THE HIERARCHAL AVERSIVENESS OF TWO SIMULTANEOUS PAINS WITHIN AN OPERANT APPROACH-AVOIDANCE PARADIGM

by

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Abstract

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Pain is a subjective, private, yet universal phenomenon that depends on a unique combination of sensory, affective, and evaluative characteristics. Although preclinical models have been used to understand much of pain physiology, the inability to communicate with animals limits affective and evaluative feedback, failing to adequately represent the entire pain experience. Therefore, this study sought to characterize the affective component of pain within an operant approach-avoidance paradigm (AAP) to determine which type of pain (inflammatory or neuropathic) may be more aversive. To reveal possible differences in pain aversiveness, animals received bilateral inflammatory and neuropathic pain conditions and were be given the choice to a) forgo appetitive reward by not receiving noxious stimulus of either inflammatory or neuropathic condition or b) receive noxious stimulus in exchange for appetitive reward. Pharmacological treatment was also assessed to reveal if selectively attenuating inflammatory or neuropathic pain via drug treatment could modulate or reverse approach-avoidance behaviors. The results revealed there was no preference in stimulation and no difference in latency to lever press and lever success rate to a specific paw in the bilateral pain condition. This suggests there was no difference in level of pain affect for neuropathic and inflammatory conditions. Pharmacological treatment of ketorolac and gabapentin did

not affect measures in the AAP and modified place escape avoidance paradigm. This research may reveal important information regarding about the lack of hierarchical aversiveness of the two most common pain models.

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Chapter 1

1. Introduction

1.1 Challenge and Dimensions of Pain

The challenge of pain primarily revolves around its multidimensional facets that influence the perception of pain. This variability has proven to be difficult in treating pain, and even more in producing an accurate definition (Mogil, 2009; Vierck, Hansson, & Yezierski, 2008; Vierck & Yezierski, 2015). Because pain is perceived only by the sufferer, specification of its features via verbal report may convey the experience of pain (Melzack, 2001; Melzack & Casey, 1968; Melzack & Wall, 1996). The specific language given to pain is typically goal-directed in trying to convince others that pain is indeed perceived and needs resolving (Eisenberger, 2012; Merskey & Spear, 1967). In fact, health care providers must attend to and use educated biases towards used language in order to produce possible cause and diagnosis of pain (Danziger, Prkachin, & Willer, 2006; de Waal, 2011; Melzack & Torgerson, 1971; Wilson, Williams, & Butler, 2009). Although seemingly trivial and common in practice, identifying semantically descriptive properties has led to a promising characterization of pain (Haefeli & Elfering, 2006; Melzack & Torgerson, 1971; Minde, 2006).

In 1971, Melzack and Torgerson took words commonly used to describe pain and asked participants to group them into categories in hopes to determine the different aspects of pain (Melzack & Torgerson, 1971). They found that pain could be classified semantically into three major divisions. The first division was derived of words describing the sensory qualities of pain and encoded stimulus parameters like size, location, and intensity of pain. The second division was characterized by affective traits that were expressed as emotional salience, unpleasantness, or distress from pain. The last division was comprised of evaluative qualities that described the holistic experience in terms of

overall intensity. The findings of this study qualified a previously proposed theory characterizing pain into those three groups, but further defining pain as multifaceted phenomenon consisting of sensory/discriminative, affective/motivational and cognitive/evaluative dimensions (Melzack & Casey, 1968). Although these dimensions may be separated from each other in characterization and neural structures, they do possess the ability to influence each other to modify the pain process (LaBuda & Fuchs, 2000; LaGraize, Labuda, Rutledge, Jackson, & Fuchs, 2004; Melzack & Wall, 1996). Due to this, the unique interactions and combinations from each dimension make up the total experience of pain and may likely drive the subjectivity of pain.

Clearly, clinical models have an extreme advantage in pain research due to the ability to communicate self-report measures of pain (de Waal, 2011; Vierck et al., 2008). Within preclinical realms, however, communicating pain remains a challenge. In fact, ensuring an animal is actually experiencing pain proves difficult without some verbal or nonverbal validation (Allen, Fuchs, Shriver, & Wilson, 2005). Yet the shared properties of nociception of humans and nonhumans, including anatomy, physiology, and behavior, along with the historical understanding of pain as a purely sensory modality, has allowed sensory reflexive information to dominate pain research (Melzack & Casey, 1968; Melzack, 1999; Mogil, 2009; Vierck et al., 2008). Sensory reflexive behaviors of both humans and nonhumans include assessments of innate withdrawal, licking, elevating, and the like towards the affected paw or body part. Although reflexive behaviors have provided critical information regarding causal neural processing of painful stimuli, they are predominately concentrated on the elucidation of peripheral and spinal mechanisms associated with pain processing. Thus, the description of sensory physiology does not provide much information beyond stimulus parameters, failing to accurately portray the

entire pain experience. As a result, the scientific community has taken steps to improve on this shortcoming.

To further characterize relevant assessments of pain processing similar to clinical realms, preclinical pain evaluations have begun to shift towards a more affective approach (LaBuda & Fuchs, 2000; LaGraize et al., 2004; McNabb, Uhelski, & Fuchs, 2012; Panksepp & Lahvis, 2011). As previously described, pain affect is comprised of the emotional understanding of pain's unpleasant and aversive qualities and the motivation to relieve pain. Emotion and motivation are tightly intertwined, where emotion, an interoceptive component, produces a behavioral motivation (Bradley & Lang, 2000; Craig, 2003; Lagraize, Borzan, Rinker, Kopp, & Fuchs, 2004). To quantify this dimension, use of non-reflexive measurements like escape and avoidant behaviors have been interpreted as indicators of the unpleasantness of pain (Fuchs & McNabb, 2012; Vierck & Yezierski, 2015). The justification arises from the assumption that since pain is an aversive, positive punishment, an animal would be motivated to terminate pain. As a result, pain would also provide a learning experience such that the animal would learn to avoid the painful environment (Fields, 2006). Various existing models for evaluating this component include conditioned place preference (Shippenburg, Stein, Huber, Millan, & Herz, 1988; Sufka, 1994), place escape/avoidance paradigm (PEAP) (LaBuda & Fuchs, 2000), two-temperature choice (Mauderli, Acosta-Rua, & Vierck, 2000), and conditioned place aversion (Johansen, Fields, & Manning, 2001), and do so by allowing an animal to choose between environments associated with either a noxious or non-noxious stimulus. For example, the PEAP utilizes instinctual preference to its advantage by stimulating an animal in the noxious paw when on the dark side of the chamber and stimulating the non-noxious paw on the light side of the chamber. If the animal shifts from the dark, preferred side to the light side (choosing which side is stimulated), it is assumed the shift in

preference indicates that the animal avoided the stimulus because pain was painful and aversive (LaBuda & Fuchs, 2000).

This measure of avoidance behavior within the PEAP has been validated as a measure of pain in subsequent studies. In fact, pharmacological work involving the use of analgesics revealed that both mechanical hypersensitivity and avoidance were attenuated with treatment revealing reduction of the intensity of pain will make stimulation less unpleasant (LaBuda & Fuchs, 2001). Furthermore, LaGraize et al., (2004) revealed lesions to the anterior cingulate reduced avoidance behaviors, but not within reflexive stimulus-evoked hypersensitivity (LaGraize et al., 2004). This finding demonstrated that the anterior cingulate cortex is involved in processing pain affect and is independent of the sensory dimension, suggesting the importance of using more than a sensory measurement to understand pain perception. Thus, these converging trends demonstrate PEAP as a valid measure.

Although the PEAP and similar paradigms have revealed critical understanding of affective mechanisms, these models only allow for escape and/or avoidance responses, which may not always be possible. Despite this limitation, these affective measures have also produced a more specific purpose of pain regarding homeostasis, especially when pain must be approached.

1.2 Pain as a homeostatic emotion

Pain has also been described a homeostatic emotion (Craig, 2003). In this context, the importance and the adaptive nature of pain are essential to the regulation of homeostasis (Salcido, Geltmeier, & Fuchs, 2018). Because homeostasis is an integral part of survival by maintaining optimal internal stability, pain stimulates homeostatic processes through sensory alarm, attention, and unpleasant affect to signal an imbalance in the body's normal set points. To promote further injury or death, motivation to relieve

the pain drives an organism to escape or avoid (Denton, McKinley, Farrell, & Egan, 2009; Kotas & Medzhitov, 2015; Salcido et al., 2018, Woolf, 2010). The rationale for this argument may be further addressed through drive reduction theory. Proposed by Clark Hull, drive reduction occurs in response to homeostatic disruption causing the organism to reduce the drive through behaviors, ensuring the organism's needs are met (Hull, 1943). As a result, reducing the drive promotes a need. Here in this case, pain (i.e. drive) is experienced by the organism and disrupts homeostasis (i.e. need), creating an unpleasant state (Figure 1). Since pain disrupts homeostasis, pain creates an imbalanced state, demands attention and requires a response that drives the organism to resolve, maintain, or revert to equilibrium. Thus, the interplay of both sensory and affective components of pain underscores the importance of homeostasis in the function of pain. In short, pain demands attention to maintain homeostasis and survival via cognitive choice and decisions (Salcido et al, 2018).

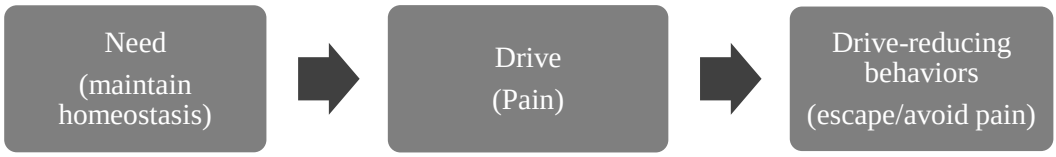


Figure 1: Drive-reduction theory (Hull, 1943). Drive-reduction theory posits all organisms contain needs primarily revolving around homeostasis. Drives change the balance of these needs promoting behavioral responses to reduce the drive and therefore fulfil needs. Essentially, motivation to survive is produced from an innate drive that ensures a constant need.

1.3 Homeostatic drives

Homeostasis is a complex system involving autonomic, neuroendocrine, and behavioral regulatory mechanisms (Cannon, 1929; McEwen & Wingfield, 2010). Minor and major disturbances such as hunger, food, and sex are constantly challenging this narrow ideal state. Yet, they rarely occur in isolation. As a result, concurrent disruption of homeostasis from different stressors can promote competition between the drives. An organism must then cognitively assess which drive to attend and satiate. To decide, Field's Motivation-Decision Model (Figure 2) posits that information regarding homeostatic status, sensory input, and prediction of future threats or rewards are all required for decision processes (Fields, 2006). As a result, decisions tend to the more salient drive. Salience is determined by focused attention and motivation for the prioritized drive. In the case of pain, this theory suggests if another drive like hunger is more salient than pain for survival, pain will be mediated by activating descending pain pathways producing anti-nociception. Conversely, if pain is more salient, the organism will relieve pain and forgo the other drive (Craig, 2003; Fields, 2006; Navratilova et al., 2014). As a result, this survival mechanism allows for the ability to attend to one of the competing motivational drives at a time, with attention given to the most aversive drive.

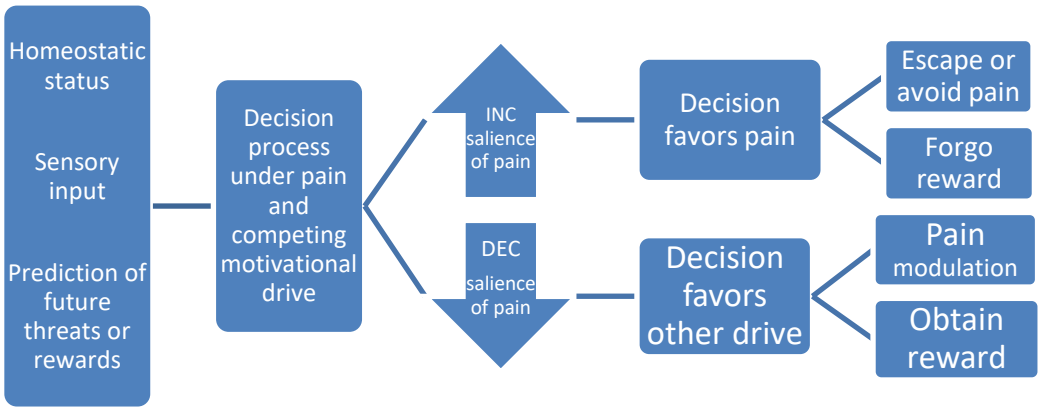


Figure 2: Schematic of Field's Motivation-Decision Model (Fields, 2006)

One common method of evaluating competing motivational drives is through the utilization of operant approach-avoidance paradigms (Higgins, 2012; Lagraize et al., 2004; Salcido, Harris Bozer, McNabb, & Fuchs, 2018). An approach-avoidance conflict poses a unique situation associated with both reward and punishment such that an animal must choose which to approach and which to avoid. By assessing value of costs and benefit to the available options, an animal will choose the most optimal outcome. Preclinical approach-avoidance assessments have previously utilized basic needs such as hunger and pain (Elliot, 2006). In such cases, these studies have revealed that pain typically more salient than hunger when animals are facing large appetitive reward in exchange for pain. In one particular study, LaGraize et al. observed differences in lever responses for food reinforcement in rodents during the separate phases of formalin (Lagraize et al., 2004). This study revealed a suppression of lever responses for appetitive reward in the first phase of the formalin test and an increase in lever responses during the second phase. Because the biphasic quality of the formalin test derives from activation of first-order neurons in the first phase and the second phase results from sensitization of dorsal horn neurons, these results may implicate that formalin pain was more salient than hunger when pain resulted from TRPA1-mediated excitation of nociceptors rather than inflammatory and central sensitization mechanisms suggesting different components of pain may differ in level of aversiveness (Mcnamara et al., 2007; Munro, 2009; Pitcher & Henry, 2002).

Thus, approach-avoidance studies such as this can provide evidence of hierarchical prioritization among conflicting drives. And although they have predominantly evaluated the relationship of hunger and pain, it may be possible to assess other conflicting drives amongst the simultaneous stress of pain. In fact, these paradigms may be useful in operationalizing the competition between two subtypes of chronic pain.

1.4 Division of chronic pain

Over 25.3 million American adults experience chronic pain at any given time (Nahin, 2015), affecting more Americans than diabetes, heart disease, and cancer combined. Chronic pain is characterized as a disproportionate pain that persists longer than the removal of the noxious stimulus, beyond tissue damage repair, and for at least 3-6 months (Woolf, 2010). Due to the maladaptive and disadvantageous nature of chronic pain, it is the leading cause for physician consultation and is associated with several inconveniences. In addition to physical distress, pain is a financial burden and is currently estimated to cost over a half a trillion dollars annually (Gregory et al., 2013; Raffaeli & Arnaudo, 2017). Other consequences of chronic pain are reduced quality of life, coping abilities, social interactions, health, sleep quality, and mood (Gagliese, Gauthier, Narain, & Freedman, 2018; Gatchel, Peng, Peters, Fuchs, & Turk, 2007; Lumley et al., 2011). As a result, the phenotype of chronic pain has distinct social, sensory, emotional, psychological, and behavioral implications that call for an overwhelming need to understand the mechanisms in order to relieve chronic pain.

In addition to the ease of manipulation and control, preclinical models have been greatly utilized to understand chronic pain due to the invasive nature and inability to treat or reverse progression of chronic pain (Barrot, 2012; Mogil, 2009; Vierck et al., 2008). Commonly used models include inflammatory and neuropathic conditions such as carrageenan and spinal nerve ligation, respectively (Barrot, 2012; McNabb et al., 2012). Each of these models have been validated and seem to produce similar results comparable to clinical pain.

Hypersensitivity, or tenderness, is a typical clinical pain response that has also been reported in inflammatory and neuropathic animals (Barrot, 2012; Gregory et al., 2013; Negus et al., 2006; Steeds, 2009; Woolf, 2010). This is observed both dependently

and independently of stimulation (Costigan, Scholz, & Woolf, 2009; Steeds, 2009). Stimulus-independent pain is likely caused by spontaneous activity of pain pathways and sensitization of neurons. Stimulus-dependent hypersensitivity has two distinct features in response to stimulus-evoked pain known as allodynia and hyperalgesia. Allodynia is characterized as increased pain response to a previously innocuous stimulus. While these sensory modalities are relatively easy to demonstrate within animal models, they only illustrate one characteristic of the pain experience and do not implicate cause (Gregory et al., 2013; Vierck et al., 2008; Yezierski & Hansson, 2018). And though these conditions may share these features, likely due to distinct neural mechanisms associated with each dimension, they do differ in etiology, progression of time course, treatment, and other characterizations (Campbell & Meyer, 2006; Costigan et al., 2009; Jensen, Gottrup, Sindrup, & Bach, 2001; Nicholson, 2006; Ren & Dubner, 1999; Xu & Yaksh, 2011).

1.4.1 Neuropathic Pain

Neuropathic pain can be described as a “disease state of the nervous system” and is the result of either damage or disease to the somatosensory nervous system (Merskey & Spear, 1967; Woolf & Mannion, 1999). Causes of neuropathy include systemic (i.e. diabetes), immune, or inflammatory disease, chemotherapy, genetic, trauma, viral infection, vitamin deficiency (Costigan et al., 2009; Woolf & Mannion, 1999; Xu & Yaksh, 2011b). Impairment is observed as either positive (gain of function) or negative (loss of function) symptoms. Positive symptoms include sensation of pain, tingling, and experience of pins and needles, as well as muscle cramps and twitching. Negative symptoms comprise numbness, deficits in balance, atrophy, and weakness. These symptoms are result from several physiological changes reflected as both peripheral and central sensitization. Altered ion channel expression, sprouting of injured axon and dorsal root ganglia, inflammatory and non-neuronal activation, and sensitization

of nociceptors are some of the peripheral implications. Pathophysiology pertaining to central mechanism include disrupted connections in the spinal cord due to neural death, reorganization of cortex, and altered spinal and brain connections (Boyce-Rustay & Jarvis, 2009; Bridges, Thompson, & Rice, 2001; Campbell & Meyer, 2006; Wall & Melzack, n.d.; Woolf & Mannion, 1999).

As previously mentioned, spinal nerve ligation is a widely used model of neuropathic pain. Developed by Kim and Chung (1992), the model involves the unilateral ligation of the L5 segmental spinal nerve of a rat. Its robust and reliable in causing hyperalgesia and allodynia and is relatively long lasting with effects persisting up to 7 weeks after surgery. The mechanism is thought to sensitize central signaling neurons via injured L5 and intact L4 spinal nerves. Injury to the L5 causes development of spontaneous activity. This enhanced activity is also coupled with projection of L5 injury to the intact L4 causing alterations in response, descending modulation, and inhibitory mechanisms (Campbell & Meyer, 2006; Kim & Chung, 1992).

Because there is no cure for neuropathic pain, drug treatments seek to reduce the sensory component of pain. Satisfactory pharmacotherapy treatments include NMDA antagonists, gabapentinoid, and anticonvulsant drugs (Woolf & Mannion, 1999; Xu & Yaksh, 2011). Of these, gabapentin is considered the leading neuropathic pain treatment (Nicholson, 2006; Xu & Yaksh, 2011). The mechanism for gabapentin is still unknown yet proves to be highly effective (Munro, 2009).

1.4.2 Inflammatory Pain

Inflammatory pain can result from neural tissue damage, like in response to neuropathic injury, but may also involve nonneural tissue damage (Melzack & Wall, 1965; Woolf, 2010). In the periphery, development of inflammation results from several cascading biochemical and immunological factors from local and migrating inflammatory

and damaged cells. Inflammatory mediators such as cytokines, chemokines, bradykinins, and prostaglandins are released, increasing blood flow and fluid leakage in the affective area as well as stimulation of nociceptors to cause pain (Barrot, 2012; Kotas & Medzhitov, 2015; Ren & Dubner, 1999). This reaction is phenotypically presented as redness, swelling, heat, hypersensitivity, and loss of function to the affected area. Though initially considered adaptive by reducing the probability of further damage via hypersensitivity promoting healing, inflammatory pain may also be nonadaptive when persistent (Costigan et al., 2009; Fehrenbacher, Vasko, & Duarte, 2012; Wall & Melzack, n.d.; Woolf, 2010). Changes in neurons of the dorsal root ganglia and dorsal horn are what mediate persistent pain. At the dorsal horn, this increases outputs facilitating projection of pain (D'Mello & Dickenson, 2008; Livingston, 2000; Millan, 1999; Xu & Yaksh, 2011).

Induction of carrageenan is a common preclinical inflammatory assessment similar to persistent injury (Ren & Dubner, 1999). Experimental application of carrageenan generally involves a subcutaneous injection to the hindpaw of a rat to produce localized inflammation. It reliably responds in a biphasic quality classified by the release of certain inflammatory mediators. Within the first hour, the early phase is characterized by histamine, serotonin and bradykinin release and does not respond to non-steroidal anti-inflammatory drugs. The second phase is primarly associated with overproduction of prostaglandin in tissues and is also accompanied by bradykinin, protease, lysosomal enzyme release. NSAIDs are effective in this stage. Due to its inflammatory qualities, it produces maximal edema at 3-5 hours after injection and may be reinjected daily to produce long term effects.

Thus, pharmacological treatments for carrageenan inflammatory pain are dependent upon the phases regarding non-steroidal anti-inflammatory drugs. NSAIDs are

widely used for chronic inflammatory pain due to their ability to inhibit cyclooxygenase. Ketorolac is a reliable NSAID due to its high therapeutic index for mild to moderate pain ((Guzmán-Priego et al., 2017; Russo et al., 2017). Other treatments include corticosteroids, antagonists of inflammatory mediator substances, and opiates (Wall & Melzack, 2005). The use of anticonvulsants, like gabapentin, as an effective analgesic proves equivocal where some studies show promising results (Hanesch, Pawlak, & McDougall, 2003), others only for certain assessments (Field et al., 1997), or no effect at all (Gould III, Gould, Minor & Paul, 2002; Patel et al., 2001). This may be largely attributed to the dose-dependent nature of the drug, administration, and the tests used to evaluate.

1.4.3 Perceived differences in humans

Fortunately, due to verbal report, humans are cognitively able to compare several types of pain. The most widely accepted and validated test for measuring clinical pain is the McGill Pain Questionnaire (Haefeli & Elfering, 2006; Melzack, 1987; Wilson et al., 2009). This measurement is effective in that it provides information from all three dimensions of pain such that differences in each modality can be assessed using language provided by the sufferer. In fact, Melzack (1975) assessed phantom limb (neuropathic pain syndrome) and arthritis (chronic inflammatory disease) according to words chosen and reported that although the two types were relatively similar there were differences in the Pain Rating Index (PRI) such that the sensory and affective components of pain were relatively lower for arthritic individuals than phantom limb patients (Ronald Melzack, 1975). Beyond this, no known comparison across comorbid subtypes of pain have been assessed such that assumptions on what type of pain is more aversive has not been evaluated. Instead, the focus rests on the similarities and differences of mechanism.

1.4.4 Perceived differences in animals

To the author's knowledge, there are only two studies that have tried to evaluate the aversive grading of these two types of pain. The published work of McNabb et al. (2012) utilized a modified PEAP (mPEAP) box to compare affective processing in L5 spinal nerve ligation and carrageenan conditions. Instead of the prototypical light/dark box implemented in the PEAP, the mPEAP designed a box with alternating white and black stripes that were horizontal on one side and vertical on the other in hopes to produce an unbiased approach by eliminating preference where neither side is preferred. This allowed a comparison of a simultaneous bilateral pain condition where a SNL was introduced to the left paw and carrageenan injection was subjected to the right paw. The results of the study revealed no preference in time spent on one side of the box over the other for bilateral conditions whereas unilateral conditions preferred stimulation of the non-noxious paw (McNabb et al., 2012). This finding suggested that pain affect may not differ across pain types in this assessment.

In a separate study, Harris (2013, unpublished) devised a novel approach-avoidance paradigm utilizing lever presses for appetitive reward to possibly establish hierarchical evaluation of inflammatory and neuropathic pain conditions. In this Multichoice Approach-Avoidance Paradigm (MAAP), animals were presented with three choices: 1) press the left lever and receive stimulation of the paw associated with the SNL condition, but receive appetitive reward 2) press the right lever and receive stimulation of the paw with a carrageenan condition, but receive appetitive reward, or 3) press no lever resulting in no stimulation and no appetitive reward. As a result, each trial allowed the animal to freely choose between the three choices ultimately indicating preference of stimulation in a paw with a neuropathic or inflammatory condition through its associative lever. Although a clever paradigm, the results of the study proved

ambiguous. Unfortunately, there was no preference for stimulation of either pain condition in the bilateral pain groups and additionally unilateral groups (sham/carrageen and SNL/saline) failed to show preference for a particular lever, unlike what washypothesized (Harris, 2013). Since studies have previously shown stimulation to the affected paws associated with those conditions was aversive, this finding was unexpected, but these results were thought to be a product of generalization due to the proximity of the levers.

1.6 Rationale and Purpose

Taken altogether, the results of research regarding levels of pain affect seem to be ambiguous. Although these two previous studies may point to no apparent differences in the affective component of these pain types, they seem counterintuitive from the results found by LaGrazie (Lagraize et al., 2004). Because differences in preference across formalin phases were revealed, this suggests that diverse causes of pain can produce different levels of pain aversion. It may be possible that the mPEAP and the MAAP may not be successful in accurately portraying the entire pain affect experience. Specifically, they may have not be able to either assess the quality of pain affect as proposed by McNabb et al. or may have been too complex such that the rodent was unable to successfully distinguish the outcomes associated with each lever/condition as within the MAAP (Harris, 2013; McNabb et al., 2012). Though these paradigms could be valuable tools, the inability to model clinical findings also poses a problem.

Evidence from clinical reports suggest that differences in the physical, emotional, and cognitive qualities of pain can be highlighted (Gatchel et al., 2007; Haefeli & Elfering, 2006; Melzack, 1975, 1987). Furthermore, based on the multidimensional approach of pain and that certain pain states involve unique profiles, individual modalities are likely to also differ across pain types. This argument may be further qualified with mechanistic differences in inflammatory and neuropathic pain that can change the perceived

experience of pain (i.e. heat versus pins and needles) (Woolf, 2010). And because there is no panacea for pain, this may pose a great advantage in possible selective treatment that may be used to determine if lack of behavioral preference still remains.

Therefore, the purpose of this study was to assess levels of aversion between the two most commonly used pain models with the aid of hunger in a competing motivational paradigm of a simplified version of Harris' approach. Instead of Harris' use where animals could choose between all three choices, this paradigm presented one lever at a time forcing the animal to choose between pressing or not pressing a lever. Thus, subjects were given the choice to forgo appetitive reward by not receiving noxious stimulus of either inflammatory or neuropathic condition or b) press the presented lever and receive noxious stimulus to the associated paw in exchange for appetitive reward. Based on previous research, it was predicted that more aversive drives would be satiated, allowing for comparison of the two conditions. Pharmacological treatment was also assessed in selectively attenuating each condition to reveal if approach-avoidance behavior could be modulated or reversed.

1.7 Specific Aims

The specific aims of this study were as follows:

(1) To demonstrate the selective analgesic effect of drugs on inflammatory and neuropathic conditions.

Hypothesis: Gabapentin would selectively attenuate mechanical hypersensitivity for SNL and ketorolac would selectively act on carrageenan-induced mechanical sensitivity.

(2) To measure the magnitude of pain affect in the approach-avoidance paradigm (AAP) in response to lever presses for each condition.

Hypothesis: Lever presses would be significantly more suppressed for SNL groups than carrageenan animals.

(3) To evaluate the effect of condition on latency to lever press.

Hypothesis: Pain animals would show increased latency to lever press compared to non-pain conditions and drug animals would decrease latency compared to control animals.

(4) To assess the differences in the AAP and mPEAP in quantifying pain affect.

Hypothesis: AAP may show differences in pain affect compared to mPEAP outcomes.

Chapter 2

2. Experimental Design of Dissertation

2.1 Materials and methods

All procedures for this dissertation were approved by the University of Texas at Arlington Institutional Animal Care and Use Committee and in accordance with the guidelines of the International Association for the Study of Pain.

2.1.1. Animals and procedures

Seventy-two adult Sprague Dawley rats were purchased from Charles River, placed in single housing in a separate colony room on a 12:12 dark/light cycle, and allowed to habituate for seven days. Afterwards, animals were placed on a food-controlled diet with a variable time feeding schedule until 85% of original weight was achieved. Water remained ad libitum.

Once at 85% of original weight, animals were trained to lever-press for appetitive reward. Animals varied on the number of training days to ensure they reached criteria for test day (80% response rate), but had a minimum of seven training days. Training occurred once a day on a variable schedule in standard operant chambers (Med

Associates, Inc.) and animals were shaped through successive approximations to press a single lever for appetitive reward (45 mg grain based pellet). On baseline day and test day (three days later), animals were subjected to lever press for appetitive reward in a modified operant box described further as approach/avoidance paradigm (AAP).

2.1.2 Training phases

Day one of training consisted of an manual training phase, where animals were exposed to the paradigm. A light above the lever, a light cue that was also used later on test day to determine which paw will be stimulated in the paradigm, began the training and was presented for 5 seconds before the lever was presented for one second and then retracted. This was automatically operated by the program such that animals did not have to perform. At the initiation of the retraction of the lever, one pellet was dispensed. This sequence occurred every 30 seconds until 60 trials had been completed. Inclusion criteria to meet the next training phase occurred when animals had successfully associated the lever retraction with appetitive reward, which was signified by the consumption of all 60 pellets from the training.

On the second phase of training, animals were subjected to another manual training where the light indicating the choice lever remained on for 5 seconds before the lever was presented. Afterwards, the lever remained out for the animal to lever press, at which, the lever retracted back in, and a new trial started with the presentation of the lever again, and one pellet was dispensed. This continued for a total of 30 minutes and animals could lever press as many times as they chose in that time. Animals who had successfully lever-pressed for 40 times or more met criteria to move onto the next phase of training.

On the third phase of training, animals were subjected to an automatic phase training. This training was characterized by the presentation of the light above the lever

that would be presented. Afterward, the lever was presented for 10 seconds before the lever retracted back in. If the animal lever-pressed within the 10 seconds, a pellet was dispensed, the lever retracted back in, the lever light was turned off and a timeout period of 25 seconds before the next trial was initiated. This occurred every 35 seconds for a total of 60 trials, where the light was presented, then the lever was presented, of which the light remained on while the lever remained out, and until the lever was pressed or the time allotted ran out, the lever was retracted back in and the light turned off and a timeout of 25 seconds began. Animals who had successfully lever-pressed for at least 80% of trial (i.e. lever-pressed for at least 48 out of the 60 trials or omitted no more than 12 trials) to move onto next level of criterion.

On the fourth training phase, animals were subjected to a dual training. In this task, it modeled the automatic training, but both levers and lever lights were presented. This training would occur until there was an no preference for a particular lever which was indicated by no more than 65% of lever presses occurring at one single bar. Animals had to meet this criteria in addition to at least 80% bar pressing success to move on to baseline avoidance approach paradigm (AAP) testing.

On baseline day, animals were placed into the AAP used on testing day. However, no pain or drug manipulations were present. To produce the AAP box, a standard operant chamber (MedPC) was removed from the sound attenuating box hub and the steel rod floor was removed. The chamber was fixed atop a mesh floor PVC platform so that a mechanical stimulus could be applied to the plantar surface of the hindpaws. The transparent outside walls of the chamber were covered in black contact paper to avoid any outside influence. Essentially, modifying an operant chamber allowed stimulation of the plantar surface of the hind paws. In this phase, the lever was presented every 35 seconds and remained out for 10 seconds. Five seconds before the lever was

presented, the light cue above the lever remained on and used to determine which paw to stimulate in response to a lever press. Assessing the behavior prior to testing conditions allowed for a baseline assessment of the paradigm. Once animals had successfully lever-pressed at least for 80% of trials (i.e. lever-pressed for at least 48 out of the 60 trials or omitted no more than 12 trials) animals moved onto surgery and then test day.

The criteria for animals to advance through the experimental protocol are described in Table 1.

Table 1: Criteria and procedure for approach/avoidance paradigm (AAP).

Table 1

Criteria and Procedure for AAP

Daily	Procedure	Inclusion Criteria
Weigh/Feed	Food-Controlled Diet	Training begins at 85% of free access weight
Weigh/Feed	Manual Training Phase 1 (MT1) (counterbalanced)	Must asociate lever presses with food reward in hopper
Weigh/Feed	Manual Training Phase 2 (MT2) (counterbalanced)	Must achieve over 40 lever presses
Weigh/Feed	Automatic Training (counterbalanced)	Must achieve 80% of presses
Weigh/Feed	Dual Train	Must achieve 80% of presses; 65% unbiased pressing
Weigh/Feed	Baseline AAP Test	Set baseline for animals under normal conditions
Weigh/Feed	Baseline MPWT	No sensitivity in hind paws
Weigh/Feed	L5 Ligation or Sham Surgery	3 days recovery
Weigh/Feed	Test Day	
	Plethysmometer Assessment	Baseline Assessment
	Saline or Carrageenan Injection	Randomly assigned
	Gabapentin, Ketorolac, or Saline Injection (2.5 hours after initial injection)	Randomly assigned
	Priming	Must lever press for 10 trials
	AAP Test	
	mPEAP Test	
	Post MPWT (three hours after initial injection)	Ensure effectiveness of carrageenan and ineffectiveness of saline to sensitivity
	Plethysmometer Assessment	Quantify inflammation of CARR

2.1.3 Mechanical Paw Withdrawal Testing (MPWT)

Once criteria were reached and prior to manipulation, animals were subjected to mechanical paw withdrawal threshold testing (MPWT). For this, animals were placed into

Plexiglas chambers, atop a mesh floor to allow access to the hind paws for tactile stimulation and left to habituate for ten minutes. Tactile sensitivity was measured using the up/down method to the plantar portion of the hind paws using a set of von Frey monofilaments (3.85, 5.68, 9.74, 18.39, 39.42, 77.3, 135.3, and 251.34 mN). Each trial of testing began with the 9.74 mN von Frey filament delivered to the left hind paw for approximately 1 second, then to the right paw, or vice versa depending on orientation of the animal. If no withdrawal response was observed (i.e. paw withdrawal or licking), the next highest force was used, whereas the next lowest force was delivered if a response was observed. This procedure was repeated until no response was made at the highest force (251.34 mN) or until five stimuli were administered in total. The 50% paw withdrawal threshold for each trial was calculated using the following formula: [Xth]log = [vFr]log + ky, where [vFr] was the force of the last von Frey used, k = 0.2593 is the average interval (in log units) between the von Frey monofilaments, and y was a value that depends upon the pattern of withdrawal responses. If an animal did not respond to the highest von Frey monofilament (251.34 mN), then y = 1.00 and the 50% mechanical paw withdrawal response for that paw was calculated to be 456.63 mN. This test was then conducted three times and the scores from each trial were averaged to determine the mean threshold to tactile stimulation for the right and left paws for each animal (Dixon, 1960). MPWT testing occurred prior to surgery (baseline) and after plantar and intraperitoneal injections (post).

2.1.4 L5 Spinal Nerve Ligation Surgery (SNL)

Once animals advanced within criteria, animals were subjected to either SNL (n = 36) or sham surgery (n = 36). To do so, animals were put under isoflourane anesthesia (3% initiation and 2 % maintenance). For SNLs, ligation procedures followed methods

previously described by Kim and Chung (1992). After shaving and skin preparation with the aseptic betadine, a 1-1.5 inch incision was made slightly left of the spinal cord. A portion of the transverse process was then removed to uncover the L5 spinal nerve which was tightly ligated using a 6-0 silk suture. After suturing of the muscle layer, the skin was be stapled (LaGraize et al., 2004). Sham surgeries were conducted in a similar fashion, apart from the removal of the transverse process and ligation of the nerve. During surgeries, the breathing rate was recorded every 15 minutes on an operative sheet. Three full days of recovery was allotted before testing occurred, and animals were monitored daily after surgeries for eating, drinking, activity, heavy breathing, chromodachyhorrea, wound status, and rough hair coat (indicating severe distress) for each day after surgery.

2.1.5 Test day

On the test day, animals were randomly assigned a subcutaneous injection into the plantar surface of the hindpaw with either 5mL of 1% carrageenan lambda (Sigma) (n =36) (or normal saline (n =36)) to induce an acute inflammatory pain condition. Animals were then allowed to habituate. 2 hours 30 minutes after injection, animals were assigned an intraperitoneal injection of either gabapentin 100 mg/kg (n = 24), NSAID 10 mg/kg (n = 24), or saline (n = 24). A MPWT test was then performed 30 minutes later to ensure the effectiveness of carrageenan and SNL to induce hypersensitivity.

Immediately after MPWT, animals were placed in the operant approach/avoidance paradigm (AAP) to quantify the animal's approach/avoidance behavior associated with the presentation or lack thereof of a noxious stimulation.

Before testing, animals were subjected to a priming, where animals had to successfully lever-press for 10 consecutive times. This was done to ensure the animal was be able to transfer performance of the task learned in the standard operant chamber

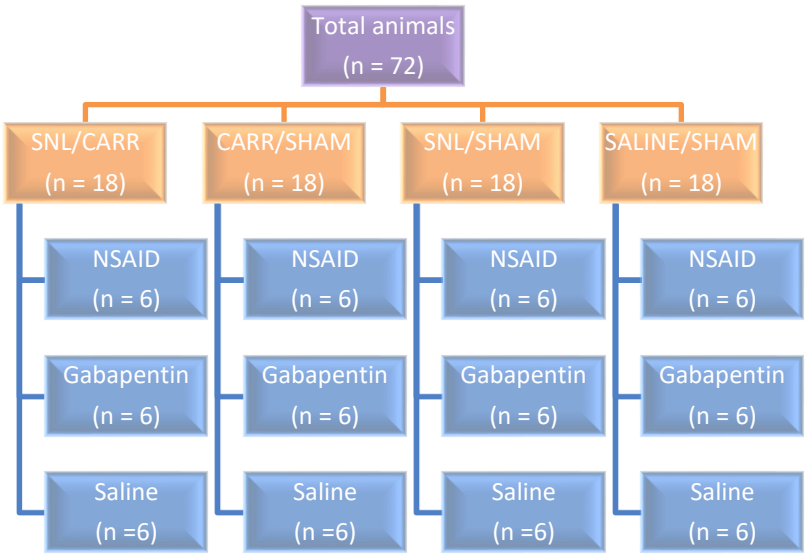
used during lever press training to the modified operant chamber and that pain had not impacted performance. During the test session, a light cue above the lever was presented for 5 seconds and then a lever was presented for 10 seconds at 30-second intervals for a total of 60 trials. Animals were able to lever press once during this 10 second interval, at which the lever retracted back in, one pellet was dispensed, and the 25 second timeout began. Thus, the single pressing of the lever signified the end of the trial. If the animal did not lever-press within the 10 second interval, the trial would end, was considered an omission, and a new trial would commence in another 25 s. This allowed for a minimum of 0 pellets and a maximum of 60 pellets within the paradigm, in which the number of lever-presses correlated with the number of pellets received. Lever presses for appetitive reward were immediately followed by stimulation of the paw associated with a suprathreshold (465 mN) Von Frey filament, while no lever responses resulted in no stimulation. The light cue was be randomly presented 5 seconds before the lever is dispensed to determine which foot was stimulated and allowed for 30 sessions for each hindpaw/lever. This allowed the rat to be presented with an approach-avoidance conflict in which animals were presented with two behavioral choices: (1) press the lever and receive noxious stimulation to the associated paw and appetitive reward or (2) not press the lever, avoid stimulation to the associated paw and forego appetitive reward. Suppression of reward seeking was viewed as an indication of the unpleasantness of the noxious stimulation. The number of trials yielding a response as well as latencies to lever-press was recorded via MED-PC operant coding by Med Associates for each trial. Therefore, the conditions were as follows:

Pain condition:

Left leg: spinal nerve ligation (SNL) or sham

Right leg: carrageenan (CARR) or saline

Drug condition: NSAID (ketorolac), gabapentin, or saline



2.1.6 Modified Place escape avoidance paradigm (mPEAP) testing

Immediately following AAP testing, animals were subjected to the mPEAP. During this 30-minute test, animals were placed into a half-vertical/half-horizonal black and white striped Plexiglas chamber (40.5 x 30.5 x 15.5 cm) atop a mesh screen to where the plantar surface of the hind paws was stimulated every 15 seconds with a suprathreshold Von Frey filament (476mN of force). The alternating black and white stripes provided a distinct, but neutral environment such that the procedure utilized an unbiased approach. Each side of the chamber was paired with stimulation of either the left or the right hindpaw, and this association was randomized between subjects. The duration of time spent within each side of the chamber was recorded as well as the total

number of line crosses from one side to the other. In this paradigm, the animal “learned” the association between paw stimulation and side of the chamber. The animal would then have the “choice” of which side to occupy and therefore which paw that was stimulated. The quantitative measure for the aversive quality of the experimental conditions was the percentage of time spent receiving stimulation of each paw.

The wire mesh platform and chamber was cleaned between test subjects to minimize scent cues and subjects were also randomly assigned to placement of the horizontal side of the chamber on either the right or left side of the mesh. Subjects that did not cross over to both sides of the chamber during the first 5 minutes were excluded.

2.2 Statistical analyses

Data analyses were performed using SPSS. To analyze mechanical paw withdrawal thresholds (MPWT) for the hindpaws, a between subjects ANOVA was used to assess then mean mechanical thresholds of pain (carrageenan and SNL) and drug (gabapentin, ketorolac, saline) treated animals. To analyze the percentage of trials that animals pressed the lever for appetitive reward, an ANOVA was performed to evaluate group differences for left and right levers. Analysis of latency to press the lever for appetitive reward utilized a mixed repeated measures ANOVA with pain condition (carrageenan, SNL, saline, sham) as the between-subjects variable and time (baseline and test) as the within-subjects variable. Avoidance behavior in the mPEAP paradigm was assessed using mixed repeated measures ANOVA with pain condition (carrageenan, SNL, saline, sham) as the between-subjects variable and time (across 30 minutes in 2-minute time bins) as the within-subjects variable.

Chapter 3

3. Results

3.1 Mechanical Paw Withdrawal Threshold (MPWT) and Paw by Condition

Left Paw (SNL or sham condition). A 2(pain condition) x 3(drug condition) between-subjects ANOVA was used to assess the group means of mechanical thresholds in pain and drug conditions on baseline and test day for the left paw. As expected, results for baseline mechanical thresholds revealed no significant differences in pain, $F(1, 66) = 1.00$, $p = .32$, or drug condition, $F(2, 66) = 1.00$, $p = .37$ (data not shown).

Data from test day revealed there was a significant main effect of pain condition, $F(1, 66) = 212.93$, $p < .001$, suggesting there was a significant decrease in paw withdrawal thresholds for SNL animals ($M = 123.89$, $SD = 15.13$) compared to sham animals ($M = 436.02$, $SD = 15.13$). There was also a significant main effect of drug, $F(2, 66) = 21.08$, $p < .001$. Simple effects revealed that animals treated with gabapentin had higher thresholds ($M = 377.79$, $SD = 18.52$) than ketorolac ($M = 238.34$, $SD = 15.13$) and saline ($M = 223.73$, $SD = 15.13$) treated animals, $p < .001$, while ketorolac treated animals similar thresholds compared to saline treated animals, $p = .58$, ns. These main effects were also qualified by a significant pain x drug interaction, $F(2, 66) = 14.66$, $p < .001$ (Figure 3).

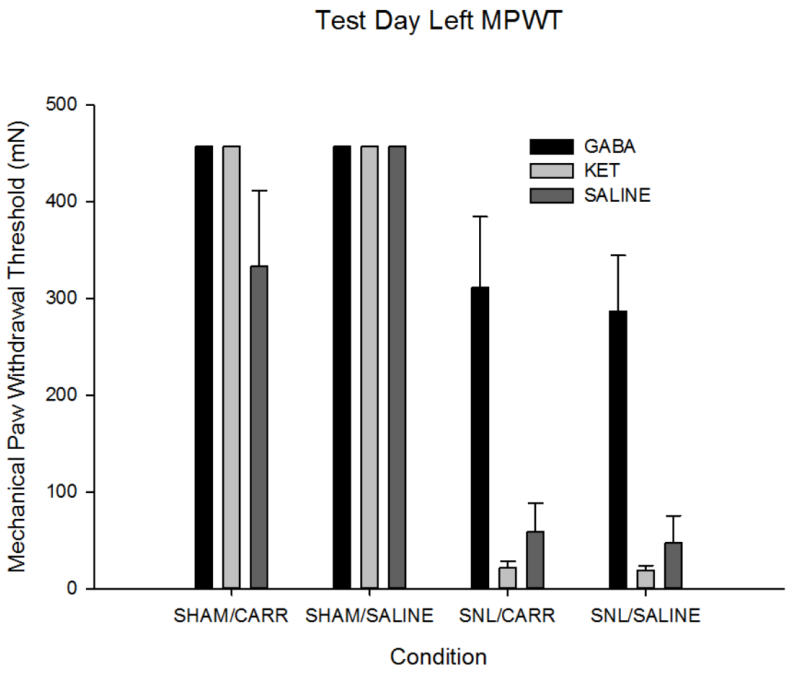


Figure 3: Mechanical Paw Withdrawal Threshold by Pain Group for Left Paw on Test Day. As expected, the neuropathic condition, SNL, reduced thresholds.

Abbreviations: GABA, gabapentin; KET, ketorolac

Right Paw (carrageenan or saline). A 2(pain condition) x 3(drug condition) between-subjects ANOVA was used to assess the group means of mechanical thresholds in pain and drug conditions on baseline and test days for the right paw. As expected, results for baseline of the right paw revealed no significant differences in pain, $F(1, 66) = 2.00$, $p = .16$, or drug condition, $F(2, 66) = .500$, $p = .61$ (data not shown).

Test day results revealed there was a significant main effect of pain condition, $F(1, 66) = 109.96$, $p < .001$, such that withdrawal thresholds for carrageenan animals were lower ($M = 165.56$, $SD = 18.28$) than sham animals ($M = 436.57$, $SD = 18.28$). There was also a significant main effect for drug, $F(2, 66) = 7.59$, $p = .01$, revealing group differences among drug treatment. Simple effects revealed that gabapentin ($M = 312.01$,

SD = 22.38) and ketorolac (*M* = 356.17, *SD* = 22.38) treated animals had higher thresholds than the saline treated group (*M* = 234.67, *SD* = 22.38), *p* = .017 and *p* > .001, respectively. Unexpectedly, there was no difference in thresholds between gabapentin and ketorolac treated animals, *p* = .16, *ns*. These main effects were also qualified by a significant pain x drug interaction, *F*(2, 66) = 6.58, *p* = .002 (Figure 4).

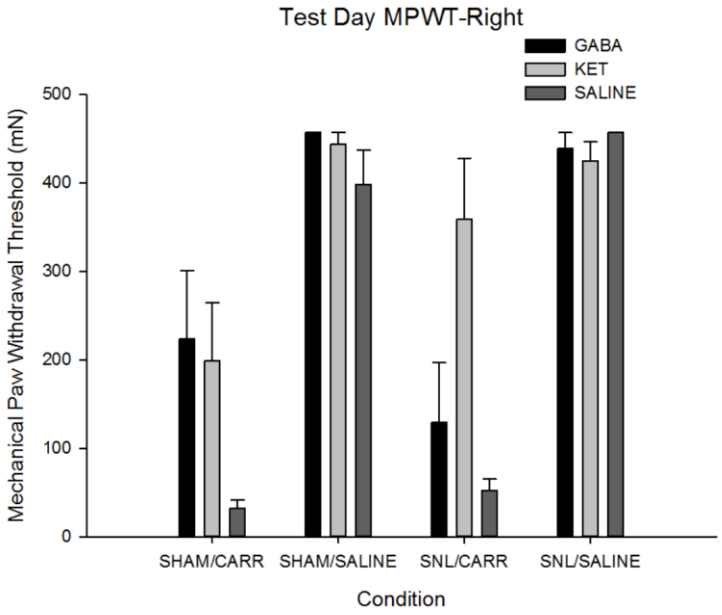


Figure 4: Mechanical Paw Withdrawal Threshold across pain Groups for right paw on Test Day. As expected, the inflammatory condition, carrageenan, reduced thresholds. Abbreviations: GABA, gabapentin; KET, ketorolac

3.2 Paw Volume and Paw by Condition

Right Paw Volume. To assess the effect of carrageenan to induce inflammation in means of right paw volume, a 2(pain condition) x 3(drug condition) between-subjects ANOVA was used.

Data for baseline revealed there was a no main effect for pain $F(1, 66) = 2.60$, $p = .08$ or drug condition $F(2, 66) = .01$, $p = .94$ (data not shown). On test day, the data revealed a main effect for pain condition, $F(3, 55) = 21.47$, $p < .001$, suggesting a significant increase in paw volume in carrageenan-treated animals over saline-treated animals, and a main effect of drug, $F(2, 55) = 5.35$, $p = .007$ (Figure 5). There was no main interaction effect, $F(3, 55) = .88$, $p = .42$.

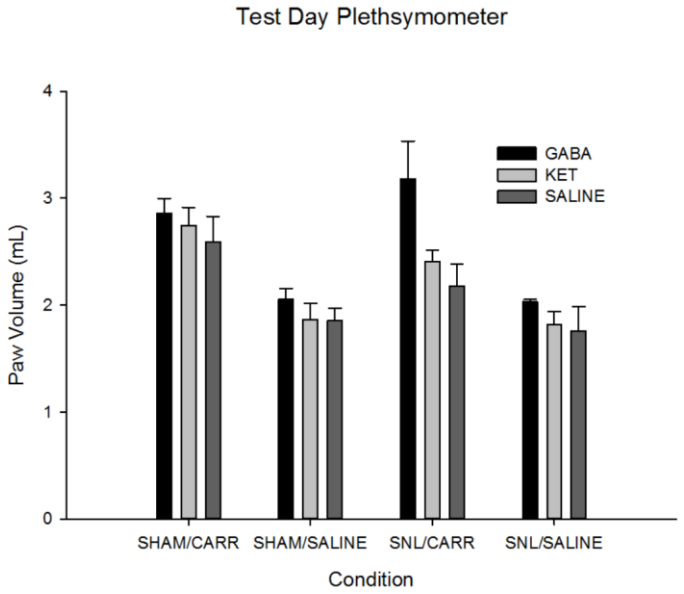


Figure 5: Paw volume across conditions for right hindpaw. As expected, there were increased paw volumes for the inflammatory condition, carrageenan.

Abbreviations: GABA, gabapentin; KET, ketorolac

Left Paw Volume. To ensure the inflammatory pain condition is isolated to the right side and did not affect the left paw, a 2(pain condition) x 3(drug condition) between-subjects ANOVA was used.

Data for baseline revealed there was a no main effect for pain $F(1, 66) = .08$, $p = .78$ or drug condition $F(2, 66) = .05$, $p = .96$ (data not shown). Data for test day also

revealed no main effect for pain, $F(1, 66) = 2.06$, $p = .16$, or drug condition $F(2, 66) = .89$, $p = .42$ (data not shown).

3.3 AAP Lever Presses by Condition

A 4(pain condition) x 3(drug condition) x 2(time) mixed-model ANOVA was conducted to assess group differences in the overall number of lever presses for appetitive reward for left or right levers across time.

Unexpectantly, left lever (associated with the stimulation of the left paw (SNL/sham)) success rate revealed there was no main effect of time, $F(1, 60) = 2.45$, $p = .12$, pain, $F(3, 60) = .27$, $p = .85$, or drug $F(2, 60) = 2.05$ $p = .14$. (Figure 6)

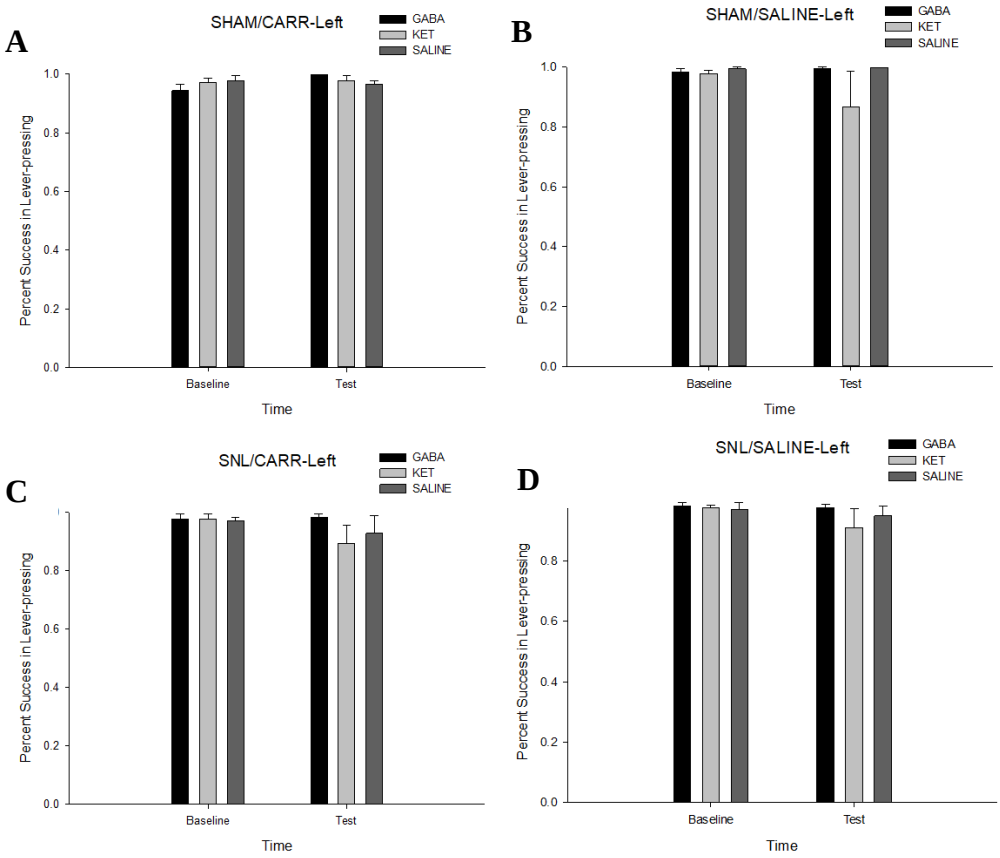


Figure 6: Percent success in lever-pressing the left lever for a) sham/carrageenan, b) sham/saline, c) SNL/carrageenan, d) SNL/saline groups.

Abbreviations: GABA, gabapentin; KET, ketorolac

For the right lever success rate, results again revealed no main effect for time, $F(1, 60) = .04$, $p = .54$, pain, $F(3, 60) = .35$, $p = .79$, or drug conditions, $F(2, 60) = .21$, $p = .81$. (Figure 7)

B

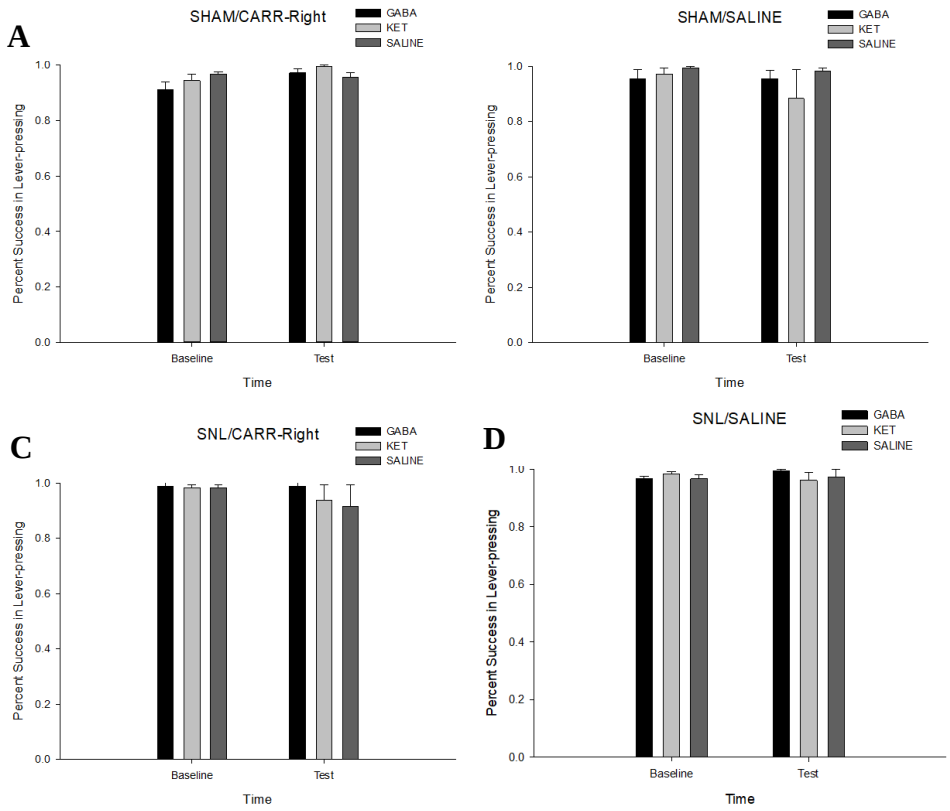


Figure 7: Percent success in lever-pressing the right lever for a) sham/carrageenan, b) sham/saline, c) SNL/carrageenan, d) SNL/saline groups.

Abbreviations: GABA, gabapentin; KET, ketorolac

3.4 Latency to Lever Press by Condition

A 4(pain condition) x 3(drug condition) x 2(time) mixed model ANOVA was conducted to assess group differences in the latency to lever presses for appetitive reward across time.

For left lever latency, results revealed no main effect for time, $F(1, 54) = .44$, $p = .51$, pain, $F(3, 54) = .29$, $p = .83$, or drug conditions, $F(2, 54) = .12$, $p = .89$. (Figure 8)

For right lever latency, results also revealed no main effect for time, $F(1, 54) = .01$, $p = .94$, pain, $F(3, 54) = .61$, $p = .61$, or drug conditions, $F(2, 54) = .04$, $p = .96$. (Figure 9)

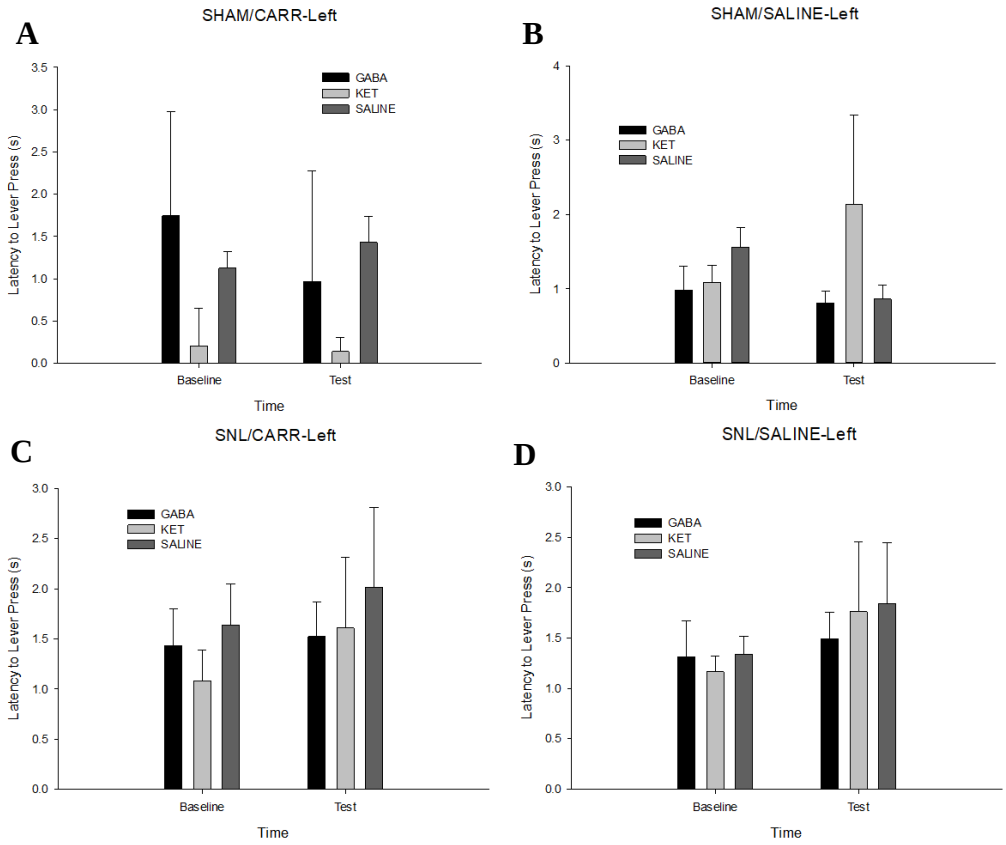


Figure 8: Latency to lever press the left lever for a) sham/carrageenan, b) sham/saline, c) SNL/carrageenan, and d) SNL/saline groups.

Abbreviations: GABA, gabapentin; KET, ketorolac

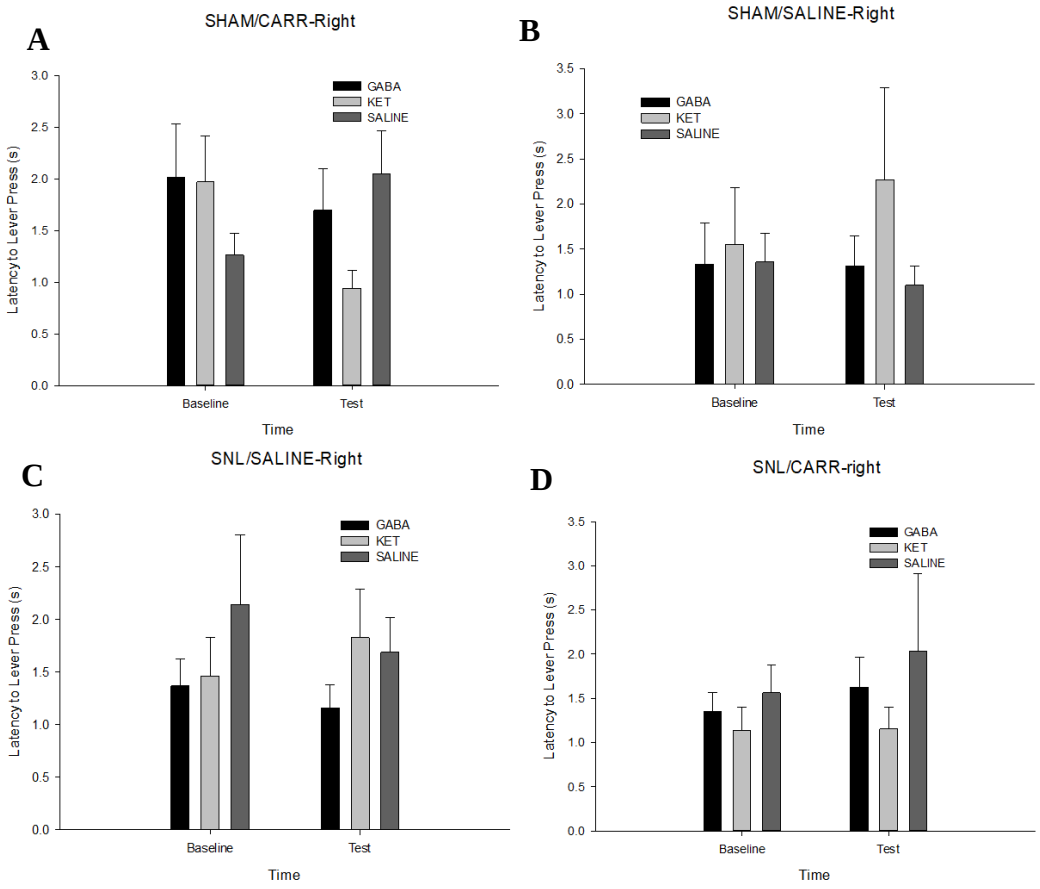


Figure 9: Latency to lever press the right lever for a) sham/carrageenan, b) sham/saline, c) SNL/carrageenan, and d) SNL/saline groups.

Abbreviations: GABA, gabapentin; KET, ketorolac

3.5 Modified Place Escape Avoidance Paradigm (mPEAP) by Condition

A 4(pain condition) x 3(drug condition) x 6(time) mixed-model repeated measures ANOVA was used to assess pain affect via the use of avoidance measures denoted by amount of time spent on the horizonal (left paw stimulated) side of the chamber. Results revealed a significant main effect for pain condition, $F(3, 60) = 3.24$, $p = .03$. Simple effects revealed that SHAM/carrageenan ($M = 64.86$, $SD = 4.81$) animals spent a

significant more amount of time on the horizonal side of the chamber compared to SNL/saline animals (*M* = 43.89, *SD* = 4.81), *p* = .03. There was no difference in average time spent in the horizonal side of the chamber for sham/carrageenan, SNL/carrageenan (*M* = 57.08, *SD* = 4.81), and SNL/saline groups compared to sham/saline animals (*M* = 55.42, *SD* = 4.81), *p* = .17, *p* = .81, and *p* = .10, respectively. Additionally, there was no main effect for drug, *F*(2, 60) = .60, *p* = .55 or time, *F*(1, 60) = .15, *p* = .70. (Figure 10)

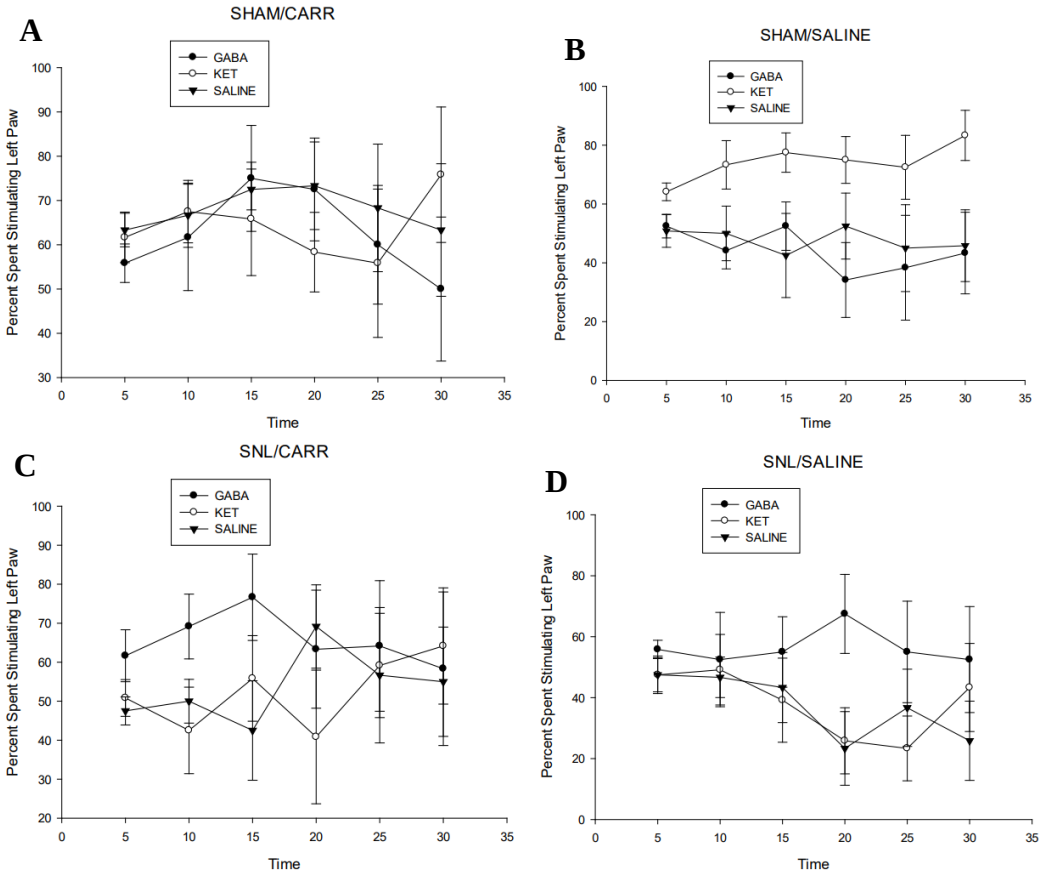


Figure 10: Percent time spent on the horizonal side (left paw stimulated) of the chamber for a) sham/carrageenan, b) sham/saline, c) SNL/carrageenan, and d) SNL/saline groups. Abbreviations: GABA, gabapentin; KET, ketorolac

Chapter 4

4. Discussion

Humans have an overwhelming privilege to be able to communicate their pain. In fact, descriptors of pain can effectively be categorized into the sensory/discriminative, affective/motivational, and cognitive/evaluative dimensions of pain(R Melzack & Wall, 1965; Ronald Melzack, 1975). These descriptors can then be quantified using the McGill questionnaire to assess Pain Rating Index (PRI), allowing individuals to provide their doctor with relative description of the quality and intensity of the pain experienced (Melzack & Torgerson, 1971; Melzack & Wall, 1988). From descriptor choice, qualitative differences in pain have been indicated in phantom limb (neuropathic pain syndrome) and arthritis (chronic inflammatory disease) such that although there were relatively similar differences in the Pain Rating Index (PRI), the sensory and affective components of pain were relatively lower for arthritic individuals than phantom limb patients (Ronald Melzack, 1975). Thus, it was assumed that these trends would also be seen in rodents.

This study sought to examine how competing motivational drives are prioritized when involving the two types of pain most commonly used in pain research: spinal nerve ligation for neuropathic pain and a carrageenan injection to the hindpaw for inflammatory pain. Through the use of a bilateral pain condition, this model allowed for a direct comparison of preference for which paw was stimulated. Furthermore, it was thought that the preference of stimulation to one side over the other would indicate which paw was less affected with stimulation. Therefore, the ultimate goal of the experiment was to examine whether inflammatory or neuropathic pain was the most aversive.

Comparing AAP and mPEAP

Fortunately, animals do not have language, a fact that forces us to develop objective tests to evaluate their psychological state, whether for pain or anything else. To assess sensory pain, we used the validated Von Frey mechanical paw withdrawal threshold testing and for elucidation of pain affect, we used a modified version of the validated place escape avoidance paradigm (mPEAP) and a novel operant approach avoidance paradigm (AAP). These two paradigms both allow animals to "choose" a preference, presenting the ability to quantify pain affect or aversiveness, but do so through different means pain motivated behavior. In the mPEAP, the paradigm allowed the animals to escape stimulation, while the AAP allowed animals to approach stimulation for food reward.

Data from mPEAP indicated no preference for stimulation of a particular paw in the bilateral pain group and no group was statistically different from the control group, sham/saline. There was, however, a significant difference in time spent receiving stimulation of the left paw for the unilateral pain condition groups where sham/carrageenan groups spent more time in the horizonal side of the chamber than did SNL/saline animals. Similar results were found in McNabb et al. (2014) where there was no preference and no difference in time spent on the horizonal side compared to sham/saline animals for the bilateral pain condition. McNabb et al. (2014) also revealed a significant difference in time spent on the horizonal side in SNL/saline and sham/carrageenan groups, but not until avoidance data was collapsed into overall average in time spent on the horizonal side. This finding is quite exciting in that results were replicated indicating that with the use of the mPEAP, there appears no difference in relative *quantity* pain affect, but as suggested by McNabb et al. (2014), it is possible that this paradigm does not tap into the *quality* of pain aversiveness. Furthermore, because

these are the only 2 known experiments that utilize these methods, the fact that these results were replicated highlights the validity of the paradigm.

One further development suggested by McNabb et al. (2014) that was implemented in this study was the use of analgesics to highlight whether pain affect would be reversed to normal behavioral trends. Unfortunately, there was no observable effect of drug condition and this may be primarily due to the fact that even without the use of analgesics, there was no discernible difference in avoidance behavior. Thus, this data may be difficult to interpret, but it appears that analgesics did not impact avoidance behavior.

Similarly, the data from the AAP revealed no significant success rate to lever press across the conditions for either paw. It was previously hypothesized that the bilateral pain condition would perform worse due to overwhelming activation of peripheral pain receptors, however this was not the case. Even more, the inability to show difference in lever-pressing success rate in the unilateral pain conditions was not expected since previous unilateral pain conditions have shown to decrease in reward-seeking behavior in animals ((Ewan & Martin, 2013; LaGraize et al., 2004; Neubert et al., 2008; Neubert, Rossi, Malphurs, Vierck, & Caudle, 2006; Neubert et al., 2005; Nolan, Hester, Bokrand-Donatelli, Caudle, & Neubert, 2011; Reina & Yezierski, 1995; Rohrs et al., 2015; Salcido, Harris Bozer, McNabb, & Fuchs, 2018). This result mirrored the results of Harris (2013, unpublished) where no preference for stimulation of one side over the other occurred. One important note is that with these data from Harris (2013, unpublished), there was a large amount of omissions from the animals despite having no main effect for condition, whereas there was almost neglectable rates of omission in the current study. This may be attributed to some environmental factor like experimenter effects or different strain of animal since the animals of Harris' study were bred within the

University of Texas at Arlington vivarium, while animals of the current study were purchased from Charles River.

The additional use of latency to quantify avoidance behavior to lever press also suggested no significant differences across conditions. This was surprising given that mPEAP avoidance behavior for horizonal side indicated a significant difference between the unilateral groups. Even more, previous research suggested that pain is associated with an increase in decision latency for unilateral pain conditions in both humans (Claes, Crombez, Meulders, & Vlaeyen, 2016) and animals (Harte, Meyers, Donahue, Taylor, & Morrow, 2016; Pahng, Paulsen, McGinn, Edwards, & Edwards, 2017; Salcido et al., 2018). Thus, the fact that there was no difference in these unilateral groups may suggest possible confounds.

Like Harris (2013, unpublished), there was no preference to lever press a particular lever. As Harris suggested, it is possible that there may be a generalization effect where animals were not able to correctly discern the association between the associated lever press and paw stimulation. This is possible, however, since the AAP simplified the Harris paradigm by allowing the animal to only be presented with one lever at a time and a light indicator present before the retraction of the lever, it may have reduced these generalized effects. In fact, during the initiation of the light cue, animals would direct their attention to that lever only suggesting that they "understood" the association between light and lever. Repeated stimulus from both AAP baseline and test days should have created an association between the light, lever, and paw stimulation given the information known about operant conditioning (Skinner, 1938a; Skinner, 1938b). However it is hard to determine that an association did in fact occur since there may not always be a perfect association between pain behavior and pain intensity (Labus, Keefe, & Jensen, 2003).

Additionally, since the rate of lever-pressing was extremely high across conditions, it is possible to consider that floor effects may have had a hand in the high success rate of lever-pressing. The requirement throughout the training maintained that animals lever-pressed at least 80% of the time. This may have weeded out poor performers and kept the highly motivated to remain in the pool increasing overall performance.

Taken all together, results from both the mPEAP and the AAP may suggest SNL and carrageenan models are comparable in quantity and suggest there are no differences in escape/avoidance and approach/avoidance paradigms in response to preference for the bilateral pain group. It is suggested that the mPEAP quantifies pain affect, but it is hard to pinpoint what exactly the AAP is quantifying since there were no suppression effects in the unilateral pain condition that was previously seen in the single lever paradigm by Salcido and colleagues (2018) that used similar aspects of the AAP. We do note, however, that mPEAP utilizes principles of negative reinforcement as an escape/avoidance paradigm (Cook & Catania, 1964), while the AAP utilizes the complex integration of both positive and negative effects on the goal of the task since it is an approach/avoidance paradigm (Aupperle, Melrose, Francisco, Paulus, & Stein, 2015; Elliot, 2006; Rohrs et al., 2015). These differences in approaches may result in different decision-making mechanisms at work in producing an outcome and may change depending upon the experience or the motivational context of the current task at hand (Van Damme, Van Ryckeghem, Wyffels, Van Hulle, & Crombez, 2012). It has been already suggested that these effects may not effectively translate in describing the quality of pain affect (McNabb et al., 2012) primarily due to the concept that preference for a side does not necessarily equate to levels of aversion.

Paw Thresholds and Paw Volume

One confound that must be noted is the lack of mechanical threshold and paw volume data before receiving drug manipulation. This increases the complexity of interpreting results for drug and pain condition. Unfortunately, without this data there is no measurement of the pain that indicates that the models did in fact induce pain. However, because these models are robust in their effect, it may be safe to assume that the pain manipulations decreased thresholds. The ability to see group effects in the mechanical thresholds suggests that on test day, animals with pain conditions did have lower pain thresholds which is exactly what we would expect. The use of selective analgesics was thought to uniquely and selectively reduce pain where ketorolac would only work on inflammatory pain and gabapentin would work only on neuropathic pain. The results suggest that ketorolac did increase pain thresholds for the carrageenan animals only, however gabapentin increased thresholds for both SNL and carrageenan animals. This result suggests that NSAIDs have limited effect in reducing hypersensitivity to only inflammatory conditions, whereas gabapentin alleviates sensory pain in both neuropathic and inflammatory pain. This may suggest that the mechanism of action for gabapentin includes both peripheral and spinal action, although as previously stated, the exact mechanism is still unknown.

As with mechanical thresholds, paw volume data may be difficult to decipher since there was no plethysmometer reading between baseline and drug injection. Although there is concern, again, this model appears to be robust in its effects and have relatively little variance so it can be assumed that inflammation was relatively similar in carrageenan groups before drug injection. As expected, right paw volume for carrageenan treated animals on test day showed an increase in paw volume compared to saline treated animals. Although there was a main effect of drug, there was no interaction

effect of drug and pain condition suggesting that paw volume was not influenced by both drug and pain conditions. A similar finding has been reported previously, suggesting ketorolac does not reverse existing edema at the time of administration (Zhang et al., 1997).

Overall Confounds

One overall confound of the study that could have possibly attributed to differences of the mPEAP and the AAP could be experimenter effects. There were three other students, one male and two female that periodically helped the experiment run through. Studies have revealed differences in data when the experimenters are different sexes (Aslaksen, Myrbakk, Høifødt, & Flaten, 2007) and have even affected pain behaviors when exposed to male-associated smells (Sorge et al., 2014). Although all the students helping in this endeavor were trained by myself, it is possible there were differences in handling and degree of stimulation. These factors could definitely drive differences across the data, however we are hopeful there was little of this influence since both major components were replicated from previous works.

One problem that was quite often experienced was problems with recovery during post-surgery. A number of animals would remove staples before fully healed which included both sham and SNL animals. Three animals had to be euthanized due to infection complications. Due to this, more enrichment was placed in the cage and checks of the animals as often as every hour were done.

Conclusion

The mPEAP and the AAP are unique means of presenting “choice” to animals. Unfortunately, the results from both paradigms seem to convey no preference for a particular pain model based on avoidance behavior or lever success rate and latency suggesting there are no differences in affective dimensions of inflammatory or

neuropathic pain. Thus, rodent pain does not vary across the subtypes of pain and does not differ in cognitive appraisal of affective measures, like observed in humans, at least for these two tasks. As a result, this finding may further suggest differences in clinical and preclinical measures or that current paradigms do not pick up on this nuance for animals. Future studies should focus on elucidating these differences through different paradigms in hopes to understand the cause of the translational gap. Thus, the need to examine the neural underpinnings is paramount so that pain pathways may be explicated and understanding on why and how perception of pain is influenced and can be different across the animal species.

Appendix A

Coding for AAP

```
\Singly presented dual lever
\Written by C. Salcido/J. Beyor (Med Associates)


\ INPUTS
^LeftLever = 7
^RightLever = 6

\ OUTPUTS
^LeftLever = 7
^RightLever = 6
^Dispenser  = 8
^LeftLight = 10
^RightLight  = 9
^HouseLight = 12


\ CONSTANTS

\ A() = Control Variables with Assigned Aliases as Defined
Var_Alias Session Time (min)                  = A(0)  \ Default = 35
Var_Alias Right Lever Reward (# of pellets)       = A(1)  \ Default = 1
Var_Alias Left Lever Reward (# of pellets)        = A(2)  \ Default = 1
Var_Alias Right Lever Time Out (sec)            = A(3)  \ Default = 30
Var_Alias Left Lever Time Out (sec)             = A(4)  \ Default = 30
Var_Alias Time to respond (sec)                 = A(5)  \ Default = 10
Var_Alias Omission Time Out (sec)               = A(6)  \ Default = 30

^Session  = 0
^RLR      = 1
^LLR      = 2
^RLTO     = 3
^LLTO     = 4
^TTR      = 5
^OTO      = 6


\ B = Trial by Trial data
\ B(E)   = Trial number
\ B(E+1) = Left Lever Presses
\ B(E+2) = Right Lever Presses
```

```
\ B(E+3) = Rewards
\ B(E+4) = Omissions


\ C(I) = Left Lever Latency
\ D(O) = Right Lever Latency

\ F = Reward counter
\ G = Total Rewards
\ H = Total Omissions
\ J = Trials
\ L = Total Left Lever presses
\ R = Total Right lever presses
\ S = Session Time
\ T = Latency Timer
\ X = List #

LIST Y =
1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,2,2,2,2,2,2,2,2,2,2,2,2,2,2,2,2,2
,2,2,2,2,2,2,2,2,2,2,2,2,2,2


DIM A = 6
DIM B = 10000
DIM C = 10000
DIM D = 10000


DISKVARS = A,B,C,D,G,H,J,L,R,S

\*************************************************
\               DEFAULTS
\*************************************************

S.S.1,
S1,
  0.01": SET A(^Session) = 41, A(^RLR) = 1, A(^LLR) = 1, A(^RLTO) = 25, A(^LLTO) = 25;
        SET A(^TTR) = 10, A(^OTO) = 25;
        SET B(E) = -987.987;
        SET C(I) = -987.987;
        SET D(0) = -987.987 ---> S2
```

```
S2,     \ First Statement: Wait for START signal, turn HouseLight and
        \ associated stimulus ON.
        \
        \ Second Statement: Update screen display with default values
        \ for Control Variables.  This will show any changes made via
        \ the "Configure | Change Variables" Window prior to START.
  #START: CLEAR 1,200 ---> S3
  1": SHOW 1,Session,A(^Session), 2,RL Rewards,A(^RLR), 3,LL Rewards,A(^LLR);
     SHOW 4,RL Time Out,A(^RLTO), 5,LL Time Out,A(^LLTO), 6,Respond Time,A(^TTR),
7,Omission TO,A(^OTO)---> SX

S3,     \ Time Session Length
  0.01": SET S = S + 0.01; SHOW 1,Session,S ---> SX
  #Z32: ---> S4

S4,     \ Wait for Screen Update and end with
        \ STOPABORTFLUSH for Automatic Data Saving
  2": ---> STOPABORTFLUSH


\*************************************************
\               MAIN PROGRAM
\*************************************************
S.S.2,
S1,
  #START: SET A(^RLTO) = A(^RLTO) * 1", A(^LLTO) = A(^LLTO) * 1";
        SET A(^TTR) = A(^TTR) * 1", A(^OTO) = A(^OTO) * 1" ---> S2

S2,
  0.01": ON ^HouseLight;
  ADD J; SET B(E) = 0, B(E+5) = -987.987, B(E) = J; ---> S3

S3,
0.01": RANDD X = Y; IF X =1 [@T, @F]
                @T: ON ^LeftLight; ---> S4 \ LEFTLEVER
                @F: ON ^RightLight; ---> S5 \ RIGHTLEVER
S4,
5": ON ^LeftLever; Z4 ---> S6

S5,
```

```
5": ON ^RightLever; Z4 ---> S6

S6,
A(^TTR)#T: OFF ^LeftLight, ^RightLight, ^LeftLever, ^RightLever; ADD B(E+4), H; Z3 --->
S7
 #R^LeftLever: OFF ^LeftLight, ^RightLight, ^LeftLever, ^RightLever; ADD B(E+1), L; Z1 -
--> S8
 #R^RightLever: OFF ^LeftLight, ^RightLight, ^LeftLever, ^RightLever; ADD B(E+2), R;
Z2 ---> S9

S7,
 A(^OTO)#T: IF S/65 >= A(^Session) [@T, @F]
         @T: Z32; ---> S1
         @F: SET E = E + 5 ---> S2

S8,
 A(^LLTO)#T: IF S/65 >= A(^Session) [@T, @F]
         @T: Z32; ---> S1
         @F: SET E = E + 5 ---> S2

S9,
 A(^RLTO)#T: IF S/65 >= A(^Session) [@T, @F]
         @T: Z32; ---> S1
         @F: SET E = E + 5 ---> S2


\*************************************************
\           Lever Rewards
\*************************************************
S.S.3,
S1,
 #START: ---> S2

S2,
 #Z1: ON ^Dispenser; ADD F, G ---> S3 \ LEFTLEVER
 #Z2: ON ^Dispenser; ADD F, G ---> S5 \ RIGHTLEVER

S3,
 0.05": OFF ^Dispenser;
      IF F >= A(^LLR) [@Done, @More]
        @Done: SET B(E+3) = F, F = 0 ---> S2
```

```
        @More: ---> S4

S4,
 0.5": ON ^Dispenser; ADD F, G ---> S3

S5,
 0.05": OFF ^Dispenser;
      IF F >= A(^RLR) [@Done, @More]
        @Done: SET B(E+3) = F, F = 0 ---> S2
        @More: ---> S6

S6,
 0.5": ON ^Dispenser; ADD F, G ---> S5


\*************************************************
\           Latency Timer
\*************************************************
S.S.4,
S1,
 #START: ---> S2

S2,
 #Z4: SET T = 0 ---> S3

S3,
 #R^LeftLever: SET C(I) = T, C(I+1) = -987.987; ADD I ---> S2
 #R^RightLever: SET D(O) = T, D(O+1) = -987.987; ADD O ---> S2
 #Z3: ---> S2
 0.01": SET T = T + 0.01 ---> SX

\*************************************************
\           Display Update
\*************************************************
S.S.5,
S1,
 #START: ---> S2

S2,
 .01": SHOW 2,LeftL Presses,L, 3,RightL Presses,R, 4,Rewards,G, 5,Omissions,H, 6,Trial
#,J ---> SX
```

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