

THE EFFECT OF SUMATRIPTAN ON CLINICALLY RELEVANT  
BEHAVIORAL ENDPOINTS IN A RECURRENT NITROGLYCERIN MIGRAINE  
MODEL IN RATS

By  
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## DEDICATION

I dedicate my thesis work to my family and friends, who have supported me throughout this entire process. I want to give a special dedication to my mom, Rhonda, who continues to be my greatest inspiration and life long cheerleader and to my dad, Jason who always encourages me to strive for greatness – I love you both.

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## ABSTRACT

MORGAN ELIZABETH DAVIS: The Effect of Sumatriptan on Clinically Relevant Behavioral Endpoints in a Recurrent Nitroglycerin Migraine Model in Rats  
(Under the direction of Dr. Kenneth Sufka)

The present research sought to determine the effects of sumatriptan on clinically relevant behavioral endpoints in migraine produced by repeated nitroglycerin (NTG) administrations in rats. Rats were given 5 NTG administrations each of which was followed by either saline, 0.3 mg/kg sumatriptan, or 1.0 mg/kg sumatriptan over a 2-week period. During their 5<sup>th</sup> NTG migraine episode, behavioral endpoints were the Rat Grimace Scale, photophobia, and movement. Sumatriptan dose dependently attenuated a) loss in weight, b) elevated Rat Grimace Scale pain scores, and c) decreased movement in the modified light-dark box. In addition, rats with the highest dose of sumatriptan tended to spend more time in the light portion of the light-dark box. We conclude from this study that sumatriptan is effective in reversing behavioral endpoints that parallel clinical symptoms of human migraineurs. These findings further validate the recurrent NTG migraine model as a clinically relevant simulation of human migraine that can be used as a drug-screening tool for novel therapeutics.

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## **1. Introduction**

Migraine is a common incapacitating neurobiological headache disorder affecting about 28 million Americans (Silberstein, 2004). In addition, 11% of the overall population in the United States and Western Europe has significant problems with migraine headache (Goadsby, Lipton, & Ferrari, 2002). Women show an increased incidence of migraine with 18% of women affected compared to 6% of men (Goadsby, Lipton, & Ferrari, 2002). Migraine is best understood as a form of neurovascular headache involving the dilation of blood vessels, which subsequently causes pain and triggers further nerve activation (Goadsby, Lipton, & Ferrari, 2002). The typical throbbing that occurs during a migraine attack is commonly aggravated by movement and accompanied by hypersensitivity to light (photophobia). Other symptoms of migraine include nausea, diarrhea, sensitivity to sound, anxiety, and depression (Silberstein, 2004).

Migraine attacks in humans greatly affect the quality of life and are severely debilitating in that of the 90% of migraineurs who report moderate or severe pain, three-quarters have reduced ability to function during the headache attacks and one-third require bed rest (Lipton et al, 2007). Migraine is also extremely costly in that each migraineur is estimated to lose 6.6 workdays per year due to absenteeism or reduced work productivity (Leonardi et al, 2010). Because of the high prevalence of migraine, this loss of productivity totals to 25-million lost workdays per year in the

United Kingdom, and migraine's yearly cost to employers reaches 13 billion dollars (World Health Organization, 2012; Silberstein, 2004). Moreover, the World Health Organization reports migraine as the 19<sup>th</sup> most common cause of disability in men and women of all ages (Leonardi et al, 2010).

Different from a typical "bad headache," migraine episodes are usually made up of four phases (premonitory, aura, headache, and resolution) characterized by a combination of neurological, gastrointestinal, and autonomic changes (Silberstein, 2004). The onset of migraine attacks are commonly marked by a premonitory phase which consists of predictable symptoms such as changes in mood, pain in the neck and back, flu-like symptoms, phonophobia (hypersensitivity to sounds), and/or fatigue that may occur hours to days before an attack (Bigal et al, 2009). For some individuals, premonitory symptoms are then followed by periods of "aura" in which migraineurs experience unusual changes in vision, sensation, speech, or movement lasting from 5 to 50 minutes (Silberstein, 2004). The headache phase of a migraine attack consists of the gradual onset of moderate to severe, typically unilateral throbbing pain lasting anywhere from 4 to 72 hours that is aggravated by movement. The resolution phase occurs after the headache and consists of a wide range of events including irritability, exhaustion, mood changes, euphoria, depression and/or malaise (Silberstein, 2004).

Migraine can be classified as either episodic or chronic. Formal diagnostic criteria for episodic migraine requires a minimum of five recurrent episodes with a combination of features that can vary among attacks (Silberstein, 2004). On the other hand, chronic migraine is classified as 15 or more headache days per month

continuing for at least 3 months (Katsarava et al, 2012). In addition, two subtypes of migraine are recognized, migraine with and without aura. Migraine with aura requires the presence of at least one neurological aura symptom that gradually develops and persists anywhere from 4 to 60 minutes followed by migraine (Ferrari, 1998). Migraine without aura, however, accounts for the majority of reported migraine episodes totaling for 75% of migraineurs (Ferrari, 1998). Despite significant efforts, migraine remains substantially under-diagnosed and undertreated (Leonardi et al, 2010).

Once an individual has been diagnosed, two standard methods of pharmacotherapy are available. Prophylactic, or preventative, treatments aim to reduce the severity and occurrence of migraine attacks. Common preventative treatments include beta-blockers, tricyclic antidepressants, and anticonvulsants (Silberstein, 2004). However, several of these drugs are compounded with adverse side effects and only moderate efficacy. As a result, only 13% of migraineurs report taking this route of action (Lipton et al, 2007).

The second method is acute treatments such as triptans, ergots, analgesics, and opioids, which attempt to abort or reverse a headache following the onset of a migraine episode. The use of triptans has resulted in a time of remarkable progress in pain modulation and treatment of migraine (Bigal et al, 2009). Triptans are effective in that they alter blood flow in the brain (Humphrey et al, 1990). In addition to relieving headache, triptans also contribute by relieving both nausea and vomiting, two symptoms frequently associated with migraine (Silberstein, 2004). Effectiveness

of triptans is greatly enhanced by early detection of migraine and fast administration of the drug.

The gold standard and first triptan to be used in treating migraine post onset is sumatriptan. There are several routes of sumatriptan administration available including oral, intranasal, suppository, and subcutaneous. Meta-analysis of subcutaneous sumatriptan, however, yields the most effective results showing that 71% of patients report improvement in headache severity to mild or no pain within 1 hour of the injection along with a therapeutic gain of 51% (Ferarri et al, 2001).

Although acute migraine treatments are moderately effective in alleviating or reducing pain associated with migraines, predicting the onset of headache is often extremely difficult, especially for patients who do not experience premonitory symptoms or aura prior to their migraine attack. In addition, current available migraine treatments are ineffective or poorly tolerated in millions of migraine patients collectively referred to as non-responders (Pradhan et al 2014). As a result, current migraine therapies remain extremely limited, leaving room for improvement in drug discovery and clinical efficacy.

Animal models represent experimental conditions produced in one species for the purpose of studying and understanding similar phenomena that occurs in another species (Willner, 1991). Because it is not always possible to study behaviors and disorders in human subjects, animal models provide a comparative mechanism for understanding and expanding upon existing knowledge of human behavior and its underlying processes (Van der Staay, 2006). When constructed and utilized efficiently, animal models attempt to simulate a symptom of a disorder, a group of

symptoms, or even a complete syndrome by addressing the etiology, treatment, physiological basis, and/or physiological mechanisms underlying successful treatment (Willner, 1991).

Animal models of human behavior should also demonstrate three types of validity. Predictive validity of an animal model shows that performance on a test accurately represents the predicted performance in the human condition being modeled. Predictive validity of a behavioral model is often addressed by testing an animal's response to clinically effective therapeutic drugs. Face validity assesses the similarity between an animal model and the disorder it simulates. This type of validity compares drug effects achieved in the model to comparable effects seen clinically. Because a given condition may vary between species, construct validity analyzes the degree of homology and significance between an identified variable relevant to the clinical condition and a behavior in the animal simulation. According to Van der Stay, to be useful, animal models should be testable, generalizable to humans, and provide a simplification of complex phenomena occurring in the clinical syndrome (Van der Staay, 2006).

One of the main difficulties in improving treatments for migraine is the absence of predictive animal models (Pradhan et al, 2014). The development of a valid animal model that accurately reflects clinically relevant endpoints of human migraine is essential for pre-clinical drug screenings to identify novel anti-migraine treatments and to gain an overall better understanding of migraine pathophysiology.

Nitroglycerin (NTG) administration in human subjects is a common experimental paradigm that produces headache in normal individuals and a delayed

migraine without aura analogous to environmentally triggered migraines in migraine patients (Pradhan et al, 2014; Iversen, 2001). The mechanism behind NTG induced migraine pain is believed to be the result of vasodilation of cranial blood vessels along with direct activation of pathways involved in nociception (Bates et al, 2010). NTG induced migraine attacks have since been reproduced in rodents in the attempt to create a valid rodent model of migraine (Iversen, 2001). These studies, however, mainly focus on two clinically irrelevant endpoints, hyperalgesia and allodynia.

One study examining the effects of acute and chronic restraint stress on pain response after NTG administration revealed that NTG produced hyperalgesia, or a heightened pain response to an already painful stimulus, after 2 and 4 hours. A tail-flick test was used as a measure of pain and NTG induced hyperalgesia was determined by the latency of response (Costa et al, 2005). Although the previous study may be useful in understanding acute and chronic stress models, hyperalgesia is associated with nonspecific pain and is rarely seen in human migraineurs. As a result, hyperalgesia is a clinically irrelevant endpoint in establishing a valid animal migraine model.

A second study demonstrated dose dependent NTG induced thermal and mechanical allodynia, or a heightened pain response to non-painful stimulus in mice, 30 to 60 minutes following NTG administration (Bates et al, 2010). Mice were injected intraperitoneally (IP) with various doses of NTG followed by assays of thermal pain thresholds using the Hargreaves test and mechanical thresholds using a von Frey apparatus. This study not only reported a significantly lower threshold to pain following NTG administration of 5 mg/kg and 10 mg/kg, but the anti-migraine

drug sumatriptan successfully reduced both thermal and mechanical hypersensitivity at 90 and 120 minutes (Bates et al, 2010).

Although allodynia is a common symptom of chronic migraine and is more clinically relevant than hyperalgesia in modeling chronic migraine, this measure remains useless when modeling episodic migraine (Marcelo et al, 2009).

Additionally, according to the diagnostic criteria for diagnosing migraine, neither chronic nor episodic migraine can be achieved clinically by a single episode. The previous study only examined the effects of a single NTG administration at various doses; therefore, the discovery of NTG-induced allodynia is not consistent with the clinical description of migraine symptoms (Marcelo et al, 2009).

Clinically relevant endophenotypes are necessary for a valid animal model of human migraine. Endophenotypes are observable, disease-specific behavioral, biochemical, endocrinological, and neuroanatomical characteristics (Van der Staay, 2006). Since hyperalgesia and allodynia do not seem to accurately model endophenotypes or clinical symptoms of migraine, previous work from this laboratory attempted to validate more clinically relevant migraine behavioral endpoints.

The first study analyzed the effects of a single NTG induced migraine episode on behavioral endophenotypes present in human migraine including pain, photophobia, sensitivity to movement, and peripheral allodynia (Staszko, 2014). Rats were IP administered 10 mg/kg NTG and assays were performed 2 hours post injection. Presence of pain was assayed using the Rat Grimace Scale, photophobia was monitored using a modified light-dark box, sensitivity to movement was assayed

using the modified light-dark box and Rotor-Rod, and peripheral allodynia was detected by a thermal tail-flick test. Although NTG animals showed slightly higher pain scores with marginal significance compared to control animals, presence of photophobia was not evident. Surprisingly, NTG animals showed less sensitivity to movement and actually had higher movement scores compared to control animals in both the modified light-dark box and Rotor-Rod test (Staszko, 2014). This study also utilized a single NTG administration producing a single migraine attack, which is problematic because human clinical diagnosis specifically requires a minimum of 5 recurrent episodes. This study suggested that multiple NTG administrations were necessary to simulate and produce clinically relevant behavioral endophenotypes associated with NTG induced migraine in rats.

The second study sought to accurately model the etiology and parallel clinically relevant symptoms of human migraine. To correct the etiology, a recurrent NTG induced migraine model was developed using both 3 NTG and 5 NTG administrations (Johnson, 2014). The various NTG administrations were necessary in determining the relationship between 5 migraine episodes in humans and the number of episodes required to produce clinically relevant endpoints in rats. Movement and photophobia were both measured using a modified light-dark box, pain was analyzed using the Rat Grimace Scale, and thermal tail dip tests determined peripheral allodynia. In addition, this study sought to determine whether or not multiple migraine episodes in rats could produce chronic comorbidity of anxiety and depression relevant to human migraineurs. The elevated plus maze and force swim tests were used to assay anxiety and depression behaviors.

Although analysis of pain scores using the Rat Grimace Scale failed to reveal significant differences among treatment groups, multiple NTG administrations significantly decreased time spent in the light chamber of the modified light-dark box, indicating photophobia; and significantly decreased movement indicating sensitivity to movement. Another measure that was not originally specifically tested was changes in weight. Animals receiving multiple NTG administrations gained significantly less weight compared to control animals, indicating the presence of nausea (Johnson, 2014). The thermal tail flick test did not produce any observable differences in latency signifying peripheral allodynia among treatment groups and the anxiety-depression assays also failed to show significance.

The previous recurrent NTG migraine model using 5 NTG administrations demonstrated the most drastic effects on photophobia, sensitivity to movement, and nausea compared to control animals; however, both 3 and 5 NTG doses failed to reveal any changes in allodynia or comorbidity of anxiety and depression (Johnson, 2014). These results suggest that the clinically relevant endpoints demonstrated in this study using 5 NTG administrations are sufficient and effective in modeling episodic migraine, but not chronic migraine.

The previous study established predictive validity in that the recurrent NTG induced migraine model illustrated performance on the modified light-dark box test that accurately represented the photophobia and sensitivity to movement reported in the human condition. To further validate the previous recurrent NTG induced migraine rodent model, in the present study, we sought to examine the effects of the commonly used acute migraine drug, sumatriptan, on a 5 NTG- induced migraine

model. All animals were treated with 5 NTG administrations. To illustrate a drug effect, we developed three conditions in which animals received saline, 0.3 mg/kg sumatriptan, or 1.0 mg/kg sumatriptan 30 minutes after each NTG injection. Animals were assayed for the presence of pain, photophobia, and sensitivity to movement 2-hours after the 5<sup>th</sup> NTG administration. The Rat Grimace Scale was used to quantify pain in rat's facial expressions and movement and photophobia were simultaneously measured using the modified light-dark box. Changes in weight were also recorded for each animal over the 2-week experimental period. Sumatriptan administration was hypothesized to dose-dependently reverse NTG migraine induced nausea, pain, photophobia, and sensitivity to movement evident in control animals. As a result, we predict that sumatriptan treated animals will maintain body weight, display lower pain scores quantified by the Rat Grimace Scale, spend more time in the light chamber of the modified light-dark box, and move more in the modified light-dark box compared to untreated animals experiencing migraine.

## **2. Materials and Methods**

### *2.1. Animals*

Experiments were conducted using 30 adult male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) initially weighing between 260 and 330 g. Two rats were housed per cage at 22° C on a 12-hour light/dark cycle. The bases of the cages were clear and plastic (Anicare) with dimensions 22.4 x 21.0 x 13.3 cm. Food (Teklad 7001, Teklad Diets, Madison, WI, USA) and water were available ad libitum via a wire lid placed on top of each cage. Additionally, each cage was given a single, circular enclosure made of PVC end caps, measuring 16.5 cm in diameter, 7.5 cm in height, and containing an arched opening. These “huts” were used to permit rats to avoid light during the NTG migraine episodes experienced in their home cages. All procedures were approved by the University of Mississippi IACUC (protocol number 13-023).

### *2.2. Experimental Design and Procedures*

Previous studies demonstrated that 5 recurrent NTG administrations in the rat produced more clinically relevant end points than a single NTG dose, making it a more valid model for human migraine. As a result, all animals in the current study

received 5 exposures of NTG intraperitoneally (IP) across a 2-week period. To illustrate a drug reversal effect this study involved three experimental conditions in which rats received saline, 0.3 mg/kg sumatriptan, or 1.0 mg/kg sumatriptan 30 minutes after each NTG injection. Pilot studies performed by Bates et. al report that NTG induced thermal and mechanical allodynia in mice begin 30-60 minutes following IP injection, which helped to determine the 30 minute time interval used in this study. 10 rats were assigned to each treatment group and each cage, containing two rats, received the same treatment in order to minimize stress and other disturbances that could occur in pair housed animals. On each treatment day, rats were placed on a scale and their weights were recorded prior to NTG administration. On the 5<sup>th</sup> treatment day, tests were run 2 hours post NTG administration and 1.5 hours post saline or sumatriptan administration. The assays performed were the Rat Grimace Scale, immediately followed by the modified light/dark box.

### *2.2.1. Drug Administration*

All injections were administered to rats IP, with the second injection administered on the opposite side of the animal's body relative to the first. The concentration of NTG (Copperhead Chemical Company Inc., SDM®27, Tamaqua, PA, USA) used was 5 mg/kg/ml dissolved in a 50% ethanol/50% propylene glycol solution. In order to achieve a final NTG dose of 10 mg/kg, a 2 ml volume of NTG was administered per kg body weight (10 mg/kg/2 ml). The original 6mg/0.5 ml preparation of injectable sumatriptan succinate (PAR Pharmaceutical, Woodcliff

Lake, New Jersey, USA) was diluted using physiological saline to attain experimental concentrations of 0.3 mg/kg and 1.0 mg/kg.

### *2.2.2. Rat Grimace Scale*

The Rat Grimace Scale is a partially automated method designed to quantify a rat's pain state by observing facial expressions (Sotocinal et al., 2011). Rats were placed in a clear Plexiglas chamber with a wire base measuring 31.1 cm x 21.6 cm x 26.0 cm and allowed to move freely throughout the apparatus. A dry-erase board was placed behind the chamber to identify each subject. Headshot pictures containing clear images of the face, ears, and whiskers were taken of each rat using a 16 GB iPad mini 5-megapixel iSight Camera with Retina display 2 hours post NTG. After pictures were obtained, each rat was transferred back to its cage, and the chamber was cleaned out using Clorox wipes before the next subject was tested. Operational definitions and guidelines outlined in studies performed by Sotocinal et al. were used to train individuals to analyze pictures of each subject and determine subsequent pain scores. Scorers viewed images, one at a time, using a Microsoft Powerpoint slide show. Pictures were randomized and identifications were removed in order to rate each animal blindly.

For each photo, pain scores of 0,1, or 2 were assigned to quantify pain in four categories, or action units. A score of "0" was given in a situation where the scorer was highly confident that the action unit was absent. A pain score of "1"

showed high confidence of moderate appearance of the characteristic and a score of “2” indicated high confidence in the obvious appearance of the action unit (Sotocinal et al., 2011). The four action units demonstrated by rats in pain included 1) orbital tightening (partial/complete eye closure), 2) nose/cheek flattening (disappearance of the crease between cheek and whisker pads), 3) ear changes (ears fold and angle forwards or outwards resulting in a pointed shape), and 4) whisker changes (whiskers move forward, away from the face and stand on end). Scores were averaged from two raters, with 72% agreement, to produce an overall pain score for each rat.

### *2.2.3. Modified Light-Dark Box with Movement*

The modified light-dark box was used to quantify avoidance of light and diminished motor activity, two measures commonly associated with episodes of migraine in humans. This device was a modified version of a conditioned place preference (CPP) apparatus (Model #ENV-013, Med Associates Inc., St. Albans, VT, USA). The CPP box was customized for this experiment by covering the tops of the black and grey chambers, which are normally clear, with dark construction paper to prevent light from coming into these sections. The white chamber was left unaltered so that light could freely pass through the clear lid. This created a distinct light and dark environment that rats could freely move throughout during testing. Following Rat Grimace pictures, rats were transferred to the grey middle chamber of the modified light-dark box. The doors of the grey chamber remained closed for one minute to allow each animal to acclimate to their new environment. After the

acclimation time ceased, the chamber doors allowing access to the black and white chambers were simultaneously opened, and the testing session proceeded for 10 minutes. Throughout the 10-minute interval, total movement throughout the apparatus was recorded as well as the amount of time spent in the white (light) chamber and the time spent in the black and grey boxes, collectively referred to as the dark chamber. The modified apparatus was cleaned using a Clorox wipe in between each subject tested.

### *2.3. Statistical Analysis and Dependent Measures*

Data collected was analyzed by one-way ANOVA using SPSS software. Planned comparisons were performed with Fisher's LSD and statistical significance set at  $p < 0.05$ . Previous work in the validation of a recurrent NTG migraine model in the rat determined the dependent measures to be examined. These variables included percent change in weight, Rat Grimace Scale overall pain scores, time spent in the light chamber of the modified light-dark box, and degree of movement recorded by the modified light-dark box.

### 3. Results

The effects of sumatriptan on NTG induced weight changes over the 2-week injection period are summarized in Figure 1. Sumatriptan dose dependently induced a change in percent weight gain compared to animals receiving saline. The control group lost about 3% of their original weight, while rats receiving 0.3 or 1.0 mg/kg sumatriptan either maintained or gained approximately 2% of their original body weight. These data were analyzed using a one-way ANOVA that revealed a main effect that approached significance,  $F(2,24)= 2.62$ ,  $p=0.094$ . Planned comparisons using Fischer's LSD revealed that the mean percent weight change of the 1.0 sumatriptan group was significantly more than the saline control group ( $p<0.033$ ).

The effects of sumatriptan on NTG induced modifications in the Rat Grimace Scale pain scores are summarized in Figure 2. Treatment with both 0.3 and 1.0 mg/kg sumatriptan reduced overall pain scores compared to animals receiving saline. Consistent with these observations, a one-way ANOVA revealed a significant main effect,  $F(2,24)= 6.23$ ,  $p=0.007$ . Planned comparison analysis also revealed that the mean RGS scores for both the 0.3 and 1.0 sumatriptan groups were significantly lower than the saline group ( $p< 0.006 - 0.005$ ).

The effects of sumatriptan on movement are summarized in Figure 3. Sumatriptan dose dependently increased average movement scores during the modified light-dark box testing periods. These data were analyzed using a one-way ANOVA variance that revealed a main effect that approached significance,  $F(2,24)=2.397$ ,  $p=0.112$ ). Planned comparisons using Fisher's LSD showed that animals treated with 1.0 mg/kg sumatriptan had significantly higher mean movement scores compared to saline animals ( $p<0.045$ ).

The effects of sumatriptan on time spent in the light chamber of the modified light-dark box are summarized in Figure 4. Treatment with 1.0 mg/kg sumatriptan produced an upward trend suggesting an increase in the amount of time spent in the light chamber. A one-way ANOVA variance of these data, however, failed to reveal a significant treatment effect,  $F(2,24)=0.636$ ,  $p=0.53$ . Further analysis using Fischer's LSD planned comparisons also failed to reveal significant group differences ( $p=n.s$ ).

#### **4. Discussion**

The current study sought to expand upon the recurrent NTG rodent model of migraine by replicating clinically relevant endpoints of human migraine demonstrated by the Johnson (2014) study and to show a drug reversal using sumatriptan. The endpoints relevant to migraine and its sensitivity to sumatriptan include weight loss, presence of pain, movement, and photophobia. Rats were administered 5 NTG injections each followed by saline, 0.3 mg/kg sumatriptan, or 1.0 mg/kg sumatriptan and then clinically relevant dependent measures were assessed during the 5<sup>th</sup> migraine episode using the Rat Grimace Scale and the modified light-dark box.

When comparing changes in body weight in NTG control and sumatriptan animals, NTG animals showed a slight decrease in mean body weight. These findings are consistent with the Johnson (2014) study that demonstrated that 5 NTG migraine episodes produced a significantly lower body weight than vehicle treated animals over the same 2-week period. In the Johnson study, rats showed a slower weight gain rather than the loss of weight experienced by rats in the present study. This difference, however, reflects the difference in weight and younger age of test animals that were approximately 100 grams lighter and in a steeper growth curve in the Johnson study.

The sumatriptan manipulation in animals dose dependently reversed changes in weight by increasing weight gain with 1.0 mg/kg sumatriptan administration

having the strongest affect. One of the common symptoms in human migraine is nausea. Experiencing nausea subsequently causes people to eat less and may ultimately result in changes in body weight, specifically weight loss in migraineurs. As a result, we interpret the previous findings along with the findings of the present research to suggest that the loss in body weight (or failure to gain weight) is likely related to NTG induced nausea that alters food consumption and impedes weight gain in rats. The reversal of migraine induced weight loss and nausea by sumatriptan parallels the clinical picture and effects of acute treatment in humans. To our knowledge this is the first report that sumatriptan alters this clinically relevant endpoint in rats.

Since the most common and defining aspect of migraine is headache pain, NTG induced pain was quantified using pain scores generated by the Rat Grimace Scale (RGS) (Sotocinal et al, 2011). Since nonverbal infants express pain using facial expressions, the idea behind this test is that rats experiencing migraine pain would display higher pain scores when rated based off of their facial features using RGS. Both sumatriptan treated groups displayed lower overall pain scores than control animals, suggesting that they were in a lower overall pain state.

Raters used in the present study quantified pain scores for the 5 NTG control animals in the 0.5 range, which was slightly higher than previously reported by Johnson et al. Presence of pain in sumatriptan animals, however, was significantly lower. These results suggest that sumatriptan may mitigate the effects of NTG induced migraine by lowering pain scores on the Rat Grimace Scale. To our knowledge, this is the first case in which sumatriptan has been reported to lower this

behavioral index of migraine related to pain quantified by facial expressions. In addition, these findings along with the similar results from the previous work in this lab indicate the reliability of this assay as a measure of pain during NTG migraine episodes and its attenuation with appropriate treatment.

Although pain scores generated by the present research are significantly lower than RGS scores from the original Sotocinal et al. study, this was expected due to the fact that pain from a NTG migraine episode is moderate compared to the types of pain assayed by Sotocinal et al. The Sotocinal study assayed spontaneous pain that reached the test's upper limit of 2. As a result, the pain assays reported by Sotocinal et al. appear to be a more severe type of pain than that produced by an NTG induced migraine episode.

In the human clinical profile, physical activity often exacerbates pain during a migraine episode. As a result, migraineurs avoid or minimize unnecessary movement. The current study replicated the previous finding of NTG migraine induced sensitivity to movement in that 5 NTG migraine episodes produced movement scores using the modified light-dark box comparable to those seen in the Johnson et al study. Additionally, our drug manipulation using sumatriptan dose-dependently increased movement scores compared to NTG saline animals, suggesting the attenuation of NTG migraine induced movement sensitivity due to migraine pain. The predictability of this behavioral endpoint under NTG migraine and treatment conditions parallels the human clinical condition and provides additional validity to our recurrent NTG migraine rodent model.

The typical throbbing that occurs during a migraine attack is also commonly accompanied by photophobia (Silberstein, 2004). To assess this in our model, time spent in the light portion of the modified light-dark box was measured. We predicted that animals experiencing migraine would mimic the human condition by avoiding the light portion of the apparatus. This study showed that under the highest sumatriptan dose animals did tend to spend more time in the light portion of the modified light-dark box, but this effect was not statistically significant.

The previous finding can be interpreted in two ways. The first suggests that the predicted effect was not evident under the current method created to test for photophobia in this study. The light source used was simply ambient light in the room coming into the box with the clear lid. Adjusting the lighting parameters and creating a brighter environment inside of the light box with greater sensitivity to light may result in a significant reversal of photophobia among sumatriptan treated animals.

The second theory suggests variability in efficacy rather than the need to alter lighting parameters in the current testing procedure. Given that only 71% of migraineurs report improvement with sumatriptan (51% therapeutic gain), we expect to have some non-responders, just like in the clinical situation (Ferrari et al, 2001). It is interesting to note, that among rats in the 1.0 mg/kg sumatriptan group, those that displayed higher RGS scores spent less time in the light portion of the light-dark box, and this correlation was -0.70. Consequently, we believe our NTG migraine animal simulation demonstrates the limited efficacy of current anti-migraine drugs in some

non-responders and thus correctly models the clinical picture on this dependent measure as well.

Another reported symptom associated with human migraine is diarrhea (Silberstein, 2004). One unexpected finding from the current study that mirrors the clinical picture is the presence of diarrhea. Although it was not systematically quantified, researchers noticed NTG saline animals having an unusual amount of diarrhea in their home cages following migraine attacks. These observations were not seen in sumatriptan treated animals. We believe that this measure might be associated with the significant amount of weight loss that we interpreted as nausea found among NTG saline animals experiencing migraine. Although this behavioral aspect was not systematically quantified across treatment conditions in the present study, this finding suggests that it might be a clinically relevant endpoint in determining the effectiveness of sumatriptan on NTG induced migraine and in future testing of drug efficacy.

The recurrent 5 NTG migraine model demonstrates two clinically relevant behavioral endpoints for migraine: sensitivity to movement and photophobia. Other noteworthy homologies demonstrated in NTG migraine induced animals are evidence of pain expression indicated by Rat Grimace pain scores and loss of weight, which was interpreted as nausea. In addition, our model shows a drug reversal effect of the previous behavioral endpoints using one of the most common pharmacotherapeutics in migraine treatment, sumatriptan. Clinical validation of an animal model requires the presence of several homologies between the animal model and the human condition, which we believe to be evident in the present NTG migraine model. Since

the present model provides predictive validity and response to clinically effective drugs, this recurrent NTG migraine model is a valid simulation of human migraine and can be used as a broad screening tool to develop novel anti-migraine treatments.

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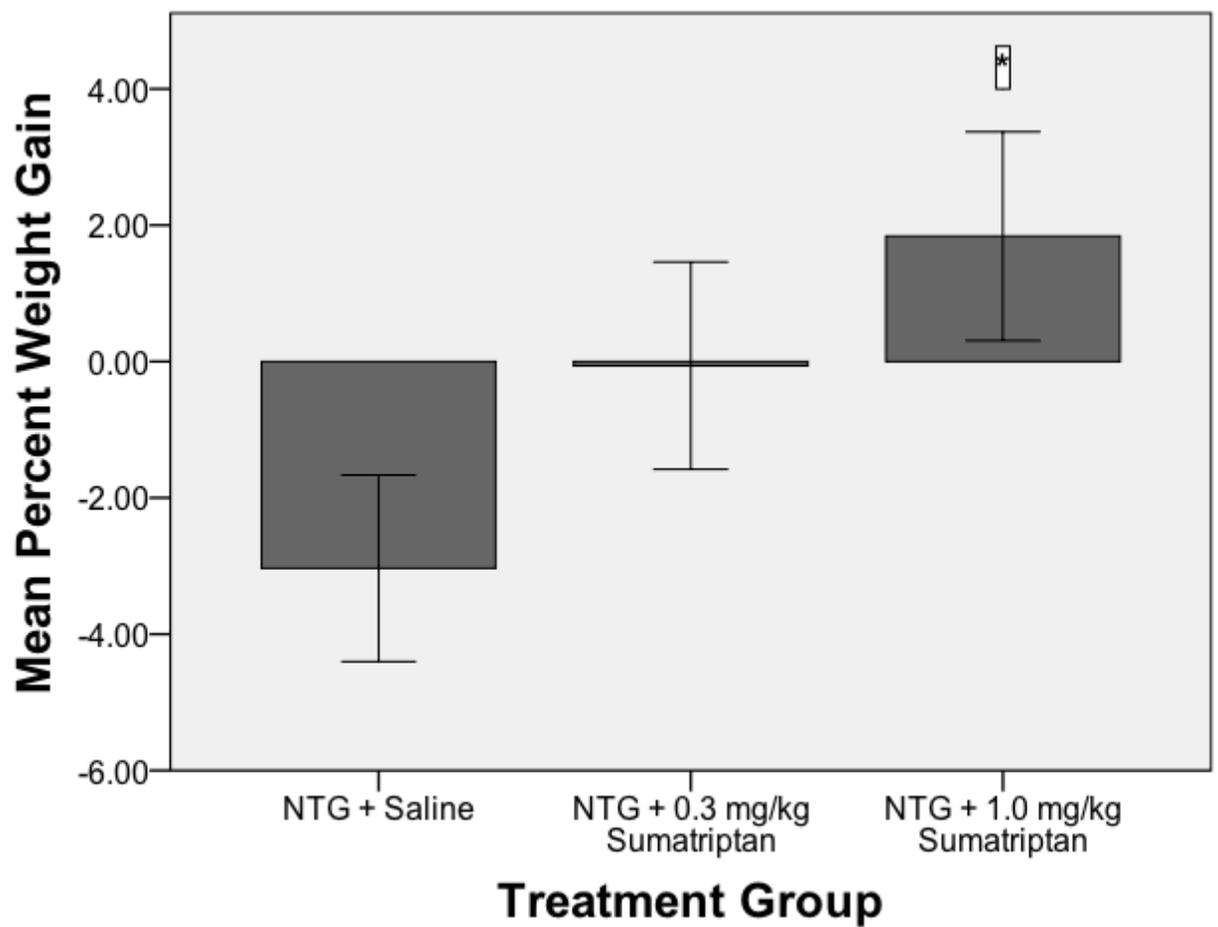
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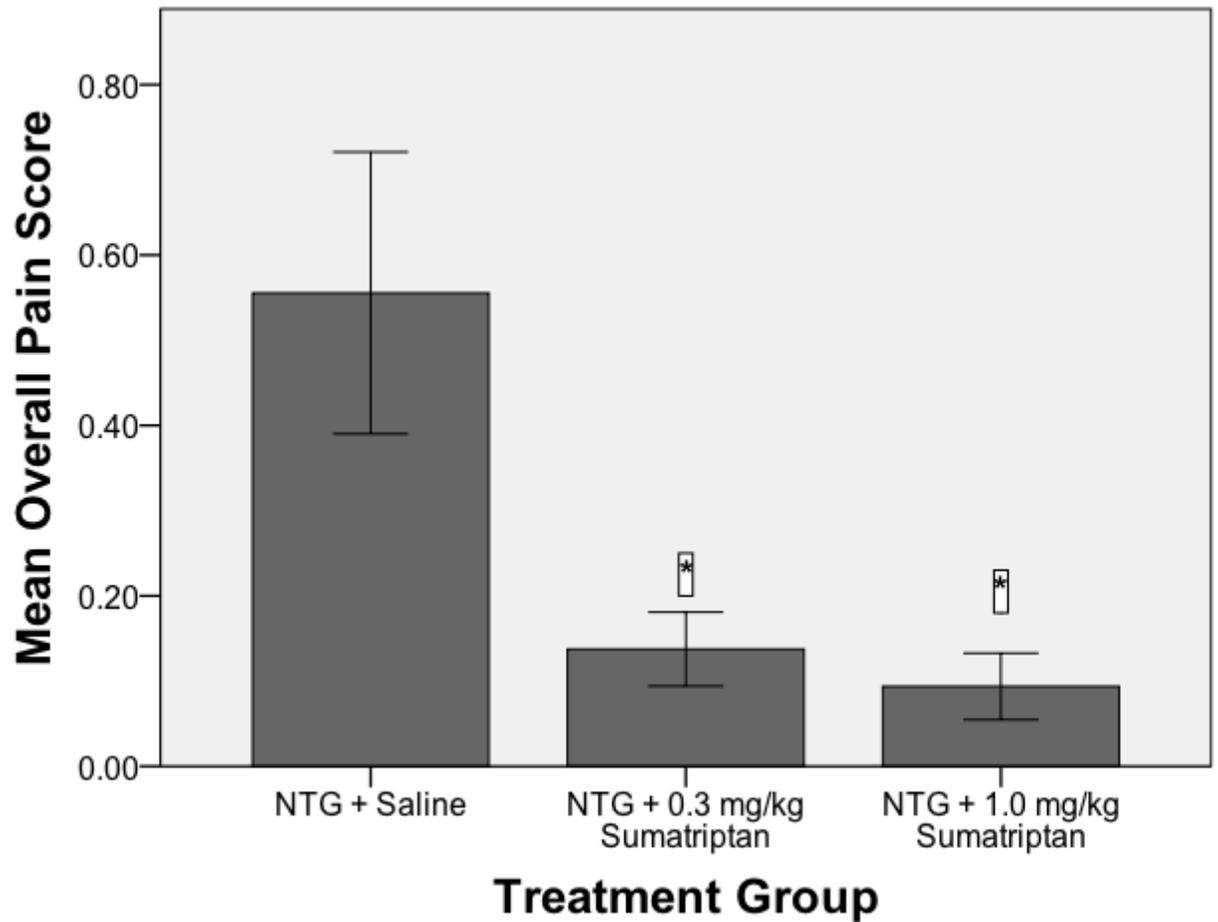
## APPENDIX

## Figures

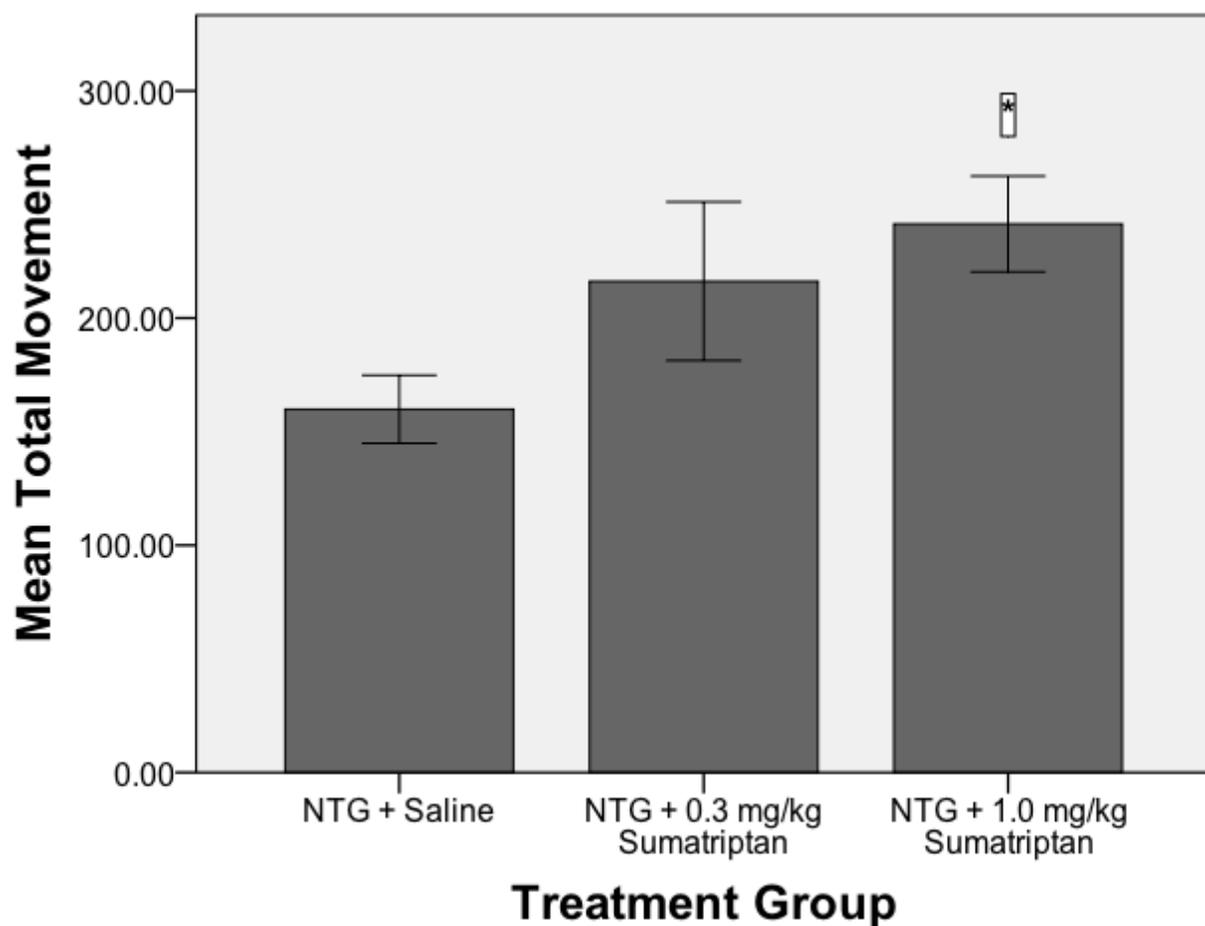


*Figure 1.* Mean percent weight gain as a function of treatment group in rats on their 5<sup>th</sup> NTG migraine episode. Bars represent group means  $\pm$  SEM. NTG + Saline animals show a slight decrease in weight compared to animals receiving 0.3 or 1.0 mg/kg sumatriptan. Animals receiving the high dose of sumatriptan gained more weight over the two week injection period compared to NTG + saline animals,

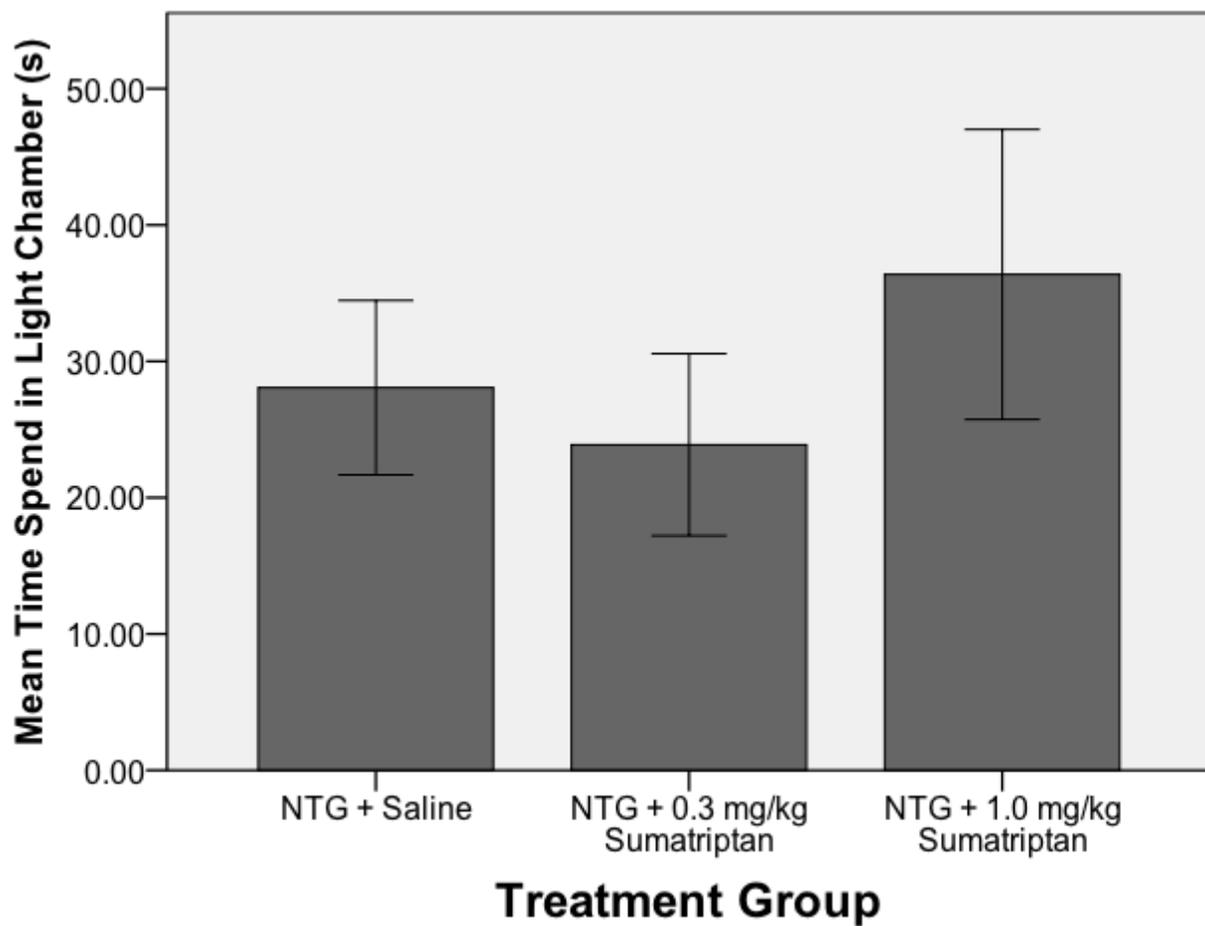
suggesting diminished nausea and increased appetite ( $p < 0.033$ ). Sample sizes were  $n = 8-10$ .



*Figure 2.* Mean Rat Grimace Scale pain scores as a function of treatment group in rats on their 5<sup>th</sup> NTG migraine episode. Bars represent group means  $\pm$  SEM. Both 0.3 mg/kg and 1.0 mg/kg doses of sumatriptan decreased overall pain scores quantified via rat's facial expressions in pictures taken during Rat grimace testing compared to NTG + saline group. Results indicate attenuation of pain associated with migraine following treatment with sumatriptan ( $p < 0.006-0.005$ ). Sample sizes were  $n = 8-10$ .



*Figure 3.* Mean movement (number of beam breaks) in the modified light-dark box as a function of treatment group in rats on their 5<sup>th</sup> NTG migraine episode. Bars represent group means  $\pm$  SEM. The correct upward trend was seen for both administration of 0.3 and 1.0 mg/kg sumatriptan. Movement was significantly increased by administration of 1.0 mg/kg sumatriptan compared to NTG + saline animals, indicating reversal of NTG induced movement sensitivity ( $p < 0.045$ ). Sample sizes were  $n = 8-10$ .



*Figure 4.* Mean time spent in the light chamber of the modified light-dark box as a function of treatment group in rats on their 5<sup>th</sup> NTG migraine episode. Bars represent group means +/- SEM. Treatment with sumatriptan following NTG produced a modest increase in time spent in the light chamber ( $p=$  n.s.). Sample sizes were  $n=8-10$ .