my goals, both inside and outside the classroom, and for funding that helped bring this project to fruition. Finally, I want to thank my family and friends for their constant and overwhelming support. I would not be in the position I am today if it were not for all of you supporting me and encouraging me each and every day.

TABLE OF CONTENTS

LIST OF FIGURES

1. INTRODUCTION

Migraine is one of the most widespread and debilitating disorders known to man. Recent estimates reveal that roughly 14% of the population should expect to contend with migraines at some point in their lifetime (Stovner et al. 2007). Migraine also has a drastic effect on the quality of life of those that suffer from it. A large-scale survey (Terwindt et al. 2000) sought to discern the differences in quality of life between migraineurs and non-migraineurs. For this survey, they administered a 36-item questionnaire measuring health quality across eight domains. This survey found a significant decrease in the quality of life of those who are affected by migraines, which was found to be inversely proportional to the frequency of migraine episodes.

Additionally, migraine has a staggering effect on society as a whole. In an epidemiological study (Leonardi et al. 2005), researchers sought to quantify migraine's effect on the population by assigning Years Lived with Disability (YLD), which takes into account the effect of disability that the disease has on population health. Utilizing the World Health Organization's global burden of disease data, migraine contributed to roughly 2% of lifetime YLDs. In a separate study, the economic burden of migraine was quantified. This study (Ferrari, 1998) relied upon data regarding patterns of healthcare use, direct costs in the form of drugs and hospital visits, and the indirect costs associated with reduced productivity at work, absence from work, and opportunity cost associated with the migraineur and their caretakers. This study found the direct cost of migraine to be roughly $9.4 billion and the indirect cost to be between $1.4 billion and $17.2 billion.

Typical migraine, as classified by the International Classification of Headache Disorders (ICHD-9), is characterized as recurrent pulsatile headaches and facial pain,

often accompanied by nausea and photophobia. As migraine trends from episodic to chronic, a number of physical endpoints present themselves. 85% of episodic migraineurs present photophobia or sensitivity to light (Rothrock et al 1996) and 79% present some form of cutaneous allodynia (Burstein et al. 2000). These clinical endpoints provide feasible measures of migraine symptoms and, as such, are relied upon experimentally.

Current treatment for migraine is problematic. To combat migraine, two main types of pharmaceuticals are utilized: abortives and prophylactics. The standard abortive used is sumatriptan, which is administered orally. In a double blind, placebo-controlled study in humans (Tfelt-Hanson, 1998), an orally administered 100 mg dose of sumatriptan was only 58% efficacious. In a separate study, a notable prophylactic, propranolol, had 79% efficacy, though with a very small sample size (n=19) (Weber et al 1972). There is room for improvement when it comes to pharmaceuticals and the screening of potential therapies.

Use of analgesics to treat migraine related pain can lead to another form of neurological pain called medication overuse headache (MOH). Roughly 4% of analgesic users overuse medication and roughly 1% of analgesic users suffer from medication overuse headache (Diener et al 2004). In fact, a separate epidemiological study (Castillo et al. 1998) found that 0.75% of migraineurs have their episodic migraine transformed into chronic daily headache by medication overuse. This transformation into chronic daily headache results from changes in the endocannabinoid system (Cupini et al. 2008). In this instance it is clear that not only are our current methods of treatment largely ineffective, but they may in fact be causing unnecessary harm to those who depend on them.

Having considered the shortcomings of the current methods of treatment, one is left to wonder where these shortcomings might originate. Largely, it is the inability of these models to relate closely to the clinical picture of migraine. This lack of translational relevance is problematic, because a model is only as good as the endpoints that it measures, and if a model does not mirror and measure the physical endpoints seen in the clinical picture, it cannot be utilized very effectively (Garner, 2014).

As migraine research has progressed, the translational relevance of available models has improved. The first generation of models sought to determine the effects of administered nitroglycerine (NTG) in rats on the outcome of some potentially relevant clinical endpoints (Tassorelli et al. 2003). For this experiment, rats were administered NTG and tested for signs of hyperalgesia, largely through the tail flick apparatus. This experiment found significant increases in hyperalgesia at 2 and 4 hours post-NTG injection. These results used clinically relevant induction and revealed the possible role of nitrovasodilators in pain modulation. Though this experimental format may have had its merits, it lacked translational relevance to the clinical picture. The clinical need focuses on episodic migraine, which is characterized by recurrent migraine episodes. Additionally, the endpoints measured in this current model of migraine are not diagnostic criteria for migraine in the clinical picture.

In the second generation of migraine modelling, translational relevance improved by relying on multiple NTG administrations and migraine inductions (Pradhan et al. 2013). In this experiment, mice were injected with NTG every other day for 9 days, resulting in 5 total NTG injections. This experiment found significant increases in hyperalgesia related to von Frey mechanical sensitivity (technically their endpoint

measured allodynia), and that these effects not only persisted, but worsened with episodic migraine induction. The implementation of recurrent NTG administration helped to improve the translational relevance of this migraine model, in regards to frequency, though they still neglected more clinically relevant endpoints of episodic migraine by focusing solely on hyperalgesia.

The third generation of migraine models sought to include diagnostic endpoints in its assessment of episodic migraine (Sufka et al. 2016). This experiment subjected rats to 5 NTG injections over the course of 15 days and measured the effect on the novel endpoints of expressed facial pain (using the Rat Grimace scale) and photophobia and decreased movement (both quantified using a Light/Dark box). The results of this experiment showed a significant decrease in locomotor activity and an increase in light sensitivity over the course of the five test sessions. The changes that this experiment introduced to the model allowed it to become more translationally relevant and come closer to modelling the clinical picture.

The final generation of modelling, and the one that is utilized in this experiment, sought to add another clinically relevant endpoint related to tactile allodynia (Harris et al. 2017). This experiment followed the recurrent administration of NTG that previous models utilized, and added a new apparatus in an attempt to quantify the development of tactile allodynia associated with chronification of migraine. This new apparatus is a circular arena consisting of two half fields of sandpaper of differing grit and seeks to quantify movement data as well as preference for one half field or the other. Through the implementation of this new apparatus, a significant increase in tactile allodynia, as measured by a decrease in movement and a preference for spending time on the smoother

sandpaper, was recorded during the course of this experiment. However, this increase only occurred during the 5th episode, which points toward tactile allodynia related to the chronification of migraine.

Throughout history, a number of natural products have been utilized in the treatment of modern diseases. Oftentimes, through long-term trial and error related to ayurvedic medicine, a botanical treatment was found to be successful for reasons that would later become apparent when laboratory science could elaborate on its mechanism of action. In the case of headache, this has been demonstrated with the oral botanical therapies of Feverfew and Butterbur (Levin, 2012), which have been shown to be generally effective in the reduction of migraine frequency and in migraine prevention, respectively (Diener et al. 2005; Lipton et al. 2004). Feverfew was discovered to have a role as an inhibitor of prostaglandin synthesis (Pareek et al. 2011), which has been previously implicated in the onset of migraine (Antonova et al. 2012).

Additionally, anecdotal evidence points to the possible efficacy of cannabis in alleviating symptoms of migraine. As early as the year 700 B.C.E., Indian ayurvedic medicine touted the ability of cannabis to be utilized for migraine pain (Russo, 1998). These anecdotal reports continued and persist even to this day, attesting to the possible benefits of cannabis use for migraine treatment (Russo, 1998).

However, reports of the analgesic properties of cannabis extend beyond anecdotal evidence and transition to experimental data. A 1972 study sought to compare the analgesic properties of THC to those of morphine (Buxbaum, 1972). To accomplish this goal, rats were administered intraperitoneal injections of THC or morphine and then

subjected to hot plate and tail flick tests. This experiment found that an intraperitoneal injection of THC had analgesic effects equipotent to morphine in rats.

Furthermore, the analgesic effects of THC have also been demonstrated in human trials (Noyes Jr et al. 1975). Patients suffering from continuous pain attributed to cancer were administered varying doses of THC, and had their pain relief compared to the effects of codeine. This experiment found that a 20 mg dose of THC was slightly more effective at relief and reduction of pain than a 120 mg dose of codeine. This experiment speaks to the analgesic properties of THC and provides some validity for anecdotal efficacy in humans.

Finally, synthetic compounds that modulate the endocannabinoid system have been shown to have a positive effect on pain states related to NTG-induced migraine (Nozaki et al. 2015). Mice presenting varying gene knockouts were administered intraperitoneal nitroglycerin and then tested for mechanical sensitivity using von Frey testing methods. This experiment found a significant increase in the threshold of mechanical sensitivity in mice who had a knockout of the gene for fatty acid amide hydrolase (which metabolizes anandamide, a portion of the endocannabinoid system that interacts with CB_1 and CB_2 receptors). This modulation of the endocannabinoid system and its positive effects on pain management again point to the possibility of utilizing THC as an analgesic in migraine related pain.

The present study aims to build upon previous research and investigate the effects of a THC pro-drug formulation on a recurrent migraine model in rats. By utilizing clinically relevant induction, frequency of administration, and endpoints, this study aims to screen a novel pharmacotherapy for possible migraine treatment.

2. METHODS

2.1. Subjects

Male Sprague Dawley Rats (225-280 g; Envigo, Indianapolis, IN) were housed in pairs in acrylic tubs with bedding material and maintained under a 12:12 hr light-dark cycle in a temperature and humidity controlled vivarium. Food and water were available *ad libitum*. Animals were handled twice daily for seven days preceding experimental manipulations to reduce experimenter-related stress. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Mississippi (Protocol #15-021).

2.2. Drug Administration

2.2.1 Migraine Induction

The NTG stock solution contained 5 mg/mL NTG dissolved in 30% alcohol, 30% propylene glycol, and water (American Regent; Shirley NY). NTG was administered in a volume of 2 mL/kg to achieve a dose of 10 mg/kg. Induction of episodic migraine episodes consisted of 4 NTG injections administered every third day over 12 days. Rats acclimated in the testing room for at least 1 hr in their home cages prior to behavioral testing. All behavioral tests were begun at 110 min post NTG injections.

2.2.2 Test Articles

The test article was a THC pro-drug, THC-mono-val mono-hemisuccinate (THC-VAL-HS), administered at doses of 0.5, 1.0, and 2.0 mg/kg/mL (purity >98%). For a positive control, propranolol was used and was dissolved in saline. For our vehicle group, 5 animals were assigned saline while 5 animals were assigned the THC-VAL-HS vehicle

of cremaphor (cremaphor/EtOH in water). All test articles were administered 60 min before NTG injection.

2.3. Rat Grimace Scale

Orbital tightening, a facet of the Rat Grimace Scale, was utilized to quantify pain by examining facial features of a rat during a pain state. Images of the rat's facial features were taken 90 min post NTG administration with a 5 megapixel, 1080p camera positioned 6 to 12 inches from the subject's home cage. During this imaging, rats remained in their home cages to reduce experimental stress. Following the experiment, unlabeled images were displayed on a computer monitor for scoring by two trained research assistants who were blinded to condition. Images were scored for orbital tightening, nose/cheek flattening, and ear and whisker changes using a scale of 0-2 (not present, moderate, obvious). Scores were averaged across the two raters to produce an overall pain score for each rat. The inter-rater reliability between the two raters was 0.840.

2.4. Light/Dark Box

Light/Dark (L/D) box testing for photophobia and movement was conducted in a two chamber condition place preference apparatus (Med-Associates, St. Albans, VT) with chamber dimensions of 25.5 cm x 21.0 cm x 20.9 cm. Black construction paper was taped over the acrylic lid of the black chamber to reduce illumination. Under these conditions, lumens measured 260-266 in the white chamber and 0 lumens in the black chamber. Each chamber was equipped with infrared photobeam detectors to track animal activity. 110 min post NTG treatment administration, rats were placed in the corner of the white chamber facing away from the experimenter. The rats were then allowed to explore

the white and dark chambers for 20 min. Time spent in the white chamber during this period was measured and expressed in seconds. Total number of photo beam breaks in both chambers quantified activity.

2.5. Arena Testing for Tactile Allodynia

Tactile allodynia was assessed using an open field arena (50.8 cm diameter with a 25.4 cm diameter sidewall). The arena floor was divided into two halves containing either 40 or 220 grit sandpaper. The 40 grit sandpaper contained low density (i.e., areas void of media) grains of large diameter (425 μm) rounded media, while the 220 grit sandpaper contained high density grains of small diameter (68 μm) jagged media. Animal activity was recorded by a camera mounted above the open field arena and analyzed on a computer equipped with Ethovisions XT software (Noldus, Holland) to track movement and time spent in each half of the arena. Ethovisions software also provided heat signal maps that tracked animal movement for the duration of the test. These tests were conducted immediately after L/D box testing. Rats were placed in the center of the arena facing away from the experimenter such that left and right paws made contact with the two grit surfaces. Animals were tracked for a 5 min period.

2.6. Statistical analyses

Data were analyzed with SPSS software using one-way (between groups) ANOVAs or two-way (between and within groups) ANOVAs with post-hoc tests for simple effects (Fisher's exact); one tailed testing with significance at $p < .05$. Because propranolol had a different vehicle from the THC-VAL-HS compound, the vehicle group for this experiment consisted of 5 animals receiving saline and 5 animals receiving the THC-VAL-HS vehicle (Cremaphor/EtOH in water).

3. RESULTS

Weight Data

A summary of weight gain across the recurring migraine protocol across treatment groups is summarized in Figure 1. Over the course of the study, rats in every treatment group consistently gained weight. In the day following a migraine episode, the average weight of every treatment group decreased before recovering its upward trend. Consistent with these descriptions, a two-way ANOVA revealed a significant main effect for Day [$F(10,40) = 83.645$, $p < 0.001$]. Additionally, there was no significant Treatment effect [$F(5,40) = 1.805$, $p = 0.134$] nor a significant Day x Treatment interaction [$F(50,40) = 0.963$, $p = 0.549$]. No further analyses were performed on these data.

Orbital Tightening

The effects of repeated migraine episodes on orbital tightening across treatment groups are summarized in Figure 2. No systematic pattern showing changes or worsening of orbital tightening nor any separation among treatment groups in a consistent manner was detected from these data. For example, the two vehicle groups differed in their progression, whereby saline animals had consistent orbital tightening scores across the 4 migraine episodes, whereas the cremaphor animals seemed to show a decrease in orbital tightening scores. Neither propranolol or THC-VAL-HS reliably reversed any patterns. Consistent with these observations, a two-way ANOVA failed to reveal a significant effect for Day [$F(3,37) = 0.963$, $p = 0.421$] or Treatment [$F(5,37)$, $p = 0.077$], nor a significant Day x Treatment interaction [$F(15,37) = 1.104$, $p = 0.363$]. No further analyses were performed on these data.

L/D Box

The effects of repeated migraine episodes on mean movement in the Light/Dark box across treatment groups are summarized in Figure 3. All treatment groups showed a decrease in movement over the course of the four test sessions, with no group showing any kind of marked difference in this endpoint from any other group. Consistent with these observations, a two-way ANOVA revealed a main effect for Day [$F(3,25) = 10.547$, $p < 0.001$] and failed to reveal an effect for Treatment [$F(5,25) = 1.210$, $p = 0.333$]. Additionally, there was no significant Day x Treatment interaction [$F(15,25) = 1.553$, $p = 0.113$]. A separate one-way ANOVA performed on the test sessions revealed no significant effect for Treatment in any session.

The effects of repeated migraine episodes on mean time in light in the Light/Dark box across treatment groups are summarized in Figure 4. Again, all treatment groups generally decreased over the course of the 4 NTG episodes. On the second migraine episode, the 0.5 THC-VAL-HS group displayed a sharp increase in time spent in light, followed by a decrease over the next two episodes. No group seems to be markedly higher than another group in this endpoint over the course of the 4 NTG episodes. Consistent with these observations, a two-way ANOVA revealed a main effect for Day [$F(3,25) = 23.405$, $p < 0.001$] while no significant effect was revealed for Treatment [$F(5,25) = 1.537$, $p = 0.215$], nor any significant Day x Treatment interaction [$F(15,25) = 0.918$, $p = 0.549$]. A separate one-way ANOVA performed on test sessions 1-4 found significant effects for Treatment on test sessions 1, 2, and 4. On test session 1, the mean time in light score of the THC-VAL-HS 1.0 group was significantly higher than the mean time in light score of the propranolol group ($p = 0.040$). On test session 2, the mean time

in light score of the THC-VAL-HS 0.5 group was significantly higher than mean time in light score of the saline ($p = 0.031$), propranolol ($p = 0.009$), and THC-VAL-HS 1.0 groups ($p = 0.045$). On test session 4, the mean time in light score of the THC-VAL-HS 2.0 group was significantly higher than the mean time in light score of the propranolol group ($p = 0.032$).

Open Arena

The effects of repeated migraine episodes on movement in the open arena across treatment groups are summarized in Figure 5. Over the course of the 4 NTG episodes, all groups decreased movement drastically from episode 1 to 2 and then slowly increased movement over the remaining episodes. No treatment group seemed to be markedly different in this endpoint from any other. A two-way ANOVA revealed a main effect for Day [$F(3,40) = 279.893$, $p < 0.001$] and a significant Day x Treatment interaction [$F(15,40) = 2.033$, $p = 0.019$] but failed to reveal a main effect for Treatment [$F(5,40) = 0.177$, $p = 0.970$]. A one-way ANOVA performed on test sessions 1-4 showed a significant Treatment difference during episodes 2 and 4. In episode 2, the mean arena activity score of the THC-VAL-HS 0.5 and THC-VAL-HS 1.0 groups were significantly higher than the mean arena activity score of the saline group ($p = 0.041$ and $p = 0.030$). In test session 4, the mean arena activity score of the THC-VAL-HS 1.0 group was significantly lower than the mean arena activity score of the THC-VAL-HS 0.5 and THC-VAL-HS 2.0 groups ($p = 0.045$ and $p = 0.002$).

The effects of repeated migraine episodes on time spent in half field (in this case, the 40 grit sandpaper half field) across treatment groups are summarized in Figure 6. All treatment groups followed the same pattern over the 4 test sessions. The first 3 sessions

remained relatively constant, followed by a sharp decrease from test session 3 to 4. There were no noticeable differences between each treatment group for this endpoint. Consistent with these observations, a two-way ANOVA found a significant main effect for Day [$F(3,40) = 17.795$, $p < 0.001$]. However, this analysis failed to reveal a main effect for Treatment [$F(5,40) = 0.735$, $p = 0.602$] nor any significant Day x Treatment interaction [$F(15,40) = 1.228$, $p = 0.263$]. A separate one-way ANOVA revealed a significant Treatment effect on test sessions 1 and 2. On test session 1, the mean 40 grit duration of the propranolol group was significantly higher than the mean 40 grit duration of the saline ($p = 0.015$) and THC-VAL-HS 2.0 groups ($p = 0.031$). On test session 2, the mean 40 grit duration of the propranolol group was significantly higher than the mean 40 grit duration of the saline ($p = 0.014$) and THC-VAL-HS 2.0 groups ($p = 0.025$) while the mean 40 grit duration of the THC-VAL-HS 1.0 group was significantly higher than mean 40 grit duration of the saline group ($p = 0.040$).

4. DISCUSSION

The goal of this research was to determine whether a recurrent NTG migraine protocol models the progression of migraine from episodic to chronic and to examine the effects of a THC pro-drug formulation in reducing migraine endpoints in this protocol. Rats received 4 recurring injections of NTG, with endpoints of facial pain, photophobia, activity, and tactile allodynia quantified after each exposure. We predicted that 1) evidence of chronification on these endpoints would be demonstrated by the recurrent NTG model, and 2) THC-VAL-HS, alongside the positive control of propranolol, would attenuate the relevant migraine endpoints.

Human migraineurs often report an increased sensitivity to light and that activity exacerbates migraine intensity. In this current study, rats demonstrated a worsening of many clinically relevant endpoints. Across the 4 NTG administrations, rats showed increasing levels of photophobia, decreasing levels of motor activity in the light-dark apparatus and decreasing motor activity in the open arena. These findings are also consistent with previous studies that show the clinically-relevant migraine related endpoints of photophobia and reduced activity worsen after multiple NTG administrations (Pradhan et al. 2013; Sufka et al. 2016). Moreoever, these results are similar to symptoms present in human migraineurs and aligns with the ICHD-9 diagnostic criteria.

Emergence of spontaneous allodynia is common among migraineurs with frequent episodes. Spontaneous allodynia is that which occurs to non-noxious stimuli from daily activities, and it is a diagnostic criterion for migraine chronification. In our recurrent NTG model, we found evidence of spontaneous allodynia in our open arena as episode frequency increased. In other NTG migraine models including single episode protocols, the most common migraine endpoint is an evoked allodynic response via von Frey procedure. However, evoked allodynia is not a clinically relevant endpoint and such measures suffer from poor translational relevance. It may be that spontaneous allodynia, as derived from the arena apparatus, offers a more translationally-relevant endpoint for migraine chronification and yield increased model validity.

A common feature of migraine in humans is the presence of nausea and vomiting. There is no direct measure capable of quantifying nausea in rats, nor does this species vomit. However, we measured weight gain as an index of nausea and a loss of appetite.

Changes in weight gain may serve as a surrogate measure of nausea, which presumably decreases appetite. We hypothesized that, if migraine were to induce nausea and a loss of appetite, it would manifest in decreased food consumption and result in decreased weight gain following an episode. Our measure of rodent weight over the entire protocol follows such a pattern and may mirror the clinical presentation of migraine. In the day following a migraine episode, the average weight of every treatment group decreased before recovering its upward trend. This "stairstep" trend signifies a decrease in appetite on days in which NTG was administered, and may reflect nausea often present during a migraine episode.

The clinically-relevant behavioral measures in this recurrent NTG protocol align well with the ICHD-9 diagnostic criteria of migraine and migraine chronification. Animal models that demonstrate the greatest number of homologies to a clinical syndrome are considered the most valid (Garner 2014), and thus the similarity in findings between this study and previous research, which demonstrated chronification of migraine related endpoints over repeated NTG administration and the emergence of spontaneous tactile allodynia, (Harris et al. 2017) serves to add construct validity to this recurrent migraine model.

Propranolol is common prophylactic in migraine, with 78% efficacy (Weber 1972). Though propranolol was predicted to act as an effective positive control and prophylactic agent, this group showed no reversal on any of the migraine-related endpoints on any episode. The failure to demonstrate propranolol's efficacy in the model may be due to differences in drug administration in the current model with what is currently done in humans. In humans, propranolol is typically administered daily,

whereas in the current study it was administered intermittently and only 30 minutes before NTG administration. Pre-loading and daily administration in the current model may yield positive effects. Support for this idea comes from research which showed that the greatest decrease in hypertension in rats occurred when they were subjected to administration of propranolol that was both chronic and delivered before experimentation began (Lin 1991). In this experiment, the rats that were pre-administered propranolol began administration three days before the protocol commenced. Our current migraine protocol may benefit from a similar "pre-loading" of propranolol and may see it act as a positive control.

THC has been shown to have analgesic actions against a wide variety of nociceptive stimuli in rodents (Buxbaum 1972). Anecdotal reports from humans suggest that cannabis use attenuates migraine (Russo 1998). In contrast to our predictions, THC-VAL-HS failed to alter migraine related endpoints in this recurrent migraine model. There are three possible explanations for THC-VAL-HS's ineffectiveness against migraine. First, it may be the case that THC is ineffective at modulating migraine endpoints in this type of model. A second possible explanation for lack of efficacy may be related to the use of a pro-drug with a different pharmaco-dynamic profile than pure THC. The absence of an effect in our model may be related to the slower onset and low overall blood levels than the same dose of the pure compound because of its rate-limited conversion from pro-drug to pure THC. Thus, it may be necessary to utilize higher doses to achieve clinical benefit. A third possible explanation for the lack of effects may relate to the use of THC alone in the test compound. Cannabis may be effective at modulating migraine, but to do so would require many cannabinoids or non-cannabinoids

constituents in the plant extract. A study of marijuana extracts and fractions in this model would address this problem.

This experiment suffers from some limitations and has some opportunities to improve. First, orbital tightening is presented as an early indicator of photosensitivity, yet this measure is not temporally connected to measures of photosensitivity in the light-dark box. This experiment would benefit from having these two endpoints more closely linked, perhaps by removing subjects from the light-dark box to assess orbital tightening scores.

Second, data about weight progression and its relation to migraine-induced nausea over the course of the recurrent migraine protocol is tenuous. Thus we can only infer that these changes in weight data are related to migraine induced nausea simply because it appears to mirror the clinical presentation of migraine. Evidence that body weight changes in this "staircase" design may be a surrogate measure for nausea could be obtained from an experiment that utilized a compound that was known to give animals nausea. Two possibilities to cross-validate this as a surrogate measure of nausea would be a) to find a similar protocol in which a feature of some administration has as a side effect of nausea or b) to utilize compounds that are commonly used to produce conditioned aversions. For the first approach, chemotherapy reveals itself as a possible solution. In this case, cisplatin is known to induce nausea in animals in a standard chemotherapy protocol should demonstrate this same "staircase" outcome in weight gain (Hesketh 2008). This standard protocol could even be modified to administer cisplatin every third day so as to more closely relate to our recurrent NTG migraine protocol. For the second approach, injections of lithium chloride may be effective, as it is often utilized for

conditioned taste aversion (Nachman 1963). Utilizing one of these approaches would function as an effective cross-reference for our use of weight data as a surrogate measure of nausea.

Third, the novel measure of spontaneous tactile allodynia in the open arena could benefit from having its findings validated with additional data. It would be beneficial to determine if this arena apparatus generalizes to other models that present spontaneous allodynia, such as a cisplatin-induced neuropathy model (Authier et al. 2000). If spontaneous allodynia in other models can be captured by the arena apparatus, the next step would be to determine whether spontaneous allodynia differs from evoked allodynia in pharmacological sensitivity. A previous experiment of this type (Cucinello et al. 2017) found gabapentin and oxycontin to be effective analgesics in the evoked allodynia measure of electronic von Frey, but only found oxycontin to be effective in the spontaneous allodynia measure. These results align with the clinical presentation in that oxycodone but not gabapentin is effective in managing allodynia.

Finally, the absence of a prophylactic agent in attenuating these migraine endpoints is a limitation when it comes to drug screening. Model validity could be greatly improved with an increase in pharmacological sensitivity. Efficacious and non-efficacious migraine therapies should screen positive and negative in the model, respectively, while also avoiding false positives and false negatives.

LIST OF REFERENCES

Antonova, M., Wienecke, T., Olesen, J., Ashina, M., Prostaglandin E2 induces migraine-like attack in migraine patients without aura. *Cephalalgia*. 2012; 32(822-833).

Authier, N., Fialip, J., Eschalier, A., Coudore, F., Assessment of allodynia and hyperalgesia after cisplatin administration to rats. *Neuroscience Letters*. 2000; 291(73-76).

Burstein, R., Yarnitsky, D., Goor-Aryeh, I., Ransil, B.J., Bajwa, Z.H. An association between migraine and cutaneous allodynia. *Annals of Neurology*. 2000; 47(614-624).

Buxbaum, D.M. Analgesic Activity of delta-9-tetrahydrocannabinol in the rat and mouse. *Psychopharmacologia*. 1972; 25(275-280).

Castillo, J., Munoz, P., Guitera, V., Pascual, J. Epidemiology of Chronic Daily Headache in the General Population. *Headache The Journal of Head and Face Pain*. 1999; 39(190-196).

Cucinello, J.A., Warren, J.D., Oellerich, P.L., Harris, H.M., Sufka, K.J., Enhancing Translational Relevance in a Murine Model of Cisplatin-Induced Neuropathy Using a Novel Arena Apparatus. Annual meeting of UMMC's Neuroscience Research Day. Jackson, MS 2017.

Cupini, L.M., Costa, C., Sarchielli, P., Bari, M., Battista, N., Eusebi, P., Calabresi, P., Maccarone, M., Degradation of endocannabinoids in chronic migraine and medication overuse headache. *Neurobiology of Disease*. 2008; 30(186-189).

Diener, H.C., Limmroth, V., Medication-overuse headache: a worldwide problem. *The Lancet Neurology*. 2004; 3(475-483).

Diener, H.C., Pfaffenrath, V., Schnitker, J., Friede, M., Henneicke-von Zepelin, H.H. Efficacy and safety of 6.25 mg t.i.d. feverfew CO_2-extract (MIG-99) in migraine prevention – a randomized, double-blind, multicenter, placebo-controlled study. *Cephalalgia*. 2005; 25(1031-1041).

Ferrari, Michel D. The economic burden of migraine to society. *Pharmacoeconomics.* 1998; 6(667-676).

Garner, J.P. The significance of meaning: why do over 90% of behavioral neuroscience results fail to translate to humans and what can we do to fix it? *Institute for Laboratory Animal Research Journal*. 2014; 55(438-456).

Harris, H.M., Carpenter, J.M., Black, J.R., Smitherman, T.A., Sufka, K.J. The effects of repeated nitroglycerin administration in rats; modeling migraine-related endpoints and chronification. *Journal of Neuroscience Methods*. Under re-review.

Hesketh, P.J., Chemotherapy-Induced Nausea and Vomiting. *The New England Journal of Medicine*. 2008; 358(2482-2494)

Leonardi, M, Steiner, T.J, Scher, A.T. The global burden of migraine: measuring disability in headache disorders with WHO's Classification of Functioning, Disability and Health (ICF). *Journal of Headache Pain*. 2005; 6(429-440).

Levin, M., Herbal treatment of headache. *Headache*. 2012; 52(76-80).

Lin, H.C., Soubrane, O., Cailmail, S., Lebrec, D. Early chronic administration of propranolol reduces the severity of portal hypertension and portal-systemic shunts in conscious portal vein stenosed rats. *Journal of Hepatology*. 1991; 13(213-219).

Lipton, R.B., Gobel, H., Einhaupl, K.M., Wilks, K., Mauskop, A. *Petasites hybridus* root (butterbur) is an effective preventative treatment for migraine. *Neurology*. 2004; 63 (2240-2244).

Nachman, M.U., Learned aversion to the taste of lithium chloride and generalization to other salts. *Journal of Comparative Psychology*. 1963; 56(343-349).

Noyes Jr, R., Brunk, S.F., Avery, D.H., Canter, A. The analgesic properties of delta-9-tetrahydrocannabinol and codeine. *Clinical Pharmacology and Therapeutics*. 1975; 18(84-89).

Nozaki, C., Markert, A., Zimmer, A. Inhibition of FAAH reduces nitroglycerin-induced migraine-like pain and trigeminal neuronal hyperactivity in mice. *European Neuropsychopharmacology*. 2015; 25(1388-1396).

Pareek, A., Suthar, M., Rathore, G., Bansal, V. Feverfew (*Tancetum parthenium L.*): A systematic review. *Pharmacognosy Review*. 2011; 5(103-110).

Pradhan, A.A., Smith, M.L., McGuire, B., Tarash, I., Evans, C.J., Charles, A. Characterization of a novel model of chronic migraine. *Pain*. 2014; 155(269-274).

Rothrock, J., Patel, M., Lyden, P., Jackson, C., Demographic and clinical characteristics of patients with episodic migraine versus chronic daily headache. *Cephalalgia*. 1996; 16(44-49).

Russo, E. *Cannabis* for migraine treatment: the once and future prescription? An historical and scientific review. *Pain*. 1998; 76 (3-8).

Stovner, L.J., Hagen, K, Jensen, R, Katsarava, Z, Lipton, R.B., Scher, A.I., Steiner, T.J. Zwart, J.A. The global burden of headache: a documentation of headache prevalence and disability worldwide. *Cephalalgia*. 2007; 27(193-210).

Sufka, K.J., Staszko, S.M., Johnson, A.P., Davis, M.E., Davis, R.E., Smitherman, T.A. Clinically relevant behavioral endpoints in a recurrent nitroglycerin migraine model in rats. 2016; 17:40

Tassorelli, C., Greco, R., Wang, D., Sandrini, M., Sandrini, G., Nappi, G. Nitroglycerin induces hyperalgesia in rats --- a time course study. *European Journal of Pharmacology*. 2003; 464(159-162).

Terwindt, G.M., Ferrari, M.D., Tijhuis, M, Groenen, S.M.A., Picavet, H.S.J., Launer, L.J. The impact of migraine on quality of life in the general population. *Neurology*. 2000; 55 (624-629).

Tfelt-Hansen, P., Efficacy and adverse events of subcutaneous, oral, and intranasal sumatriptan used for migraine treatment: a systematic review based on number needed to treat. *Cephalalgia*. 1998; 18 (532-538).

Weber, R.B., Reinmuth, O.M. The treatment of migraine with propranolol. *Neurology*. 1972; 22(366-369).

APPENDIX

Figure 1.

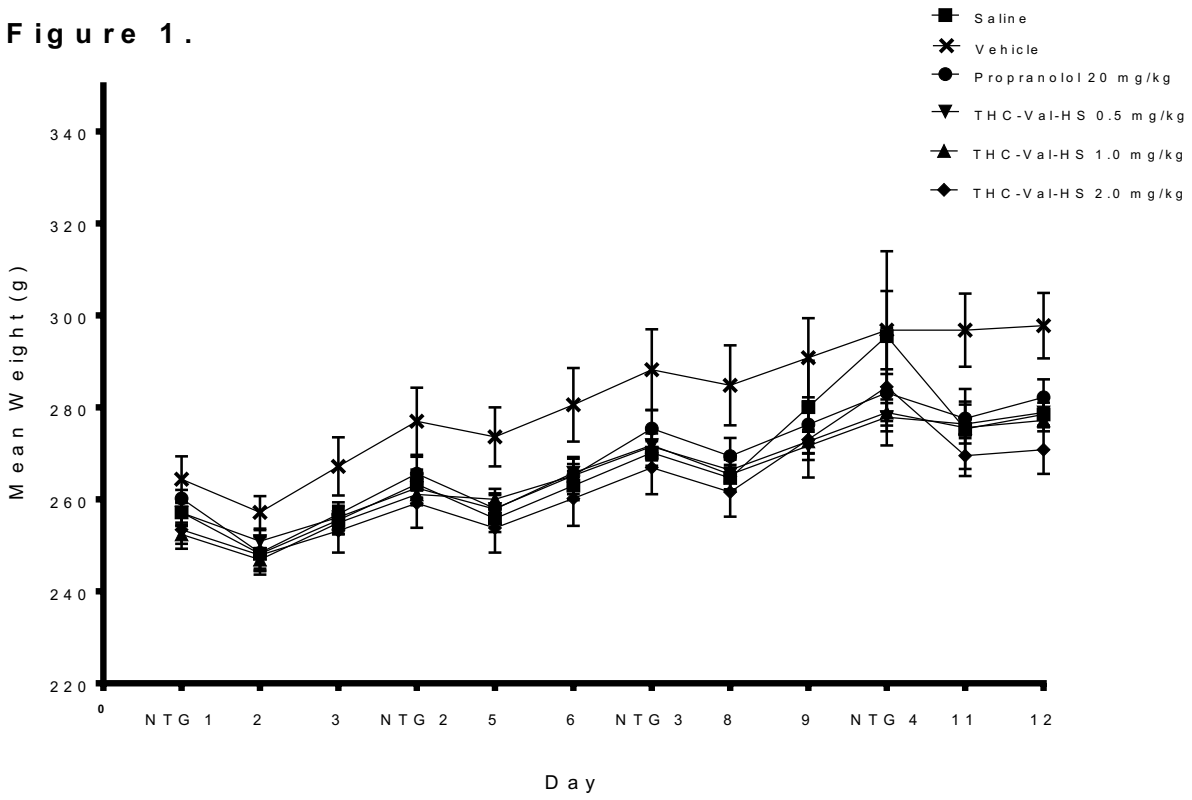

Figure 1. Mean body weight across treatment groups during migraine induction protocol. Each data point represents mean group body weight ± SEM.

Figure 2.

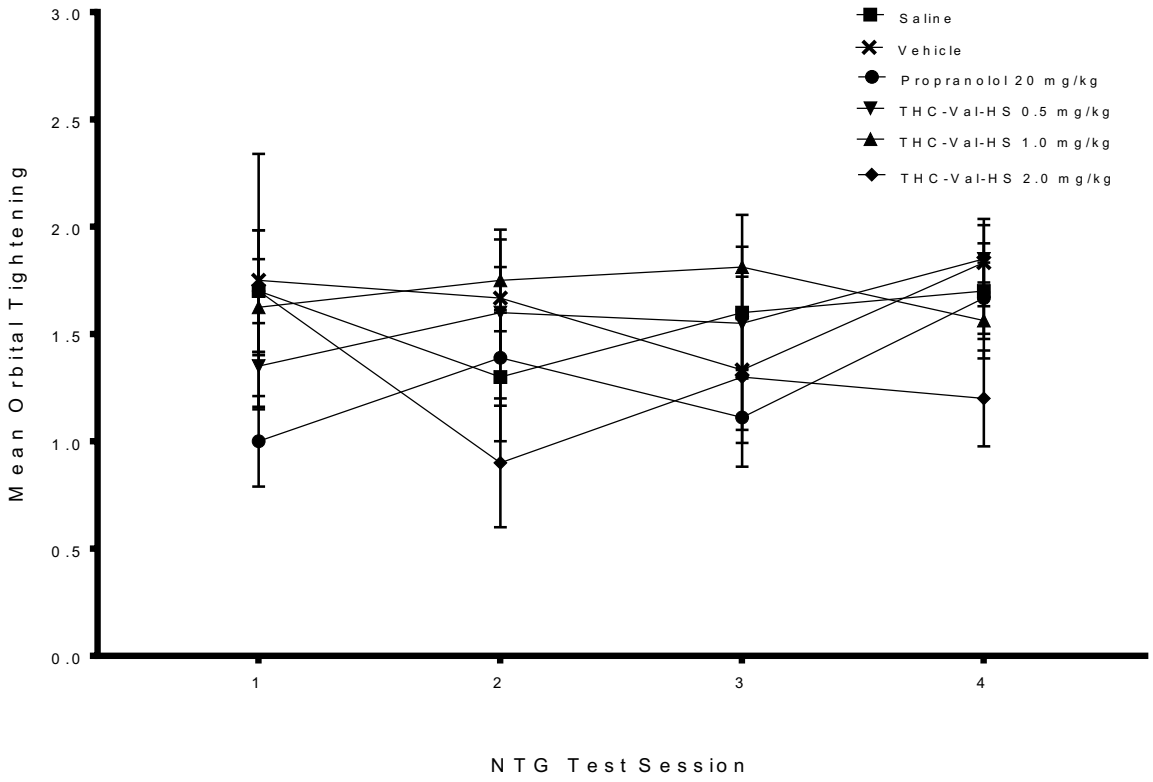

Figure 2. Mean orbital tightening score across treatment groups over four NTG test sessions. Each data point represents group mean orbital tightening score ± SEM. Orbital tightening was assessed 30 min post NTG administration (20mg/kg).

Figure 3.

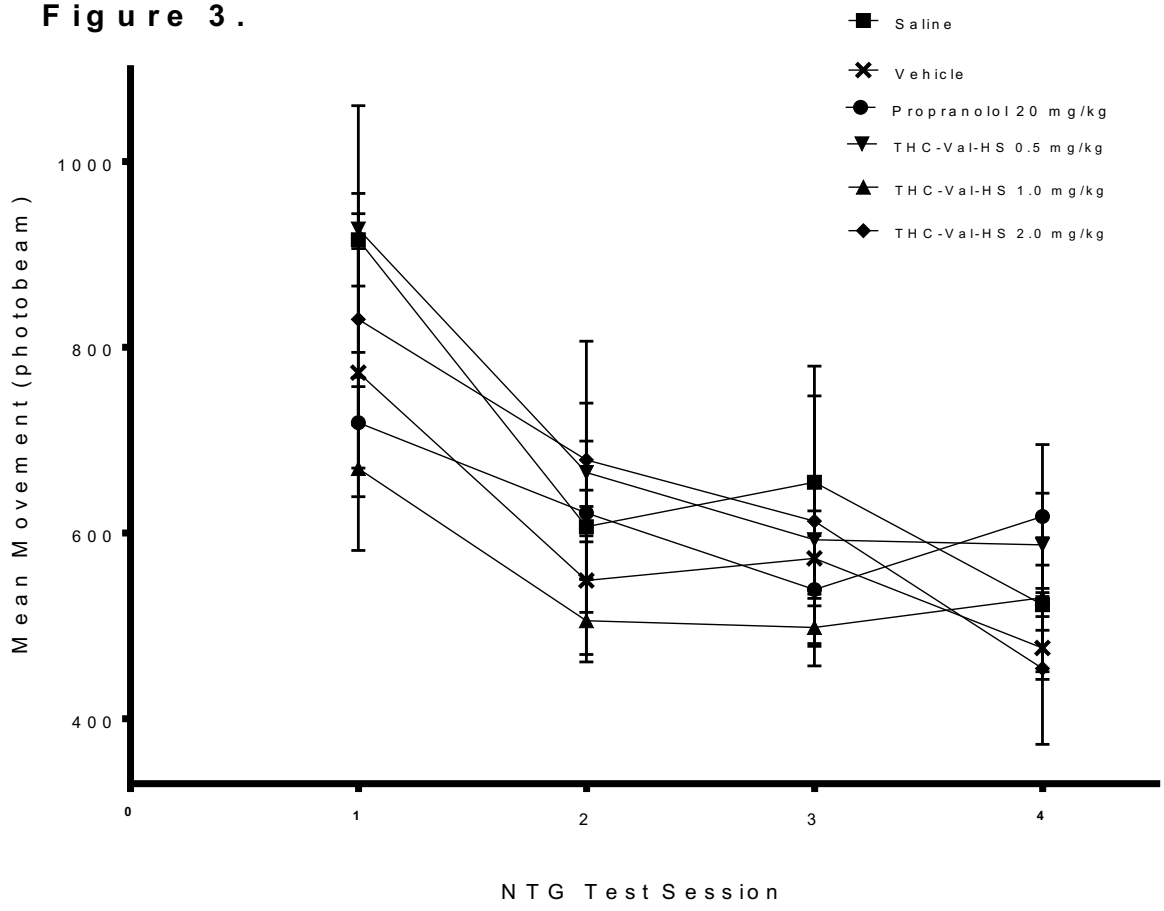

Figure 3. Mean movement in Light/Dark box across treatment groups over four NTG test sessions. Each data point represents group mean movement (quantified as number of photobeam breaks) ± SEM. Movement data was collected 110 min post IP NTG administration (20 mg/kg) and was collected over a 20 minute time period.

Figure 4.

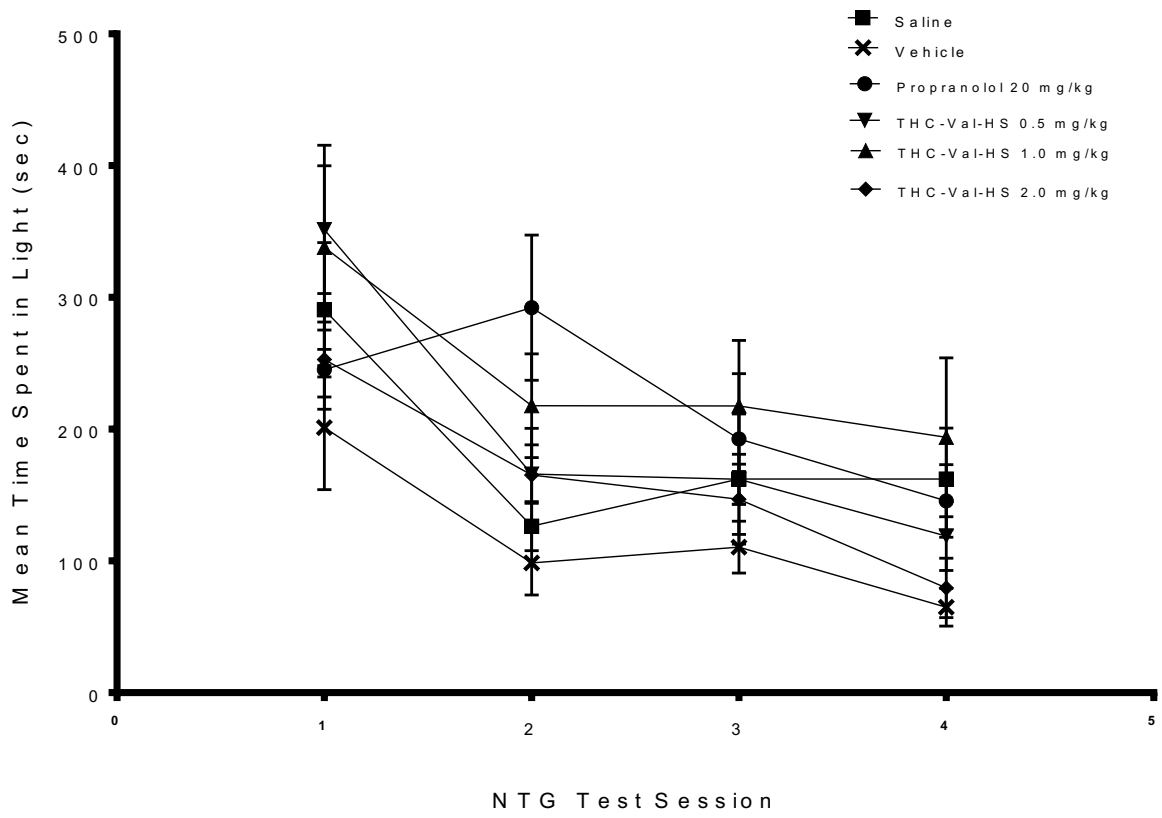

Figure 4. Mean time spent in light portion of Light/Dark box across treatment groups over four NTG test sessions. Each data point represents group mean time spent in light ± SEM. Light data was collected 110 min post IP NTG administration (20 mg/kg) and was collected over a 20 minute time period.

Figure 5.

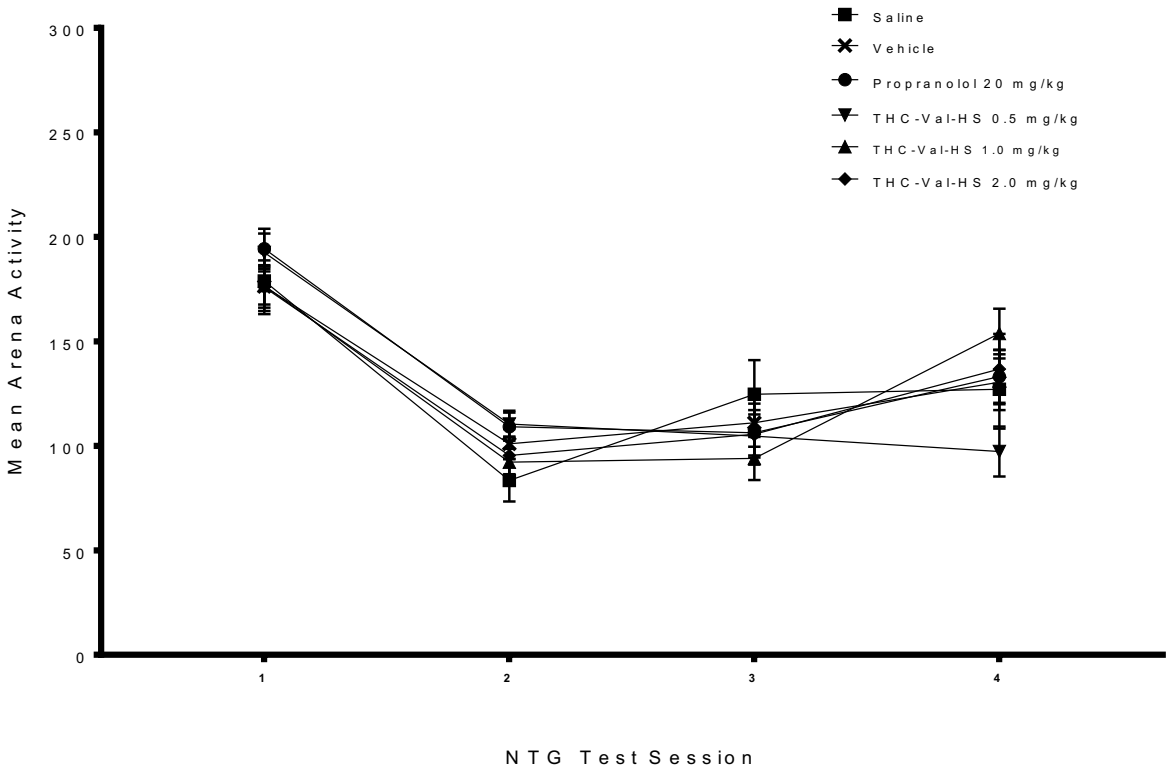

Figure 5. Mean activity in the open arena across treatment groups over four NTG test sessions. Each data point represents group mean arena activity (quantified in cm traveled) ± SEM. Arena activity data was collected 130 min post IP NTG administration (20mg/kg) and was collected over a 5 minute time period.

Figure 6.

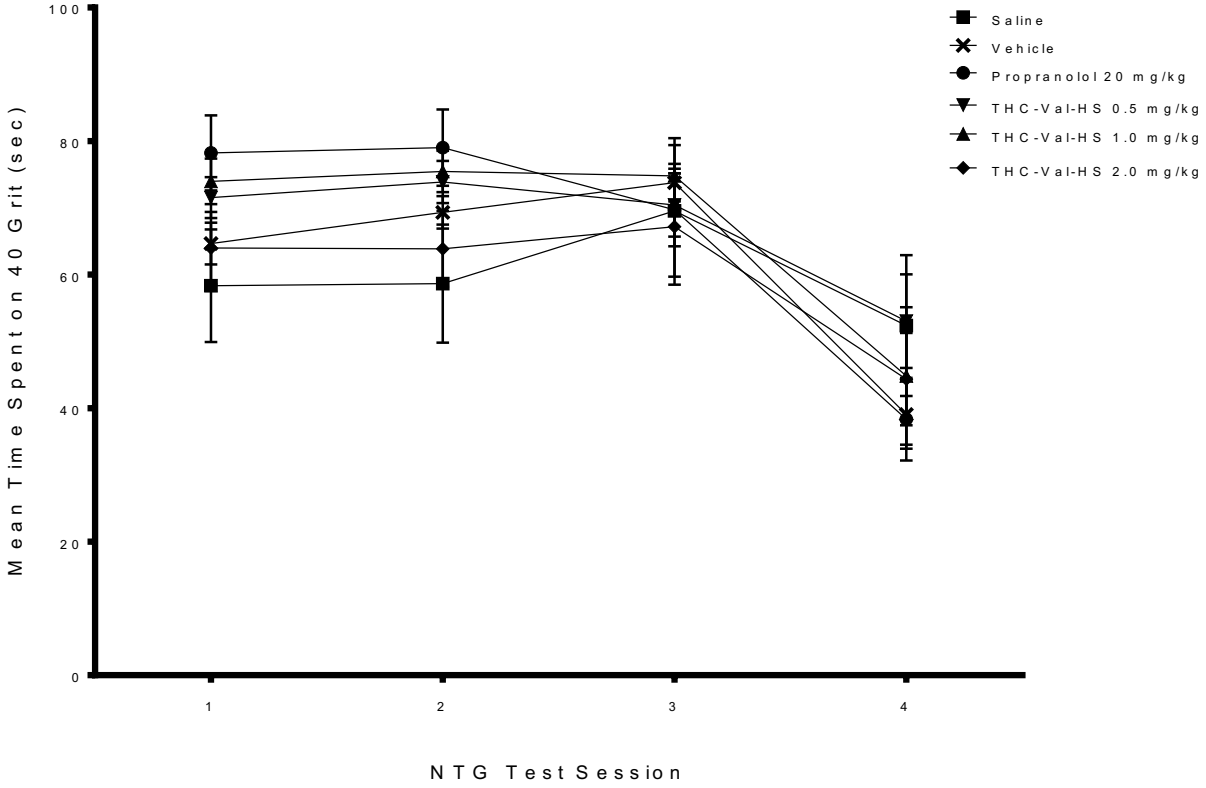

Figure 6. Mean time spent on 40 grit half field across treatment groups over four NTG test sessions. Each data point represents group mean time spent on the 40 grit half field ± SEM. Data was collected 130 min post IP NTG administration (20mg/kg) and was collected over a 5 minute time period.