2012

CHRONIC OROFACIAL PAIN INFLUENCES SELF-REGULATION IN A RODENT MODEL

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Arts and Sciences
at the University of Kentucky

By

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2012

ABSTRACT OF THESIS

CHRONIC OROFACIAL PAIN INFLUENCES SELF-REGULATION IN A RODENT MODEL

Self-regulation is the capacity to exert control over cognition, emotion, behavior, and physiology. Since chronic pain interferes with the ability to self-regulate, the primary goal of this study was to examine, in rodents, the effects of chronic pain on self-regulation processes. Sixteen male Sprague-Dawley rats were divided into two groups: (1) chronic constriction injury of the infraorbital nerve (CCI-ION) and (2) naïve. Testing confirmed that CCI-ION animals had significant mechanical allodynia compared to naïve animals ($p<0.001$). A two-part self-regulation behavioral paradigm consisting of a cued go/no-go task and a subsequent persistence task was developed based on human paradigms. In the cued task, both groups made fewer incorrect lever presses in post-surgery trials ($p<0.001$); naive animals had a greater decrease in number of incorrect presses than CCI-ION animals ($p=0.06$). Similarly, both groups had a larger correct to total lever presses ratio in post-surgery trials ($p<0.001$); naïve animals had a greater increase than CCI-ION animals ($p=0.06$). In the persistence task, naïve animals experienced a greater decrease in lever presses ($p=0.08$) than did CCI-ION animals ($p=0.66$). These results suggest that animals experiencing chronic pain were not able to learn as well as naïve animals, and may have difficulty responding to novel environmental demands.

KEYWORDS: self-regulation, learning, chronic pain, chronic constrictive injury, infraorbital nerve

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ACKNOWLEDGMENTS

I would like to express my sincerest appreciation for everyone who provided support and insight as I completed this thesis. First, I would like to acknowledge my committee members, Charles Carlson, Craig Miller, Karin Westlund High, and Robert Danaher. Without their encouragement and guidance this project would not have been possible. A special thank you also goes to my colleagues, including Fei Ma, Liping Zhang, Chunmei Wang, and Sabrina McIlwrath, who have taught me a great deal. Finally, I would like to thank my family and friends for their unwavering love and support.

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Chapter One: Introduction

Chronic Pain

Chronic pain is a major health problem in the United States and throughout the world and is one of the primary reasons that people seek medical treatment (Gureje, Von Korff, Simon, & Gater, 1998; Schappert & Burt, 2006). The International Association for the Study of Pain (IASP) defines chronic pain as "pain without apparent biological value that has persisted beyond the normal tissue healing time (usually taken to be 3 months)" (Harstall & Ospina, 2003).

Clinical Importance

A meta-analysis of studies examining the prevalence of chronic pain found that those studies which used the IASP definition of chronic pain estimated a mean prevalence of 35.5% (Harstall & Ospina, 2003). Additionally, a recent, large, cross-sectional survey estimated the prevalence of chronic pain in the United States population at 30.7% (95% CI, 29.8–31.7) where chronic pain was defined as pain that is not fleeting or minor and lasts at least six months (Johannes, Le, Zhou, Johnston, & Dworkin, 2010). Johannes and colleagues found that the majority of individuals with chronic pain had experienced the pain for a year or more, most experienced pain frequently (two to three times per week), and about a third reported severe average pain intensity (2010). Clearly, chronic pain is a disabling and costly condition that is prevalent among adults both in the United States and worldwide.

One form of chronic pain that many individuals struggle with is neuropathic pain. Neuropathic pain is defined as "pain initiated or caused by a primary lesion or dysfunction in the nervous system;" and trigeminal neuropathic pain is a frequently

occurring condition in humans (Merskey, 1986). Although some therapies such as antidepressants and anticonvulsants have been found to benefit individuals suffering from neuropathic pain, a large portion of patients become unresponsive to these drugs (Swerdlow, 1984). Thus, there is a need for studies that explore the nature of neuropathic pain and potential treatment strategies.

Animal Models

A rat model of trigeminal neuropathic pain that involves a chronic constriction injury of the infraorbital nerve (CCI-ION) shares many characteristics with the clinical disorders seen in humans suffering from trigeminal neuralgia or trigeminal neuropathic pain (Vos, Strassman, & Maciewicz, 1994). The infraorbital nerve forms almost the entire maxillary division of the trigeminal nerve in the rat (Greene, 1955). It innervates the mystacial vibrissae, vibrassal pad, part of the rhinarium, the upper teeth, and part of the dorsal section of the oral cavity (Vincent, 1913; Greene, 1955; Fink, Aasheim, Kish, & Croley, 1975). Behavioral studies have demonstrated that the CCI-ION model induces mechanical allodynia, as measured with von Frey fibers, of this area beginning two to three weeks post-surgery and lasting up to 11-12 weeks post-surgery (Ma, Zhang, & Westlund, in press; Vos, Strassman, & Maciewicz, 1994).

This model of neuropathic pain is beneficial for several reasons. First, similar to clinical findings, pain-related behaviors observed in rats with CCI-ION are difficult to treat with tricyclic antidepressants and single or repeated administrations of morphine (Idänpään-Heikkilä & Guilbaud, 1999). Additionally, although other studies of chronic pain have used models which last only up to two weeks, most criteria associated with clinical diagnoses of chronic pain conditions require that the individual have experienced

at least three months of symptoms before a diagnosis can be made. The CCI-ION model of neuropathic pain is particularly useful for this study since the effects of CCI-ION can be maintained for at least 12 weeks post-surgery. Finally, unlike acute pain models, current, unpublished studies conducted in our lab have shown that CCI-ION does not change feeding behavior (Thut et al., 2007). This is important to the current study since food pellets were used as a reward; and we wanted to ensure that the effects measured were not simply changes in feeding behavior caused by pain.

Self-regulation

Self-regulation involves the capacity to exert control over cognition, emotion, behavior, and physiology (Baumeister, 1998; Carver & Scheier, 1998; Higgins, 1996). Baumeister, Schmeichel, and Vohs (2007) define self-regulation as the "self altering its own responses or inner states…this takes the form of overriding one response or behavior and replacing it with a less common but more desired response…self-regulation also includes the ability to delay gratification." The authors argue that choice and self-regulation are intertwined and work together to create the novelty and diversity observed in behavior. In fact, Baumeister, Schmeichel, and Vohs (2007) demonstrate that making choices and showing self-regulation draw on a common resource, such that making choices temporarily reduces one's ability to self-regulate and vice versa.

Many research articles on self-regulation describe this limited resource from which one must draw to self-regulate and which may become depleted after use. For example, Baumeister and Alquist (2009) describe self-regulation as a muscle, after the muscle has been used it becomes fatigued and time must pass until its energy source has been replenished before using it to its full capacity again. In sum, self-regulation requires

an individual to exert control over some physiological, behavioral, or cognitive capacity and draws on a limited energy resource.

Self-regulation is also intimately related to executive functions. Solberg Nes, Roach, and Segerstrom (2009) reviewed this relationship and concluded that "self-regulation appears to rely on executive functions" since a deficit in executive functioning may result in problems controlling and regulating behavior. Furthermore, research indicates that performing an initial self-regulatory task may cause fatigue which results in poorer subsequent performance on executive tasks (Schmeichel, 2007; Schmeichel, Vohs, and Baumeister, 2003). Thus, self-regulatory fatigue and executive capacity covary inversely in a way that can lead to a potential downward spiral where "self-regulatory demands cause self-regulatory fatigue, reduce executive cognitive resources for further self-regulation, and thereby increase difficulty in meeting further demands" (Solberg Nes, Roach, and Segerstrom, 2009).

Importance in Chronic Pain Population

Chronic pain conditions are challenging to live with and are often referred to as "stress-associated conditions or syndromes or as chronic multisymptom illnesses" (Solberg Nes, Roach, & Segerstrom, 2009). These conditions are characterized by complex interactions between cognitive, emotional, and physiological disturbances and therefore their demands are wide-reaching. Individuals with chronic pain must learn to manage the pain itself, to negotiate relationships affected by the limitations associated with chronic pain, to suppress ruminative thoughts, and to regulate moods such as depression and anxiety that are often found to be comorbid with chronic pain disorders.

As Solberg Nes, Roach, and Segerstrom point out in their 2009 review of the topic, all of these demands require self-control or self-regulation.

Although self-regulation has been implicated as important in the management of chronic pain conditions, several studies show that chronic pain itself can interfere with the ability to self-regulate. In 2010, Solberg Nes and colleagues found that patients with chronic pain conditions, including fibromyalgia and temporomandibular disorders, have less capacity to persist on a task following an initial self-regulation task than persons without chronic pain. In this study, participants were asked to watch a movie while ignoring words flashing on the screen, and then asked to complete an unsolvable anagram. The researchers recorded how long the two groups (pain and control) persisted in trying to solve the anagram. As expected, the participants with chronic pain conditions persisted for a shorter amount of time. These results suggest that chronic pain patients may suffer from chronic self-regulatory fatigue. Thus, while self-regulatory ability can be fatigued in control groups, there is evidence showing that participants with chronic pain conditions have an even greater deficit in self-regulatory ability.

It is not surprising to learn that deficits in self-regulatory capacity, or executive functioning, have also been linked to patients with chronic pain conditions. Although the cause of these deficits is unclear, several researchers have hypothesized that pain operates as an additional processing burden or a constant cognitive distraction (Eccleston & Crombez, 1999; Sanchez, 2011). Karp and colleagues (2006) found that pain severity is associated with decreased mental flexibility and that the cognitive difficulties experienced by chronic pain patients are usually worse during times of extreme pain (i.e. flare-ups). Another example of these deficits is "fibrofog," a controversial topic which

refers to a variety of cognitive and executive functioning problems that may accompany fibromyalgia (Katz, 2004; Landro, Stiles, and Sletvold, 1997; Park, Glass, Minear, & Crofford, 2001).

Other research on executive functioning in chronic pain patients has focused specifically on deficits in memory and attention. Studies in human clinical populations have demonstrated that chronic pain patients usually suffer from memory deficits, and also that around two-thirds of these patients have disrupted working memory (Dick & Rashiq, 2007; Hart, Martelli, & Zasler, 2000; Legrain, Damme, Eccleston, Davis, Seminowicz, & Crombez, 2009). Ren and colleagues further examined this effect in a rodent model, and found that peripheral nerve injury was associated with memory impairment and dysfunction of the hippocampus (2011). Additionally, it has been observed that patients with fibromyalgia have reduced attentional resources for processing information other than pain and a diminished ability to inhibit the processing of irrelevant information (Grisart & Van der Linden, 2001; Leavitt & Katz, 2006).

Although chronic pain can be difficult to treat, several interventions have been successful in improving functioning; specifically cognitive-behavioral therapy (CBT) which aims to alter perception of and behavioral responses to the pain itself (Beck, 1976; Turk & Sherman, 2002). Another intervention strategy which has been successful in the treatment of some chronic pain conditions is relaxation therapy. Interestingly, both of these strategies require the individual to self-regulate, as well as requiring a certain degree of executive capacity. Even if persons with chronic pain show a willingness to engage in these treatments, it may be very difficult for them to remain engaged and

persist due to lack of regulatory resources (Baumeister, Bratslavsky, Muraven, & Tice, 1998; Muraven, Tice, & Baumeister, 1998; Solberg Nes et al., 2009).

While self-regulation seems to act as a muscle whose energy can be depleted with use, it may also be possible to increase self-regulatory capacity through practice over time (Solberg Nes et al., 2009). Thus it is reasonable to conclude that self-regulatory exercises completed during therapy may work to increase self-regulatory strength. However, this does not help with the problem of how to initiate therapy with a group of individuals suffering from chronic self-regulatory fatigue. If we could find a way to increase ability to self-regulate, especially at the beginning of therapy for patients with chronic pain conditions, we may help to jump-start therapy for these individuals until they increased their self-regulatory ability through repeated practice.

Physiological Factors

Although the causes and etiology of many chronic pain disorders are not fully understood, it appears that some sort of central nervous system (CNS) dysfunction is involved in the onset and progression of these conditions (Crofford & Demitrack, 1996; Giovengo, Russell, & Larson, 1999; Gur & Oktayoglu, 2008; Larson, Givengo, Russell, & Michalek, 2000). It could be that self-regulatory deficits in physiological systems are also involved. Studies have indicated that heart rate variability (HRV), an index of fluctuation in the time interval between normal heartbeats, is an index of self-regulation capacity (Thayer & Lane, 2000). In several studies, chronic pain patients have shown lower HRV compared with controls (Cohen, Neumann, Shore, Amir, Cassuto, & Buskila, 2000; Martinez-Lavin, Hermosillo, Rosas, & Soto, 1998; Schmidt & Carlson, 2009; Stewart, Weldon, Arlievsky, Li, & Munoz, 1998). Although the cause of this difference

is not clear, it is possible that chronic pain patients, who are already experiencing self-regulatory deficits, may have HRV that differs from healthy controls because of dysregulation of physiological systems (Martinez-Lavin et al., 1998).

Other abnormalities that can occur in chronic pain patients include dysregulation of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal axes (Crofford & Demitrack, 1996). In some cases chronic pain conditions have also been linked to hypocortisolism (Korszun, Young, Engleberg, Masterson, Dawson, Spindler, McClure, Brown, & Crofford,, 2000; Korszun, Young, Singer, Carlson, Brown, & Crofford, 2002; Ehlert, Gaab, & Heinrichs, 2001). Glucocorticoids are secreted by the adrenal cortex and are essential for the production and metabolism of blood glucose. Since the activities of the brain rely heavily on blood glucose for energy, a range of cognitive and behavioral deficits may occur if the flow of blood glucose to the brain becomes insufficient (Solberg Nes et al., 2009). Interestingly, recent research indicates that self-regulation efforts might rely on glucose as a limited energy resource (Benton, Parker, & Donohoe, 1996; Galliot & Baumeister, 2007; Gailliot, Baumeister, DeWall, Maner, Plant, Tice, Brewer, & Schmeichel, 2007; Miller, et al., 2010). It follows that self-regulatory deficits observed in chronic pain patients may be influenced by blood glucose levels.

Recent research has shown that self-regulation can be depleted and that self-regulatory capacity is related to glucose level in the bloodstream. For example, Galliot et al. (2007) conducted nine different studies looking at the effects of fatigue and glucose on self-regulation. After examining the data collected, the authors came to three main conclusions. First, initial exertion on a self-regulation task significantly dropped

participants' blood glucose levels. Second, low blood glucose levels after the first task were related to poor performance on a subsequent self-regulation task. Finally, administration of glucose reduced or eliminated the effects of the initial self-regulation task on subsequent performances. It seems that glucose plays a role in participants' ability to self-regulate and that it may be possible to treat deficits in self-regulatory ability with glucose administration. Currently, the literature demonstrates that administration of glucose can combat self-regulatory fatigue after an initial task; however, there have not been studies examining the effects of glucose on different types of self-regulatory deficits particularly those arising from chronic pain conditions.

Animal Models

Although past research has focused on the study of self-regulation in human populations, it would be beneficial to study the effects of chronic pain on self-regulation in an animal model. An animal model of chronic pain allows for greater experimental control and fewer threats to internal validity. For example, in human populations with chronic pain, other diseases and disorders are commonly found to be comorbid to the presenting pain condition. Studying chronic pain conditions in a controlled setting allows investigators to observe only the effects of chronic pain itself on self-regulation. Additionally, studying this phenomenon in an animal model allows the experimenter to explore the physiological pathways responsible for this self-regulatory deficit and to examine possible treatments such as glucose administration. Although many researchers have argued that self-regulation only occurs in humans, a recent study with dogs has shown that it is possible to examine these effects in other species (Miller et al., 2010).

The study conducted by Miller et al. (2010) examined the effects of self-regulatory fatigue and glucose administration in dogs. In the first experiment, dogs participated in a primary self-regulation task that required them to sit and stay for a certain period of time. After the initial task, the dogs were given a toy containing a piece of hot dog that they were not able to remove from the toy. Persistence on this task was measured as the amount of time the animal continued to try to remove the food. As expected, animals in the self-regulatory condition performed worse on the persistence task (i.e. persisted for a shorter amount of time) then animals that had not been cued to sit and to stay in one place. In the second experiment, the experimenters demonstrated that administration of glucose after the initial self-regulatory task eliminated the negative effects of prior self-regulatory exertion. These findings support previous studies showing that self-regulation draws on a limited resource and that glucose counteracts the effects of an initial self-regulation task. Additionally, this paper demonstrates that self-regulation can be examined in species other than humans.

The main goal of the proposed study was to explore whether self-regulation can be studied in a rodent model. As mentioned previously, the establishment of a self-regulation model in rodents would be beneficial in allowing greater experimental control as well as the ability to examine physiological mechanisms underlying self-regulatory processes. Additionally, numerous pain models are well established in rodents and can be used to examine the effects of chronic pain conditions on self-regulation. Specifically, in this study it was anticipated that animals experiencing pain would perform more poorly on a subsequent task after initial self-regulatory depletion than control animals This is based on the findings of numerous researchers who examine self-regulation in

human populations as well as the results of Miller and colleagues (2010) who were the first to examine these effects in a non-human population (i.e., dogs).

Chapter Two: Methods

Animals

Eighteen male Sprague-Dawley rats, weighing 200 – 300 grams (g) on arrival were used in the study. Due to time and space limitations in the vivarium, animals were run in two groups (N = 8 and N = 10). Animals were singly housed throughout the duration of the experiment. Low soy content diet (Harlan Teklab 8626, Madison WI) was provided and the animals were maintained under a reverse 12:12 light:dark cycle (lights off at 7:00 am, lights on at 7:00 pm). Adequate measures were taken to minimize pain or discomfort in this study. Experiments were carried out in accordance with the Guidelines of the National Institute of Health regarding the care and use of animals for experimental procedures. Experiments were approved by the Institutional Animal Care and Use Committee at the University of Kentucky, Lexington, Kentucky. All animals were housed in AAALAC and USDA approved facilities.

Surgical Model

Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg, i.p.) and the head of the rat was fixed in a stereotaxic frame. The surgery was performed under direct visual control using a Zeiss operation microscope (6−40X). Surgical ligation of the infraorbital nerve was completed using procedures developed by Gregg (1973) and Jacquin and Zeigler (1983). First, lidocaine (2%) was injected at the site of surgical incision. A midline scalp incision was then made, exposing the skull and nasal bone. The infraorbital part of the left infraorbital nerve was exposed using a surgical procedure adapted from Gregg and Jacquin and Zeigler (1973; 1983). The edge of the orbit, formed by the maxillary, frontal, lacrimal, and zygomatic bones, was

dissected free. To provide access to the infraorbital nerve, the orbital contents were gently deflected. The infraorbital nerve was separated from other structures at its most rostral extent on the orbital cavity, just caudal to the infraorbital foramen.

In order to ligate the infraorbital nerve, a suture was looped over a small neural hook (2 mm) instrument with a blunt tip inserted under the nerve and gently pulled under the nerve. Two chromic gut (5−0) ligatures were loosely tied (with about 2 mm spacing) around the nerve. To obtain the desired degree of constriction, a criterion formulated by Bennett and Xie (1988) was used; the ligations reduced the diameter of the nerve by a just noticeable amount, but did not interrupt the epineural circulation. Blood circulation through epineural vessels was visually observed in each animal undergoing surgery. The scalp incision was closed using PDSII absorbable suture and the wound treated with triple antibiotic ointment (polymycin B sulfate, bacitracin zinc, and neomycin-pramoxine HCl) and 2% lidocaine.

All animals in the CCI-ION group underwent ligation of their left-side infraorbital nerve; the right-side nerve remained untouched. The control group of rats remained naïve, and did not undergo surgical procedures. Due to the possibility of damaging the nerve and surrounding tissue during sham surgery, and thus producing some degree of pain, it was decided that completely naïve animals should be used as the control group. Animals were allowed seven days to recover from surgery with food and water available ad libitum.

Behavioral Measure

In order to ensure the effectiveness of the CCI-ION model of chronic pain, von Frey filaments (also referred to as Semmes-Weinstein (S-W) monofilaments) were used

to assess mechanical sensitivity on the whisker pad. Although Randall and Selitto (1957) originally developed a method for evaluating mechanical sensitivity in inflamed rats, the major disadvantage of this method is that it requires the rat to be forcibly restrained. This restraint of the animal results in a stress response that may significantly confound the measure of mechanical sensitivity (Ren, 1999). To improve the Randall-Selitto method, increased handling of the animal can be introduced so that the animal becomes familiar with the testing situation and the stress response is reduced (Taiwo, Coderre, & Levine, 1989).

In the current study, methods adapted from those developed by Ren (1999) were used to test mechanical sensitivity of the whisker pad under non- or minimal-restrained conditions. Animals were habituated to stand against the experimenter's hand wearing a regular leather work glove. Additionally, instead of standing on a meshed metal surface, the rat stood on a soft pad. Each animal was handled and habituated to the experimental procedure twice for 30 minutes each during the week prior to the first baseline trial. Additionally, animals were habituated on each trial day for a period of 15 minutes prior to testing.

The modified up-and-down method utilized in the study to determine the 50% withdrawal threshold is detailed in Ma, Zhang, and Westlund (in press). Briefly, mechanical sensitivity was measured with eight von Frey fibers (0.4, 0.6, 1, 2, 4, 6, 8, 15 g; Stoelting, Wood Dale, IL) by modified up-and-down method with a default maximal 50% withdrawal threshold at a gram force of 18.72. Mechanical stimuli were applied within the infraorbital nerve innervated region, near the whisker pad centers, both ipsilateral and contralateral to the surgery site. Responses to von Frey filaments applied

to the rat whisker pad determined the threshold required for 50% head withdrawals. Each filament was applied five times at intervals of a few seconds. If head withdrawal was observed at least three times after probing with a filament, the rat was considered responsive to that filament. Whenever a positive response to a stimulus occurred, the next smaller von Frey filament was applied. Otherwise, the next higher filament was applied. Behavioral changes to mechanical stimuli were tested once a week for two weeks prior to surgery and five weeks after surgery (i.e. days 7, 14, 21, 28, and 35 post-surgery).

Self-regulation Model

The self-regulation model utilized was based on the models used in previous human studies. In the majority of human experiments, self-regulatory fatigue is studied by exposing participants to two tasks. The first task requires participants to complete some activity which requires self-regulation. For example, participants are asked to watch a video while ignoring words that are flashing on the bottom of the screen. The second task exposes participants to an unsolvable or impossible activity and measures the amount of time they are willing to persist. Many past self-regulation studies have utilized an unsolvable anagram task to measure persistence.

The current study modified this design to examine self-regulation in rats. Animals were exposed to two tasks, an initial activity requiring self-regulation and a subsequent impossible task to measure persistence. The self-regulation portion of the experiment consisted of a cued go/no-go task. During this task, animals were placed into a test chamber for 21 minutes and allowed to press a lever four times to obtain a food reward. However, animals were only rewarded for pressing the lever when a cue light

was illuminated (the cue light cycled on and off every three minutes, beginning with a light on cycle). The second portion of the experiment began immediately following the initial self-regulation task (i.e., immediately following a light off cycle). During this persistence task, the cue light remained illuminated for ten minutes, but animals were not rewarded for any lever presses. The time duration of these tasks were selected based on pilot data which determined animals would not become satiated during the total 21 or 31 minute task and would continue pressing the lever to receive food rewards throughout the entire task.

Data recorded included number of lever presses during the initial 21 minute task (differentiating between lever presses when the cue light was on and off), number of lever presses during the subsequent 10 minute persistence task, and the time of each animal's last lever press during the 10 minute persistence task. Lever presses made while the cue light was illuminated are referred to as "correct presses" and lever presses made while this light was off are referred to as "incorrect presses." Thus, analyses were run on correct and incorrect lever presses made during the initial self-regulation task, the ratio of correct to total lever presses made during the initial task, total number of lever presses made during the subsequent persistence task, and total time (i.e. time of the last lever press) spent on the persistence task.

Shaping and Training

In order to train the rats to receive a food reward by pressing a lever on a 4:1 fixed ratio schedule only when a cue light was illuminated, training and shaping techniques adapted from Thut, Hermanstyne, Flake, and Gold (2007) were used. These techniques required that animals be restricted to 10 g of food on days immediately preceding trial or

training tasks. Animals were always allowed access to water ad libitum, and were allowed access to food a libitum on all days not preceding trials or training tasks. Animal body weights were annotated daily to ensure proper health during food restriction. Animals that experienced a 10% or greater decrease in body weight within any seven day period were to be seen by a staff veterinarian for evaluation; however, none of the animals needed to be seen for weight loss and all animals gained weight over the duration of the experiment.

All animals underwent two weeks of training and one week of baseline testing prior to surgery. On Monday of week one, animals were restricted to 10 g of food pellets and water ad libitum in their home cages. On Tuesday through Friday of week one, animals were shaped to press a lever for 45-mg food pellet rewards on a fixed ratio schedule of 4:1. On Tuesday, the animals were placed into the testing chambers (MedPC Associates) and underwent magazine training, during which animals automatically received 20 pellets of food regardless of lever pressing behavior. On Wednesday, the animals were placed in the testing chambers for 60 minutes and received food pellet rewards on a fixed ratio schedule of 1:1. The ratio of required lever presses was increased so that animals received food pellet rewards on a fixed ratio schedule of 4:1 on Friday of week one. After the training session on Friday, rats were placed in their home cages with food and water available ad libitum until Monday morning.

Food was again restricted to 10 g daily beginning the following Monday. On Tuesday of week two, rats received another training session for 60 minutes at a fixed ratio of 4:1. Beginning Wednesday of week two, animals were trained in the cued go/no-go task. This task required that animals learn to press the lever only when a cue light,

placed immediately above the lever in the testing chamber, was illuminated. Cued go/no-go task days consisted of 21 minute trials, split into eight phases of 3-minute intervals. The intervals alternated between light-on and light-off phases (4 intervals with the light off and 3 intervals with the light on) and began and ended with a light-off phase. Animals were rewarded on a 4:1 basis only during light-on phases, but when the cue light was off animals did not receive pellets regardless of number of lever presses. Animals were placed in the test chambers for 21 minute training sessions for the cued go/no-go task on Wednesday - Friday of week two. After the training session on Friday, rats were placed in their home cages with food and water available ad libitum until Monday morning. Following the two week training period all animals ate pellets as they were earned, and an average of only 0-2 pellets were not eaten by the end of the 21 minute task.

Experimental Procedure

During week three, animals received a cued go/no-go training session on Tuesday and Thursday, and baseline data was obtained on Wednesday and Friday. During trial days, animals were placed into the test chamber for the 21 minutes cued go/no-go task followed immediately by the persistence task (during which the cue light remained illuminated for 10 minutes, but animals did not receive food rewards regardless of lever presses). After the trial session on Friday, rats were placed into their home cages with food and water available ad libitum until Monday morning.

On Monday of week four, half of the animals underwent CCI-ION surgery, and half of the animals remained naive. Animals were allowed to recover with water and food ad libitum for seven days following this surgery. During weeks 5 – 7 (i.e. week 1-3

post-surgery), animals were restricted to 10 g of food per day each Tuesday, and received a cued go/no-go training session each Wednesday. After this session, rats were returned to their home cages with food and water available ad libitum until the following Tuesday morning.

Post-surgery trial data were collected after the animals developed mechanical sensitivity, during week eight and week nine (i.e. week 4 – 5 post-surgery). Animals were restricted to 10 g of food per day beginning each Tuesday on testing weeks. On each Wednesday, animals received a cued go-no training session; and on each Thursday, animals received a full trial containing both tasks with the same procedures listed above. After the trial session on Thursday, rats were placed in their home cages with food and water available ad libitum until Tuesday morning. A full schedule of the training and experimental techniques that were used in the current study can be seen in Table 2.1.

Tissue Collection

Upon completion of testing, rats were anesthetized by intraperitoneal injection of sodium pentobarbital (70 mg/kg) and perfused transcardially with heparinized saline followed by 4% ice-cold paraformaldehyde in 0.1 M phosphate buffer solution (pH 7.4). Infraorbital nerves were dissected out and placed in 4% paraformaldehyde in 0.1 M phosphate buffer solution (pH 7.4) at room temperature overnight. Samples were then switched to 70% ethanol and stored at 4°C. The pons, trigeminal nuclei, and trigeminal ganglion were dissected out and placed in 4% paraformaldehyde in 0.1 M phosphate buffer solution (pH 7.4) for an additional 4 hours at room temperature. Samples were then switched to 30% sucrose in PBS at 4°C for 24 hours, following which standard paraffin embedding procedures were carried out to prepare the tissue blocks. These

Table 2.1

Summary of Shaping and Experimental Methods

	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**
Week 1	Restrict Food	Magazine, training	1:1, training	2:1, training	4:1, training
Week 2	Restrict Food, von Frey	4:1, training	4:1 cued, training	4:1 cued, training	4:1 cued, training
Week 3	Restrict Food, von Frey	4:1 cued, training	Baseline	4:1 cued, training	Baseline
Week 4	Surgery	OFF	OFF	OFF	OFF
Week 5	von Frey	Restrict Food	4:1 cued, training	OFF	OFF
Week 6	von Frey	Restrict Food	4:1 cued, training	OFF	OFF
Week 7	von Frey	Restrict Food	4:1 cued, training	OFF	OFF
Week 8	von Frey	Restrict Food	4:1 cued, training	Trial	OFF
Week 9	von Frey	Restrict Food	4:1 cued, training	Trial	OFF

tissues were stored for investigation of additional physiological, neuroanatomic and molecular issues that will be reported in a subsequent paper.

Statistical Analyses

An *a priori* power analysis was conducted using Faul, Erdfelder, Lang, and Buchner's G*Power 3 (2007) software to compute the sample size needed to achieve a power of 0.80. This analysis was conducted based on the results of the Miller et al (2010) experiment. Using their effect size ($d = 1.55$) and a two-tailed, t-test to analyze the data, it was determined that a total sample size of 16 animals (8 animals per group) was needed (power = 0.80, $\alpha = 0.05$). Based on this analysis, a total of 18 animals were run through the experimental procedures. The naïve group consisted of eight animals, while the pain group consisted of ten animals to compensate for a less than 100% surgical success rate.

Statistical analyses were conducted on animal body weight, mechanical allodynia as measured by von Frey fibers, and performance in the initial self-regulation and subsequent persistence tasks. Outliers, defined as animals having a score greater than or less than two standard deviations away from the mean, were identified separately in each analysis and were excluded. Body weights were analyzed at arrival, baseline trials, surgery, and post-surgery trials using univariate analysis of variance (ANOVA) tests to check for differences between the two groups. Analyses of mechanical allodynia and performance in the self-regulation and the persistence tasks were completed using repeated measures ANOVA tests where appropriate and followed up with specific contrasts.

Chapter Three: Results

Outliers, Baseline Differences, and Normality

Animals were tested with von Frey fibers for evidence of mechanical allodynia following CCI-ION surgery. The highest obtainable von Frey value for 50% withdrawal was 18.72 gram force; and all animals during baseline trials, as well as all animals in the naïve group during post-surgery trials, remained constant at this value. Two animals in the CCI-ION group were excluded from all analyses because their von Frey values remained equal to a gram force of 18.72 during post-surgery trials, indicating that these animals did not experience mechanical sensitivity. Thus, a total of 16 animals (8 CCI-ION, 8 naïve) were available for each analysis.

Outliers, defined as animals having a score greater than or less than two standard deviations away from the mean, were identified separately in each analysis and were excluded. For each analysis, a univariate ANOVA was used to check for differences between the two groups during baseline trials. Significant differences were not observed between groups during baseline trials for any of the analyses conducted. Normality was also tested for each analysis using the Shapiro-Wilk test. This test was not significant for any of the analyses at the initial baseline.

Body Weight

Significant differences in body weight between the two groups were not observed at any time point throughout the study (arrival, $F(1,14) = 1.06$, $p = 0.32$; average baseline trial, $F(1,14) = 0.49$, $p = 0.50$; surgery, $F(1,14) = 1.77$, $p = 0.21$; average trial, $F(1,14) = 3.43$, $p = 0.09$). Characteristics of animal body weights are presented in Table 3.1

Table 3.1

Means and Standard Deviations for Animal Body Weight

Variable	CCI-ION	Naïve	p Value
Arrival Weight	248.43 (6.85)	252.74 (9.70)	0.32
Average Baseline Weight	292.30 (8.27)	296.47 (14.73)	0.50
Surgery Weight	318.60 (11.87)	330.99 (23.52)	0.21
Average Trial Weight	366.76 (13.38)	385.84 (25.90)	0.09

Mechanical Allodynia

Animals in the CCI-ION group experienced a statistically significant decrease in the gram force of von Frey fibers in post-surgery trials compared with baseline, whereas naïve animals did not experience a change in their response to the von Frey fibers. Means and standard deviations for von Frey testing can be seen in Table 3.2. A repeated measures ANOVA indicated that there were significant effects of group, trial, and the interaction of trial by group at day 21, 28, and 35 post-surgery (day 21, $F(1,14) = 25.29$, $p < .001$; day 28, $F(1,14) = 3{,}563.76$, $p < .001$; day 35, $F(1,14) = 14{,}679.70$, $p < .001$; see Figure 3.1). Analyses of mechanical allodynia on day 7 and 14 post-surgery were determined not to be significantly different (day 7, $F(1,14) = 2.00$, $p = 0.18$; day 14, $F(1,14) = 1.00$, $p = 0.33$).

Self-regulation Task

Performance in the self-regulation task was analyzed with repeated measures ANOVA tests comparing the number of correct and incorrect lever presses and the ratio of correct to total lever presses made during the initial self-regulation task to check for effects of group (i.e. CCI-ION versus naïve), trial/time (pre- or post-surgery), and the interaction of group by time. Mean and standard deviations for the self-regulation task can be seen in Table 3.3.

For correct lever presses, no outliers were identified and thus none were excluded from the analyses. It was determined that there was no significant effect of group ($F(1,14) = 0.30$, $p = 0.59$), trial/time ($F(1,14) = 0.01$, $p = 0.91$), or the interaction of group by time ($F(1,14) = 0.51$, $p = 0.49$). These results indicate that there was no significant

Table 3.2

Means and Standard Deviations for von Frey Data

Trial	CCI-ION	Naïve
Average Baseline	18.72 (0)	18.72 (0)
Day 7, post-surgery	15.94 (5.56)	18.72 (0)
Day 14, post-surgery	18.58 (0.41)	18.72 (0)
Day 21, post-surgery	9.92 (4.95)	18.72 (0)
Day 28, post-surgery	2.73 (0.76)	18.72 (0)
Day 35, post-surgery	1.87 (0.39)	18.72 (0)

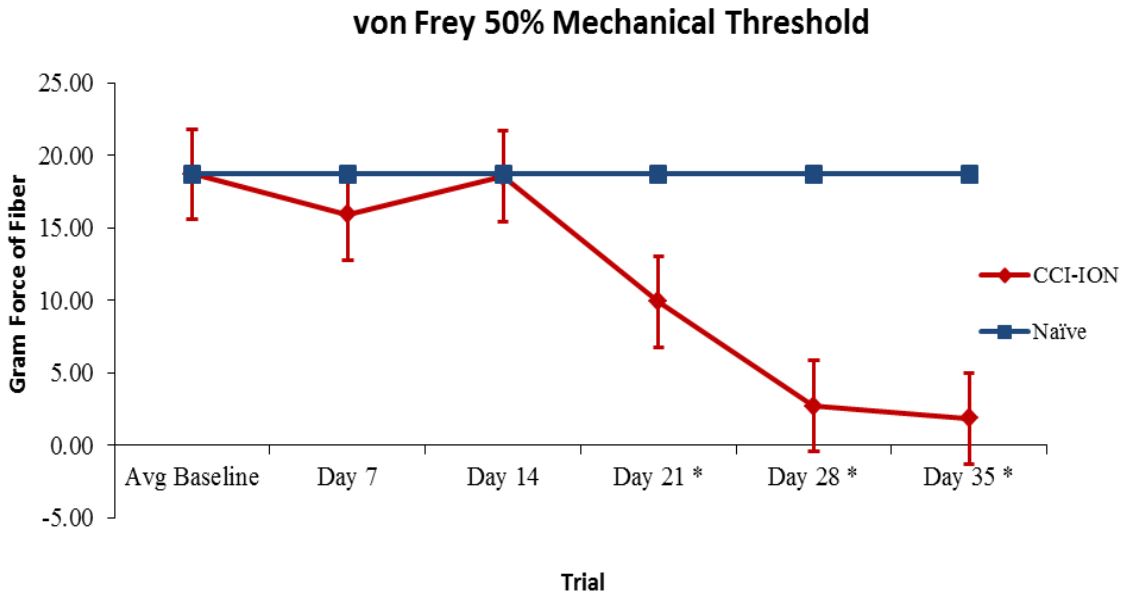

Figure 3.1, *von Frey 50% Mechanical Threshold*

Table 3.3

Means and Standard Deviations for Self-Regulation Task Analyses

Statistical Analysis	CCI-ION	Naïve
Correct Lever Presses – Baseline	174.00 (48.86)	178.06 (37.23)
Correct Lever Presses – Trial	166.50 (43.70)	183.56 (40.25)
Incorrect Lever Presses – Baseline	41.07 (14.03)	51.21 (11.01)
Incorrect Lever Presses – Trial	29.00 (13.41)	23.14 (8.86)
Correct:Total Ratio - Baseline	0.81 (0.05)	0.76 (0.06)
Correct:Total Ratio - Trial	0.85 (0.06)	0.87 (0.06)

difference between the two groups and no significant difference produced by trial/time (i.e. pre- to post-surgery trials) in number of correct lever presses.

For incorrect lever presses, two animals were identified as outliers and excluded from the analyses; therefore, the total N = 14 (7 animals in naïve group, 7 animals in CCI-ION group). A significant effect of group was not observed ($F(1,12) = 0.18$, $p = 0.68$). However, there was a significant effect of trial/time, such that animals in both groups produced significantly fewer incorrect lever presses in post-surgery trials than they did in pre-surgery baseline trials ($F(1,12) = 26.10$, $p < 0.001$). In addition, the interaction of group by time approached significance, indicating that animals in the naïve group experienced a greater decrease in number of incorrect lever presses from pre- to post-surgery trials than did animals in the CCI-ION group ($F(1,12) = 4.15$, $p = 0.06$; see Figure 3.2).

In the analysis of the ratio of correct to total lever presses in the self-regulation task, one animal was identified as an outlier and excluded; therefore, the total N = 15 (8 animals in naïve group, 7 animals in CCI-ION group). A significant effect of group was not observed ($F(1,13) = 0.40$, $p = 0.54$). However, there was a significant effect of trial/time, such that animals in both groups had a significantly higher ratio of correct to total lever presses in post-surgery trials than they did in pre-surgery baseline trials ($F(1,13) = 22.39$, $p < 0.001$). In addition, the interaction of group by time approached significance, indicating that animals in the naïve group experienced a greater increase in their ratio of correct to total lever presses from pre-to post-surgery trials than did animals in the CCI-ION group ($F(1,13) = 4.32$, $p < 0.06$; see Figure 3.3).

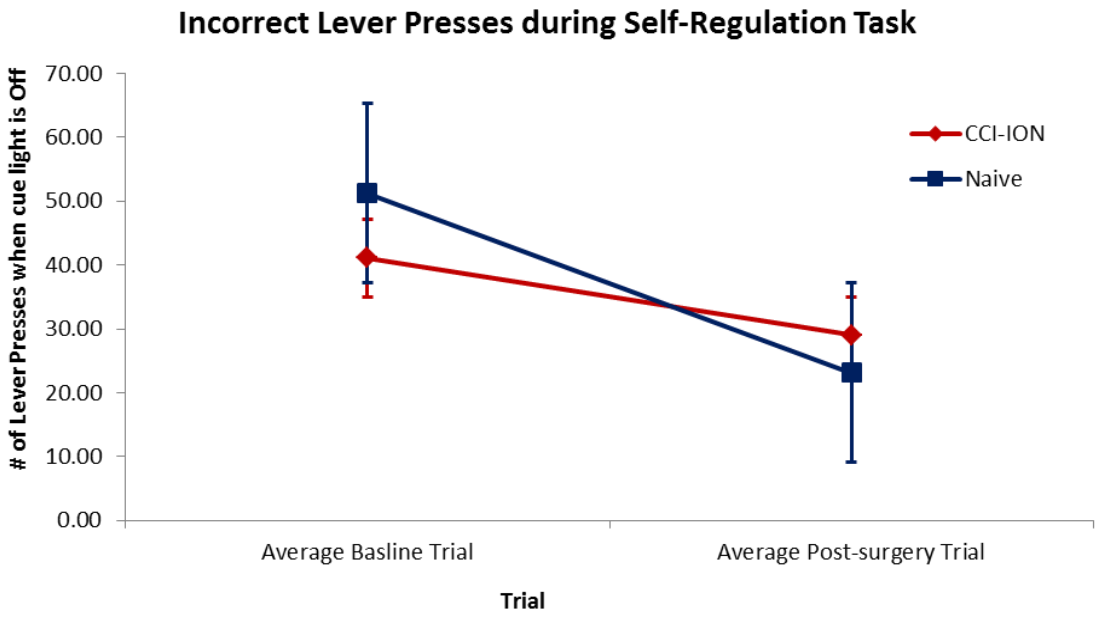

Figure 3.2, *Incorrect Lever Presses during Self-Regulation Task*

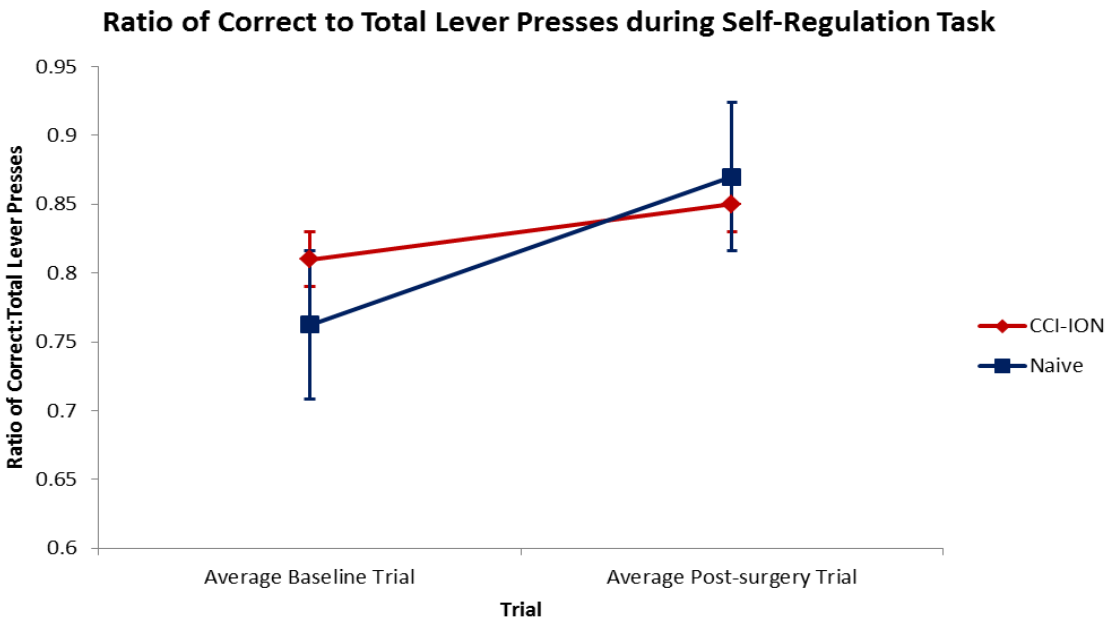

Figure 3.3, *Ratio of Correct to Total Lever Presses during Self-Regulation Task*

Persistence Task

In order to evaluate the a priori hypothesis, performance in the persistence task was analyzed with t-tests comparing total number of lever presses made and total time persisted (time of the last lever press) during the 10 minute persistence task. Follow-up analysis were run using repeated measures ANOVA analyses to compare these two measures to check for effects of group (i.e. CCI-ION versus naïve), trial/time (pre- or post-surgery), and the interaction of group by time. Mean and standard deviations for the persistence task can be seen in Table 3.4.

For total time persisted, one animal was identified as an outlier and excluded; therefore, the total N = 15 (8 animals in naïve group, 7 animals in CCI-ION group). In order to test the hypothesis that animals in the CCI-ION group would persist for a shorter amount of time than animals in the naive group, two-tailed, paired-samples t-tests were run on the total time persisted during the persistence task. Significant differences were not observed between pre- and post-surgery trials for either animals in the CCI-ION group ($t(5) = -1.49$, $p = 0.18$) or animals in the naïve group ($t(6) = 0.86$, $p = 0.42$).

Follow-up analysis with a repeated measures ANOVA indicated that there was no significant effect of group ($F(1,13) = 0.07$, $p = 0.80$), trial/time ($F(1,13) = 0.47$, $p = 0.55$), or the interaction of group by time ($F(1,13) = 3.02$, $p = 0.11$). These results indicate that there was no significant difference between the two groups and no significant difference produced by trial/time (i.e. pre- to post-surgery trials) in the total time animals persisted.

For total number of lever presses, one animal was identified as an outlier and excluded; therefore, the total N = 15 (7 animals in naïve group, 8 animals in CCI-ION group). In order to test the hypothesis that animals in the CCI-ION group would

Table 3.4

Means and Standard Deviations for Persistence Task Analyses

Statistical Analysis	**CCI-ION**	**Naïve**
Total Time – Baseline	378.86 (193.39)	477.56 (137.73)
Total Time – Trial	498.57 (58.16)	425.63 (120.27)
Total Lever Presses – Baseline	44.88 (12.28)	47.79 (23.73)
Total Lever Presses – Trial	41.94 (14.82)	31.07 (7.60)

fewer lever presses during the persistence task than animals in the naive group, two-tailed, paired-samples t-tests were run on the total number of lever presses made during the persistence task. These analyses showed that there was no significant difference for animals in the CCI-ION group between the number of lever presses made during the pre-surgery baseline trials and the post-surgery trials ($t(6) = 0.47$, $p = 0.66$). There was a nearly significant difference for animals in the naive group such that they pressed the lever fewer times in the post-surgery trials than they did in pre-surgery baseline trials ($t(5) = 2.13$, $p = 0.08$; see Figure 3.4).

Follow-up analyses indicated that there was no significant effect of group ($F(1,13) = 0.39$, $p = 0.54$) or the interaction of group by time ($F(1,13) = 1.91$, $p = 0.19$). However, the trial/time approached significance, indicating that animals in both groups made fewer lever presses in post-surgery trials than they did in pre-surgery baseline trials ($F(1,13) = 3.89$, $p = 0.07$).

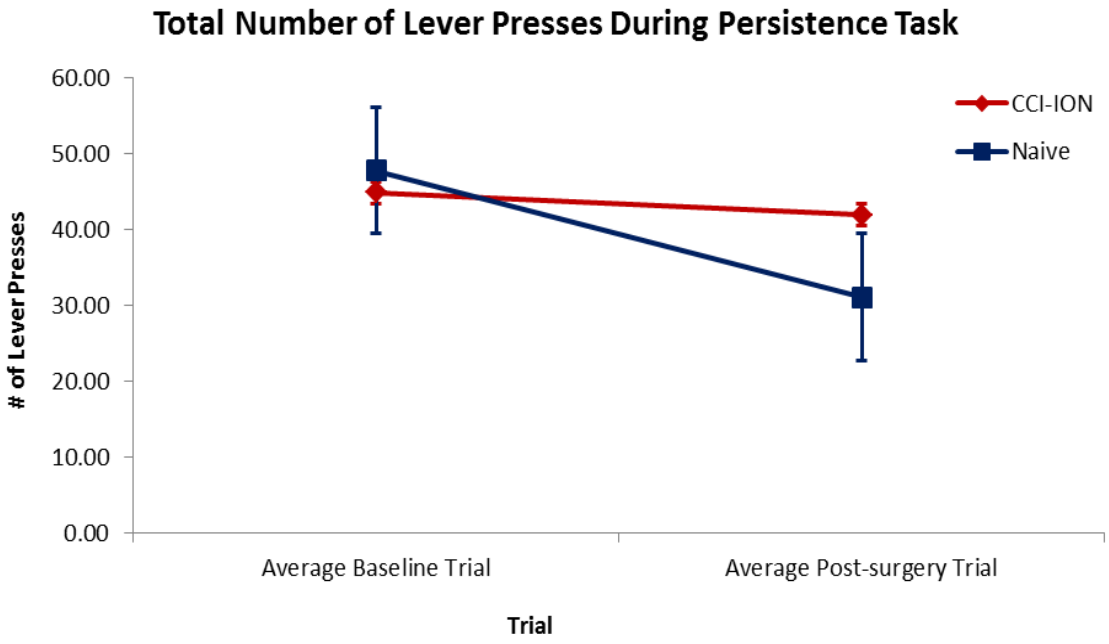

Figure 3.4, *Total Number of Lever Presses During Persistence Task*

Chapter Four: Discussion

The primary goals of the study were: (1) to demonstrate that self-regulation can be studied in a rodent model and (2) to study, in rodents, the effects of chronic pain on self-regulation. As mentioned previously, the establishment of a self-regulation model in rodents would be beneficial in allowing a greater amount of experimental control, as well as the ability to examine physiological mechanisms underlying self-regulatory processes. This model would further allow for pre-clinical trials of different treatments both of chronic pain and of the self-regulatory fatigue associated with chronic pain.

The present study was successful in utilizing the CCI-ION model of chronic, neuropathic pain as a manipulation. CCI-ION surgery had an 80% success rate of producing mechanical sensitivity of the whisker pad within five weeks post-surgery (i.e. eight of ten animals who underwent surgery developed mechanical sensitivity). Furthermore, statistical analyses of the eight animals that developed mechanical sensitivity demonstrate that animals in the CCI-ION group experienced significantly more mechanical sensitivity than animals in the naïve group.

It was expected that during the persistence task, animals in the CCI-ION group would persist for a shorter period of time and would press the lever fewer times than naïve animals. Statistical analyses found no significant effect for time persisted. In fact, the data obtained for total time persisted were highly variable with large standard deviations. Interestingly, there was a nearly significant effect of surgery on the number of lever presses made during the persistence task; however, this effect was in the opposite direction of our hypothesis. In other words, unlike CCI-ION animals, naïve animals had

a significant decrease in the number of lever presses made during the persistence task from pre- to post-surgery trials.

Although these findings were not anticipated, it seems that they may be related to the animals' capacity to learn. The persistence task utilized in the study is essentially an extinction trial, during which animals are no longer rewarded for previously rewarded behavior. Typically, animals would learn that they were no longer being rewarded and they would adjust their behavior by ceasing to press the lever. Our findings suggest that naïve animals behaved in this manner, and continued to improve from pre- to post-surgery trials (i.e., made fewer lever presses each time they were exposed to the persistence task). However, animals experiencing chronic pain did not show this improvement and continued to press the lever about the same number of times from pre- to post-surgery trials. Thus, the animals experiencing chronic pain may have been less able to learn that they were no longer being rewarded.

Results from the self-regulation task further support this interpretation. First, it was determined that animals in the naïve group experienced a greater decrease in number of incorrect lever presses from pre- to post-surgery trials than did animals in the CCI-ION group. This may be because animals in pain did not learn that lever presses when the cue light was off would not be rewarded as well as animals that were not in pain. Second, a difference was found in the ratio of correct to total lever presses such that animals in the naïve group experienced a greater increase from pre-to post-surgery trials than did animals in the CCI-ION group. This may be explained by the results above (i.e. because animals in the naïve group made fewer errors, they also had a greater ratio of correct to total lever presses).

In summary, the findings indicate that animals experiencing pain may be less capable of learning than animals in the naïve group, and thus slower or unable to figure out that they are no longer being rewarded for lever presses and to adjust their behavior accordingly. What remains unclear is whether these findings are attributable simply to a deficit in learning, to a deficit in self-regulation, or to a combination of the two. These two phenomena are closely related; and, as previously stated, a deficit in one domain can lead to further deficits in the other (Solberg Nes, Roach, and Segerstrom, 2009). Therefore, one explanation is that animals experiencing pain experienced a deficit in learning causing them to persist in pressing the lever even though no rewards were received. An alternative explanation is that animals experiencing pain were able to learn, but not able to regulate their behavior to reflect this learning. Finally, a third explanation is that animals experiencing pain experienced a deficit in self-regulation which in turn caused the deficit in learning. Thus further research is necessary to tease apart the effects seen in this study.

Limitations

First, the current study utilized a sample size of 16 animals (eight per group) based on an *a priori* power calculation. However, this calculation was based on a study of self-regulation in dogs. A new power analysis conducted using results from the current study and predicting the use of a repeated measures ANOVA, indicate that a total sample size of 24 animals (12 per group) is needed to achieve a power of 0.80 in the measure of total lever presses during the persistence task ($f = 0.38$, $\alpha = 0.05$). It is therefore reasonable to conclude that sample size utilized in the current study may have been too small to fully capture all of the significant effects.

Another limitation of this study is that it is currently unclear whether the effects observed in the current study are due to a deficit in learning, to a deficit in self-regulation, or to a combination of the two. To address this issue, it would be beneficial to follow-up this study with experiments that look for a deficit in learning following CCI-ION in a different behavioral protocol. One possibility for such research would be to examine the behavior of animals that underwent CCI-ION in a conditioned place preference task. Animals could be exposed to an aversive stimuli in one chamber of the apparatus, and then capacity for learning could be determined by observing how readily the animals learned to avoid that chamber in future trials.

Future Directions

As stated above, it would be beneficial to replicate these findings with a larger sample size and with hypotheses based on the findings of this study to allow for focused comparisons. It would also be beneficial to complete follow-up studies that use alternative learning paradigms to examine how chronic pain in animals influences their learning outcomes. For example, it would be beneficial to examine whether a deficit in attention, memory, or other cognitive ability was responsible for hindering the animals' ability to learn. These studies may be further extended by examining anatomical, physiological, and molecular differences between the two groups as a way of identifying the underlying processes associated with learning deficits. Finally, it is interesting to note that in previous human research relief of pain in chronic pain patients with opioid medications has failed to improve their cognitive functioning (Dick and Rashiq, 2007). The current behavioral model offers a unique opportunity to examine the effects of

medications on cognitive functioning (i.e., learning) in animals experiencing chronic pain.

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VITA

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Date of Birth: March 20, 1987
Place of Birth: Independence, Missouri

EDUCATION

August 2010 – Present	**University of Kentucky** Clinical Psychology Doctoral Program Current GPA: 4.0/4.0
August 2005 – May 2010	**Texas A&M University** B.S. in Psychology and B.A. in English Minor in Neuroscience Professional Writing Certificate (January 2009) GPA: 4.0/4.0 Honors Thesis: Impact of ghrelin and cocaine on intracranial self-stimulation in rats.

RESEARCH POSITIONS

July 2010 - Present	**Department of Physiology, University of Kentucky** Graduate Research Assistant
June 2009 – June 2010	**Department of Psychology, Texas A&M University** Undergraduate Research Assistant (Neuroscience)
January – May 2009	**Department of English, Texas A&M University** Undergraduate Research Assistant
August – December 2007	**Department of Psychology, Texas A&M University** Undergraduate Research Assistant (Social Psychology)

CLINICAL POSITIONS

July 2011 - Present	**Orofacial Pain Center** Behavioral Medicine Resident
August 2011 - Present	**Jesse G. Harris Psychological Services Center** Therapist to Individual Clients

TEACHING POSITIONS

August – December 2011	**Department of Psychology, University of Kentucky** Grader for Developmental Psychology Course

HONORS AND AWARDS

August 2010 - Present	Daniel R. Reedy Quality Achievement Award
August 2009 - May 2010	Janet Reed Schalit Scholarship
June – July 2009	Summer Undergraduate University Research Funding
August 2008 - May 2009	Stewart and Anna Morgan Scholarship
August 2005 – May 2009	Lechner Fellowship

MEMBERSHIP IN PROFESSIONAL ORGANIZATIONS

May 2012 – Present	Society for Neuroscience
August 2011 - Present	Kentucky Psychological Association
July 2011 - Present	American Psychological Association of Graduate Students
May 2009 – Present	Phi Beta Kappa

PUBLICATIONS

Clifford, P. S., Rodriguez, J., Schul, D., Hughes, S., **Kniffin, T.**, Hart, N., Eitan, S., Brunel, L., Fehrentz, J. A., Martinez, J. and Wellman, P. J. (2011). Attenuation of cocaine-induced locomotor sensitization in rats sustaining genetic or pharmacologic antagonism of ghrelin receptors. *Addiction Biology.* doi: 10.1111/j.1369-1600.2011.00339.x.

PRESENTATIONS

Yoder, W. E., Danaher, R. J., Westlund, K., Ma, F., Zhang, L., Wang, C., **Kniffin, T.**, Carlson, C., and Miller, C. S. (2012, March). *Viral vector delivery to trigeminal sensory neurons.* Poster presented at American Association for Dental Research Annual Meeting, Tampa, Florida.

Kniffin, T. C., Wellman, P. J., and Clifford, P. S. (2010, March). *Impact of ghrelin and cocaine on intracranial self-stimulation in rats.* Poster presented at Texas A&M Student Research Week, College Station, Texas. Received 2nd place in the Anatomy, Physiology, and Kinesiology Studies Taxonomy.

Kniffin, T. C., Wellman, P. J., and Clifford, P. S. (2009, November). *Impact of ghrelin and cocaine on intracranial self-stimulation in rats*. Poster presented at Texas A&M University System Pathways Student Research Symposium, Laredo, Texas.

PROFESSIONAL MEETINGS AND WORKSHOPS

Kentucky Psychological Association, Annual Convention. "Core Competencies in Psychology for the 21st Century." November, 2011. Lexington, Kentucky.

University of Kentucky, Markey Cancer Center. "Chemo Brain: Mechanisms and Assessments." October, 2011. Lexington, Kentucky.