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Targeting the trigeminal nerve system for orofacial pain treatment

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TARGETING THE TRIGEMINAL NERVE SYSTEM FOR OROFACIAL PAIN TREATMENT

by

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

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has been approved by the Examining Committee
for the thesis requirement for the Master of Science
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To my parents for their immense support and guidance

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ABSTRACT

Orofacial pain is associated with various pathologies such as headache, dental pain and ophthalmic pain. The trigeminal system innervates a large section of the head, including the nasal and oral cavities, the cornea and facial skin, and is responsible for the transmission of pain signals from the orofacial regions to the brain.

These investigations were undertaken to study the effect of intranasal delivery of analgesics on orofacial pain using an operant testing method in mice. Doses of either lidocaine HCl or butorphanol tartrate were administered to mice, and the analgesic effectiveness was measured using a thermal operant behavior test involving a facial heat stimulus. Two parameters were measured in the operant assay: the number of licks and the duration of facial contact. Pain response was measured at two different temperatures: 37 ºC and 49 ºC. The magnitude of analgesic response was also compared between intranasal and intraperitoneal drug administration at 49 ºC.

Mice showed a significant decrease in the number of licks and duration of facial contact for both treatment and control groups as the temperature was increased from 37 ºC to 49 ºC. A significant difference in the duration of facial contact was observed following either lidocaine or butorphanol by nasal administration. One group of animals receiving intranasal lidocaine did exhibit an increase in the duration of facial contact compared to the control. Two doses of butorphanol were tested and increases in the duration of facial contact were observed at both levels, but no significant difference was observed in the number of licks recorded.

No convincing differences were observed in the mice behaviors for intranasal or intraperitoneal dosing of lidocaine or butorphanol. This suggests that nasal administration

of these two analgesics at the doses tested did not provide superior pain relief compared to systemic delivery of the agents.

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CHAPTER 1: INTRODUCTION

Intranasal Administration of Drugs

Intranasal delivery of drugs allows local as well systemic delivery of drugs resulting in effective mucosal or systemic effects. It is a convenient route since it is non-invasive, avoids hepatic first pass metabolism and affords a rapid onset of action. Therefore intranasal administration can maximize patient compliance by allowing easy administration. It is also useful for patients who have limited abilities to use oral medication, including those with nausea.[1,2,3] The intranasal route of administration represents a potential route for delivery of drugs directly into the brain while bypassing the blood brain barrier (BBB). The blood brain barrier is mediated by tight junctions and membrane proteins which limit the entry of foreign particles, high molecular weight substances and many hydrophilic and moderately hydrophobic materials from reaching the central nervous system from the systemic circulation. Nasal delivery is a preferred route of administration compared to other invasive methods such as intracerebroventricular or intraparenchymal injections for the delivery of drugs into the brain. They are not beneficial for multiple dosing as they are invasive, expensive methods and require surgical expertise.[4] Thus, the intranasal route has been considered as a potential alternative delivery method for transport of drugs to the central nervous system (CNS). Intranasal delivery can bypass the BBB and this transfer pathway appears to involve the olfactory and trigeminal pathways. In addition to this, pathways involving the vasculature, cerebrospinal fluid and lymphatic systems may also be involved in transport of the molecules from nasal cavity to brain.

Intranasal delivery has been shown to enhance the delivery of small molecules, polynucleotides, small proteins and even viral vectors into the brain via the olfactory pathway. Earlier studies showed the transport of tracers like wheat germ agglutinin conjugated with horseradish perioxidase, colloid gold, ferrocyanide and iron ammonium citrate through the olfactory bulbs into the CNS in neurons.[5,6,7,8] Researchers have also shown that small lipophilic molecules such as cocaine, morphine and testosterone reach the brain after intranasal administration to rodents.[4,9,10,11,12 ,13] Even large macromolecules such as plasmid DNA ranging in size from 3.5 to 14.2kb was delivered to the brain of rats.[14] Researches have also shown that intranasal administration of nerve growth factor can attenuate memory deficits and neurodegeneration in transgenic animal models of Alzheimer's disease, and administration of IGF-1 was helpful in significantly decreasing ischemic brain damage.[15,16 ,17] Thus, intranasal delivery shows to be a promising route of delivery for brain enhanced drug delivery.

Although nasal delivery has several advantages, it also has some disadvantages such as the limited reproducibility and variation in dosing due to clearance of the dose from the nasal cavity. Although intranasal delivery avoids hepatic first pass metabolism, there are also enzymes and secretions in the nasal mucosa that can degrade drugs, and more research is required to understand this limitation. The dose of a drug able to reach the brain may be limited due to small area for absorption of drugs from the nose to CNS.

Nasal Anatomy

Each nasal passage is composed of lateral wall, septal wall, a roof and a floor. The total surface area of nasal cavity is approximately150 cm^2 and, in adults, has total volume of 15 mL. [18] The nasal cavity is divided into two halves, separated by the septum, and

extends from the nostrils to the nasopharynx. The nasal cavity is divided into three primary regions: nasal vestibule, olfactory region and respiratory region. Turbinates are the bony structures that are covered by the vascularized mucosal tissue. These turbinates increase the surface area of the nose and are helpful in humidifying, filtering and warming the inhaled air.[19] The olfactory region has total surface area of approximately 15 cm^2 (3-5%) and is located near the superior turbinate. The respiratory region covers the remainder of the nasal surface area (approximately135 cm^2) and is present in the region of the middle and inferior turbinate.[18,19] (Figure 1-1)

The vestibule region of the nose is lined with keratinized, stratified squamous epithelium. This region is also lined with hairs that are responsible for filtration and cleaning and sebaceous glands are also present. The transition region between the vestibule and respiratory region is covered initially by stratified squamous epithelium and then by pseudostratified columnar and epithelium. This region is also lined by the cilia that allow the movement of mucous into the throat. The respiratory mucosa is predominant in inferior, middle and part of superior turbinate and olfactory region is predominant in superior turbinate. These tissues contain olfactory receptors that respond to odor producing substances.[1,20]

The nose itself has a rich blood supply. The arterial blood supply to the nose comes from branches of the internal and external carotid arteries. Externally, the nose is supplied by the facial artery, while the internal section of the nose is supplied by the sphenopalatine artery posteriorly and by the anterior and posterior ethmoid arteries superiorly. The arterial blood supply pattern is also followed by the venous return pattern

in the nose. The blood supply may also support the systemic absorption of drugs following intranasal administration.[1]

There are many similarities between the nasal cavity of humans and other animal species, but there are also distinct differences. Human noses are quite simple structurally with air humidification and cleansing as the primary function, while in other animals, the nose is more complex and olfaction serves as a primary function. The turbinates of many animal species have more folding and branching patterns as compared to humans. The maxiloturbinates in the nasal cavity of the rodents is more complex and more efficient in filtration, absorption and airstream cleansing than the human nose. Surface of human nose is lined by squamous epithelium and has hair follicles while no hair follicles are present in rodents. Another significantly different feature is the tissue composition of the mucosa with the respiratory region, where total mucosal surface area in rats is approximately 50% while in humans the composition increases to 80-90%.[19,21]

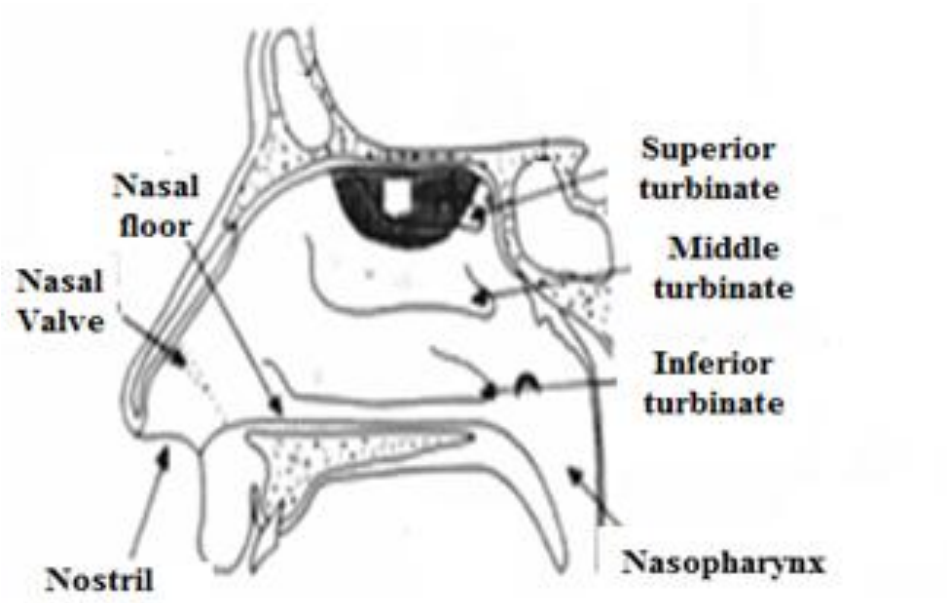

Figure 1-1: Human Nasal Anatomy[22]

Trigeminal Nerve Pathway

The trigeminal nerve (cranial nerve V) innervates large sections of not only the head (oral and nasal cavities) but also the corneas, conjunctiva and the skin covering much of the face. The meninges are also densely innervated by the trigeminal axon that bifurcates with the small blood vessels branching from the cerebral and middle meningeal arteries. These are the pain-sensitive tissues within the cranium.[23] It is composed of both large sensory and small motor roots and is, hence, regarded as a mixed nerve. The motor fibers are responsible for transmission of the signals to the mastication muscle responsible for chewing while the sensory fibers transmit their signals from the face, mouth and nasal cavities.[24] There are three major sensory branches of trigeminal nerve: ophthalmic (V1), maxillary (V2) and mandibular (V3). These branches innervate distinct regions of the face and head.V1 and V2 are only sensory nerves while V3 is the mixed portion of the trigeminal branch. V1 enters the cranial compartment in humans through the superior orbital fissure, V2 through the foramen rotundum and V3 through the foramen ovale. However, in rats, V1 and V2 enter the cranial compartment through the anterior lacerated foramen while V3 enters through the foramen ovale.[25] The ophthalmic nerve branch also innervates the dorsal nasal mucosa and anterior section of the nose, the maxillary nerve innervates the lateral wall of the nose and the mandibular nerve innervates the lower jaw and teeth.[26,24,27] Branches of trigeminal nerves innervate the respiratory epithelium and the fibers from ganglionic cells of the trigeminal nerve extend to respiratory and olfactory region to an extent that the nerve endings lay very close to the epithelial surface. All the branches of the trigeminal nerve also innervate parts of meninges. These trigeminal fibers enter the brain at the same level as the pons and

through the cribriform plate through the olfactory bulbs that permits entry to the brain through posterior and anterior entrances. The trigeminal nucleus then extends to the upper portions of the spinal cord.[28] Figure 1-2 shows the projections of trigeminal system along the nasal passage of a rat.

Several investigators have shown trigeminal nerve pathway as a potential pathway for transport of drugs to the brain. High concentrations of radioactive ^{125}I-labeled proteins such as Insulin like Growth Factor (IGF-I) were observed in the trigeminal branches, pons and olfactory bulb following nasal administration first. Others have also showed that interferon- β1b, hypocretin-1 and peptoids rapidly distribute along the trigeminal nerve system.[27,29,30,31] Wheat germ agglutinin-horseradish peroxidase (WGA-HRP) was also seen to be transported not only to olfactory region internally but also to the trigeminal nerves and then to the brainstem.[30] However, since the branches of the trigeminal nerve extending to the olfactory bulbs, when a drug is given intranasally one cannot say assuredly whether it has been transported to the brain through the olfactory pathway, the trigeminal pathway or both.[4] (Figure 1-3).

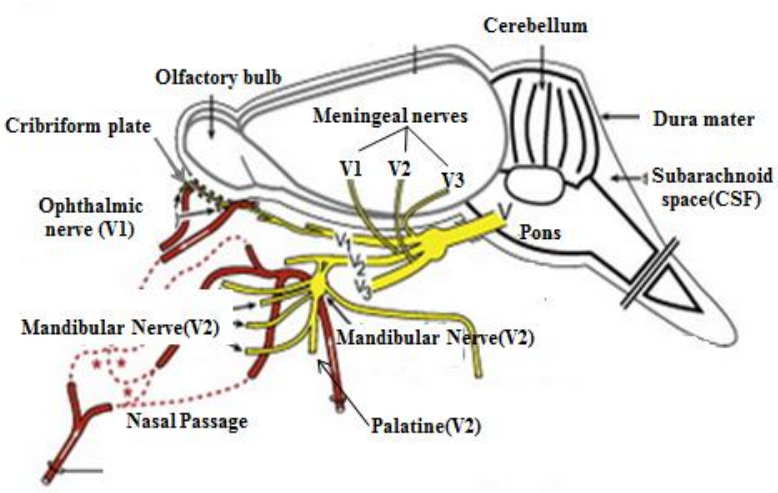

Figure 1- 2: Nasal passage and three major sensory branches of trigeminal nerve: ophthalmic (V1), maxillary (V2) and mandibular (V3) trigeminal nerve system[24]

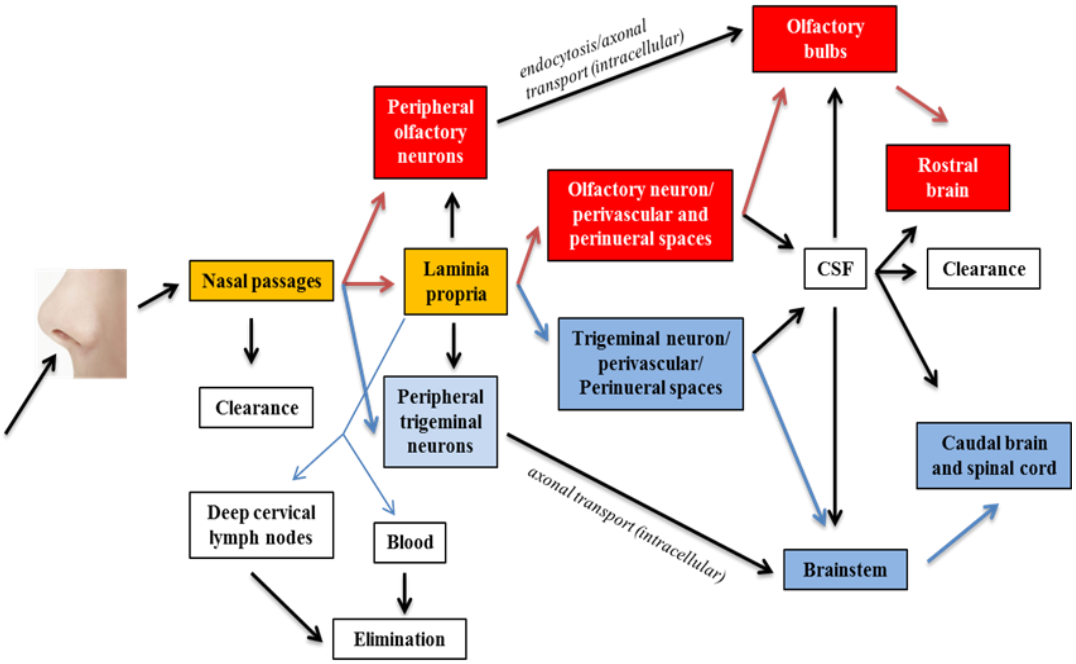

Figure 1- 3: Different pathways after intranasal administration[24]

Orofacial Pain

Orofacial pain relates to pain caused by a broad range of disorders. The orofacial region is innervated predominantly by trigeminal nerve branches which transmit sensory signals to the brain from the teeth and jaw. Orofacial pain is associated with several chronic conditions, such as temporomandibular disorders (TMD), trigeminal neuralgia, migraine, headaches and myofasical pain.[32] Other nerves, such as cranial nerve VII (facial), cranial nerve IX (glossopharyngeal) and cranial nerve X (vagus) are also involved in transmitting the impulses from facial regions including the skin of the face, forehead, scalp; skin of hollow of auricle of external ear; mucosa of the pharynx, palatine tonsils, and the lateral and posterior neck.[20]

It is observed that 20% of the population is affected by some type of acute orofacial pain and 33% affected by chronic pain each year.[32,33] The pain transmission pathway that is associated with the trigeminal nerve is shown in the Figure 1-4. Sensory neurons associated with pain are defined by axons with slow conduction velocities (i.e. finely - myelinated A delta fibers and unmyelinated delta C fibers). Physical stimuli (thermal and mechanical) and chemical stimuli (gaseous, liquid or solid) can trigger the pain receptors to send signals to the brain regarding smell, taste, temperature and pain. Pain information is transmitted by the three divisions of the trigeminal nerve to the sensory ganglion located in the dura mater which is then transmitted to the pons region of the brain which further descends into the brainstem as the spinal trigeminal tract. Spinal trigeminal tract fibers conduct pain responses to the adjacent trigeminal nucleus and then spread parallel to the spinal trigeminal tract in the brainstem. The trigeminal nucleus is a long nucleus that extends into the upper cervical spine, but it does not transmit signals to

the spinal cord. Axons from the nucleus cross to the opposite side of medulla and ascend into the thalamus where the pain stimuli finally terminate at the somatosensory cortex.[28,34]

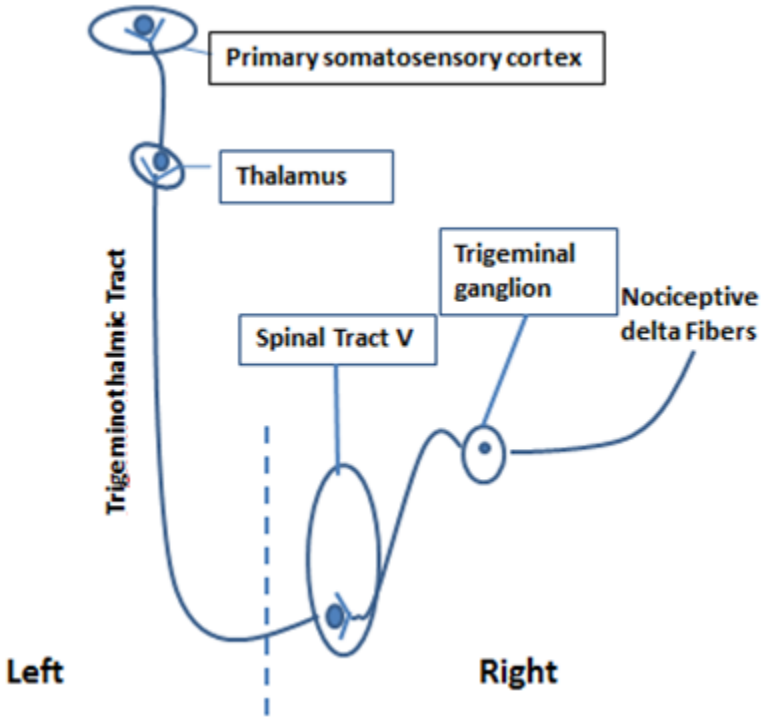

Figure 1- 4: Transmission of orofacial pain across the trigeminal nerve

2.5 OROFACIAL PAIN MODELS

Orofacial pain is a common and restricting problem that can tremendously affect patient's quality of life. Many previous studies in humans were aimed at evaluating the verbal pain response and it was shown that verbal pain response assessments due to painful stimulation can be increased or decreased by operant techniques.[34,35,36,37] Verbal pain behavior response may be an important pain assessment, nonverbal behavior can also be beneficial for studying pain behavior. The facial pain behavior response has been used as an important predictor in acute pain tests. It is regarded as an automatic, reflexive and inborn response that provides distinct facial pain responses relating muscle movements that are distinct from other facial expression.[34,35,36] The drawback of facial pain response behavior condition is that the response, when observed, may not be a result of the operant conditioning but an overlying result of emotional effects of the painful stimulus. Thus, development and continued improvement of the existing pain methods can provide essential knowledge regarding the neurobiology of orofacial pain and animal models have been extensively used to understand this kind of pain.[38]

Pain is mainly classified as neuropathic or inflammatory pain. Neuropathic pain involves direct damage to a nerve by cutting, ligating or injury causing an immediate neuropathic pain. An example of a neuropathic pain is demonstrated by the chronic constriction injury (CCI) of the infraorbital nerve (ION) a model in which loose ligatures are placed around the nerve. It was seen that rats showed significant signs of neuropathy after induction of injury and the behavior included abnormal positioning of the paw and continuous shaking, licking and decreased grooming behavior.[39] The disadvantage of this method is that it requires habituation and a significant amount of training and skill of the

investigator in identifying pain due to the evoked response. Another model to study neuropathic pain in the facial region is the compression of the trigeminal nerve and its demyelination, which then exhibits features that characterizes that mimics those of trigeminal neuralgia (TN).[40]

The inflammatory pain models involve use of injection of an inflammatory agent into the area under study. Inflammatory agents can be irritant chemicals (carrageenan, formalin), microbial cell wall fragments or toxins (Complete Freund's Adjuvant-CFA) or agents that activate the sensory neurons (mustard oil, capsaicin). There are inflammatory responses such as edema, fever, allodynia or hyperalgesia, that can be studied by different orofacial pain models using rodents.[40,41] These models use electrical, thermal and mechanical stimuli for observing pain (nociceptive) responses. Each method has its own advantage and disadvantage in evaluating the pain response and, hence, it is difficult to identify the most appropriate orofacial pain operant assay.

In the electrical stimulation method, animal can easily adjust to the temporal behavior as it can distinguish between a withdrawal response and elimination of adverse response when electricity is turned on and off rapidly. It creates local hot spots in the superficial tissues. If there is no proper adaptation to the stimulus by the animals, increased level of stress and anxiety is observed, which causes variation in the result of pain sensitivity.[39,42,43] Insertion of bipolar stimulating electrodes in the pulp of the incisors of rats is an example of this type of stimulation. It was shown that this method activated the periodontal nerve fibres, opening of jaw reflex and nociceptive responses were increased.[36]

Mechanically stimulated pain response is frequently studied using Von Frey filaments, which are thin plastic filaments pressed into the plantar surface of the hind paw of the rodents causing mechanical stress. The stiffness of the filament and the force applied determines the paw-withdrawal response.[44,45] An air puff method is also helpful to study the pain induced by mechanical stimulus. Ahn et.al have explained the facial neuropathy and inflammation using this method and tested if animals develop mechanical allodynia. These methods are easy to perform but are not truly representative of orofacial studies of nociception.[40,46,47] Experimenter bias is also a factor to be considered, especially when the handling is done manually when the operator observes a painful response, this response can be confused with the freezing response that is indistinguishable to a fear response.[34,38]

Thermal stimulation is one of the most commonly used pain test. Radiant heat is used in the tailflick and hindlimb withdrawal test. One significant advantage is the absence of concurrent mechanical stimulus.[42,48] In a hot plate test, thermal stimulation is generated by transmitting the signal from the floor of the compartment to the paw of the moving animals. Hotplate tests introduce rat or mouse onto the floor of an open-ended cylinder where the floor consists of a metal plate supplied with heat from the base by a thermode or a boiling liquid.[49,50] Through continuous training, the animals learn to avoid the stimulus by moving into another, non-heated, compartment of the cage. The Hargreaves plantar test is one of the methods to evaluate pain response where pain response due to thermal signaling was induced by moving an infrared source placed underneath the targeted hind paw and the latency in withdrawing the paw was observed.[51,52] The advantage of the Hargreaves test is that if allows independent measures

in each paw of the same animal. Thermal stimulation does possess the advantage of a constant threshold across body sites, and extensive research has been conducted in understanding the physiological and psychological aspects of the thermal studies in order to define temperatures that induce pain while limiting physical damage. However, such tests are also not useful for measuring facial pain.[53,54]

Chemical stimuli can also induce nociceptive pain and methods have been developed to assess this pain stimulus in animals. The formalin test is an example which is performed in rodents and involves the injection of formalin into the upper lip followed by observation of the animals licking and scratching behavior.[55,56] A head flinching behavior and chewing like motions of the mandible are observed after temperomandibular joint (TMJ) injections of the irritant to mice for assessing the pain behavior.[57] Capsacin and mustard oil are other substances that induce nociceptive pain response when applied to the orofacial region of rats and mice.[58,59,60] However, the assessment and interpretation of the behavior studies is a limiting factor. Thus, testing in the orofacial region is of a more true representation of nociception.

Trigeminal Operant Pain Method

Operant term relates to a response that occurs spontaneously and is identified by reinforcing effects or inhibiting effects. The frequency of the behavior is influenced by environmental incidents. Operant pain behavior paradigms show that positive consequences (i.e. reward following an experiment or withdrawal from the painful response) lead to an increase in frequency of the behavior while negative responses such as behavior after an aversive stimulation or avoidance of the reward lead to reduction in frequency. [34 ,35,36,38,61] The advantage of this assay is that the “operant measure” are

observed by conditional reward activity. Operant behavior more closely represents the clinical conditions. For example, operant measures are better indicators of the effect of analgesics than are evoked response assay.[37] Fordyce, however, claimed that pain on induction can be expressed by observable actions such as rubbing, licking or groaning. He also stated that once pain behavior was constant by continuous training, it could later be just an operant response rather than a response due to pain induction.[36,53,54]

A method of understanding operant orofacial pain involving the trigeminal system is described by Neubert et al.[32] (Figure 1-5) It is based on the use of thermal and mechanical stimuli followed by observation of the nociceptive stimulus responses. For this model, the rodent is initially trained. It is place in an acrylic box and then the rodent is free to choose a reward in the form of sweet tasting (condensed) milk or receive an aversive signal. In order to receive the reward, the rodent places its snout between two rods that can be heated to controlled temperature. As the temperature of the rods is increased, the capacity to reach the reward is reduced due to the pain stimulus induced by the heated rods. When different analgesics or anesthetics are administered, the reward seeking behavior is again increased. When the licks and contacts with the rods are recorded digitally, the data can be quantitatively obtained and analyzed. Different parameters such as number of licks and duration of facial contact are studied as they important parameters to study this pain model. Neubert and et al. conducted experiments using this method on mice and showed that TRPV1-KO mice were insensitive temperature between 37-52 ºC. It was seen that the mice licking events were lowered when capsaicin was applied and this effect was reversed (i.e. the mice licking events increased) after administering analgesics.[32]

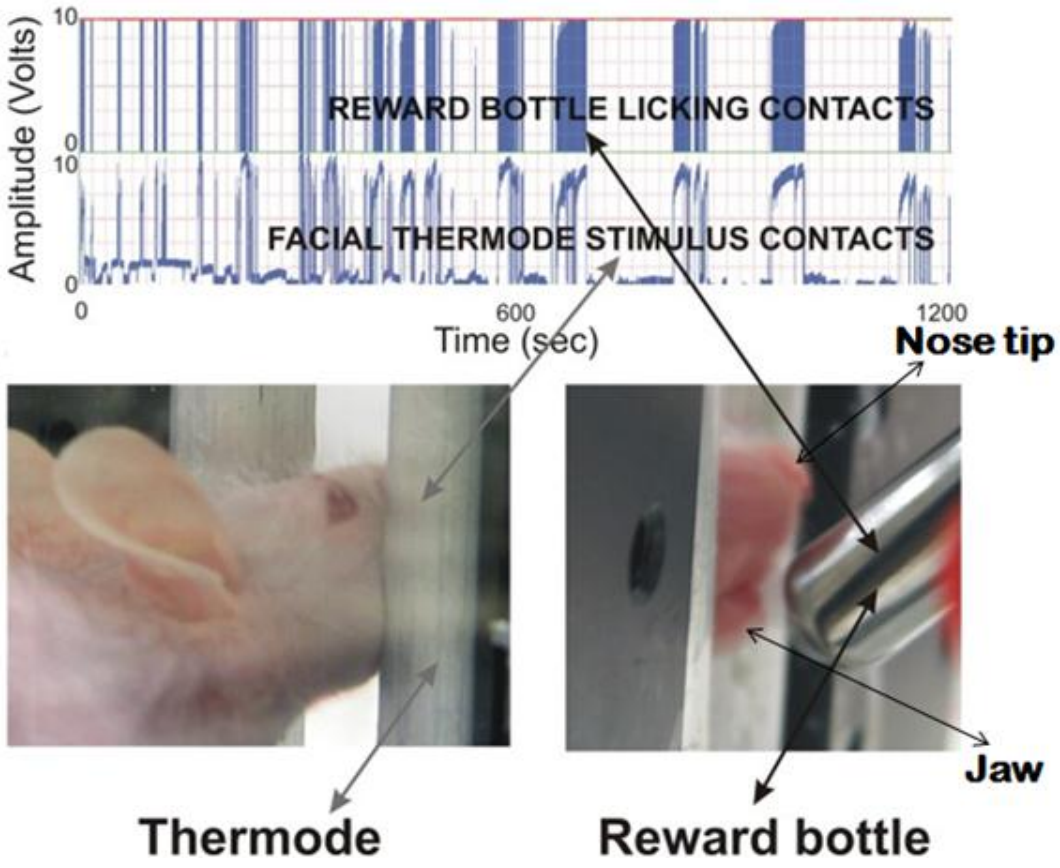

Figure 1- 5: Number of licks and facial contact was seen by using DATAQ software. SKH1E mice licking the milk from the reward bottle[32]

Another alternative method to measure trigeminal pain is based on the ability of rodents to chew on objects obstructing their passage in a narrow tube. Pain inducing a gnawing dysfunction is an indication of orofacial pain and by using a dolognawmeter. The researcher showed a resemblance of this trigeminal pain to the pain induced by chewing in humans. However, this method involves observing grimacing, and it is hard to see the grimace in an inflamed condition. This method is useful for studies involving short-moderate pain and not for chronic conditions.[40]A summary of inflammatory pain models is presented in Table 1.

Table 1- 2: Inflammatory models for assessing pain behavior.

Animal	Induction of Inflammation	Stimulus or Observed behavior	Reference
Rat	Formlin	Spontaneous grooming	Clavelou et al. (1989)[48]
Rat	CFA	Von Frey	Ren and Dubner(1999)[56]
Rat	CFA	Thermal(radiant)	Imbe et al. (2001)[57]
Rat	CFA	Operant behavior(food intake)	Thut et al. (2007)[58]
Rat	IL-1β	Air Puff	Ahn et al.(2004)[41]
Rat	Menthol	Cold Operant behavior	Rossi et al. (2006)[59]
Rat	Capsaicin cream	Mechanical Operant behavior	Nolan et al. (2011)[39]
Mouse	TRPV1 k/o	Heat Operant behavior	Neubert et al. (2008)[29]
Mouse	CFA	Gnawing	Dolan et al. (2010)[60]
Mouse	CFA	Von Frey and Air puff	Krzyzanowska et al.(2011)[61]
Rat	IoN-CCI	Von Frey	Vit et al.(2008)[62]
Rat	TG compression	Air Puff and Pin prick	Ahn et al.(2009)[63]
Rat	Injection of LPA	Air Puff and Pin prick thermal radiation	Ahn et al.(2009)[64]

*CCI-constriction injury model, IoN- Infraorbital nerve, TG- trigeminal ganglion, CFA-Common Freund's Adjuvant, IL-1β-Interleukin β, LPA- Lysophosphtidic acid, TRVP 1-Transient receptor potential cation channel sub family V member 1

Intranasal Administration of Lidocaine

Lidocaine is a local anesthetic which has been used for many years in the treatment of many conditions including neuropathic pain. It has been administered intravenously as an antiarrhythmic.[61,62] It has 35% oral bioavailability, a logP value of 2.44 and pKa of 8.01.[63] (Figure 1-6) Its half –life following intravenous injection is about 1.5-2 hours. Maximum recommended human dose for lidocaine HCl should not exceed 4.5mg/kg irrespective of the route of administration. Lidocaine inhibits the ionic fluxes which are needed for initiation and conduction of impulses and thus stabilizes the neuronal membrane and produces the required local anesthetic action. It blocks the fast voltage gated sodium (Na^+) channels in the neuronal cell membrane and alters signal transmission. Due to this blocking of the membrane, the postsynaptic neuron does not depolarize, and it fails to transmit the action potential producing an anesthetic effect.[61,63]

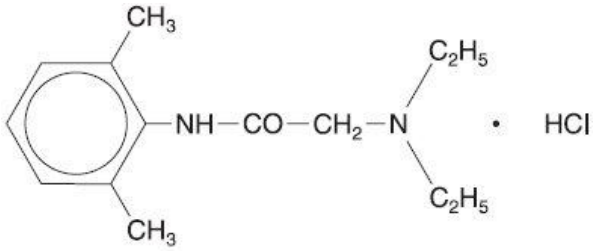

Figure 1- 6: Chemical structure of lidocaine hydrochloride[63]

Lidocaine patches (5%) have been shown to be useful and safe for the treatment of post-herpetic neuralgia, painful diabetic neuropathy and low back pain. Trigeminal nerve block with 10% lidocaine has shown to be beneficial for intermediate duration pain relief for trigeminal neuralgia treatment.[64] Lidocaine is also frequently used as a local anesthetic in preparation for nasal or oropharyngeal procedures. In the study reported by Chou and Donovan, lidocaine was used as a model compound to study its direct transport from the nasal cavity to the brain. It was observed that lidocaine was well absorbed from the nasal cavity and showed high concentrations in the cerebrospinal fluid and increased CSF uptake (Figure 1-7).[65]

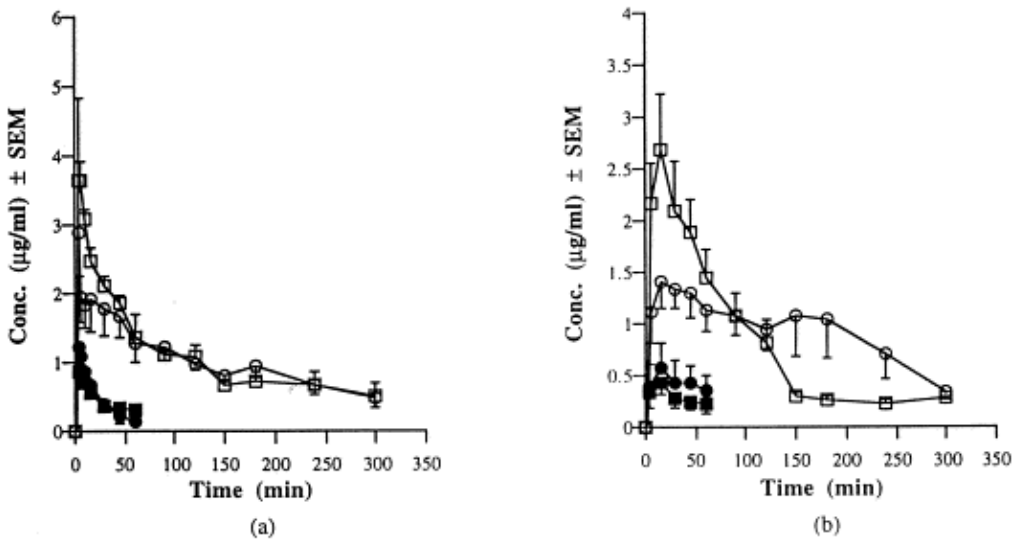

Figure 1- 7: Lidocaine concentrations in (a) plasma and (b) CSF following intra-artial (□, ■) and nasal (○, •) administration (open symbol: 17.14 mg/kg, closed symbol: 2.86 mg/kg)[65]

In addition to local treatment, lidocaine administered via nasal cavity has been used therapeutically for the treatment of headache, especially migraine and cluster headaches, which suggests pharmacologic effects beyond local V2 action. A recent report describes the measurement of lidocaine concentrations in specific tissue regions following intranasal administration. These investigators showed that there was a significantly higher nasal cavity and orofacial region tissue concentration following intranasal delivery as compared to intravenous administration. This showed that, following nasal instillation, lidocaine can distribute to and affect nearby tissues, including the trigeminal nerve branches.[30]

High concentrations of lidocaine (10%) were directly injected into the trigeminal nerve (nerve blocking) for assessing its efficacy in chronic pain treatment of trigeminal neuralgia in patients with short duration pain attacks. It was observed that this procedure could block the pain-producing signals through the nerve axons for long relatively periods. This was a better method to provide prolonged pain relief and was found to be useful in surgical procedures. There are studies showing that, although lidocaine was not indicated for migraine or trigeminal neuralgia, it was therapeutically effective when given intranasally.[64]

Thus based on the distribution pattern along the branches of the trigeminal nerve and into the trigeminal nucleus one can suggest that drugs administered via the nasal cavity may exert enhanced effects at the level of the tissues innervated by the trigeminal nerve system either because of the drug present in the individual nerve branches or drug presented in the trigeminal nucleus which can inhibit further signaling to the brain. Therefore, we decided to use lidocaine for our research in order to further understand the

trigeminal uptake mechanism in mice after nasal administration to evaluate whether it provides targeted, local pain relief. Lidocaine was also selected to assist in the validation of the thermal operant pain testing method since it had shown previous positive results using rat inflammatory pain method.

Intranasal Administration of Butorphanol

Opioids are extensively used in anesthesia, analgesia and drug abuse treatment. The natural opioid, morphine, and a synthetically- generated opioid, butorphanol, are used for analgesic effects while some other synthetically produced opioids, such as fentanyl and sufentanil are used for anesthetic effects.[66] The half – life of butorphanol following intravenous administration is about 5.6 hrs and following nasal administration is about 6 hrs. Butorphanol, being an opioid analgesic has side effects such as drowsiness, lethargy, mood changes. FDA first approved transnasal butorphanol tartrate for relief of pain in humans caused by post-operative pain and migraine headache pain. The chemical structure of butorphanol tartrate is shown in Figure 1-8.[67]

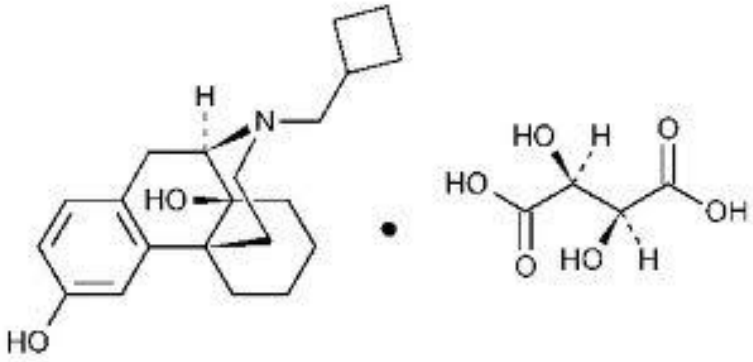

Figure 1- 8: Chemical structure of butorphanol tartrate[63]

Butorphanol has logP = 3.3, thus it is highly lipophilic.[63] It is a weak base with pKa of 8.6. It is biologically related to morphine but is five to seven fold more potent. Butorphanol is an opioid, partial agonist, narcotic analgesic.[68] Its mode of action is of mixed type where it acts on the μ and κ opioid receptors.[69,70,71] Scientists using a tail-flick method showed that μ agonist action are responsible for generating butorphanol's antinocieceptive activity in mice.[70] A study conducted by Souza et.al also showed that nalbuphine (an analgesic) had higher affinity for μ and κ receptors and also showed that high concentrations of this drug was seen at the substansia gelatinosa of the trigeminal nerve indicating presence of μ receptors in the trigeminal nerve.[72] Immunoreactivity experiment conducted on the C-terminus of the rat μ receptor showed differential pattern and high distribution of μ receptors at superficial layers of the spinal cord dorsal horn and nucleus caudalis of the spinal tract of the trigeminal nerve.[73]

Butorphanol is available in various formulations such as parenteral injection and nasal spray. The nasal preparation has a pH- 3-5.5. The nasal spray formulation is effective for moderate to severe pain conditions like migraine, dental and surgical pain. It has nasal bioavailability of 60-70% and oral bioavailability of around 5-17%.[68 ,66 74]

There has been research done showing successful delivery of analgesics such as morphine and fentanyl following nasal administration, improving the safety, onset of action and reducing side effects and avoiding the discomfort associated with other routes of administration.[14] As we know, those oral or intravenous analgesic administrations are associated with side effects such as drowsiness and GI irregularities. Butorphanol is primarily a centrally-acting analgesic and has fewer side effects than the above mentioned analgesics. It was thus selected to decide whether this drug, following

intranasal administration, could potentially be acting on the trigeminal nerve or whether it uses other pathways (systemic circulation, olfactory pathway) to stimulate central effects. And provide improved therapies with minimal side effects.

CHAPTER 2: OBJECTIVES

Effective treatments for painful conditions in the cranial and facial region remain elusive. Branches of the trigeminal nerve are responsible for all the sensory perception from the facial region and meninges, so treatments targeted to this nerve represent a promising therapeutic goal. Since many current pain therapies use therapeutic agents that are associated with significant central and systemic side effects, targeted therapies with improved efficacies are needed for head-associated pain conditions.

It was previously proposed that transmission of an analgesic agent along the trigeminal nerve pathways following intranasal delivery can mediate the perception of orofacial pain while minimizing side effects, yet those initial studies were performed with a locally acting anesthetic, and its use may also be associated with unwanted side effects. The objective of this study is to investigate the effect of centrally and peripherally acting analgesics, butorphanol and lidocaine, to determine their effects on the trigeminal nerve following intranasal administration. Operant behavior testing in mice was used to compare the effect of the two analgesics following nasal and intraperitoneal administration. Operant methods, which enable the subject to choose to participate in a neuronal and painful stimulus combination, have been developed for the investigation of the orofacial pain. Modifications of the standard method were also investigated to reduce the number of animals needed for evaluation and to reduce their stress during the trigeminal testing period.

CHAPTER 3: METHODS

Thermal Operant Assay

Pain behavior was assessed using an operant based assay developed by Neubert et al. where the mice were allowed to choose between a positive reward or avoid a painful facial stimulus. SKH1-E hairless mice were placed in an acrylic box for the testing (Figure 3-1) and the mice were trained according to the protocol detailed in Section 3.2. Prior to testing, the mice were trained in the testing chamber for 10 sessions under alternate fasting and non-fasting conditions and allowed to voluntarily drink 1:3 diluted sweetened condensed milk (Nestle) from the watering bottle. Phosphate buffer (PBS) (Table 2) (4 μL/nostril) was administered to the mice at the beginning of each training period. To obtain the milk, they placed their head through the opening of the cage that was lined with grounded aluminum tubing and touched their face against the stimulus thermo-electrode while receiving the reward. The temperature of the metal tubing was varied to the desired temperature range between 37-49 °C controlled by a water bath and circulating pump (Model RTE-7 D+, Thermo Electron). The temperature of the tubes was measured using a digital thermometer (Fluke, Model 54II).[75]

The metal spout of the bottle was connected to a multi-channel data acquisition module (WinDaq Data Acq DI-710-UH, DATAQ Instruments, Inc). When the mouse made facial contact with the tube, the circuit was completed and a facial contact was recorded by the data acquisition software. The distance between the heated tubing was adjusted to calibrate the system for each mouse in each testing box. A second circuit was completed when the mouse licked the metal spout (lick response). The level of detection

was adjusted to account for background noise by visual comparison of mouse lick and the data system response. This improves the accuracy and reproducibility of the results. In order to increase the magnitude of the response, the plate under the mouse was wet with Q-tips dipped in water. This provided higher conductivity which resulted in more distinct orofacial responses.

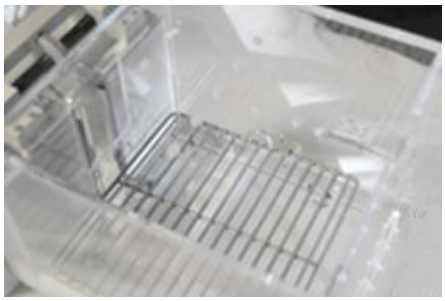

Figure 3 - 1: The text box consists of a small acrylic box insert (7 cm w × 7 cm d × 8 cm h) placed on an elevated platform (6cm) placed within a larger, clear acrylic box having dimensions 20.3 cm w × 20.3 cm d × 16.2 cm h.

Table 3 - 1 Formula for Phosphate Buffer Solution (PBS) (pH – 7.4 adjusted using NaOH/HCl if needed)

Compound	Name	500mL	1L
NaH_2PO_4	Sodium phosphate, monobasic	1.6g	3.2g
K_2HPO_4	Potassium phosphate, dibasic	8.4g	16.8g
NaCl	Sodium chloride	4.5g	9.0g
dd H_2O	Double distilled water	500mL	1L

The data were analyzed using Microsoft Excel 2010 and GraphPad Prism 6.0 for various parameters such as number of licks, number of facial contacts, and duration of contact. The results were compared for each group of the 16 mice, and the average of the results was noted for that day.

Control groups received 4µL/nostril of PBS, and for the experimental groups, two different classes of analgesic drugs were administered either intranasally or intraperitoneally to measure their ability to affect the animal's response to pain resulting from an increased thermal stimulus. New groups of mice were used for each analgesic: A dose of 0.16mg/8µL (4µL/nostril) for lidocaine hydrochloride (HCl) (2% lidocaine HCl, APP Pharmaceuticals, LLC, Schaumburg, IL)) and 0.08mg/8µL (4µL/nostril) of butorphanol tartarate (10mg/ml intranasal spray (Roxane Laboratories Inc., Cleveland, OH)) was administered into both nostrils of each mouse during the course of the experiment. Each mouse was administered 4µL of drug solution with a time interval of 5 minutes between the two nostrils. The mice were placed in the test cages immediately after completion of dosing and testing was initiated with simultaneous data recording for the following 20 minutes. Only one testing session was conducted each day. Two sessions of the experiment were initially conducted at 37 ºC; data was analyzed and then temperature was increased to 46 ºC for another 2-3 sessions. When the temperature of the tubing was further increased to 49 ºC, the mice were first trained at this temperature for 2-3 sessions since at high temperature, thermal pain is induced which causes a significant decrease in the number of licks and duration of facial contact. The experiment was conducted with analgesic administration for additional 2 more sessions at 49 ºC. In this experiment, two groups of mice received lidocaine HCl intranasally. The first set

received the dose and were tested temperatures: 37 ºC and 49 ºC. As the first was over-trained and the results were not accurate, the second set of mice was also administered lidocaine HCl intranasally and was tested only at 49 ºC.

To test the hypothesis that intranasal administration of the analgesic is more effective in reducing facial pain response than intraperitoneal injection, mice were injected intraperitoneally with 100 µL of the drug and tested for 20 minutes with the tube temperature at 49 ºC. For the intraperitoneal injection, 0.5 mL of 2% lidocaine hydrochloride was diluted with 0.95 mL of PBS (dose = 0.16 mg/ 8µL). The injection was made on the right side of the mouse's abdomen to avoid damage to the liver or essential organs. Intraperitoneal injection was performed on two study days and the average of both days was taken to obtain the results and calculation of various parameters. Prior training for intraperitoneal injection was not performed and the intraperitoneal testing was done in the previously lidocaine/ butorphanol tested mice.

For the investigations testing butorphanol, an initial set of experiments using a 10 mg/mL butorphanol tartrate solution was conducted. However, based on the initial results, a higher concentration (50 mg/mL) was also tested in the same group of mice. A suspension of 50 mg/mLof butorphanol tartrate (Sigma Aldrich, St. Louis, MO) was prepared by dispersing 50 mg of the drug in 1mL of 0.9% Sodium Chloride solution, USP (Hospira Inc., IL). For intraperitoneal injection, a 0.1 mL (40 mg/mL) (total dose= 0.08mg) near-saturated clear, solution was injected. A similar dilution procedure as with lidocaine was used for preparation of the intraperitoneal butorphanol tatrate solution. Butorphanol tartrate was dissolved in 0.9% Sodium Chloride Solution, US and the

solution was passed through a 0.2 μm filter Table 3-2 summarizes the different doses of the analgesics given.

Table 3 - 2: Doses of analgesics administered to the mice

DOSE	2 % Lidocaine HCl	10 mg/mL Butorphanol Tartrate	50 mg/mL Butorphanol Tartrate
Dose/nostril (mg/4 μL)	0.08	0.04	0.2
total mass delivered (mg)	0.16	0.08	0.4
Dose(mg/kg)	4.5	2.28	11.1

Animal Methodology

Young hairless SKHE-1 mice (25-30g- 5-6 weeks old) were ordered from Charles River Laboratories, Wilmington, MA, USA and were placed in groups of four in four cages. They were housed under a 12 hour light and dark cycle and were provided with food and water. SKH1E hairless mice (30-40g, n=16) were trained 10 sessions with alternate fasting and non-fasting condition. Mice were fasted for 17-18 hours prior to each testing period. Water was available ad libitum before and after the fasting period and the animal's weight was recorded daily during the training period. These mice were brought into a behavior room for testing and kept in the room for 30 minutes prior to testing to allow them to adjust to room's temperature and noise level. All of the procedures were approved and performed in accordance to the Institutional Animal Care and Use Committee at University of Iowa. The testing period lasted 20 minutes.

Mice, during their initial training period, were placed in the cage within the acrylic box very close to the metal spout of the milk bottle in order to allow them to drink freely. This training was conducted for three sessions until the mice drank milk voluntarily from the bottle. The temperature of the tubing during this phase was maintained at body temperature (37 °C). As the mice became habituated to the drinking process, the distance between the metal tubing and the milk spout was increased to an extent that still allowed access to the reward but required the mouse to place its face between the thermodes to reach the spout. Similarly, before conducting the experiments at 49 ºC, the mice were habituated to the 49 ºC temperature condition for 2 sessions. During the training period, all the mice received PBS intranasally to also train to accept placement of the drops within their nostrils without significant effects on their behavior

during testing. The final two training sessions were averaged together and the number of licks and duration of facial contact values were set as the baseline values for later drug administration experiments at both 37 ^{0}C and 49 ^{0}C.

Animals were grouped into control and treatment groups based on their licking performance during the training period in order to avoid bias in results. This was done by taking the average number of licks during the previous two training sessions such that the average number in the treatment and control group was similar. During the course of the experiment, the control group was administered phosphate buffer solution (PBS) intranasally while the treatment group was administered lidocaine HCl or butorphanol tartarate. After the entire testing was completed within 6-8 sessions, the mice were euthanized by asphyxiation with carbon dioxide.

The apparatus for the experiment is shown in Figure 3-2. While a mouse-specific apparatus was used, when the mice were placed in the small box within the cage to restrict their movement, some learned to escape by pushing on the box. A small weight was placed at the back of the cage to prevent the mice from pushing the box. Also, since the mice could move around in the box, their tails would touch the thermodes and these contacts would be mistakenly counted as facial contacts. Hence, continuous monitoring of behavior of the mice during the experiment was performed to obtain accurate results. Tail flicks and facial testing could be easily distinguished based on the pattern of the digitized output. While the tail flick responses were not removed from the dataset, all data were examined for the presence of a number of tail flicks sufficient to affect the resulting statistical analysis. None of the datasets used for the quantification were significantly affected by errant tail flicks.

Statistical Analyses

The data collected i.e. the mean values for number of licks and duration of facial contacts was compared using GraphPad Prism 6.0 and Microsoft Excel 2010 (Redmond, WA). Two - way ANOVA was used to evaluate the effects of temperature on treatment groups and control groups. Unpaired t-tests were used to compare the effect of treatment groups and control. If significant differences were observed in the ANOVA analysis, a post - hoc comparison was evaluated using the Bonferroni corrections. *$p<0.05$ was considered to be statistically significant for all of the evaluated variables.

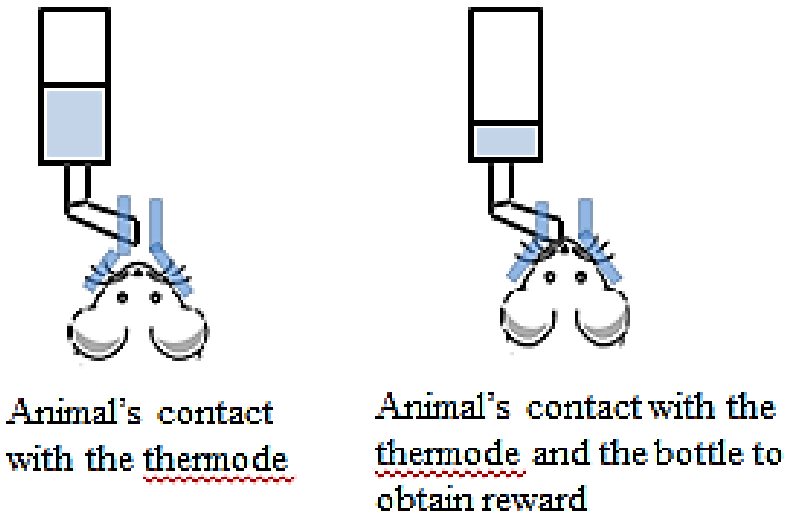

Figure 3 - 2: Detection sequence for animal seeking reward of sweetened milk. When the animal touches the thermode and the bottle, it will be recorded as a lick response and a facial contact.

CHAPTER 4: RESULTS

Intranasal Administration of Lidocaine in Mice

Treatment vs Control

The effect of lidocaine was tested on one set of mice (set 1) at 37 ºC, 46 ºC and 49 ºC, and the number of licks and duration of facial contacts were measured. A second set of mice (set 2) were tested using lidocaine only at 49 ºC. For set 1, the number of mice in the treatment group was 7 (n=7) and control group had 8 (n=8). For set 2, the number of mice in the treatment group was 8 (n=8), and there were 5 controls (n=5).

Effect of Thermode Temperature

From the results obtained at 37 ºC, it was observed that the control group and treatment group did not show significant differences in the number of licks ($p=0.4841$) (Figure 4-1a). As the temperature was increased from 37 ^{0}C to 49 ^{0}C, the number of licks significantly decreased for the treatment as well as the control group ($p < 0.0001$). When the duration of facial contact was evaluated for the set 1 and set 2 mice, it was observed that the duration of facial contact was also significantly decreased as the temperature was increased from 37ºC to 49 ºC ($p < 0.0001$) (Figure 4-1b).

Effect of Treatment

Unpaired t-test results also showed that treatment and control groups showed no significant differences for the number of licks for either set 1 ($p = 0.7090$) and set 2 ($p = 0.3757$) mice (Figure 4-1a) at 49 ºC. For set-1 mice at 49 ºC, the treatment group did not show any significant changes in the duration of facial contacts as compared to the control group ($p=0.3302$) (Figure 4-1b). The number of licks was also not significantly

different for both sets at 49 °C. No significant increase in the duration of facial contact was also observed in the treatment group for both set of mice (Figure 4-1b).

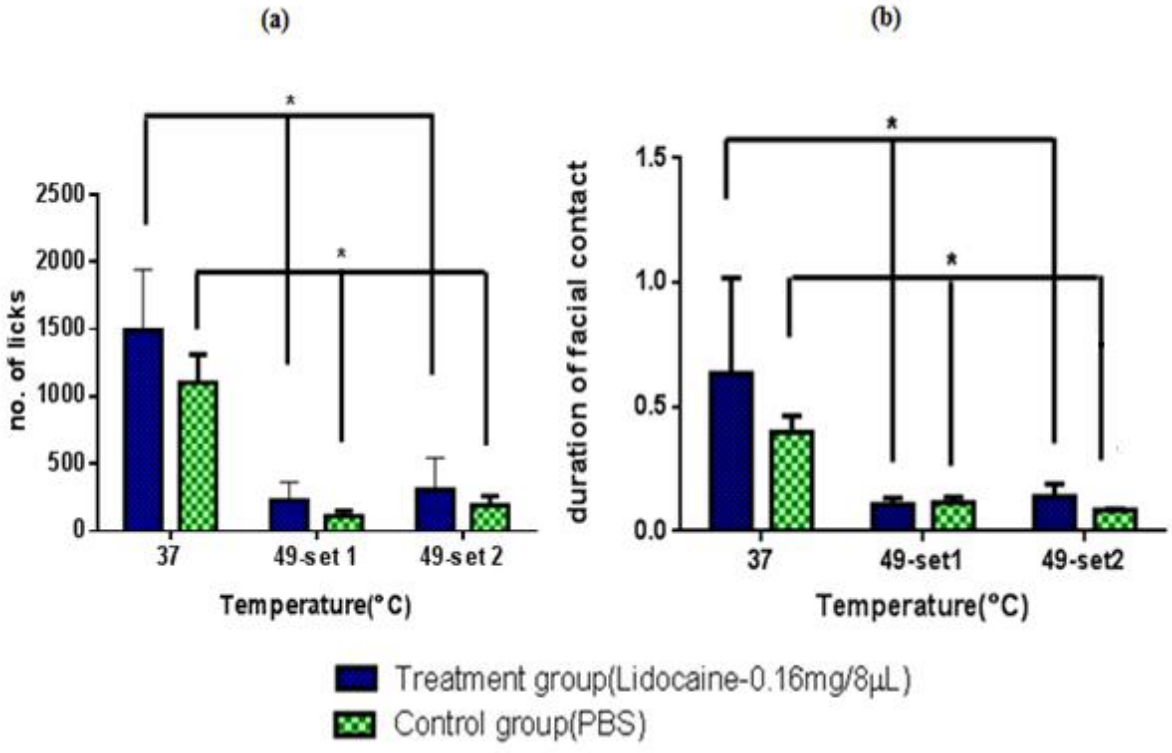

Figure 4 - 1: **Comparison of number of licks and duration of facial contacts between treatment and control group at different temperatures for hairless SKH1-E mice - (a)** Significant decrease in the number of licks was observed between treatment and control as the temperature was increased from 37 ºC to 49 ºC ($p < 0.0001$). **(b)** Significant decrease in duration of facial contact in the treatment group and control group was also observed as the temperature increased from 37 ^{0}C to 49 ^{0}C ($p < 0.0001$). A significant change in duration of facial contact was not observed between the treated mice than the control mice in both set at 49 ºC.

Baseline vs Experimental Behavior

Mice were trained for 10 sessions and once the mice were trained, the analgesic demonstration experiments were conducted. The final two training sessions were averaged together to determine a baseline for the experiments at both 37 ^{0}C and 49 ^{0}C. In order to be sure that there weren't any significant differences in baseline values at 37 ºC for the treatment and control groups, a paired t-test was used. It was found that there were no significant differences between the groups and their baseline (treatment = p = 0.0503, control= p= 0.6503) at 37 ºC (Figure 4-2). Similarly, when comparisons between the treatment and baseline were evaluated for set 1 mice at 49 ºC, there were no significant changes in the number of licks (p = 0.4246). Comparison of baseline and control group also showed that at 49 ºC, there was no significant difference in the number of licks (p = 0.0588) (Figure 4-4). Set 2 mice also showed no change in the number of licks between treatment and baseline at 49 ºC (p = 0.9410) and also no significant difference in number of licks was observed between control and baseline (p = 0.6400) (Figure 4-6). For the duration of facial contacts, no differences at either 37 ºC or 49 ºC was observed between baseline and treatment/control (Figure 4-3, 4-5, 4-7).

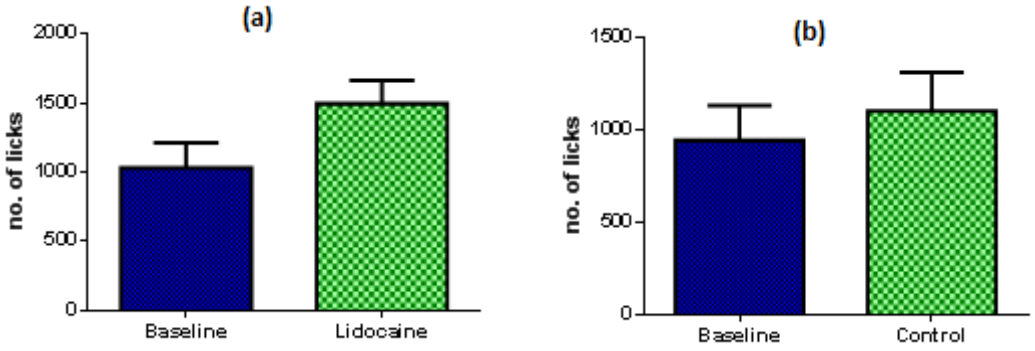

Figure 4 - 2: **Comparison of number of licks in set 1 hairless SKH1-E mice at 37 ^{0}C** – No significant change in the number of licks between **(a)** treatment and baseline values (p=0.0503) and **(b)** control and baseline values (p=0.6503). The baseline values were obtained from the final two days of training.

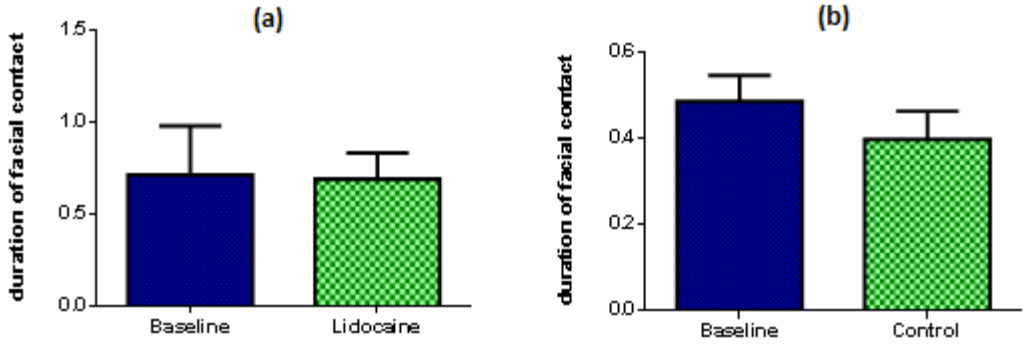

Figure 4 - 3: **Duration of facial contact comparison for set 1 mice at 37 ^{0}C**- No significant change in duration of facial contact was observed between **(a)** treatment and baseline values (p=0.7991) and **(b)** control and baseline (p=0.3566)

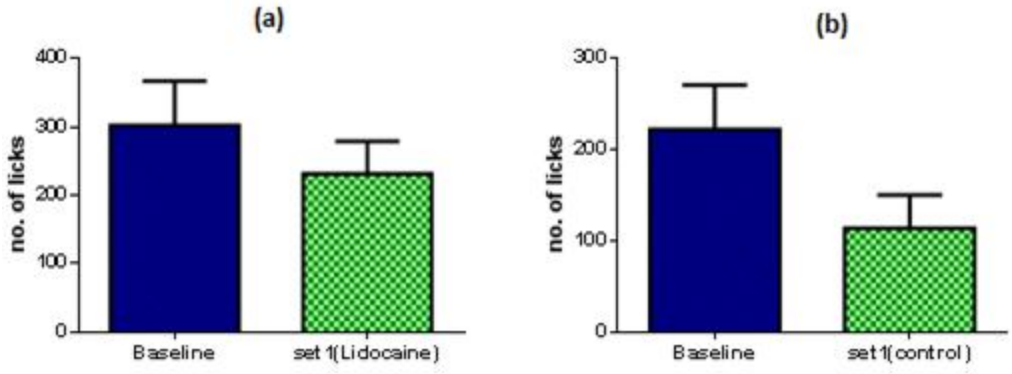

Figure 4 - 4: **Comparison between treatment/control values and baseline values for set 1 hairless SKH1-E mice at 49 ^{0}C** – No significant decrease in number of licks between **(a)** treatment and baseline values (p=0.4246) and **(b)** control and baseline values (p = 0.0588).

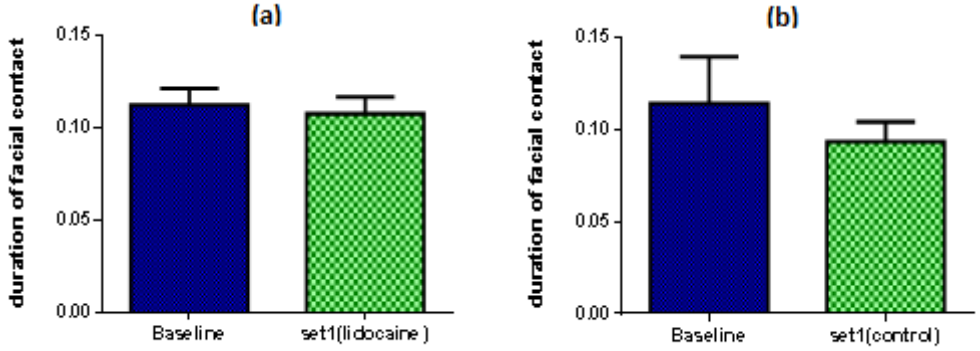

Figure 4 - 5: **Duration of facial contact comparison between treatment/control and their baseline for set 1 mice at 49 ^{0}C** – No significant decrease in duration of facial contact between **(a)** treatment and baseline values (p = 0.3466) and **(b)** control and baseline values (p = 0.5603).

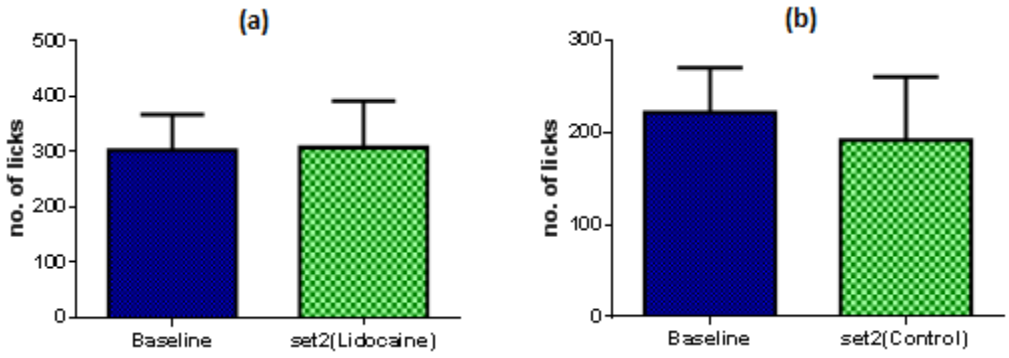

Figure 4 - 6: **Comparison of number of licks for set 2 mice at 49 ^{0}C** – No significant difference in number of licks between **(a)** treatment and baseline values (p = 0.9410) and **(b)** control and baseline values (p = 0.6400).

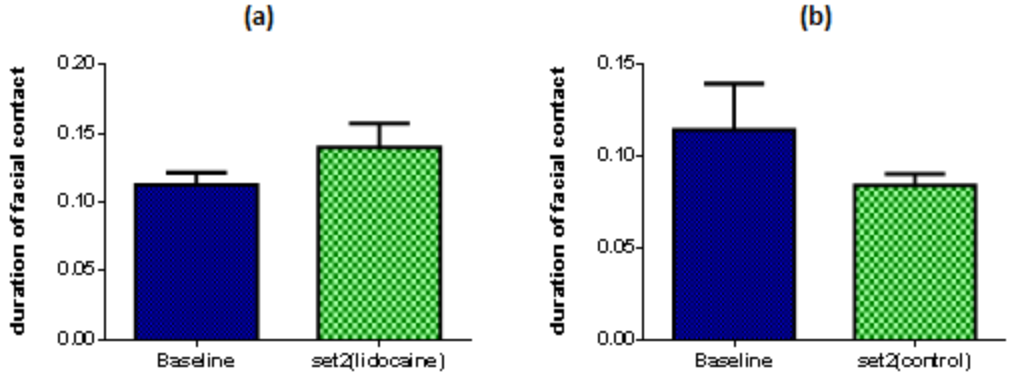

Figure 4 - 7: **Analysis of duration of facial contact for set 2 mice at 49 ^{0}C** – Duration of facial contact did not significantly change between **(a)** treatment and baseline values (p = 0.3904) and **(b)** control and baseline values (p = 0.3622).

Intranasal vs Intraperitoneal Administration

After testing sessions were completed for the intranasal administration of lidocaine (0.16mg/ 8µL), a wash out period of 3-4 days was allowed and then intraperitoneal testing was performed. A testing temperature of 49 ºC was used for intraperitoneal testing. The change in number of licks was evaluated using two - way ANOVA to determine whether any differences in the pain threshold existed based on the route of analgesic administration. From Figure 4-8a, it can be seen that the number of licks was not significantly different for intranasal to intraperitoneal administration of lidocaine. There was also no significant difference in number of licks between the treatment and control groups receiving intraperitoneal injection ($p = 0.4015$).

When measuring the duration of facial contact however, the results showed that there was a significant difference in the duration of facial contact when comparing the treatment groups receiving intranasal lidocaine to those of the controls in set 1 mice ($p = 0.0242$) (Figure 4-8b). No significant change in the duration of facial contact was observed between the treatment and control groups for intraperitoneal administration of lidocaine ($p = 0.3302$) (Figure 4-8b).

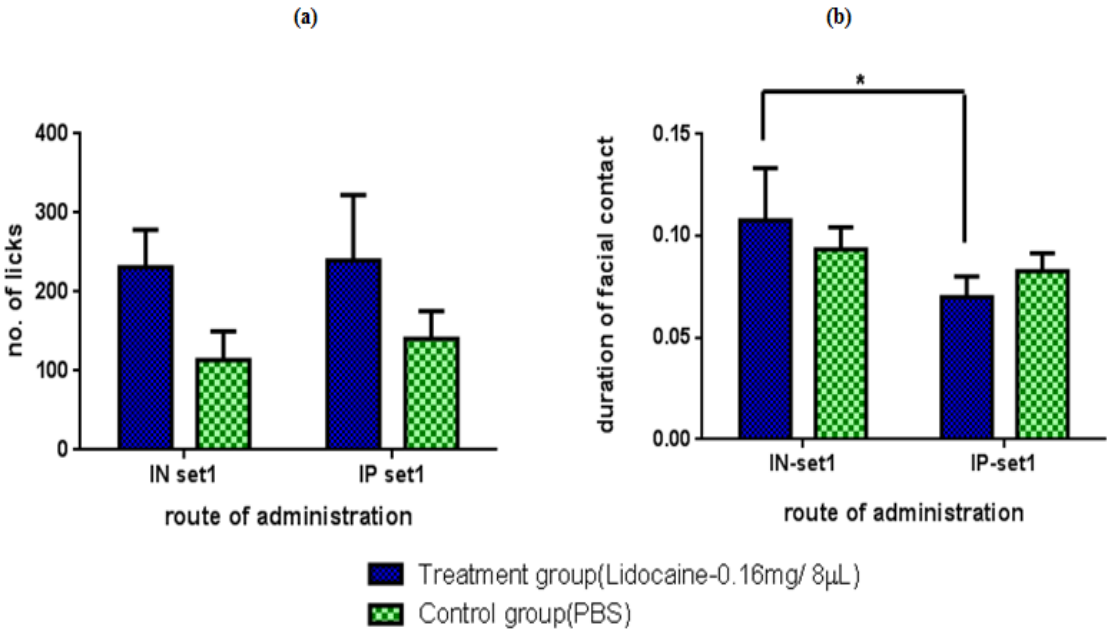

Figure 4 - 8: **Operant testing at 49 ˚C for mice administered lidocaine HCl** – **(a)** No significant difference was observed in the number of licks when comparing the two routes of administration for set 1 mice. Also no significant difference was observed in the number of licks between treatment and control groups following intraperitoneal administration (p =0.4015). **(b)** Significant difference in duration of facial contact between the treatment group of IN set 1 and IP set 1 (p=0.0242) was observed. No significant difference was observed between the control group and treatment group after intraperitoneal injection (p = 0.3302).

Intranasal Administration of Butorphanol in Mice

Treatment vs Control

During the initial testing, mice were administered a 10 mg/ml (0.04mg/4µL/nostril) solution of butorphanol tartrate intranasally. After conducting preliminary data analysis, the results showed that there were indications of positive differences between the treatment (n=8) and control groups (n=5), but they hadn't reached statistical significance. To improve the ability to show statistical differences between the groups, the experiment was repeated and the dose was increased to 50mg/mL suspension (0.2 mg/4 µL/ nostril).Two way ANOVA with Bonferroni corrections was were used for the data analysis.

Effect of thermode temperature

When the mice were initially tested at 37 ºC, control and treatment groups showed significant differences in the number of licks ($p=0.0151$) (Figure 4-9a). As the temperature was increased from 37 ^{0}C to 49 ^{0}C, the number of licks significantly decreased for both the treatment as well as the control group ($p < 0.0001$) (Figure 4-9a). No significant differences were observed between the treatment and control groups for duration of facial contact ($p = 0.0951$) at 37 ºC. The duration of facial contact was significantly decreased as the temperature was increased ($p < 0.0001$) (Figure 4-9b). No significant difference in number of licks between treatment and control group was observed at higher dose (0.4mg) ($p = 0.7563$). However, differences between treatment and control group continued to be observed at higher dose level (0.4mg) when analyzing duration of facial contact at 49 ºC ($p = 0.0006$). There was also significant difference between the treatment groups when comparing the two doses ($p = 0.0413$). (Figure 4-10)

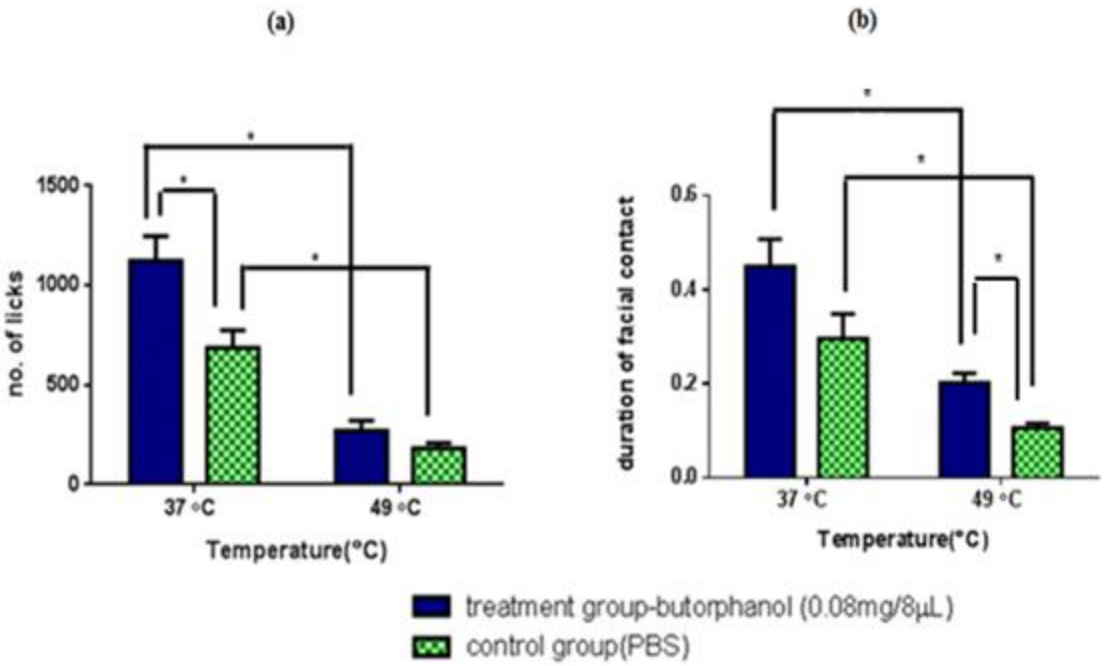

Figure 4- 9: **Temperature effect on the number of licks and duration of facial contact in the treatment and control group- (a)** Significant increase in the number of licks in the treatment group as compared to the treatment group was observed at 37 ºC ($p=0.0151$). The number of licks was also significantly decreased in both the treatment and control groups as the temperature was increased from 37 ºC to 49 ºC ($p<0.0001$). **(b)** No significant decrease in duration of facial contact between treatment and control group at 37 ºC was observed ($p = 0.0951$). Duration of facial contact significantly decreased in both treatment and control groups as the temperature was raised from 37 ºC to 49 ºC ($p <0.0001$). At 49 ºC, a significant increase in duration of contact was observed for the treatment group than the control group ($p =0.0051$)

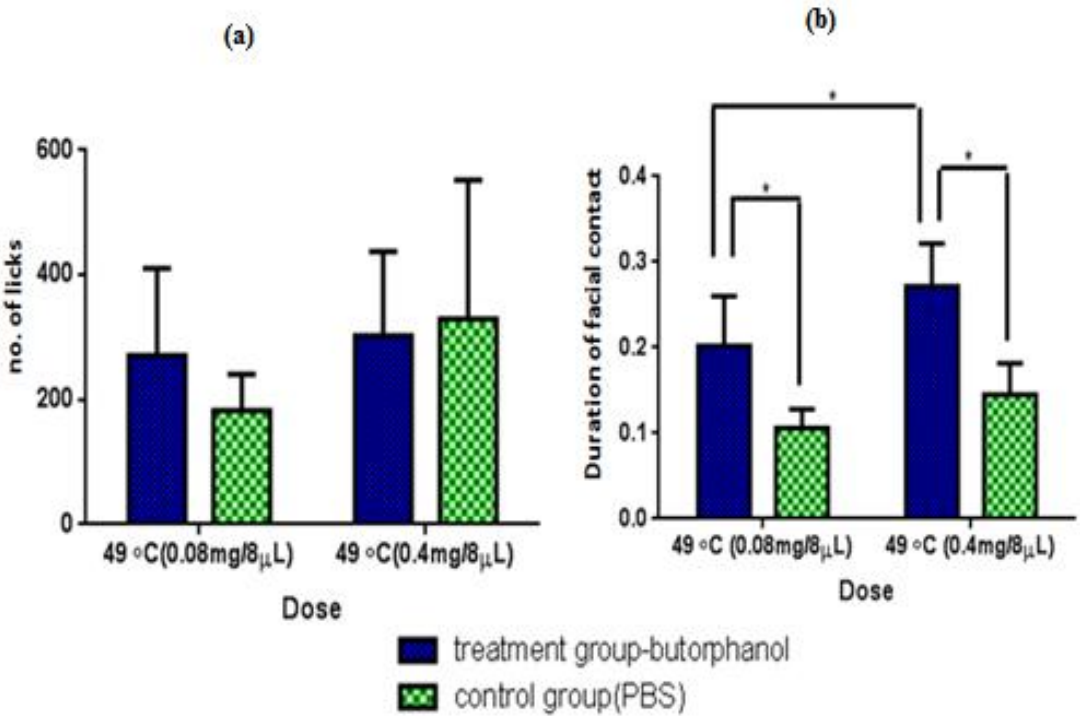

Figure 4 - 10: **Dose effect on the number of licks and duration of facial contact in the treatment and control group at 49 ºC- (a)** No significant change in the number of licks in the treatment group as compared to the control group at higher dose ($p = 0.7653$). **(b)** A significant increase in duration of contact was observed for the treatment group than the control group ($p = 0.0051$) at lower dose. This effect was enhanced when the mice were administered a higher dose ($p = 0.0006$). Significant difference between the treatment groups when comparing the two doses was also observed ($p = 0.0413$).

Baseline vs Experimental Behavior

Similar to the experiments where mice were administered lidocaine HCl, the final two training sessions were averaged together to set a baseline for the experiments for butorphanol tartrate administration. In order to be sure that there was no significant difference in baseline values at 37 ºC and 49 ^{0}C for the treatment and control groups, paired t-tests was performed between the baseline and experimental day data. The results showed that for the number of licks at 37 ºC, there was no significant difference between the treatment group and baseline ($p = 0.9364$) but, the treatment group showed a significant decrease compared to the control group and their baseline ($p = 0.0274$) at 37 ºC when evaluating the number of licks (Figure 4-10). It was also observed that there was no significant difference between the control and baseline for the duration of facial contact ($p = 0.1806$), but the treatment group's duration of facial contact significantly decreased compared to the baseline ($p = 0.0217$) at 37 ºC (Figure 4-11). Results obtained from comparisons between treatment and their baseline or control and baseline at 49 ºC showed that there were no significant differences in the number of licks between the groups and their baseline values at either dose level. (Figure 4-12, 4-14)

Similarly, when comparisons were made between treatment and baseline or control and baseline for the lower dose (0.08) at 49 ºC, there were significant differences in duration of facial contact between the treatment and their baseline ($p =0.0092$) but no significant change in duration of facial contact between the control and their baseline was observed ($p = 0.8314$) (Figure 4-13). When the mice were administered the higher concentration suspension of drug at 49 ºC, the results showed a significant difference in the duration of facial contact for the treatment compared to their baseline ($p = 0.0008$)

and no significant difference between the control group and their baseline (p = 0.3004). (Figure 4-15).

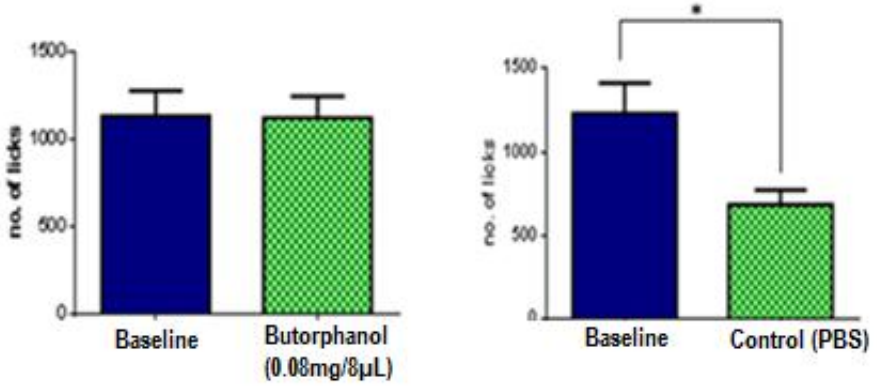

Figure 4 - 11: **Comparison of number of licks in set 1 hairless SKH1-E mice at 37 ^{0}C** – No significant change in the number of licks between treatment and baseline values (p = 0.9364) was observed and there was significant change between control and baseline values (p = 0.0274).

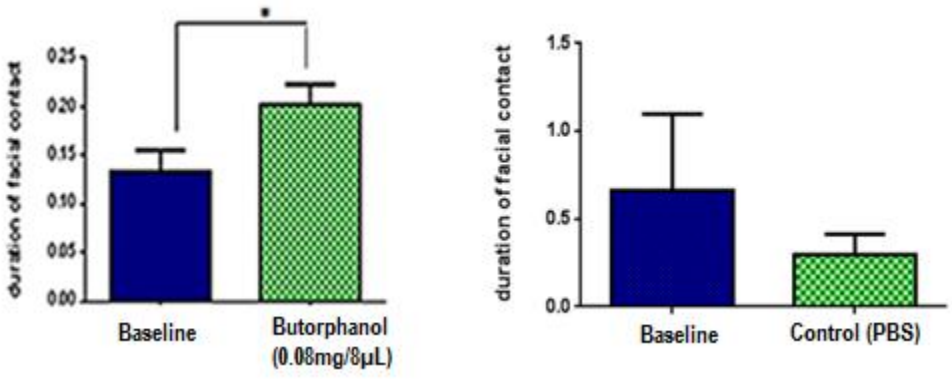

Figure 4 - 12: **Duration of facial contact comparison for mice at 37 ^{0}C -** Significant increase in duration of facial contact was observed at 37 ºC between treatment and baseline values (p = 0.0217). No significant change was observed for the same parameter between control and baseline (p = 0.1806).

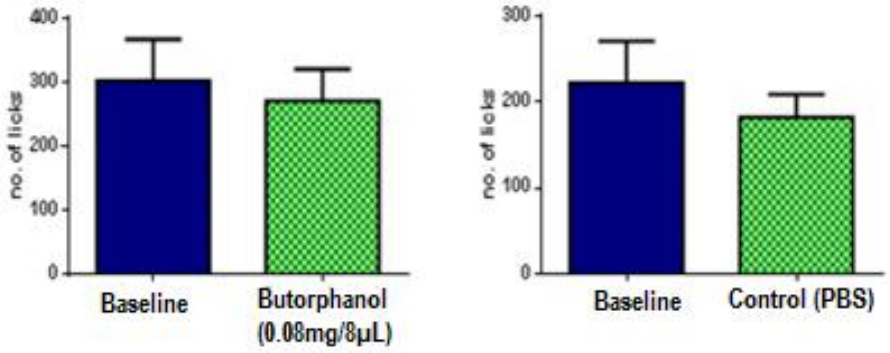

Figure 4 - 13: **Comparison of number of licks in set 1 hairless SKH1-E mice at 49 ^{0}C** - No significant change in number of licks at 49 ºC was observed between treatment and baseline values (p = 0.7020) and control and baseline values (p = 0.5894).

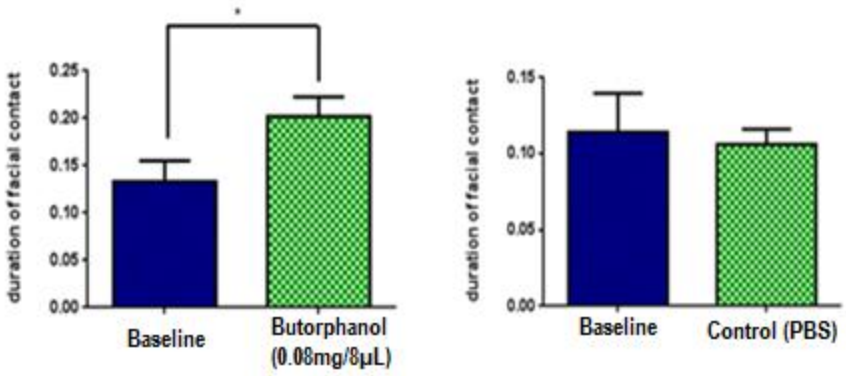

Figure 4 - 14: **Duration of facial contact comparison for lower dose data values in mice at 49 ^{0}C** – A significant increase in duration of facial contact was observed in the treatment group values in mice following 0.08 mg of butorphanol tartrate at 49 ºC administered intranasally (p = 0.0092). No significant change in duration of contact was observed between the control and their baseline values (p = 0.8314).

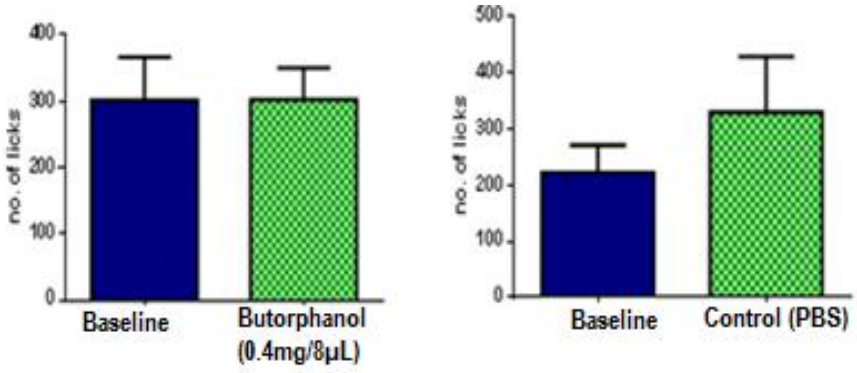

Figure 4 - 15: **Comparison of number of licks at 49 ^{0}C** - Number of licks was not significantly different between treatment and control group from their baselines respectively following 50 mg/mL (0.4mg) intranasal butorphanol tartrate (p = 0.9962, p = 0.4987).

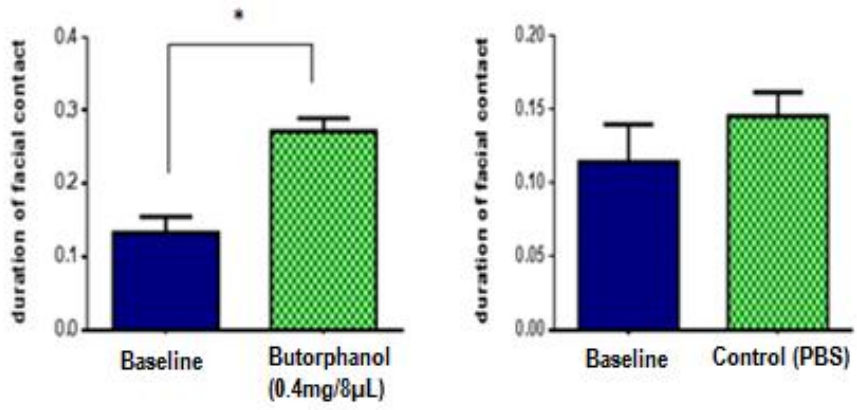

Figure 4 - 16: **Duration of facial contact comparison in mice at 49 ^{0}C** – A significant in duration of facial contact was observed in the treatment group compared to the baseline at 49°C (p = 0.0008). No significant change in duration of facial contact was observed between control and baseline values (p = 0.3004) following intranasal butorphanol tartrate solution (0.4 mg).

Intranasal vs Intraperitoneal Administration

Following the completion of intranasal testing, a washout period of 3-4 days was provided and then intraperitoneal testing was performed at 49 °C. The change in number of licks and duration of facial contacts was measured to understand whether there were any differences in effect based on the route of administration. From Figure 4-17, it can be seen that the number of licks was not significantly different when comparing intranasal and intraperitoneal administration for both the treatment ($p = 0.4258$) and control group ($p = 0.9233$). An increase in the number of licks by the control group following injection of intraperitoneal saline suggests that the new route of administration caused some behavioral changes in the animals.

No difference in the duration of facial contact was observed between the treatment groups ($p = 0.9434$) and control groups ($p = 0.1865$) in the mice when comparing intraperitoneal administration and intranasal administration. Following intranasal administration, however, there was a significant increase in the duration of facial contact for animals administered butorphanol compared to untreated controls ($p = 0.0067$) (Figure 4-16).

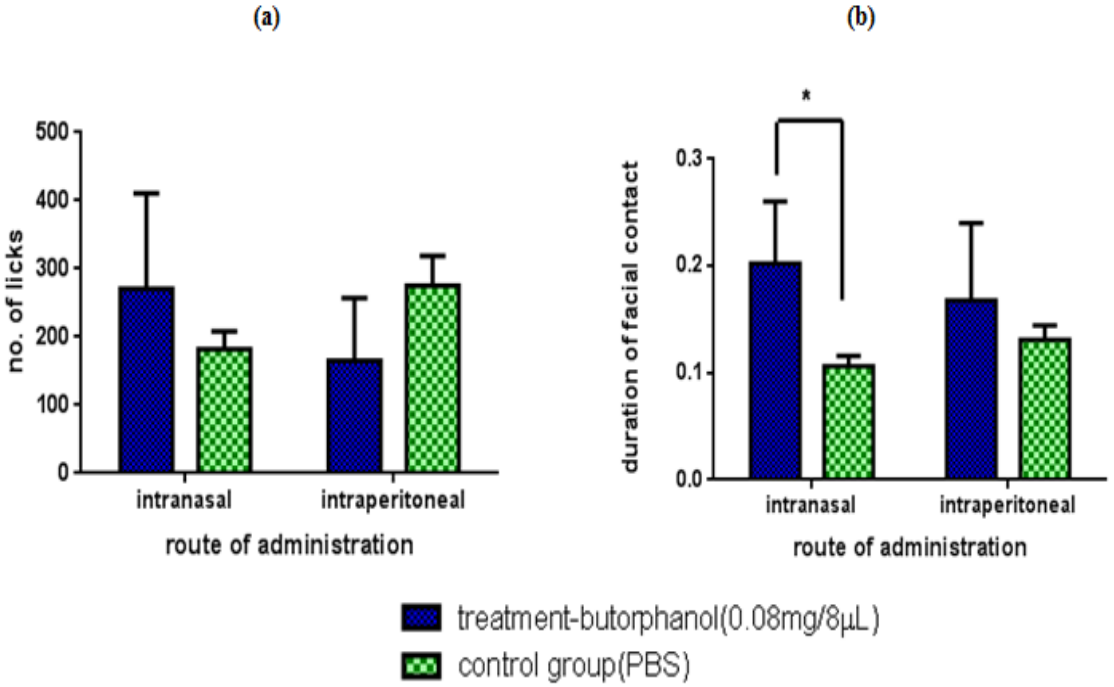

Figure 4-17: **Number of licks and duration of facial contact comparison in mice intranasal and intraperitoneal administration at 49 ºC** – **(a)** Number of licks was not significantly different when comparing intranasal and intraperitoneal administration for both treatment groups ($p = 0.4258$) as well as control group ($p = 0.9233$), respectively. Number of licks increased for the control group than the treatment group after intraperitoneal injection. **(b)** No significant difference between the duration of facial contact was observed between the treatment group and control group in the mice when comparing intraperitoneal administration and intranasal administration. Duration of facial did significantly differ for the treatment group than the control group following intranasal administration of butorphanol tartrate (0.08mg) ($p = 0.0067$).

CHAPTER 5: DISCUSSION

Orofacial pain is a major concern in public health, and advances continue to be made in the understanding the mechanisms of pain signal transmission involving this region. The goal of these experiments was to determine whether intranasally administered lidocaine or butorphanol could directly access the trigeminal nerves within or near the nasal mucosa to reduce pain signal propagation in the treatment of orofacial pain.

The advantage of the operant method selected for use in these studies is that the mice can decide between receiving a painful stimulus prior to a reward or to not pursue the reward. The results can provide an indication of the severity of the pain experienced by the animal under the experimental conditions. In current experiments, the ability of analgesics to diminish the level of pain experienced was used to evaluate whether intranasal delivery of the analgesic provided pain control which may indicate this would be an effective method to provide local treatment of orofacial pain. This method is also simple and easy in operation and requires minimal training and no surgeries compared to the other operant techniques or pain models. The ease and reproducibility of automated data collection provides another advantage for this operant method.[76] Operant orofacial pain methods are also better than traditional hind paw inflammation models as those methods are not true representatives of orofacial pain.

Research conducted by Neubert et al. showed equivalence for the use of shaved, Sprague Dawley rats in this operant method and the use of SKH1-E mice, although this strain may not be typically used for pain models. The advantage of using mice is they are quick and efficient learners, are easy to handle and can adapt well to their surroundings. Hence, mice were considered to be a suitable species for these preliminary experiments.[32]

The current experiments also differed from those of Neubert et al. in that we only used the thermal stimulus induced by the aluminum thermode as the source of nociceptive pain. In Neubert's model, inflammation was induced by first applying capsaicin to the whisker pad and using higher temperatures to measure the pain responses. We chose not to use capsaicin treatments in these preliminary studies to minimize the discomfort experienced by the animals. However, use of only thermal pain may not have been sufficient to produce statistically significant results.

Initially, the mice were trained using body temperature thermodes (37 ºC). After training for several sessions at this temperature, the temperature of the aluminum thermode was increased to 46 ºC. However, the results obtained for the number of licks and duration of facial contact were not significantly different from the 37 ºC results and, consequently, the temperature was increased to 49 ºC. At this temperature, significant differences in reward seeking behaviors were observed. Hence no further increase the temperature was evaluated for testing. Since SKH1-E mice are hairless, testing at increased temperatures increases the likelihood for damage to the skin (burns) and alteration of the pain nerve receptors. Neubert's et al. conducted experiments using 52 ºC, and the results showed even greater decreases in the number of licks and duration of facial contacts between treatment and control group compared to the 37 ºC condition. These investigators do not report any injuries to the mice using 52 ºC, and it is likely that if our experimental temperature was increased to 52 ºC, similar results to Neubert's report may be observed.

Orofacial pain treatment using lidocaine

Previous research showed uptake by the trigeminal nerve of intranasally administered lidocaine in rats using an inflammatory pain model.[77] The investigators concluded that intranasal delivery can mediate the perception of the orofacial pain as well as minimize systemic exposure.[30] Their method involved the use of the TrigeminAir device to assess the sensitivity of the orofacial region. Carrageenan (2% or 4%) was injected into the whisker pad region and the animals needed to pass their noses through a concentric airstream (15 psi unit pressure) to reach a feed bottle containing sweetened milk. Their results showed a 20 - fold increase in tissue concentration of intranasally administered lidocaine (8 mg/80 µL dosed over 18 minute intervals) in the trigeminal nerve regions compared to the brain and blood. Their experiment involved the assessment of inflammatory pain by using air; it may not be a truly representative of all types of orofacial pain. The stream of air may be irritating to the mice but not very painful, as opposed to mechanical, thermal or chemically induced pain used in other orofacial structures. Hence, the operant method selected for these studies was more useful to assess orofacial pain since the mice felt thermal pain directly in orofacial region when they touched the heated aluminum bars. They were able to select between the thermal stimulus and the positive reward, which provides additional information about the animals' subjective experience.

The studies conducted by Frey et al. used either 4% or 10% lidocaine in their work as the dosing solutions. However, for animal safety we attempted to start with a lower concentration and used 2% lidocaine. The results obtained from the thermal operant assay did not show any significant increase in the number of licks or in the

duration of facial contact between the treatment and control groups at 49 ºC for the initial (set 1) group of animals. This lower dose (0.16 mg/8 µL) of the drug may not have been sufficient to reach a therapeutic concentration at the nerve. Another reason for the lack of difference could be that as this group of mice was over- trained since they were used over 2 month period with intermittent breaks of several weeks between testing sessions. The longer the mice were used, the more variable their responses became and the variability diminished the ability to detect difference in the animals' behavior. Hence, the same study was repeated in a second set of mice where a strict testing schedule was followed. No significant increase in the number of licks and duration of facial contact was observed for this group of mice, either. Since the mice showed no ill-effects due to the lidocaine, testing another group of animals using the 4% or 10% lidocaine concentration as used by the previous researchers could be investigated to determine whether the lack of response was due to an "under- dosing" of the anesthetic.

Since the trigeminal nerve is responsible for facial sensation, a comparison of intranasal delivery with intraperitoneal administration was conducted to evaluate the effect of increased local lidocaine tissue concentrations following intranasal administration compared to the potentially higher systemic concentrations anticipated from intraperitoneal administration. However, the results for the number of licks and duration of facial contact obtained following intraperitoneal injection of 2% lidocaine were not significantly different from those following intranasal administration. Therefore, the current experiments were not able to show that intranasal administration was more effective in reducing the orofacial response than the systemic administration. Again, over-exhaustive experimentation with the set 1 mice may have influenced this

comparison such that by the end of the experiment, the mice adopted a strategy to obtain the reward by touching the thermode for shorter durations while still drinking a sufficient amount of milk.

Orofacial pain treatment by butorphanol

There has not been any operant pain methods used to study orofacial pain reduction after administration of butorphanol. Butorphanol is primarily a centrally-acting analgesic. It was selected for these investigations to determine whether this drug, following intranasal administration, it could potentially directly act on the trigeminal nerve or whether it uses other pathways (systemic circulation, olfactory pathway) to elicit central effects. Our results showed that when a 0.08 mg/8 µL (10 mg/mL) or 0.4 mg/8 µL dose (50mg/mL) butorphanol tartrate were administered intranasally, the duration of facial contact with the thermodes was significantly longer in the treatment group. This suggested that the nociceptive pain response induced by the high temperature was diminished by the analgesic. Intranasal administration of butorphanol produced significant reductions in orofacial pain measures and it may suggest that the drug is effectively absorbed and acts centrally to diminish pain. Since butorphanol tartrate is available as an intranasal spray for pain control in the US, the systemic route of absorption has been previously demonstrated.

Under ideal behavior, the group of mice receiving the butorphanol and the group of control mice receiving PBS at 37 ºC should show a similar number of licks and duration of facial contact. However, the control group for this set of the experiments showed a significantly lower in the number of licks and duration of facial contact during testing compared to their baseline (training) activity. During the period for this testing,

there were thunderstorms and this change in the environmental conditions may have caused the decrease in the parameters for the control group. However, the results from the treatment groups were not as affected. This may have been the result of the pharmacodynamics activity of the drug itself, where the animals' receiving the butorphanol were less disturbed by the environmental changes due to the sedative action of the drug.

When the mice were tested at 49 ºC using 0.4 mg dose of butorphanol tartrate intranasally, the number of licks was decreased for the treatment group compared to the control group, in contrast to the previous behavior. This may be the effect of the analgesic which caused the treatment group to drink less milk from the bottle. The higher dose of butorphanol may have caused drowsy effects in the treatment group which caused less desire to reach the milk. The clear interpretation of the results is difficult; however, the treatment group had longer facial contact with the thermodes as compared to the control group indicating that this drug was capable of reducing the orofacial pain.

Conclusion

When drugs are given by intraperitoneal injection, they undergo an absorption phase and enter the systemic circulation and reach the brain by crossing the BBB. Intranasally administered drugs, on the other hand, can potentially reach the brain either through systemic absorption or directly through the nasal passages where the nerves might act as conduits for their transport. The results obtained from these operant testing experiments did not show significant changes in the number of licks or duration of facial contact when comparing the two routes of administration. This suggests that intranasal

administration of lidocaine HCl or butorphanol tartrate may not be more effective than intraperitoneal for treatment of orofacial pain conditions.

Further experiments will need to be conducted to elucidate the uptake pathway and target effect sites for analgesics administered intranasally. Additional experiments with lidocaine, beyond those described here, will be required to show the potential dose dependence of its activity in orofacial pain.

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