OROFACIAL MECHANICAL SENSITIVITY IN RATS

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

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To my wife

ACKNOWLEDGEMENTS

I thank Jesus for giving me peace, and my wife for always supporting me. I thank Drs. Neubert and Nolan for their wealth of knowledge and advice. I thank Vitaly for his technical assistance in making the testing plates, and I thank my research committee for their guidance. I thank my parents for encouraging me to always be excellent.

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LIST OF ABBREVIATIONS

NiTi	Nickel Titanium wires
OFP	Orofacial Pain
PBS	Phosphate Buffered Saline
S.C.	Sub-Cutaneous

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
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Chair: John Neubert
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Objectives: The goal was to assess the behavioral responses to mechanical facial stimulation under normal (baseline), analgesic and hyperalgesic conditions. Methods: Animals were trained to place their face against looped nickel titanium wires (0.008", 0.010", 0.012", 0.014", and 0.016") providing access to positive reinforcement in a reward conflict paradigm. Rats were tested under naïve, analgesic (morphine 1 and 3 mg/kg, SNC-80 5 and 10 mg/kg, s.c.) and algesic (capsaicin, 0.075%, s.c.) conditions, under conditions of gas anesthesia (isoflurane, 2.5%), and with isoflurane, capsaicin, and morphine 3mg/kg together. We evaluated the reward stimulus:contact ratio (mean±s.e.m.) as our pain outcome measure.

Results: Wire diameters of 0.014" and 0.016" produced significantly lower stimulus:contact ratios compared to wires with a diameter of 0.010" under naïve conditions. Morphine treatment at 1mg/kg did not result in a significant increase from naïve values using the 0.010" diameter (t-test, p=0.1087) wire, nor did it show an increase with the 0.016" (p=0.5006) diameter wire. Morphine (3mg/kg) response tested on 0.014" showed a significant increase in the success ratio for morphine treatment (p= 0.0073) as compared to the vehicle group. Facial injection of capsaicin induced a

significant decrease (p=0.0180) in the success ratio for wire diameter 0.010". Morphine 3mg/kg reversed the nociceptive effect of the capsaicin injection (p=0.0370). SNC 80 (10mg/kg) showed a significant increase in the success ratio (p= 0.0161) while 5mg/kg caused no significant difference from vehicle injection.

Conclusion: Radially arranged looped nickel titanium wires provide an effective mechanism for operant evaluation of facial pain.

CHAPTER 1
INTRODUCTION

Research Problem

Operant models for assessing mechanical orofacial pain in rodents are not well characterized. If we can validate a mechanical operant model for behavioral assessment in rodents, the result will have a significant impact on the way new analgesics are screened and evaluated. Making this validated model available to the scientific community will help to screen drugs more efficiently, speeding the process of getting new pain therapies on the market for patients who need relief.

Literature Review

Orofacial pain disorders have been well described in humans; however, evaluation of orofacial pain in animals has been difficult[1]. There are few animal models in existence that can replicate the human pain experience, especially for the facial region. This lack of testing devices is a problem because it slows drug development and evaluation.

Neubert et al. have developed a novel thermal operant behavioral assay for characterization of orofacial pain sensitivity[1]. They have also demonstrated the utility and innovativeness of the operant assay, using a variety of pain conditions (e.g., inflammation, heat, cold), pain states (e.g., allodynia), environmental conditions, and pharmacological agents[1-5]. The development of this assay was a major advance in OFP translational studies because it provided the first thermal operant model to quantitatively evaluate pain within the trigeminal system. The benefit of this model compared to reflex-based models is that it more closely represents the human pain experience through

emotional and motivational inclusion, incorporation of higher level cognitive processing in evaluating pain.

A significant problem for many patients with orofacial pain is that they experience mechanical pain, including touch-evoked allodynia, a condition characterized by pain associated with normally non-painful stimuli. Mechanical allodynia affects many patients with burn and trauma, as well as specific disorders such as trigeminal neuralgia. For example, patients with trigeminal neuralgia could find simple mechanical stimuli such as tooth brushing or face washing to be unbearable. Or another example, putting on a shirt is normally not a painful experience; however, after having a severe sunburn, putting on a shirt can be extremely painful. In dentistry, patients undergoing active orthodontic treatment can have facial pain to normal stimuli due to the sensitivity of the teeth. All of these problems mentioned involve mechanical sensitivity. Although the thermal operant behavioral assay has been thoroughly validated, an operant model for assessing mechanical orofacial pain in rodents is not well characterized. Previous work in our lab has assessed punctate sensation in a mechanical facial operant assay[6].The current study uses a mechanical facial operant assay to assess pressure sensation in facial pain. It is important to distinguish between the two different mechanical stimuli because they represent different sensations and may involve different pain pathways.

Background

The development of novel analgesics is a lengthy process involving a number of different stages, including animal modeling, measurement, and assessment of efficacy. Animal models are intended to replicate human pain conditions, including acute inflammatory pain, chronic arthritic pain, and neuropathic pain conditions. Given that the human pain condition can be induced in the rodent, the next hurdle is to utilize a reliable

method for assessing the pain. Drug testing is the final step, whereby new pharmacological agents are then evaluated against standard analgesics using these models.

Pain assessment in rodents

The pain produced in animal models has been evaluated by assessing: 1) simple reflexes, 2) unlearned or innate behaviors, and 3) learned or operant behaviors[7]. No single assessment can evaluate all aspects of pain, with each assessment having advantages and disadvantages. Simple reflexes, such as paw withdrawal and tail-flick, do not require learning and can occur automatically[8]. During the reflex response, sensory signaling to the spinal cord along sensory neurons initiates a reflex arc that triggers a signal directly back to the involved muscles. The advantages of simple reflex assessments are that they are easy to perform and the results can be related to similar human studies. However, the simple reflex is not directly assessing the amount of pain, it measures the segmental connection between the sensory, intermediate, and motor nerve cells to deliver a response[7].

Reflex response testing has been shown in both spinalized and decerebrated animals. One study demonstrated that after mid-thoracic spinalization, both thermal and mechanical stimuli were able to elicit tail-flick and hindlimb withdrawal reflexes[9]. Another study showed that chronic decerebrate animals with aspiration of all the cranial contents rostral to the mesencephalon have intact spinal reflexes in response to mild noxious thermal and mechanical stimuli[10].

While reflex responses depend on segmental processing, unlearned or innate behaviors such as paw licking, face-rubbing, limb guarding, vocalization, grooming, escape responses, chewing/biting, or a combination of these behaviors are mediated by

supraspinal or suprasegmental processing[11-17]. Some methods for assessing these behaviors include the hot plate test[17], the Hargreaves test[13], and Von Frey filament[34] testing which allow a variety of the behaviors to be monitored. Cold allodynia was also demonstrated in a neuropathic pain model that measured innate behaviors such as flicking and guarding[18]. Innate behaviors are useful because they are relatively easy to perform and deliver reliable results; however, they are not able to discriminate allodynia from hyperalgesia.

In developing more ideal testing conditions, one must consider that the experimenter performing the tests and the testing lab have a significant impact on measuring nociception[19,20]. Also, restraint stress, which is common in many cases of behavior analysis, can be a confounding factor. For example, the magnitude and duration of analgesia produced by opioid agonists was dose-dependently increased in restrained vs. unrestrained rats[21,22]. Therefore, removing the variable of experimenter manipulation and developing independent measures of assessing pain behavior is highly important**.** Operant response assays provide the conditions to allow for independent testing of animals where the animal chooses the amount of nociception they endure[23].

In reward/conflict paradigms, the animal decides how much nociceptive stimuli it will endure and is able to modify its behavior based on several factors involving cortical processing. Additionally, the lack of animal restraint in these models removes the confounding variable of restraint stress inherent to other pain-testing techniques. Another significant benefit of these strategies is that the procedure can be automated after the animal is placed in the testing box. The automation allows for a high

throughput of behavioral data to be obtained because several animals can be tested simultaneously.

Preclinical orofacial pain models

Orofacial pain models do exist, however, the difficulty in making a good model lies in translating from the hindpaw to the facial region[23-29]. These few assays include assessment of mechanical sensitivity using von Frey filaments[30,32] and thermal sensitivity[31], using unlearned behaviors such as grooming[31,32], or via indirect measures such as food intake[33]. The problems with these assays are stress and anticipation of the stimulus, as the animal is either aware of the stimulus or is restrained. Even when the animal is unrestrained, it may be able to see the stimulus coming and experience an anticipatory effect.

Recently, Neubert et al. have utilized an operant design to assay orofacial pain in rodents[1,2]. They used a reward/conflict paradigm to assess thermal hyperalgesia/allodynia in a novel assay in which animals endured painful stimuli to receive a positive reward[1,2]. In this assay, animals were fasted and trained to voluntarily place their face against a stimulus thermode at different temperatures, which provided access to positive reinforcement- sweetened condensed milk.

The outcomes measured include number of licking contacts on the dropper with the milk reward, number of contacts on the thermode bars, and duration of facial contact on the thermode bars. As the temperature increased, facial thermode contacts increased and the duration decreased, replacing long periods of contact/drinking with more numerous short drinking attempts. Also, the ratio of reward licks/facial contact events was significantly decreased as the temperature increased[1]. When an inflammatory response was induced, significant thermal allodynic and hyperalgesic

effects were seen. These exaggerated responses were blocked with morphine pre-treatment[2].

Animal pain assessment and drug testing

Developing a standard for testing is important to allow pharmacological agents to properly characterize nociceptive pathways. Uniform animal testing is needed[34] because different measures of pain, such as reflex and operant, can produce different results using similar nociceptive stimuli. The differing results make interpretation of drug efficacy and applicability unclear. Systemic use of morphine, the "gold standard" drug for controlling clinical pain, has been used in many animal pain models and demonstrates the need for a "gold standard" test. However, when considering the use of drugs, it is important to understand that different behavioral assays can give different outcomes to the same dose of drug. For example, while high doses of morphine (> 3mg/kg) [35-38] can inhibit a reflex-based outcome, these doses can also impare motor and motivational responses[39-42] and can compromise interpretation of results, as animals can become immobilized or unresponsive[43]. It has been shown that low doses (<1 mg/kg) of morphine can decrease the sensitivity to painful stimuli and enhance unlearned behaviors when tested at the same stimulus temperatures[45]. These results show that operant assays allow for assessing drugs within their clinically relevant dose range, while minimizing negative side effects.

Opioids have long been used and are among the world's oldest drugs. However, it has only been since the 20th century that major advancements in the knowledge of how opiates like morphine exert their powerful and selective effects[44]. It was discovered in the 1960s and 70s that different opioid receptors existed. Since then, much work has been done to characterize these receptors and to create agonist drugs that would

activate analgesia while minimizing negative side effects. The classic opioid drug morphine, which targets the μ receptor, has been shown to exhibit a good anti-nociceptive profile in many animal models; however, morphine also exhibits many negative side effects, including constipation, respiratory depression, and loss of motor coordination[45]. δ-opioids have been shown to have fewer side effects after systemic administration in rats, although their analgesic efficiency has been reported to be inferior to μ-opioids[45]. SNC-80 is a δ-opioid agonist that has potential to be a good analgesic drug and needs to be characterized in operant testing.

Summary

The most important aspects in developing a "gold standard" orofacial pain behavioral model are to remove confounding factors, such as restraint and anticipation, and have the ability to observe behavior indicative of pain intensity. Neubert et al. have developed such an operant test to examine pain behavior with a thermal stimulus[1]. After validating the operant assay, it has been used to study thermal allodynia and hyperalgesia[2], cold allodynia[4], receptors' involvement in pain[3], the effect environment plays on thermal sensitivity[5], thermal sensitivity[46], the effect of lesioning neurons on pain[47], and animal thermal preference[4]. The operant test validated in 2005 has made a large impact on how thermal pain is studied. However, a similar mechanical operant test still needs to be developed to study pressure sensitivity. To address this current deficit, we propose an innovative, automated, software-controlled operant behavioral approach for assessing mechanical pressure induced orofacial pain. This approach takes advantage of the operant model, using higher level processing to more convincingly evaluate human pain conditions in rodents, and applies its benefits to testing and treating mechanical allodynia. Additionally, the speed with which drugs can be

evaluated is greater using an automated operant system as compared with reflexive testing with von Frey filaments, translating into quicker development of novel drugs at a reduced cost.

CHAPTER 2
MATERIALS AND METHODS

Animals

Experiments were performed on adult male Sprague-Dawley rats (200-470g). Animals were housed two per cage, under a 12 hour light/dark cycle, in a temperature and humidity controlled environment. Animals were fasted for 15-18 hrs prior to each testing day and were provided with standard food chow and water ad libitum following each session. All testing was done between 9:00am and 1:00pm. All experimental protocols conformed to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the University of Florida Institutional Animal Care and Use Committee.

Mechanical Operant Testing System

The testing device consists of a testing cage (20.3 cm W x 20.3 cm D x 16.2 cm H) with acrylic walls constructed with an opening on the back wall (4 x 6 cm). On the back of the cage, a hard circular plastic plate was secured over the opening that has embedded in it eight looped nickel titanium wires arranged in a radial fashion to act as the mechanical stimulus. We chose nickel titanium (NiTi) wires due to their high elasticity and ability to deliver the same force reliably, and the radial design because it allowed us to have contacts all around the face. When the animals are placed in the clear acrylic box, they have access to a standard rodent watering bottle that contains a diluted (1:2 with water) sweetened condensed milk solution (Nestle, room temp) by placing their head through the opening between the wire arrangement.

The hard plastic plates have a 42mm diameter circular hole cut out of the center. Eight NiTi wires of the same diameter are cut at 10cm in length and folded over on

themselves with stainless steel crimpable stops cinched over the two wire ends. The wires are secured in place, leaving a 14mm diameter opening for the rats to push through in getting the reward. After the wires are secured, copper is wrapped around the plate, contacting all the niti wires (Figure 2-1). The free end of the copper wire connected to the plate has a female adapter that connects to a male adapter on a copper wire that is plugged into the multi-channel data acquisition module (WinDaq Lite Data Acq D148U, DATAQ Instruments, Inc).

The reward bottle was positioned just outside of the opening through the wire arrangement (Figure 2-2). The metal spout on the watering bottle was connected to a DC power supply and, in series, to the multi-channel data acquisition module. When the animal drank from the bottle, the tongue contacted the metal spout on the water bottle, completing an electrical circuit (Figure 2-3). The closed circuit was registered in the computer and each spout contact was recorded as a “licking” event. A separate circuit was established from the metal wire to the animal by grounding the floor with an aluminum sheet for recording of “facial contact” events. The latter circuit was necessary to determine if the animal was discouraged by the mechanical stimulus. The investigator monitored online data acquisition to ensure that each recorded licking event from the first circuit corresponded to a recorded facial contact on the wires (the second circuit). This ensured that the animal was not accessing the reward without contacting the wires, thus eliminating false-positive recordings of licks.

A complete testing session lasts for twenty minutes. For each behavioral experiment, we evaluated the following operant outcome measures: total number of licking events, total number of facial contacts, and the lick/facial contact ratio. We

classify the lick/facial contact ratio as our pain indices and lower values are representative of a painful behavior[1].

Mechanical Stimulus Response Study

Rats (N=8) were first trained to the operant task by using non-nociceptive plates with .008” and .010” wire sizes. After the animals achieved >1000 licking events within a testing period (20min), we determined that they were trained to do the task of accessing the milk. We then evaluated the effects of increasing wire diameter on the operant measures. The animals were tested on each diameter (0.008”, 0.010”, 0.012”, 0.014”, and 0.016”) wire. The animals were tested on an every other day basis with maximum of three testing sessions/week to minimize excessive fasting of the animals in any given week. All the animals were fasted for 15-16 hours and at the same time to control for level of appetite.

Analgesia response study

Animals were injected with morphine (1mg/kg, and 3mg/kg, s.c.), and SNC-80, a delta opioid receptor agonist (5mg/kg, and 10mg/kg, s.c.). Each testing session where a drug was used had half of the animals injected with the drug and the other half injected with phosphate buffered saline (PBS, 0.9%, s.c.) as a control for the effects of the injection. The analgesic drugs and PBS were administered between the scapulae 30 min prior to mechanical testing. The original animals were tested on both the 0.010” and 0.016” wires with morphine (1mg/kg). In addition, all original animals were tested with just isoflurane on 0.010” to determine the effects of anesthesia on mechanical sensitivity. Another set of rats (N=12) were trained and baselined on the 0.014” wire. They were then tested with SNC80 (5mg/kg and 10mg/kg, s.c.) and morphine (3mg/kg) on the 0.014” wire.

Inflammation response study

The original eight animals were anesthetized with isoflurane for delivery of a capsaicin injection. Each rat was injected (s.c.) with 20ul of either capsaicin (0.075%) or PBS bilaterally over the center of the body of the superficial masseter. Half of the rats tested received the capsaicin and the other half received PBS 30 minutes prior to mechanical testing on the 0.010" wires.

The new set of 12 rats was also tested with capsaicin on 0.014". All animals were anesthetized with isoflurane and received the same capsaicin injections mentioned above, while half of the animals received morphine (3mg/kg, s.c.) and the other half PBS (s.c.).

Statistical analysis

Statistical analysis included an unpaired t-test to compare between two different treatment groups. Additionally, comparison of multiple wire types was performed using a one-way ANOVA with Tukey's post-hoc comparisons. All statistical evaluations were made using GraphPad Prism (v. 4.02, GraphPad Software, San Diego, CA). Significance was set at $p < 0.05$.

CHAPTER 3
RESULTS

Mechanical Stimulus Response Study

Wire diameters of 0.014” and 0.016” produced significantly lower lick/facial contact ratios of 2.8±0.6 and 1.8±0.4, respectively (ANOVA, $p<0.001$) as compared with a baseline diameter of 0.010” under naïve conditions producing a 8.3±1.2 ratio (Figure 3-1). This data indicates that the larger diameter wires produced an aversive response.

Analgesia Response Study

Morphine (1mg/kg) response was tested on 0.010” and 0.016” (Figure 3-2 a and b) wires.Morphine (1mg/kg) did not produce a significant increase in the success ratio on either 0.010” or 0.016” wires. Morphine (3mg/kg) response was tested on 0.014” (Figure 3-3) wires. There was a significant increase in the success ratio for morphine treatment ($p= 0.0073$) as compared to the vehicle group. Isoflurane administration alone on 0.010” wires showed a significant increase in the success ratio compared to naïve on the same wire size ($p= 0.0016$) (Figure 3-4).

SNC-80 was tested with 5mg/kg and10mg/kg on the 0.014” wire. SNC-80 at 5mg/kg did not show a significant increase in the success ratio compared to PBS (Figure 3-5a). SNC-80 at 10mg/kg did show a significant increase in the success ratio compared to PBS, $p= 0.0161$ (Figure 3-5b).

Inflammation Response Study

When both capsaicin and saline animals were anesthetized with isoflurane, capsaicin showed a significant reduction in success ratio ($p=0.0180$) on 0.010” wires (Figure 3-6). Morphine (3mg/kg, s.c.) was significant in reducing the pain response created by capsaicin injections ($p= 0.0370$) on 0.014” wires (Figure 3-7).

CHAPTER 4
DISCUSSION

Mechanical Stimulus Response Study

We were successful in validating a new mechanical operant testing model in rodents evaluating pressure mechanical sensation. We showed that as the force levels upon the face increased with the increasing wire diameter, success ratios decreased. This indicates that the animals sensed the aversive stimuli and decided that the reward was increasingly not worth the sensation they would have to endure when the larger wires were in place.

The 0.008” wires produced a lower success ratio than the 0.010” wires. We believe this was because the 0.008” wires did not offer enough resistance. The rats were able to penetrate too much of the hole and may have become distracted with the task at hand. In addition to low resistance, another factor influencing the lower ratio on 0.008” is that there could be a tickle sensation involved. Because the tickling sensation depends in part on the nerves that transmit pain[48], the 0.008” may evoke a dysesthetic or uncomfortable condition. Since the 0.010” wire provided the highest success ratios, we chose it for our baseline measurements.

Analgesia Response Study

We chose the 0.016” wire diameter initially to assess our analgesic drug effects due to its lowest success measurement in baseline testing; however, this wire seemed to be too rigid to allow for an increased response even with analgesic drugs. For that reason successive tests were performed with the 0.014” diameter wires to assess the analgesic drugs against in the new batch of animals.

Our data shows that morphine 1mg/kg did not have a significant increase on the success ratio of the animals on either the 0.010” or 0.016” wire diameters. This indicates that morphine at 1mg/kg was not efficacious at reducing OFP from pressure sensation in our operant model. However, at 3mg/kg morphine produced a significant difference in the success ratio ($p=0.0073$). Although these results are consistent with other projects showing that morphine is dose dependent in relieving mechanical pain[49,50], we anticipated that our pressure operant testing model would be similar to the punctate model in the morphine response. This difference may be accounted for by differing numbers of mu-opiod receptors on the afferent pain fibers transmitting the pressure versus punctate mechanical pain.

Although isoflurane has been used as an inhaled anesthetic for nearly thirty years[51], we did not anticipate that anesthetizing the rats briefly thirty minutes prior to testing would not have an effect on the results. However, isoflurane was recently shown to significantly suppress spontaneous paw flinches and paw withdrawal in rats induced by thermal and mechanical stimuli in a dose-dependent manner[51]. While this study was analyzing the anti-nociceptive effects of an intrathecal injection of isoflurane, our study shows that a brief period of inhaled isoflurane thirty minutes prior to testing had a significant anti-nociceptive effect specific to mechanical testing. This was an interesting finding that should be addressed in future studies.**Error! Bookmark not defined.**

After studying SNC-80 at 5 and 10 mg/kg, we found that at 10mg/kg, there was a significant increase in the success ratio and that 5mg/kg caused no significant difference from vehicle injection. Sluka et al. assessed the effects of opioid agonists on mechanical hyperalgesia induced by repeated intramuscular injections of acid and

found that SNC80 dose dependently increased the mechanical withdrawal threshold back toward baseline responses[52]. The significant increases in threshold did not occur until a higher dosage of 30nmol was reached. Another experiment showed SNC80 produced neither a blockade nor a prolongation of the tail withdrawal response latency even at the highest dose tested (80 mg/kg)[53]. The supraspinal methods used to assess pain in these tests require higher concentrations of SNC-80 to produce analgesia. We have shown in our operant assay that SNC-80 is dose dependent in relieving pressure mechanical pain and that 10mg/kg is capable of providing significant analgesia without negative side-effects.

The ability of opioid agonists to block nociception is influenced by many factors, as opioids have different profiles in different pain models[54,55]. The test used to assess nociception also may have a dramatic effect on opioid potency[55]. We have characterized both mu and delta opioid efficacy in the operant assay to be used as baseline data to test new drugs against in the future.

Inflammation Response Study

Capsaicin significantly reduced the success ratio as compared to saline. This is similar to previous studies that have also shown capsaicin reducing mechanical success measurements [2,6,56]. Intraplantar injection of capsaicin in rats was shown to increase the frequency of withdrawal to von Frey filaments[58]. Facial application of capsaicin cream was shown to reduce the success outcome in a punctate mechanical operant model[6]. Our tests have shown a capsaicin injection superficial to the masseter muscles can reduce success ratios in a pressure mechanical operant model, thus this provides a simple, reproducible model of neurogenic inflammation.

Morphine 3mg/kg reversed the nociceptive effect of the capsaicin injection. Another study analyzing the effects of morphine injection on the inflammatory response induced by intraplantar capsaicin injection showed dose dependent attenuation of pain responses with morphine[57]. They found that morphine induced pain attenuation was dose dependent and significant at 1mg/kg, 3mg/kg, and 10mg/kg, measuring the paw withdrawal threshold to von Frey filaments. Quantifying the effective morphine dose in each animal model is important to accurately relate to human studies, which have shown the ability of morphine 1mg/kg in humans to reduce capsaicin-induced mechanical hypersensitivity[58].

CHAPTER 5
CONCLUSION

Our novel mechanical operant testing assay will fill the current void of validated automated *in vivo* assays for measuring mechanical pressure pain and provide a key to the advancement of translational pain research. This is accomplished because the assay provides behavioral measures that require expression of both the physiological and cortical aspects of pain. As pain spans any number of diseases, ranging from diabetes to cancer, quickly identifying an analgesic to treat the pain would provide a tremendous societal benefit. After hundreds of years using the same analgesic drugs and billions of dollars spent every year on treating pain patients, new methods of assessing pain are requisite if we hope to discover new effective treatments. The successful translation of even one novel therapy that demonstrates significant analgesic properties without compromising other normal functions would provide an avenue for significant commercial success of our operant assay.

Future studies will evaluate wire density, structural arrangement, and differing pain conditions. We will also further adapt this operant facial testing paradigm for use with mice. This line of research is significant because it allows for investigator-independent measures of facial pain, which can lead to better assessments of novel analgesic therapies.

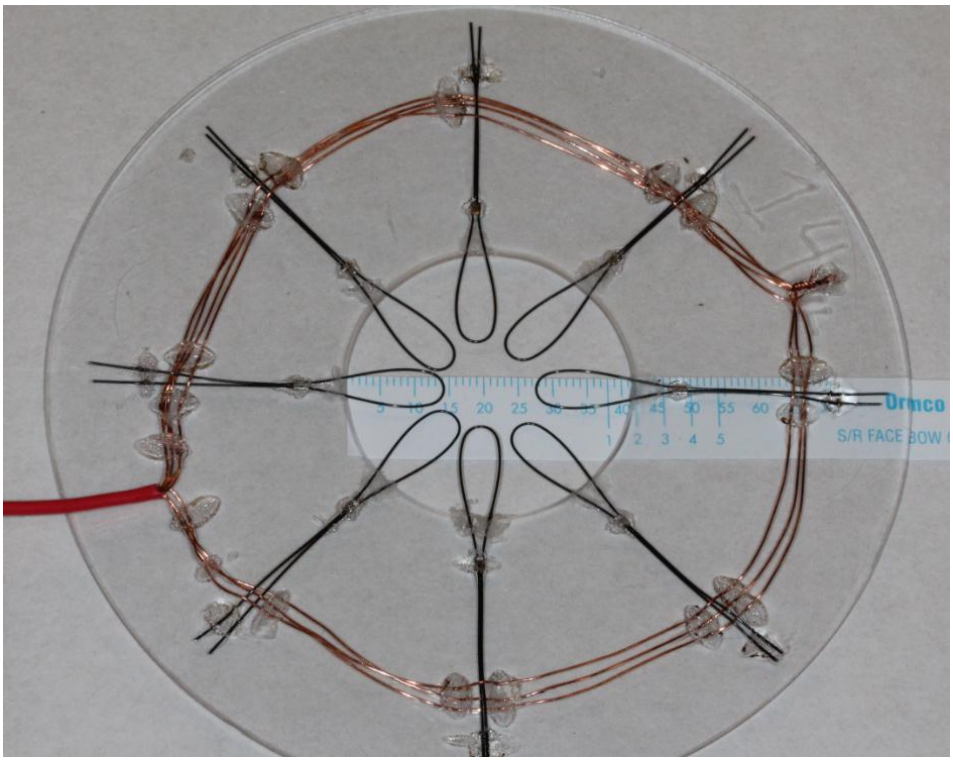

Figure 2-1. Picture of the plate that is attached to the testing box. 42mm diameteropening in the plate with a 14mm diameter opening between the wires.This plate has 0.014” wires.

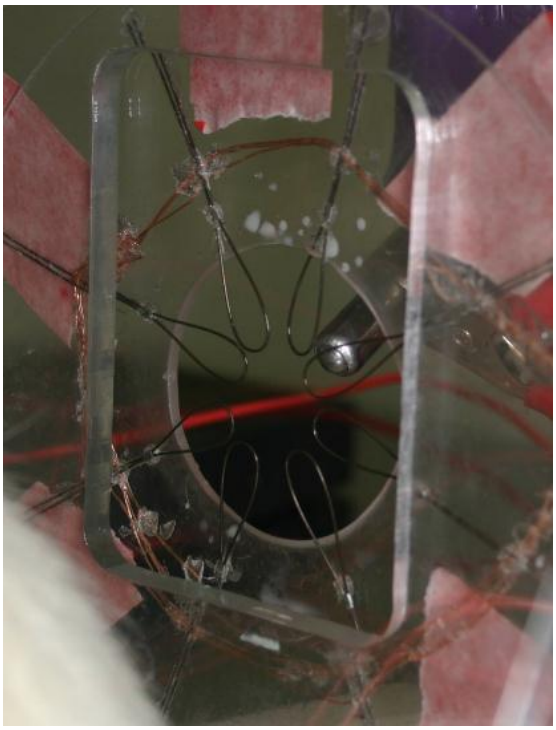

Figure 2-2. Picture showing the relationship of the milk dropper just outside of the looped wire arrangement. Animals must insert their faces through the wire arrangement in order to obtain the sweetened milk reward.

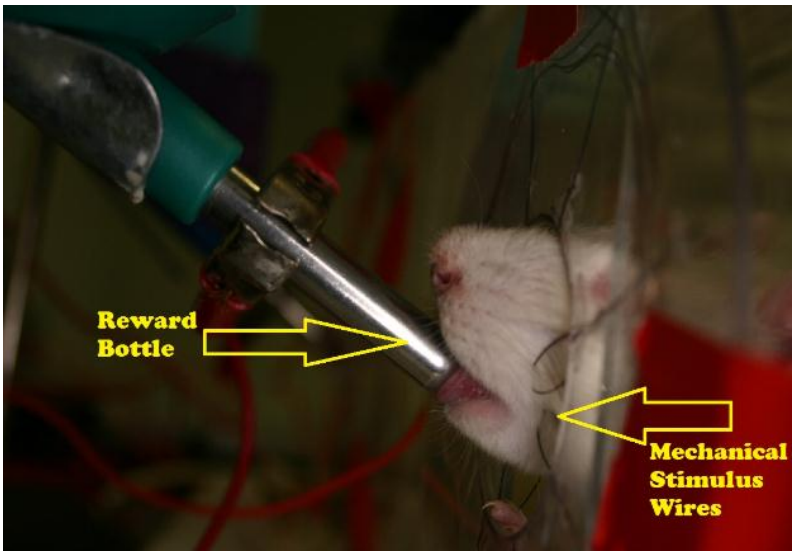

Figure 2-3. Picture showing the tongue contacting the milk dropper to record a “licking” event. Facial contacts are also recorded as the face touches the grounded Niti wires.

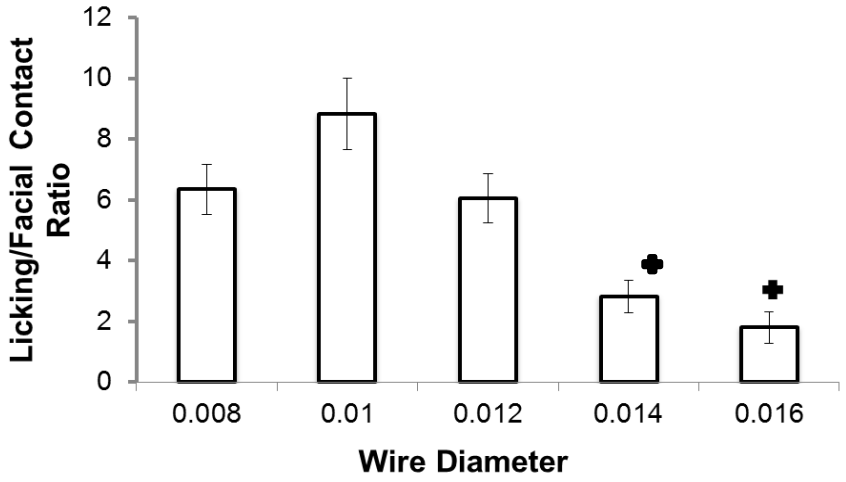

Figure 3-1. Graph showing the success ratios of naïve testing with the different Niti wire diameters. Significant decrease between 0.010" and 0.014"/0.016"(ANOVA, $p<0.001$).

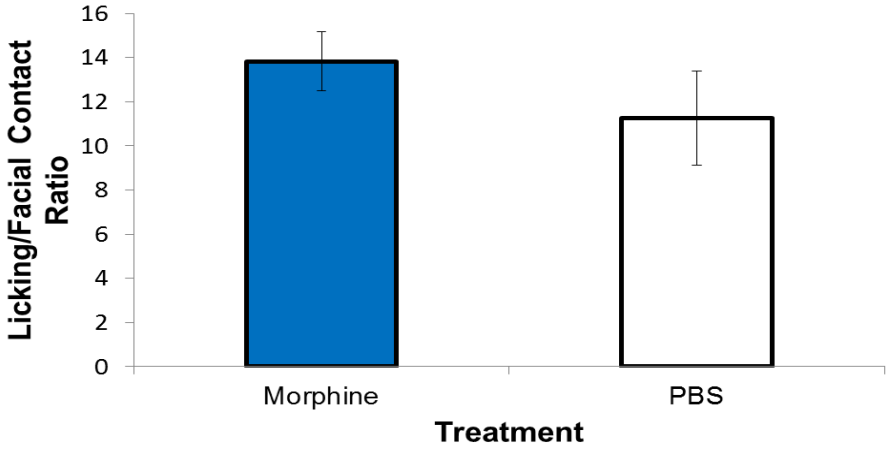

Figure 3-2 a. Graph showing the success ratio of morphine (1mg/kg, $p= 0.1087$) at0.010" wire size.

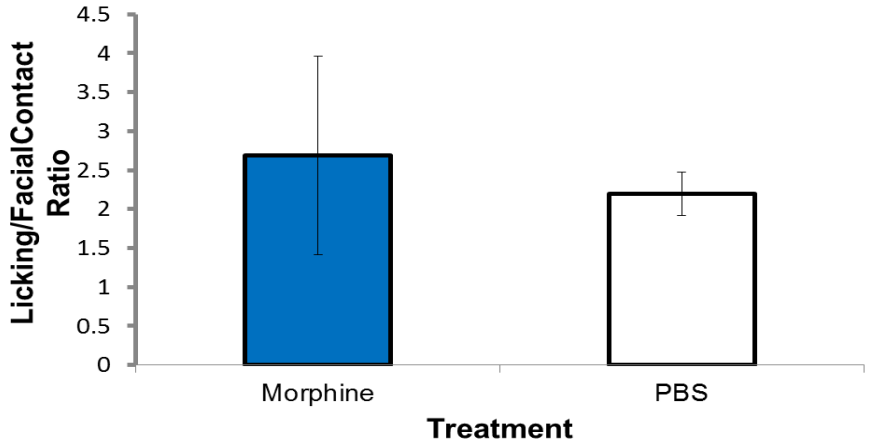

Figure 3-2 b. Graph showing success ratios of morphine (1mg/kg, p=0.5006) at 0.016" wires.

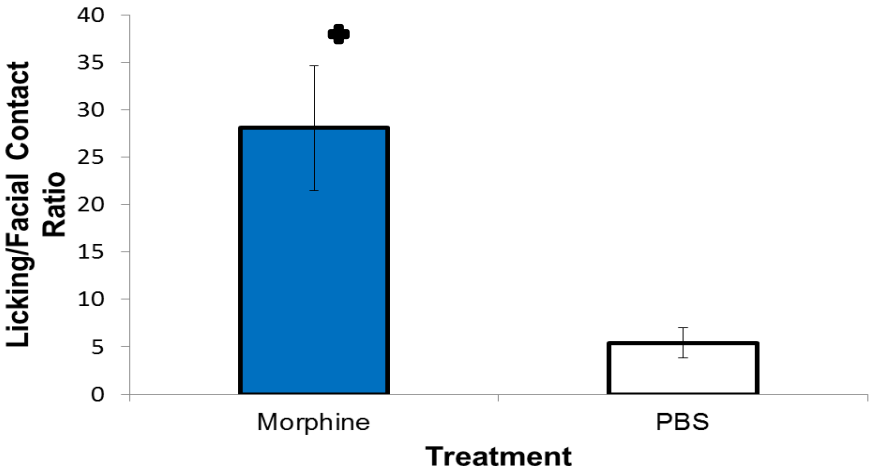

Figure 3-3. Graph showing success ratio of morphine (3mg/kg, p= 0.0073) at 0.014" wires.

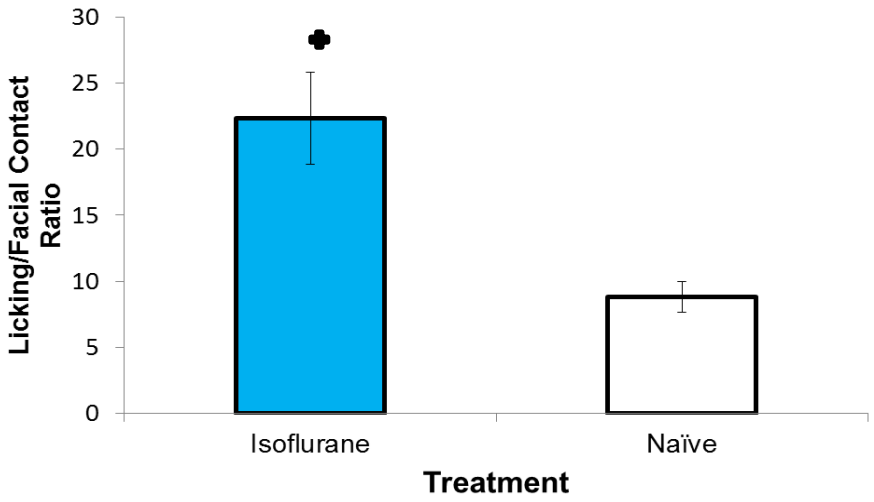

Figure 3-4. Graph showing success ratios of naïve vs naïve having been under isoflurane anesthesia at 0.010" wires (p= 0.0016).

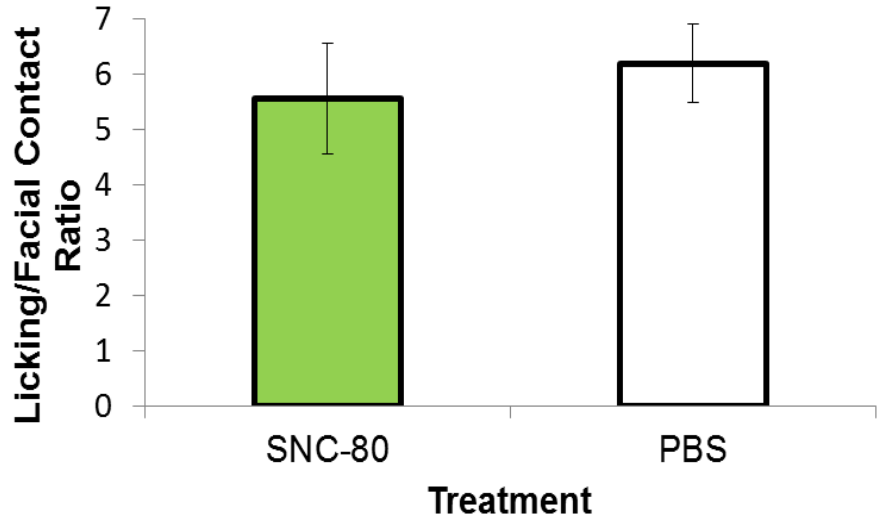

Figure 3-5a. Graph showing success ratios of SNC-80 (5mg/kg) vs PBS at 0.014"wires.

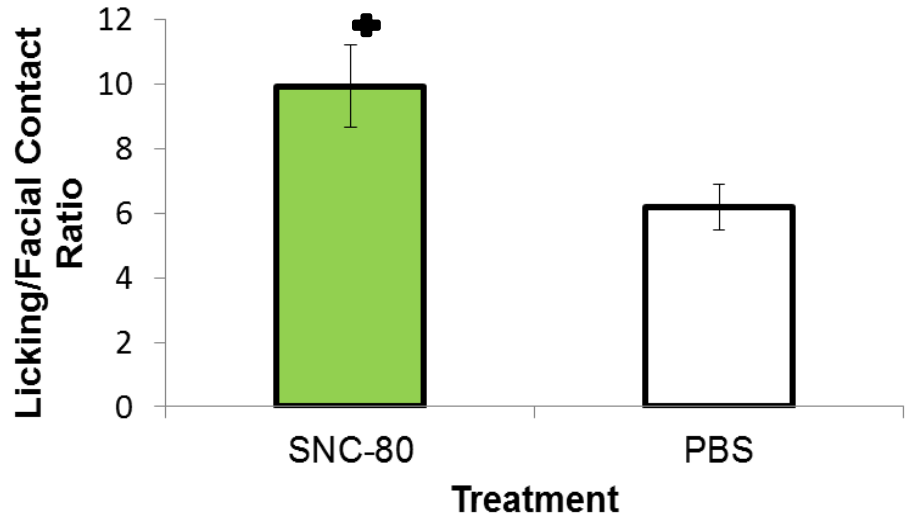

Figure 3-5b. Graph showing success ratios of SNC-80 (10mg/kg) vs PBS at 0.014”wires (p= 0.0161).

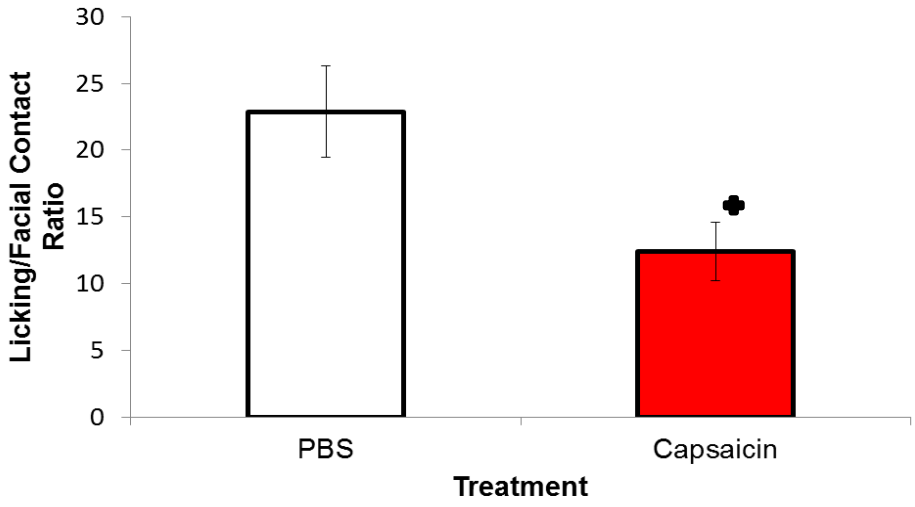

Figure 3-6. Graph showing success ratio of capsaicin under isoflurane at 0.010” wires (p= 0.0180).

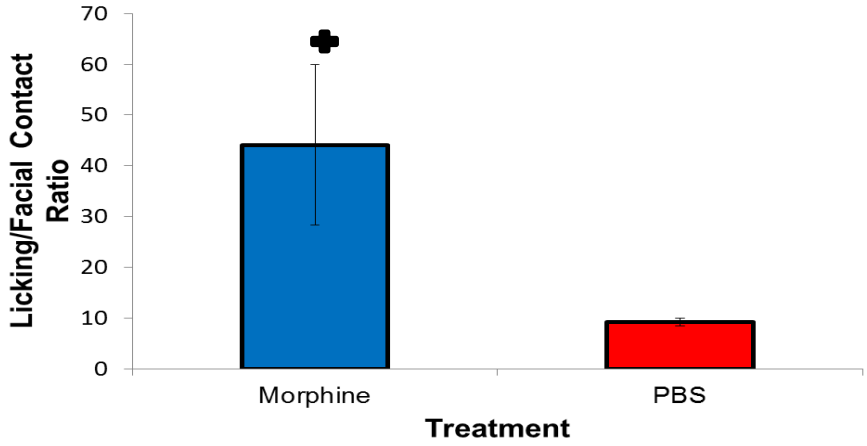

Figure 3-7. Graph showing success ratios of morphine (3mg/kg, p= 0.0370) and PBS,both having isoflurane anesthesia and capsaicin injection

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BIOGRAPHICAL SKETCH

Daniel Bass was raised in Sarasota, Florida where he attended Pine View School for the Gifted from 2nd grade through high school. After graduating high school, he dawned the Orange and Blue colors on a journey towards a Bachelor of Arts in Business Administration. He enjoyed the University of Florida so much that after graduating with a bachelor's degree, he decided to stay for another four years to complete dental school. He was surely glad that he stayed and got to witness all of the Gator national championship victories! After graduating dental school and getting married all in the same weekend, he was prepared and excited to stay another three years at the University of Florida to complete his post-doctorate training in the dental specialty of orthodontics. After an amazing eleven years at the University of Florida, he became a husband, father of a beautiful one year old princess, and a certified orthodontist.

Dr. Bass opened an orthodontic practice in Wellington, FL. He was sad to leave the home town of his alma mater, but he is happy to live close to his wife's family, and to the Atlantic ocean, where he enjoys surfing.