PAIN PROCESSING IN THE ISOLATED SPINAL CORD:

ADAPTIVE NOCICEPTIVE MODIFICATIONS

A Dissertation

by

DENISE ALEJANDRA PUGA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2011

Major Subject: Psychology

Pain Processing in the Isolated Spinal Cord: Adaptive Nociceptive Modifications

Copyright 2011 Denise Alejandra Puga

PAIN PROCESSING IN THE ISOLATED SPINAL CORD:

ADAPTIVE NOCICEPTIVE MODIFICATIONS

A Dissertation

by

DENISE ALEJANDRA PUGA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Chair of Committee,	James Grau
Committee Members,	Mary Meagher
	Rajesh Miranda
	Michelle Hook
Head of Department,	Ludy Benjamin

May 2011

Major Subject: Psychology

ABSTRACT

Pain Processing in the Isolated Spinal Cord: Adaptive Nociceptive Modifications.

(May 2011)

Denise Alejandra Puga, B.A., New Mexico State University;

M.S., Texas A&M University

Chair of Advisory Committee: Dr. James W. Grau

We utilize a simple instrumental (response-outcome) learning task to measure spinal plasticity in the isolated spinal cord. Peripheral uncontrollable nociceptive input has been shown to disrupt spinal instrumental learning and induce enhance tactile reactivity. In contrast, 1.5mA of continuous shock has been found to induce antinociception and protect spinal plasticity from the detrimental consequences of uncontrollable stimulation. The experiments of this dissertation examined the link between the beneficial effects of continuous stimulation and antinociception.

The results replicated previous work examining the protective and antinociceptive effect of 1.5mA of continuous shock (Experiments 1-2). Novel to this research was the inclusion of a lower (0.5mA) intensity continuous stimulation. Results revealed that 0.5mA of continuous shock induced a comparable antinociception to that seen with 1.5mA of continuous shock (Experiment 1). At this lower intensity, however, continuous shock was unable to protect the isolated spinal cord from the detrimental effect of intermittent stimulation (Experiment 2). Further examination revealed that co-

administration of intermittent and continuous shock did not affect continuous shock-induced antinociception. This was true at both the higher (1.5mA) and lower (0.5mA) intensities of continuous shock (Experiment 3).

When 0.5mA of continuous shock was administered prior to intermittent shock, this intensity of continuous shock was better able to immunize the spinal cord from the induction of the learning deficit than 1.5mA (Experiment 4). Further analysis called into question the link between antinociception and the protective effect of continuous shock, as the beneficial effect of continuous shock outlasted the expression of antinociception (Experiment 5). Moreover, 0.5mA of continuous shock was found to reverse the expression of the learning deficit, when continuous stimulation was given after intermittent shock treatment (Experiment 6).

While blocking the induction of antinociception was not sufficient to prevent the immunizing effect of continuous shock, data suggest that the mu opioid receptor is implicated in the beneficial impact of continuous stimulation (Experiments 7 and 8). Endogenous brain derived neurotrophic factor (BDNF) release was also found to play a role (Experiment 9). Moreover, continuous shock was found to down-regulate the expression of early genes implicated in the development of central sensitization, *c-fos* and *c-jun*. Finally, we found that while continuous stimulation was detrimental to locomotor recovery after spinal cord injury, the combined treatment of continuous and intermittent shock did not negatively affect recovery (Experiments 11 and 12).

DEDICATION

The joy of dreaming is waking up to realize that life is more beautiful than that imagined. This work is dedicated to the guardians of my dreams, Roberto and Alba Puga, and the joy in my life, Christina Norman and Pamela Puga.

ACKNOWLEDGEMENTS

I would like to thank Dr. James W. Grau for being a kind and patient guardian through this process, and for always having the time to share his intellectual foresight and love of science. I would like to thank Dr. Michelle A. Hook for her mentorship and friendship throughout my graduate studies. I would like to thank the members of my dissertation committee, Drs. Mary W. Meagher and Rajesh C. Miranda. I would like to express my heartfelt gratitude to Dr. John R. Huie for being my teacher and friend. On that same note, I want to thank my best friend Sarah A. Woller for being an outstanding confidant and travel companion. For her incredible availability and know-how, I am greatly indebted to Dr. Sandra Garraway. Finally, I would like to thank Drs. Kyle M. Baumbauer and Stephanie Washburn, Kevin Hoy, and Milly Lee for their friendship and support.

TABLE OF CONTENTS

CHAPTER Page

LIST OF FIGURES

FIGURE Page

FIGURE Page

CHAPTER I

INTRODUCTION

Dissertation Overview

Primary interest in spinal plasticity is motivated by the clinical application of extensive laboratory studies that have examined the functional capabilities of spinal circuits. A number of currently utilized clinical therapeutic interventions for the treatment of spinal cord injury (SCI) originated from laboratory experiments delineating the ideal conditions under which the spinal cord responds to environmental manipulations. Countering this excitement, however, is the ever-growing literature that highlights spinal plasticity as mediating the development of maladaptive pain after injury. More importantly, there is new research suggesting that uncontrollable nociceptive information disrupts the adaptive potential of spinal neurons (Baumbauer et al., 2008; Ferguson, Crown, & Grau, 2006; Grau el al., 1998; Hook, Huie, & Grau, 2008). The significance of these findings becomes apparent when we consider the high incidence of pain reporting after SCI (Anderson, 2004; Siddall & Loesser, 2001). Not only are SCI patients afflicted with the affective component of pain, but also, evidence indicates that chronic pain can interfere with rehabilitation efforts to promote functional recovery after injury.

It is, thus, essential to identifying therapeutic interventions that can both alleviate

---

This dissertation follows the style and format of *Behavioral Neuroscience.*

injury induced pain and promote functional recovery. Previous work in our laboratory has identified a promising candidate, continuous electrical stimulation, which both a) protects spinal plasticity from uncontrollable stimulation and b) causes a decrease in thermal responding to radiant heat (antinociception) (Crown et al., 2002). In the subsequent sections, leading up to the findings of the current dissertation, a brief introduction into the behavioral potential of the spinal cord will be presented. This effort will include research on the well-documented capability of spinal neurons to adapt to environmental manipulation, and the role of spinal plasticity in the development of maladaptive pain. Moreover, research will be presented that highlights the potential of electrical stimulation as a therapeutic intervention in the treatment of SCI-associated pain, with the added benefit of harnessing spinal plasticity.

Spinal Plasticity: Functional Recovery and Chronic Pain after SCI

Plasticity is ubiquitous in the central nervous system (CNS); this intrinsic characteristic of all neuronal systems is evident in spinal circuits, as so: spinal neurons are able to undergo both functional and structural changes in response to descending and ascending information. Interest in spinal plasticity has centered on both understanding the role of spinal neurons in the acquisition and maintenance of motor skills, as well as the ability of spinal circuits to not only transmit but also, more interestingly, alter pain signals at the level of the spinal cord. Efforts to delineate the underlying mechanisms responsible for the behavioral potential of spinal neurons, both as it pertains to motor and sensory processing, are especially important in instances where the normal function

of the CNS has become compromised. Although this question is relevant to a number of CNS disorders, our current focus is on identifying how changes in sensory processing can alter motor acquisition learning in spinal neurons after SCI.

The question at hand is rich in complexity, as the onset of SCI initiates both adaptive and maladaptive mechanisms at the level of the spinal cord that have the potential to influence long-term recovery after injury. For our purposes, it is important that we first move away from the larger picture (i.e. the anatomical, immunological, etc. consequences of SCI) and focus on a fundamentally simpler system/question; more precisely, before we can begin to understand how complex factors (such as the immune system) impact recovery of function, we must first acquire a basic understanding of the neurobiology and plasticity of spinal neurons. Fortunately, decades of research have paved the way to our current understanding of how spinal neurons function in the absence of supraspinal input. As already discussed, spinal plasticity underlies both sensory and motor adaptations, which is true not only in intact organisms but also in the isolated spinal cord. This latter finding has opened the door to research identifying how environmental manipulations can be utilized to foster adaptive plasticity in the injured spinal cord.

A popular approach to assess how environmental cues affect spinal function involves a surgical transection of the lower lumbar-sacral region of the spinal cord, thereby severing communication between the lower spinal cord and the brain. This procedure produces a paraplegia in rats that blocks the affective sensation of pain below the waist, and negates the ability to voluntarily initiate motor movement. Research in our

laboratory has focused on characterizing the conditions under which the spinal cord adapts to new environmental relations in the absence of supraspinal input (for review see Grau et al., 2006). Using an instrumental paradigm, we have shown that the isolated spinal cord is sensitive to response-outcome relations (controllable shock), whereby spinally transected rats that receive shock to one hindlimb contingent on leg placement, over time, acquire a target response (an increase in flexion duration) that minimizes shock exposure (Grau et al., 1998). However, if shock is non-contingent (uncontrollable shock) to leg position, subjects fail to display an increase in flexion duration. Furthermore, animals initially exposed to uncontrollable shock fail to learn when later tested with response-contingent shock on both the ipsilateral and contralateral limb.

Of interest, both behavioral and pharmacological data suggest that the induction of this spinal learning deficit is related to the development of neuropathic pain (Ferguson, Crown, & Grau, 2006). Neuropathic pain is thought to result from a diffuse over-excitation of nociceptive neuronal circuits within the spinal cord, a phenomenon known as central sensitization (Coderre & Melzack, 1992; Campbell & Meyer, 2006; Ji et al., 2003). Evidence indicates that central sensitization depends on many of the same neurochemical systems implicated in hippocampal long-term potentiation (LTP), such as NMDA receptor (NMDAR)-mediated plasticity (Ji et al., 2003; Woolf & Thompson, 1991). In accordance with this, data collected in our laboratory has found that pharmacologically antagonizing the NMDA receptor disrupts both spinal learning and the induction of the learning deficit (Ferguson, Crown & Grau, 2006; Joynes, Janjua, & Grau, 2004). As well, exposure to uncontrollable nociceptive information (provided by

intermittent shock) has been shown to enhance reactivity to mechanical stimulation (allodynia) (Ferguson, Crown, & Grau, 2006). Moreover, there are data suggesting that uncontrollable nociceptive information disrupts sensory and locomotor recovery after SCI (Grau et al., 2004). For these reasons, it is pressing that we identify therapeutic interventions that can counter the detrimental consequences of uncontrollable nociceptive information in the compromised spinal cord.

Therapeutic Interventions: Harnessing Spinal Plasticity

Our laboratory has sought to identify both behavioral and pharmacological interventions that can be used to prevent and/or reduce the impact of uncontrollable nociceptive input in the injured spinal cord. One approach that has yielded promising results is the use of controllable shock (instrumental training) (Crown & Grau, 2001). Spinalized rats that receive 30 minutes of controllable shock prior to uncontrollable intermittent shock to the tail, when tested on the contralateral hindlimb, do not show the learning deficit. Moreover, controllable shock has been found to restore the behavioral potential of the isolated spinal cord. Spinally transected rats that were treated with controllable shock after intermittent shock treatment, in the presence of intrathecal naltrexone, showed a reversal of the intermittent shock-induced learning deficit. Thus, controllable shock can be used to both immunize against the learning deficit and restore the potential of the isolated spinal cord.

The beneficial impact of instrumental training appears to be mediated by the extent to which spinal neurons can control and predict the onset and termination of

noxious stimulation. Interestingly, new research suggests that spinal neurons are also sensitive to temporal relations, whereby temporal distribution of stimuli differentially affect behavior (Baumbauer, Huie, Hughes, & Grau, 2009). For instance, spinally transected rats that received 180 variable-spaced pulses applied to the tail, when later tested with controllable shock, exhibit a learning deficit. The same results are observed when rats are treated with 180 (ITI = 2s) fixed-variable pulses. However, spinalized rats treated with 900 fixed-spaced pulses to the tail do not exhibit the learning deficit. Extending the number of pulses from 180 to 900 changes the impact of fixed-spaced, but not variable-spaced, stimulation on spinal learning. More importantly, treatment with 900 fixed-spaced pulses both prevents and reverses the spinal learning deficit (similar to what is seen with controllable shock). Evidence indicates that the beneficial impact of fixed-spaced stimulation and controllable shock are mediated by the release of endogenous brain derieved neurotrophic factor (BDNF) (Baumbauer, Huie, Hughes, & Grau, 2009; Gomez-Pinilla et al., 2007). This is of particular importance, given that exogenous BDNF treatment has been found to have protective and therapeutic effects against the detrimental consequences of uncontrollable stimulation in the isolated spinal cord (Huie et al., 2006).

Another form of protection, and the topic of the current set of experiments, was discovered in studies examining the impact of continuous (360s of continuous 1.5 mA tailshock) versus intermittent uncontrollable shock on spinal learning (Crown et al., 2002). Prior work has shown that 15-360s of continuous 1.5 mA tailshock induces a robust antinociception in spinally transected rats (Crown et al., 2002). Interestingly, in

contrast to uncontrollable intermittent shock, continuous shock does not induce a spinal learning deficit. In fact, when continuous shock is administered at the same time as intermittent shock, continuous shock protects against the adverse effects of intermittent shock on spinal plasticity (Crown et al., 2002). These findings have led us to question whether the onset of antinociception mediates the protective effect of continuous shock against intermittent shock by silencing uncontrollable nociceptive signals at the level of the spinal cord. For instance, we know that intermittent shock causes an allodynic response in spinally transected animals (Ferguson, Crown, & Grau, 2006), which is contrary to what is seen in intact animals.

In intact animals, exposure to intermittent uncontrollable shock leads to a decrease in nociceptive responding. This effect has been linked to the release of spinal dynorphin and descending serotonin tracts (Drugan, Ader, & Maier, 1985; Grahn et al, 1999; Jackson, Maier, & Coon, 1979; Watkins, Wiertelak, & Maier, 1992). While in spinally transected animals, the release of endogenous spinal dynorphin has been linked to the expression of the learning deficit, such that: pretreatment with the kappa opioid receptor antagonist nor-BNI has been found to attenuate the expression of the learning deficit, and the treatment with the kappa-2 receptor agonist GR89696 has been shown to produce a dose-dependent inhibition of learning (Joynes & Grau, 2004; Washburn, Maultsby, Puga & Grau, 2008). Hence, it would appear that removing descending serotenergic fiber function after transection blocks the ability of uncontrollable intermittent shock to induce antinociception in the isolated spinal cord, removing a

dampening effect that may normally counter the development of over-excitation (Crown & Grau, 2005).

There is one more piece of evidence underscoring the importance of silencing uncontrollable nociceptive signaling at the level of the spinal cord, as a means of protecting spinal plasticity. Fixed spaced stimulation, in addition to promoting spinal learning, has been found to block the negative effect of capsaicin-induced inflammation on spinal plasticity (Baumbauer et al., 2009b). Spinalized rats treated with capsaicin showed both allodynia and the learning deficit. In contrast, animals treated with 900 fixed spaced tailshocks showed hyporeactivity to mechanical stimulation. More importantly, treatment with fixed space stimulation prior to and after capsaicin treatment prevented and reversed, respectively, the expression of the capsaicin-induced learning deficit. It is important to note, however, that fixed spaced stimulation did not lead to changes in thermal responding, suggesting an alternative mechanism of action for the protective effect of fixed spaced stimulation that is independent of antinociception. Nonetheless, these data support the hypothesis that by silencing the effects of uncontrollable stimulation at the level of the spinal cord, it is possible to protect spinal neurons from the deleterious effects of uncontrollable stimulation.

Clinical Application

There is an interesting gap in the study of how pain mechanisms and factors that influence recovery of function interact after SCI. Both elements are critical to our understanding of spinal plasticity. However, in clinical and laboratory research, there

appears to be an either/or situation: researchers either focus on the causal mechanisms of pain with the aim of alleviating the affective component of injury, or center their efforts on improving recovery of function by promoting endogenous and exogenous mediators to that effect. Few studies have ever examined how the development of chronic pain after injury impacts functional recovery.

Coming to terms with the dual nature of plasticity after SCI may serve to open a fruitful avenue of research. For instance, it is now widely accepted that the immune system both protects and harms the morphology of the injured spinal cord, thereby both promoting and limiting functional recovery after SCI (Donnelly & Popovich, 2008; Stoll, Jander, & Schroeter, 2002). With this in mind, many researchers are now attempting to disrupt the maladaptive component of the immune system, while enhancing the beneficial impact of the immune-mediated response. Similarly, by acknowledging that not all neural modifications after injury are beneficial, we can better design therapies to both promote functional recovery and decrease the incidence of pain after injury.

Transcutaneous electrical stimulation (TENS) is currently available for the treatment of chronic pain. TENS is defined by the American Physical Association as the application of electrical stimulation to the skin for pain control. Several theories support the use of TENS to produce pain relief, including the gate control theory and release of endogenous opioids (for review see Sluka & Walsh, 2003). In rats, TENS has been shown to reduce hyperalgesia after carrageenan administration (Ainsworth et al., 2006), and to decrease the release of the excitatory neurotransmitters glutamate and aspartate in

animals with joint inflammation (Sluka, Vance, & Lisi, 2005). In clinical studies, peripheral electrical stimulation has been shown to significantly decrease morphine requirements post-operatively (Burchiel et al., 1996). As well, there is data supporting the use of TENS as a potent analgesic in the treatment of chronic back pain, with the added benefits of increasing physical activity and improving quality of sleep (Ghoname et al, 1999).

Given the data that have been reviewed up to this point, a convincing argument can be made that in instances when inhibitory supraspinal systems become compromised, such as after a SCI, therapeutic electrical stimulation might be useful in lessening the detrimental effects of nociceptive insult to the spinal cord. Ideally, if TENS-like stimulation can inhibit the adverse effects of uncontrollable afferent input, it can be utilized in the clinic to attenuate over-excitation and cell death, as well as chronic pain, after SCI.

Specific Aims

The experiments of this dissertation are designed to further examine the behavioral consequences of continuous shock stimulation in the isolated spinal cord, as it pertains to nociceptive processing and spinal plasticity. Experiments will be conducted to examine under what circumstances the protective effect of continuous shock is observed. As well, we will measure if continuous shock has a lasting effect against the induction of the learning deficit (Aim 1). Through the use of pharmacological and molecular techniques, data will be collected to further our understanding of what

neuronal changes underlie the protective effect of continuous stimulation. An initial investigation will be conducted to assess if the protective effect of continuous shock is mediated by the release of endogenous opioids (Aim 3). Subsequent experiments will be conducted to determine if continuous shock shares a similar neurobiological profile with other shock schedules (instrumental training and fixed variable shock) known to block and reverse the induction of the learning deficit (Aim 3). Finally, we will examine if continuous stimulation can be utilized as a therapeutic tool in the treatment of uncontrollable nociceptive stimulation after a contusion injury in the rat, an animal model of incomplete SCI (Aim 4).

CHAPTER II

GENERAL METHODS

Subjects

Male, Sprague-Dawley rats obtained from Harlan (Houston, TX) were utilized as subjects for these experiments. Animals were approximately 100-120 days old and weighed between 310 and 410 grams. Subjects were individually housed with water and food available *ad libitum*, and maintained on a 12 hour light-dark schedule. Behavioral testing was conducted during the light portion of the cycle. All animal care protocols are in accordance with the Texas A&M University Laboratory Animal Care and Use Committee.

Surgery

Spinal Transection

Surgeries consisted of a complete transection of the spinal cord at the second thoracic vertebra (T2). Animals were anesthetized with isoflurane, and the area surrounding the shoulders was shaved and sterilized with iodine. An anterior-posterior incision approximately 1.5 cm in length was made over the second thoracic vertebra, and the tissue immediately anterior to T2 was cleared to expose the spinal cord. The exposed cord was transected with cauterization, and the ensuing space was filled with Gelfoam (Harvard Apparatus, Holliston, MA). Next, a cannula (25 cm of polyethylene tubing) fitted with a stainless steel wire (0.09 mm diameter) was inserted into the subarachanoid space on the dorsal surface of the cord. The cannula was inserted 9 cm down the ventral

column, and the exposed end of the tubing was secured with the use of an adhesive to the skin. The incision was closed with Michel Clips (Fine Science Tools, Foster City, CA), and immediately thereafter, the animals received an injection of 0.9% saline (2.5 ml, i.p.) to maintain hydration.

During recovery, animals were maintained in a temperature-controlled environment (25.5 °C) with food and water available at ad libitum. Bladders were expressed at least twice a day, and immediately before performing any behavioral procedures. To confirm full transection of the cord: a) a visual inspection was performed during surgery, b) animals were monitored to ensure complete paralysis below the forelimbs and a lack of vocalization during shock exposure, and c) cords were examined in a randomly selected subset of post-mortem subjects.

Contusion Injury

Subjects received a contusion injury using the MASCIS device developed by Gruner (1992) and Constantini and Young (1994). Subjects were anesthetized with isoflurane. After a stable, and comparable, level of anesthesia is achieved, a 7.0 cm incision was made over the spinal cord. The vertebrate dorsal and medial to T10-T11 were cleared and the spinal tissue was exposed. The vertebral spinal column was fixed within the MASCI device and a moderate injury was produced by allowing the 10g impactor (outfitted with a 3.0 mm tip) to drop 12.5 mm. After injury, the subject were removed from the device, placed on a heating pad, and the wound was closed with Michel clips. To help prevent infection, subjects were treated with 100,000 units/kg

Pfizerpen (penicillin G potassium) immediately after surgery and again 2 days later. For the first 24-hrs after surgery, rats were placed in a recovery room maintained at 26.6 degrees C. To compensate for fluid loss, animals were given 2.5 ml of saline after surgery. Michel clips were removed 14 days after surgery. Bladders were expressed morning and night until subjects voided on their own for 3 consecutive days.

Apparatus

Instrumental Training and Testing

Instrumental testing was conducted while rats were loosely restrained in Plexiglas tubes (23.5 cm [length] and 8 cm [diameter]). Two slots in the tube, (5.6 cm [length] and 1.8 cm [diameter]), 4 cm apart, 1.5 cm from the end of the tube, allowed for both hind legs to hang freely. To minimize the effects of upper body movement on leg position, a wire belt was used to secure the rat's trunk within the tube. Leg shock was delivered using BRS/LVE (Laurel, MD) constant current (60Hz, AC) shock generator (Model SG-903). Two electrodes inserted over the tibialis anterior muscle were connected to a computer-controlled relay to regulate the application of leg shock.

Leg position was monitored during testing using a contact electrode constructed from a 7 cm long, 0.46 mm diameter stainless steel rod taped to the foot. The last 2.5 cm of the electrode was insulated from the foot with heat shrink tubing. A fine wire (0.01 sq mm [36 AWG] (20cm) attached to the end of the rod was extend from the rear of the foot and was connected to a digital input monitored by a Macintosh computer. A plastic rectangular dish (11.5 [w] x 19 [l] x 5[d]) containing a NaCl solution was placed

approximately 7.5 cm below the restraint tube. A drop of soap was added to the solution to reduce surface tension. A ground wire was connected to a 1 mm wide stainless steel rod, which was placed in the solution. When the contact electrode attached to the rat's paw touched the solution, it completed the circuit monitored by the computer, delivering a shock to the tibialis anterior. The state of this circuit wase sampled at the rate of 30 times/s.

Flexion force was measured by attaching a monofilament plastic line ("4 lb test" Stren, Dupont, Wilmington DE) to the rat's foot immediately behind the plantar protuberance. The 40 cm length of line passed through an eyelet attached to the apparatus directly under the paw, 16 cm beneath the base of the tube. The end of the line was attached to a strain gauge (Fort-1000, World Precision Instruments, new Heaven, CT) fastened to a ring stand. After the line was connected to the rat's paw, the ring stand was positioned so that the line was taut, just barely registering on the gauge. The strain gauge was calibrated by determining the relationship between voltage and force in Newtons. This data revealed a linear relation, which allowed us to convert voltage to force.

Tailshock

During tailshock delivery, rats were loosely restrained in opaque black Plexiglas tubes (22 cm [length] and 6.8 cm [diameter]). A 660-V transformer was used to generate tailshock. AC shock was administered through electrodes constructed from a modified fused clip covered in electrode paste, and taped to the rat's tail approximately 7.5 cm

from the tip. A computer was used to control the onset and offset of tailshock.

Nociceptive Reactivity

Nociceptive reactivity to radiant heat was accessed with an automated tail-flick device. Heat was provided by a 375-W movie light that was focused onto the rat's tail by means of a condenser lens positioned 8 cm below the light source. The light source illuminated approximately 2 cm of the rat's tail. Light intensity was controlled by an AC potentiometer (#6681-W, Leviton, Little Neck, NY), and the rat's tail was rested on a 0.5cm deep groove embedded on an aluminum block positioned 4.7 cm below the condenser lens. If the subjects failed to respond, the test trial was terminated after 8 s of heat exposure to avoid tissue damage.

Procedures

Instrumental Learning Testing Procedure

All subjects were allowed to recover for 24-hrs following surgery and the hindlimbs were shaved and marked for electrode placement prior to testing. A wire electrode was then inserted through the skin over the distal portion of the tibialis anterior (1.5 cm from the plantar surface of the foot), and one lead from the generator was attached to this wire. A contact electrode was secured to the foot between the second and the third digits with a piece of porous tape. The shock generator was set to deliver a 0.4 mA shock, and the proximal portion of the tibialis anterior (approximately 1.7 cm proximal to the wire electrode) was probed with a 2.5 cm stainless steel pin attached to a

shock lead to find a robust flexion response. The pin was then inserted 0.4 cm into the muscle. A strain gauge was utilized to verify that a single, intense (1.6 mA, 0.3 s) test shock could elicit at least a 0.8N flexion force, and to determine the amount of shock necessary to elicit a 0.4 flexion force. This amount was recorded.

To minimize lateral leg movements, a 20 cm porous tape was wrapped around the leg and attached to a bar extending across the apparatus directly under the front panel of the restraining tube. The tape was adjusted so that it was taught enough to slightly extend the knee. Finally, three short (0.15s) shock pulses were applied and the level of the salt solution was adjusted so that the tip of the contact electrode (attached to the rat's foot) was submerged 4 mm below the surface. A rat's capacity to perform the instrumental response was then tested with exposure to 30 min of controllable shock. Whenever the rat's leg fell below the level of the salt solution, the electrodes delivered a shock to the tibialis anterior muscle causing the ankle to flex. Leg position was monitored using a Macintosh computer at a sampling rate of 30 Hz.

Behavioral Measures

Three behavioral measures were used to assess a subject's capacity to perform the instrumental response: response number, response duration and time in solution (see Grau et al., 1998). Performance was measured over time in 30 1–min time bins. The computer monitoring leg position recorded an increase in response number whenever the contact electrode left the salt solution. Response duration was derived from time in

solution and response number using the following equation: Response Duration^{i} =(60 s – time in solution^{i})/(Response Number^{i} +1) where *i* is the current time bin.

Monitoring Recovering After a Contusion Injury

Locomotive performance was assessed in an open field using the procedure and apparatus described by Basso et al., (1995). Following Basso et al., a circular plastic chamber (99 cm diameter, 23 cm wall height) served as the open field enclosure. Prior to surgery, subjects were acclimated to transport, handling and the open field apparatus (15 min/day) for 4 days. During testing, subjects were placed in the open field and observed by two experimenters (blind to the subject's pretreatment condition) for 4 min. Intermediate milestones include: slight movement of the joint (1), extensive movement of the three joints (7) occasional weight supported stepping in the absence of coordination (10), and consistent weight supported stepping with consistent FL-HL coordination (14). Working with Beattie and Bresnahan, our laboratory has shown how a simple transformation improves the metric properties of the BBB scoring procedure (Ferguson et al., 2004).

Assays

RNA Extraction and RT-PCR

At 30 minutes following shock/unshock treatments, the subjects were anesthetized with pentobarbital (50mg/kg) and 1 centimeter of lumbar spinal cord was rapidly removed. To determine the spatial changes in the expression of genes of interest, the spinal cord was hemisected dorsa-ventrally to yield dorsal and ventral portions. The

cord was processed for the extraction of both total RNA (RNeasy Mini Kit; Qiagen, Valencia, CA) and total protein. Total RNA (100 ng) was converted into cDNA using TaqMan EZ RT-PCR Core reagents (Applied Biosystems) and the mRNA levels of (Include targets) were measured by TaqMan real-time quantitative RT-PCR using an StepOnePlus™ Real-Time PCR System (Applied Biosystems, Carlsbad, CA.). β-actin served as a control gene. The sequences of probes, forward and reverse primers for all targets were obtained from Applied Biosystems.

Statistics

All data were analyzed using an analysis of variance (ANOVA), with an *a priori* alpha value of .05. Group differences were further evaluated using Duncan's New Multiple Range *post hoc* tests.

CHAPTER III

BEHAVIORAL CONSEQUENCES OF CONTINUOUS STIMULATION

Previous work has shown that continuous shock induces both antinociception in spinally transected animals and protects against the learning deficit (Crown et al., 2002). The experiments of the current chapter were designed to examine if there is a relationship between the induction of antinociception and the protective effect of continuous shock.

Experiment 1

Treatment with continuous shock affords the isolated spinal cord protection from the detrimental effects of intermittent shock on spinal learning. One potential mechanism underlying the beneficial impact of continuous shock is the induction of antinociception. Treatment with 15-360s of continuous 1.5mA tailshock has been shown to decrease tailflick latencies in response to radiant heat in spinally transected rats (Crown et al., 2002). In contrast, intermittent shock has been found to induce both the learning deficit and cause enhanced responding to mechanical stimulation (Ferguson, Crown, & Grau. 2006). The current experiment is designed to replicate previous findings by measuring the impact of continuous shock on thermal responding in spinally transected rats. Novel to this study is the inclusion of a lower intensity (0.5 mA). By including a lower intensity of continuous shock, we hope to identify a form of electrical stimulation that can a) afford the spinal cord protection from uncontrollable intermittent stimulation and

b) induce antinociception, while producing minimal discomfort/pain if utilized in spinally contused animals.

Procedure

Prior to shock treatment, baseline tailflick data (3 tail-flick scores, 2min apart) were collected for all subjects. Immediately thereafter, subjects received 360s of 0, 0.5, or 1.5mA of continuous shock to the tail (n=8 for all groups). After shock treatment, all subjects were assessed for changes in thermal reactivity. Five tail-flick scores were collected, two minutes apart, over a 10 min period.

Results

The results are presented in Figure 1. The mean baseline scores are shown on the left of the graph. An ANOVA revealed no statistical difference in tail-flick latencies among groups prior to shock treatment, all Fs <1.76, $p > .05$.

Mean test tail-flick latencies are presented to the right of the baseline scores. Treatment with continuous shock (0.5 or 1.5mA) produced an increase in tail-flick latencies as compared to unshocked animals. An ANCOVA controlling for baseline scores revealed a significant main effect of shock, $F(2,20) = 3.77$, $p < .05$, while no other term reached significance, all Fs <1.0, $p > .05$. *Post hoc* comparisons of the group means showed that both 0.5 and 1.5mA of continuous shock produced a statistically significant increase in tail-flick latencies, as compared to unshocked animals. There was, however,

no significant difference between the groups that received 0.5mA and 1.5mA of continuous shock, $p < .05$.

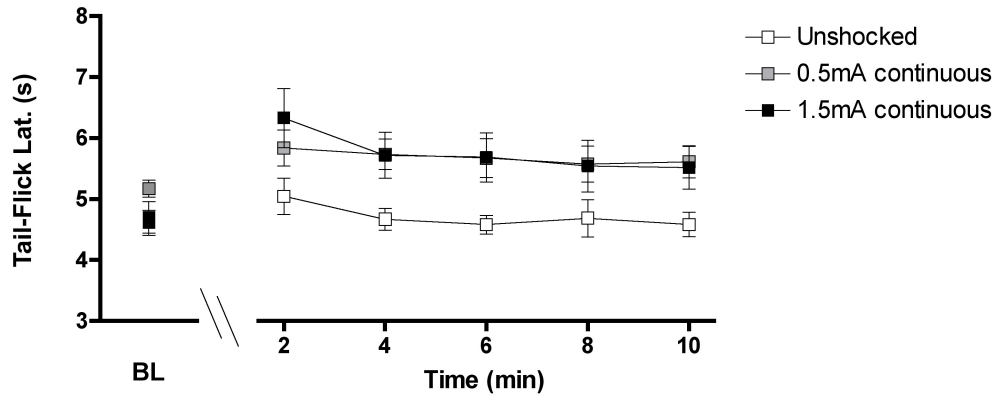

Figure 1. Exposure to continuous shock (0.5 or 1.5mA) induces an increase in thermal responding in spinally transected rats.

Summary

As previously reported, exposure to 1.5mA of continuous tailshock induced a decrease in thermal responding in spinally transected rats. Of interest, current results indicate that a lower intensity (0.5mA) of continuous shock produced comparable antinociception in the isolated spinal cord. This latter finding suggests that, if in fact, the induction of antinociception is what mediates the protective effect of continuous shock, then 0.5mA of continuous shock should be as efficient as 1.5mA of continuous shock in protecting against the intermittent-shock induced learning deficit.

Experiment 2

Treatment with 360s of continuous 1.5mA tailshock protects against the effects of intermittent uncontrollable stimulation on spinal learning. The current experiment was designed to examine if a lower intensity (0.5mA) of continuous shock, which has been shown to induce a comparable antinociception to 1.5mA of continuous shock, can protect against the intermittent shock-induced learning deficit.

Procedure

Spinally transected rats were exposed simultaneously to both intermittent leg shock and 360s of 0, 0.5, or 1.5mA of continuous tailshock (n=8 for all groups). All rats were instrumentally tested 24hrs after shock exposure.

Results

Rats exposed to intermittent uncontrollable shock, in the absence of continuous shock, failed to display a progressive increase in response duration, our index of spinal learning (Figure 2). Only animals treated with 1.5mA of continuous shock acquired the instrumental response, independent of intermittent shock treatment. Rats treated with 0.5mA of continuous tailshock and intermittent shock were unable to acquire the instrumental response. An ANOVA revealed a significant main effect of shock, $F(2,15) = 4.10$, $p < .05$, and trials, $F(29, 435) = 2.45$, $p < .05$. *Post hoc* comparisons of the group means revealed that there was no significant difference between animals that received 0

and 0.5mA of continuous tailshock. Only animals were that were treated with 1.5mA of continuous tailshock significantly differed, $p < .05$.

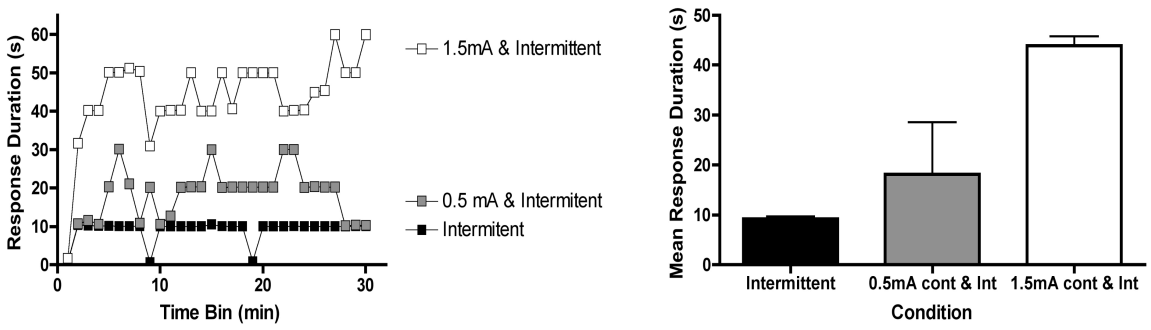

Figure 2. Treatment with 1.5mA, but not 0.5mA, of continuous shock protected the isolated spinal cord from the intermittent shock-induced learning deficit.

Summary

As expected, 360s of continuous 1.5mA tailshock blocked the induction of the intermittent shock-induced learning deficit. Results, however, revealed that 0.5mA of continuous shock failed to protect against the induction of the learning deficit. In Experiment 1, we found that 0.5mA of continuous shock elicited a comparable antinociception to that previously shown with 1.5mA. One question that the current experiment was designed to answer was whether the induction of antinociception was sufficient to prevent the effects of intermittent shock on spinal learning. Given that 0.5 and 1.5mA of continuous shock produced comparable antinociception, and only 1.5mA

of continuous shock was found to be protective, it appears that the expression of antinociception is not sufficient to protect against the learning deficit.

Experiment 3

Before we can discount that the expression of continuous shock-induced antinociception is not sufficient to protect against the learning deficit, we must first examine whether the co-administration of continuous and intermittent shock impacts nociceptive processing in the isolated spinal cord. Previous work has shown that intermittent shock induces bilateral allodynia in spinalized animals. In contrast, we have shown here and elsewhere that continuous shock (0.5 and 1.5mA) produces a decrease in thermal responding. Given the opposite impact that intermittent and continuous shock have on spinal nociceptive processing, the current experiment was designed to examine if intermittent shock affects the expression of continuous shock-induced antinociception.

Procedure

Baseline tailflick scores were collected for all subjects. Thereafter, spinalized rats received continuous tailshock (0, 0.5, or 1.5mA) and intermittent shock to the leg simultaneously (n=8 for all groups). After shock treatment, all rats were examined for changes in tailflick latencies.

Results

Mean baseline scores are shown on the left of the graph in Figure 3. An ANOVA revealed that there was no statistical difference in baseline tailflick latencies, prior to shock treatment, all *F*s <1.0, $p > .05$.

Mean test tail-flick latencies are presented to the right of the baseline scores. Spinalized rats that received continuous (0.5 or 1.5mA) shock to the tail showed increased tailflick latencies, independent of intermittent shock treatment. An ANCOVA controlling for baseline tailflick scores revealed a significant main effect of shock $F(2, 20) = 5.86$, $p < .05$, while no other term reached significance, all *F*s <1.0, $p > .05$. *Post hoc* comparisons of the group means showed that both 0.5 and 1.5mA of continuous shock produced a statistically significant increase in tail-flick latencies, independent of intermittent shock treatment. Rats that only received intermittent shock were statistically different from rats that received both intermittent and continuous (0.5 or 1.5 mA) shock, $p < 0.5$.

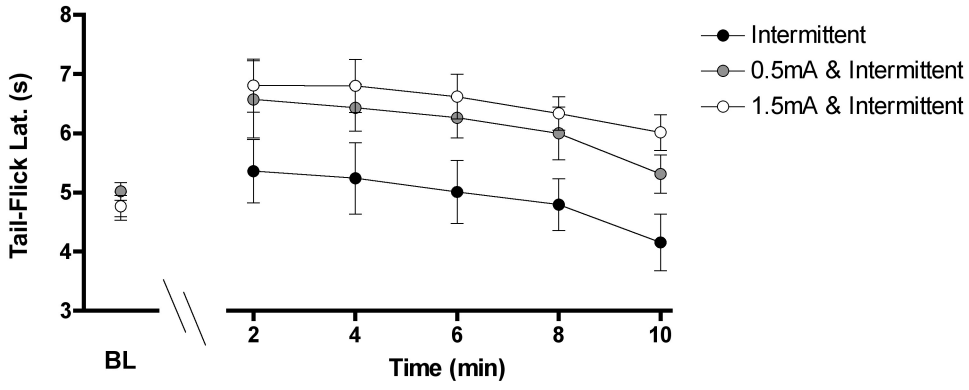

Figure 3. Continuous shock at both high (1.5mA) and low (0.5mA) intensities induced a comparable increase in thermal responding. Intermittent shock treatment failed to block continuous shock-induced antinociception.

Summary

Continuous tailshock, at both high and low intensities, causes comparable antinociception in spinalized animals (Experiment 1). In the current experiment, we further found that continuous shock-induced antinociception is not blocked by the co-administration of intermittent leg shock. This is true at both low and high intensities of continuous shock. Importantly, data collected here, together with the results of Experiment 2, suggest that the expression of continuous shock-induced antinociception is not sufficient to protect against the learning deficit. While not sufficient, it may be necessary. In Chapter II, we will further examine the necessity of continuous shock-induced antinociception in mediating the protective effect of continuous stimulation.

Experiment 4

Evidence, thus far, suggests that continuous shock has two important consequences. The first is the ability of continuous shock to induce antinociception, even in the presence of a stimulant that parallels the effects of inflammation (i.e. intermittent shock) on both spinal plasticity and nociceptive responding. Secondly, it has been shown here and elsewhere that continuous shock protects against the induction of the intermittent shock-induced learning deficit. The results of Experiment 3, however, suggest that the induction of antinociception is not sufficient to mediate the protective effect of continuous shock, thus differentiating the protective effect of continuous shock from its ability to induce antinociception. An alternative possibility is that 0.5mA of continuous shock induces a slower antinociceptive response, in comparison to 1.5mA of

continuous shock. In the current experiment, we examined the possibility that giving continuous stimulation prior to intermittent shock permits the protective effect of 0.5 mA of continuous shock to emerge. Given that the antinociceptive effect of continuous shock is observed up to 10 minutes after treatment, one prediction is that by giving continuous shock before intermittent stimulation, we can enable the protective effect of the lower intensity of continuous shock.

Procedure

Spinalized rats were first exposed to continuous (0, 0.5 or 1.5mA) tailshock (n=8 for all groups). Immediately thereafter, all animals were treated with intermittent shock to the leg. Twenty-four hours later, all rats were instrumentally tested.

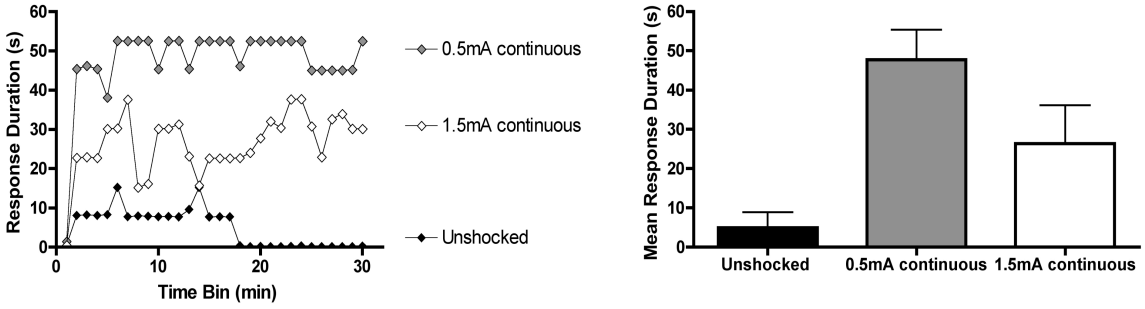

Figure 4. Intermittent shock disrupted spinal plasticity. Prior exposure to continuous shock immunized against the intermittent shock-induced learning deficit. A lower intensity of continuous stimulation (0.5mA) was found to be more efficient at protecting spinal plasticity than 1.5mA.

Results

Subjects exposed to intermittent shock alone failed to display an increase in response duration (Figure 4). Rats treated with either 0.5 or 1.5mA of continuous shock, prior to intermittent leg shock, were able to acquire the instrumental response. However, the group that received 0.5mA of continuous shock showed improved performance over rats that received no tailshock or 1.5 mA of continuous shock. An ANOVA revealed a significant main effect of shock, $F(2,21) = 10.66$, $p < 0.5$, and trials, $F(29, 609) = 2.99$, $p < 0.5$. The trial X shock interaction was also significant, $F(58, 609) = 1.55$, $p < 0.5$. A trend analysis showed a significant quadratic contrast of Condition, $F(1, 21)=15.97$, $p<0.5$. *Post hoc* comparisons of the group means revealed that rats that received only intermittent shock were significantly different from groups that received continuous shock (0.5 or 1.5 mA). Rats that received 0.5mA of continuous shock prior to intermittent shock, significantly differed from animals that received 1.5mA of continuous shock, $p < .05$.

Summary

Previously, we have shown that co-administration of continuous and intermittent shock protects against the learning deficit. Here, we have found that continuous shock can immunize against the induction of the intermittent shock-induced learning deficit. Interestingly, a lower intensity (0.5 mA) of continuous shock was found to be more efficient than 1.5mA of continuous shock. One reason for this may be that antinociception induced with a higher intensity of continuous shock (1.5mA) develops

more rapidly. Conversely, a lower intensity of continuous shock (0.5) may engage a slower developing antinociception. Allowing for the full antinociceptive response of 0.5mA of continuous shock to develop, before administering intermittent shock treatment, could explain why continuous shock at 0.5mA was better able to immunize the spinal cord from the induction of the learning deficit.

Experiment 5

Work from our laboratory has shown that the protective effect afforded to the spinal cord by both controllable shock and fixed spaced stimulation lasts 24 hours. The current experiment was designed to examine if continuous shock can similarly inhibit the induction of the learning deficit when continuous shock is given 0, 3, 6, or 24 hours prior to intermittent shock. From evidence collected up to this point, we know that the antinociceptive effects of continuous shock is transient and fades soon after exposure. Thus, we expect the protective effect of continuous shock to outlast the expression of antinociception.

Procedure

After baseline tail-flick scores were collected, spinally transected rats received 0.5mA of continuous shock 0, 3, 6, or 24 hrs prior to intermittent leg shock treatment (n=8 for all groups). Test tailflick scores were collected before intermittent shock administration in each time condition. All animals were instrumentally tested 24 hours after thermal test tailflick scores were collected.

Results

Thermal

The results are presented in Figure 5. An ANOVA revealed no statistical difference in tail-flick latencies prior to shock treatment, all Fs <1.0, $p > .05$.

Continuous shock induced an increase in tail-flick latencies that was evident immediately after shock termination. This effect was not present 3 hours after continuous shock treatment. An ANCOVA controlling for baseline scores revealed a significant main effect of Condition, $F(4,34)=12.97$, $p<0.5$, and a significant Trials X Condition interaction, $F(16, 136)=3.99$, $p<.05$. *Post hoc* comparison of the group means revealed that increased tail-flick latencies were only present in the group that was assessed immediately after continuous shock treatment, as compared to all other groups, $p<.05$.

Instrumental

No changes in instrumental responding were detected as a consequence of shock condition, including both continuous and intermittent shock (Figure 5B). There was, however, evidence to suggest that the time between continuous stimulation and intermittent shock influence instrumental learning. Though the overall ANOVA did not yield a significant main effect of Condition (all F's<1.0, p>0.5), trend analysis showed that the linear component of the interaction term reached significance, $F(1,28)= 4.09$, $p<0.5$.

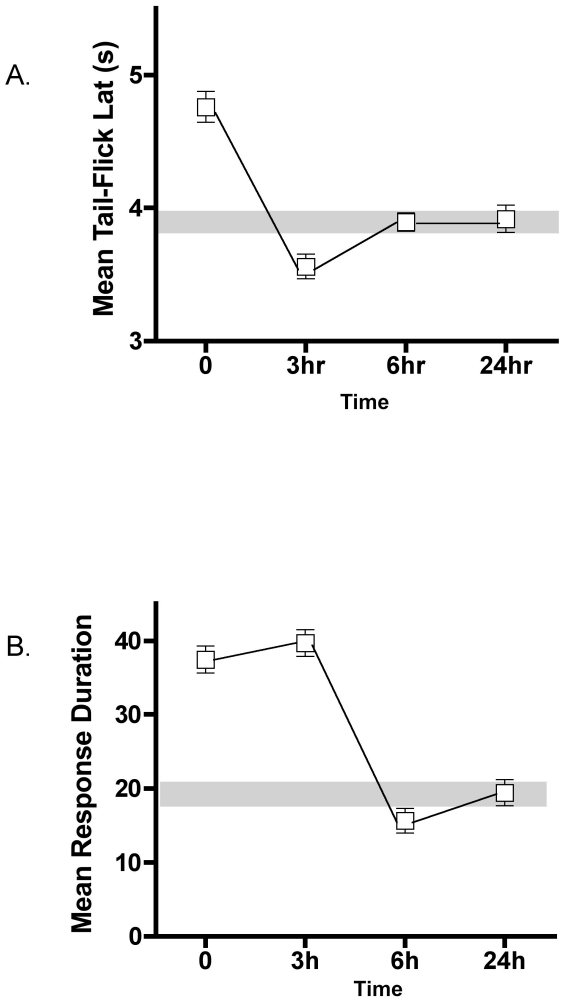

Figure 5. Intermittent shock alone did not cause changes in nociceptive responding (gray bar denotes mean scores for this group). Continuous shock (0.5mA) induced an increase in tail-flick latencies that waned soon after shock presentation, and was completely lost 3hrs later (A). Intermittent shock disrupted spinal learning (gray bar denotes the mean response duration for this group). Exposure to 0.5mA of continuous shock prevented the induction of the learning deficit. This effect was evident up to 3hrs post-continuous shock presentation, but was completely lost 6hrs later (B).

Summary

Continuous shock induces a transient antinociception that begins to wane soon after shock termination, and is completely lost 3 hours later. Re-exposure to shock in the form of intermittent stimulation does not reinstate continuous shock-induced antinociception. Interestingly, evidence suggests that the immunizing effect of continuous shock is still present three hours after continuous shock treatment is given. These results suggest that the presence of antinociception, at the time of intermittent shock treatment, is not necessary for the protective effect of continuous shock to be observed. These results, however, do not discount the possibility that the induction of antinociception- transient as it may be- is necessary for the short and long-term beneficial impact of continuous shock.

Experiment 6

Controllable shock and fixed spaced stimulation have been found to reverse the effects of uncontrollable stimulation in the isolated spinal cord. From work presented here, we know that continuous shock both protects and immunizes the spinal cord from the effects of intermittent shock. The current experiment assessed whether 0.5mA of continuous tailshock could be used to reverse the expression of the learning deficit. In addition, we examined if prior treatment with intermittent shock affected the induction of continuous shock-induced antinociception.

Procedure

Baseline tailflick scores were collected prior to intermittent leg shock treatment, or a comparable period in which the animals remained unshocked. Immediately thereafter, rats in each shock condition received either 0 or 0.5mA of continuous tailshock (n=8 per group). Tail-flick latencies were reassessed. All animals were instrumentally tested 24 hrs later.

Results

Thermal

Mean baseline tailflick scores are presented in Figure 6. An ANOVA revealed no statistical difference in tail-flick latencies between groups prior to shock treatment, all F's<2.64, $p>.05$.

Treatment with continuous tailshock caused an increase in tail-flick latencies. Prior exposure to intermittent shock did not affect continuous shock-induced antinociception. An ANCOVA controlling for baseline tail-flicks scores revealed a significant effect of Tail Shock, $F(1,27)=25.44$, $p<0.5$ and a significant Trials X Tail Shock interaction, $F(4, 108)=8.74$, $p<.05$. Neither the main effect of Leg Shock, nor the Leg Shock X Tail Shock interaction reached significance, F's<1.0, $p>.05$. *Post hoc* comparisons of the group means revealed that the groups that received continuous tailshock, independent of leg shock treatment, had significantly higher tail-flick latencies than the unshocked control group, and rats that received intermittent leg shock but not continuous electrical stimulation, $p<.05$.

Instrumental

Exposure to intermittent leg shock, without subsequent continuous tailshock treatment, disrupted spinal learning (Figure 6). Treatment with continuous electrical stimulation reversed the induction of the intermittent shock-induced learning deficit. Both unshocked control groups, and rats that received continuous shock without intermittent shock treatment, were able to acquire the instrumental response. An ANOVA revealed a significant Leg Shock effect, $F(1,28)=7.29$, $p<.05$ and significant

Trials effect, $F(29, 812)=5.49$, $p<.05$. The Trials X Tail Shock interaction, $F(29, 812)$, $p<0.5$ and the Trails X Leg Shock X Tail Shock interaction, $F(29, 812)$, $p<.05$, were also found to be significant. Post hoc comparison of the group means revealed that rats that received intermittent shock, but not continuous tailshock, had significantly lower response durations than all other groups, $p<.05$.

Summary

Continuous electrical stimulation was found to reverse the expression of the learning deficit when given after intermittent shock. This finding parallels what is seen with controllable shock and fixed space stimulation. Just as importantly, we found that prior treatment with intermittent shock did not attenuate continuous shock induced antinociception.

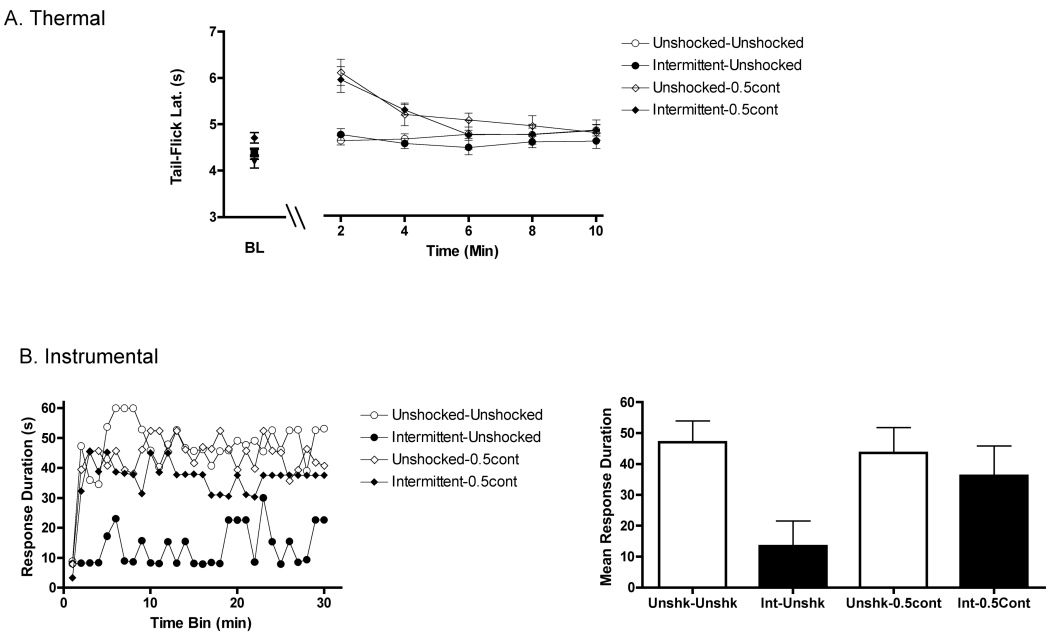

Figure 6. Continuous shock induced an increase in thermal tail-flick latencies. Prior treatment with intermittent shock did not prevent continuous shock-induced antinociception (A). Unshocked rats and those that received continuous shock alone were able to acquire the target response. Intermittent shock induced the learning deficit. Continuous shock (0.5mA) reversed the expression of the learning deficit induced by intermittent shock (B).

Chapter Summary

The findings of this chapter replicated initial work by Crown et al. (2002). Continuous shock (1.5mA) was found to both protect spinal plasticity and induce a robust antinociceptive response. Novel to these findings was the inclusion of a lower intensity of continuous shock (0.5mA). Evidence presented here revealed that, while

0.5mA of continuous shock was able to induce comparable antinociception to that seen with 1.5mA, 0.5mA of continuous shock did not have a protective effect against the learning deficit. One possible explanation for this discrepancy is that 0.5mA of continuous shock engaged a slower antinociceptive response than that seen with 1.5mA. This notion predicts that 1.5mA of continuous shock would be protective against intermittent shock when both shock treatments were simultaneously administered, but the protective effect of 0.5mA of continuous shock would not be evident unless the full antinociceptive consequence of this shock intensity were allowed to emerge. Indeed, when 0.5mA of continuous shock was administered prior to intermittent shock, the lower intensity of continuous shock (0.5mA) was better able to prevent the induction of the learning deficit than 1.5mA.

Furthermore, we found that the immunizing effect of continuous shock was evident up to 3 hours later. However, 0.5mA of continuous shock induced a transient antinociceptive response that started to wane soon after shock termination. These results suggest that the antinociceptive consequence of continuous shock and its protective effect are independent of each other, as the protective effect of continuous shock outlasts the antinociceptive effect of 0.5mA of continuous shock. In our last experiment, we found that exposure to continuous shock after intermittent shock reversed the learning deficit. Prior treatment with intermittent shock did not affect continuous shock-induced antinociception. The persistence of antinociception in both the immunizing and therapeutic effects of 0.5mA of continuous shock raises the possibility that, although continuous shock-induced antinociception is not sufficient to mediate the protective

effect of continuous shock, the induction of antinociception may somehow be linked to the beneficial effect of continuous stimulation. This possibility was further examined in Chapter II. Because the lower intensity (0.5mA) of continuous is more clinically relevant, and because it had both an immunizing and therapeutic effect, subsequent experiments focus on this treatment condition.

CHAPTER IV

THE ROLE OF THE OPIOID SYSTEM IN CONTINUOUS SHOCK-INDUCED ANTINOCICEPTION

The present chapter used pharmacological techniques to explore the link between the protective effect of continuous shock and antinociception.

Experiment 7

Exposure to peripheral stimulation can lead to the release of endogenous opioids; this holds true for both intermittent and continuous shock. For instance, spinally transected rats treated with 3 long, 25s of continuous tailshock exhibit an increase in tailflick latencies that is naltrexone-reversible (Meagher et al., 1993). However, not all opioid release, at the level of the spinal cord, is accompanied by the expression of antinociception. Indeed, intermittent shock treatment fails to induce a change in thermal responding in spinally transected rats. Furthermore, pharmacological data have shown that the intermittent-shock induced learning deficit is attenuated by the kappa receptor antagonist, norBNI (Joynes & Grau, 2004). We have also shown that the kappa-2 receptor agonist, GR89696, produces a dose-dependent inhibition of spinal learning (Washburn et al., 2008). These findings raise an interesting question concerning what role, whether beneficial or detrimental, opioid release plays in promoting and/or inhibiting spinal plasticity. The current experiment was designed to examine if continuous shock-induced antinociception is opioid-mediated by selectively antagonizing the kappa, mu and delta opioid receptors. More importantly, we examined

if the induction of antinociception is necessary for the protective effect of continuous shock against the learning deficit.

Procedure

During spinal transection surgery, all rats were fitted with an intrathecal cannula. Twenty-four hours post-surgery, baseline tailflick latencies were collected for all subjects. Thereafter, spinally transected rats received an intrathecal injection of saline vehicle, nor-BNI (kappa), CTOP (mu), or naltrindole (delta) at a dose of 10nmol/μl, followed by a 20μl saline flush. Ten minutes after drug treatment, all rats received 6 minutes of 0.5mA continuous shock to the tail or remained unshocked (n=8 per group). Tailflick latencies were, then, collected to measure changes in thermal responding. Following this, all rats were treated with intermittent leg shock and, 24 hours later, all rats were instrumentally tested.

Results

Thermal

Mean baseline scores are presented on the left of Figure 7. An ANOVA revealed no statistical difference in tail-flick latencies between groups prior to drug and shock treatment, all F's<1.0, $p>.05$.

Mean test tail-flick latencies are shown to the right of the baseline scores. Treatment with 0.5mA of continuous tailshock induced an increase in tail-flick latencies in saline-treated rats. Treatment with CTOP, naltrindole, or norBNI attenuated

continuous-shock (0.5mA) induced antinociception. Unshocked rats, independent of drug treatment, did not show a significant change in tailflick latencies. An ANCOVA controlling for baseline scores revealed a significant main effect of Drug, $F(3, 55)= 6.88$, $p<.05$ and Tail Shock, $F(1, 55)=38.00$, $p<.05$. A significant Drug X Tail Shock interaction was similarly found, $F(3,55)=6.72$, $p<0.5$. The ANCOVA also revealed a significant Trials X

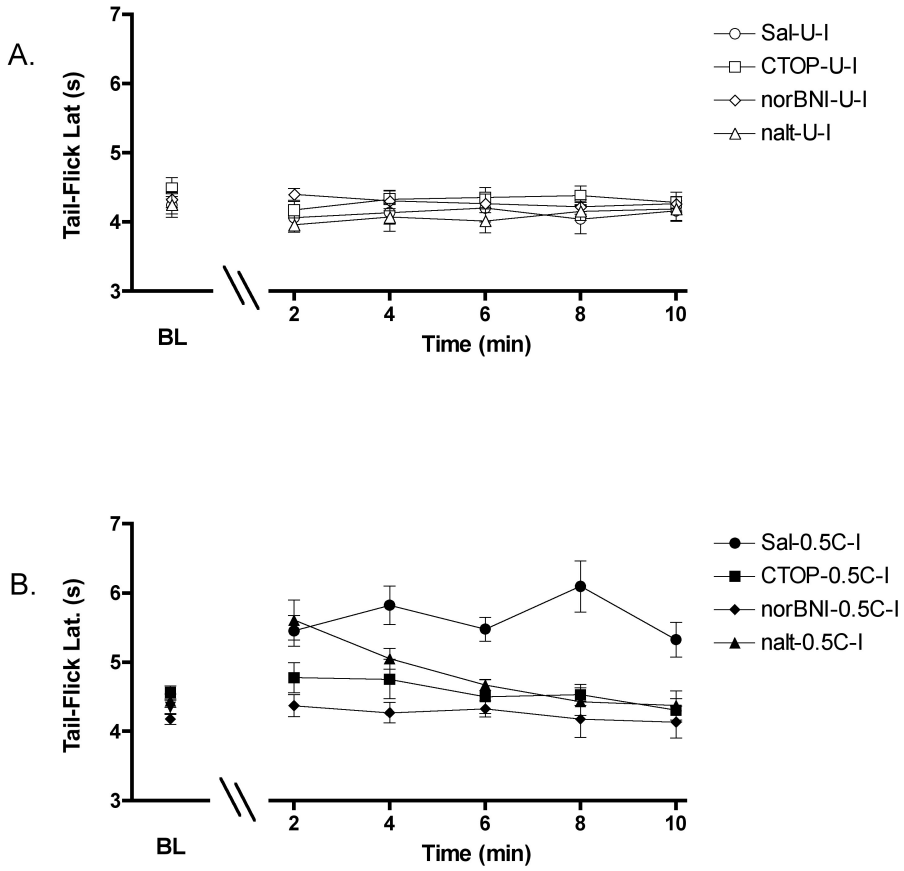

Figure 7. There was no effect of prior treatment with intrathecal CTOP, norBNI, or naltrindole in rats that received intermittent shock (A). Prior exposure to intrathecal CTOP, norBNI, or naltrindole significantly decreased continuous shock-induced antinociception (A).

Tail Shock interaction $F(4, 220)=5.74$, $p<.05$ and a significant Trials X Drug X Tail Shock interaction, $F(12,220)=2.24$, $p<.05$.

Post hoc comparisons of the group means revealed that 0.5mA of continuous tailshock induced a statistically significant increase in tailflick latencies in saline-treated rats, as compared to rats that remained unshocked (across all drug groups). Rats treated with norBNI, CTOP, or naltrindole prior to 0.5 mA of continuous tailshock showed significantly lower tailflick latencies, as compared to saline-treated rats that received continuous tailshock. Rats that received naltrindole prior to continuous shock showed significantly higher tailflick latencies than all unshocked groups, independent of drug treatment. This group (naltrindole-continuous shock) also showed significantly higher tailflick latencies than rats that received norBNI prior to continuous shock. Lastly, rats that received CTOP prior to continuous shock showed significantly higher tail-flick latencies than naltrindole, unshocked rats, $p<.05$.

Instrumental

Prior treatment with CTOP blocked the immunizing effect of continuous electrical stimulation against the intermittent shock induced learning deficit, results are shown on Figure 8. An ANOVA revealed that there was no significant effect for Drug or Tail Shock, all *F'*s<1.0, $p>.05$. There was a significant effect for Trials, $F(29, 1629)= 5.92$, $p<.05$ and a significant Trials X Drug X Tailshock interaction, $F(87, 1624)=1.28$, $p<.05$. A post hoc comparison of the group means showed a significant difference between rats that received CTOP prior to continuous shock, and subsequently

intermittent leg shock, and rats that received saline and continuous shock prior to intermittent shock treatment, $p<.05$.

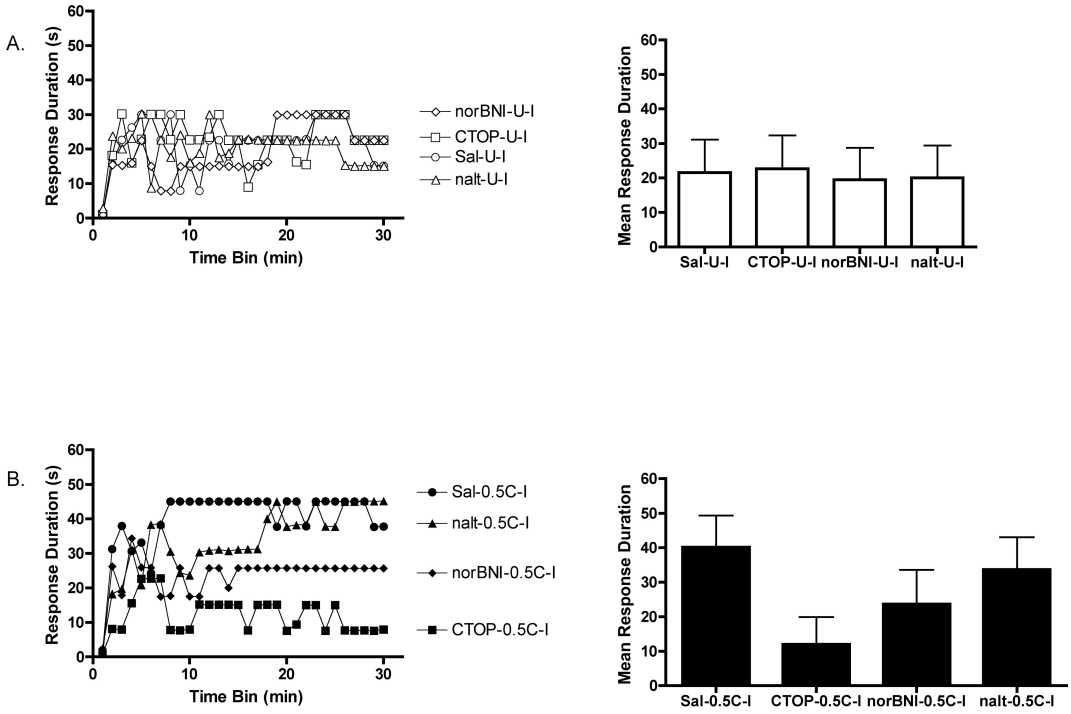

Figure 8. Prior treatment with intrathecal CTOP, norBNI, or naltrindole did not block the effects of intermittent shock on spinal learning (A). Intrathecal CTOP significantly prevented the immunizing effect of 0.5mA of continuous shock against the learning deficit. Neither norBNI nor naltrindole blocked the beneficial effects of continuous shock (B).

Summary

The results of the current experiment suggest that the mu, kappa, and delta opioid receptors are all implicated in the induction of continuous shock-induced antinociception. However, as evident by the inability of naltrindole to completely block the induction of antinociception, the involvement of each of the opioid receptors in

mediating the antinociceptive consequence of continuous shock differs. More interestingly, only the mu opioid receptor antagonist, CTOP, significantly prevented the immunizing effect of continuous shock. The results of Experiment 2 revealed that the induction of continuous shock-induced antinociception was not sufficient to protect against the learning deficit. The results presented here, in part, suggest that the induction of antinociception is necessary for the immunizing effect of continuous shock. More importantly, it appears that the activation of the mu opioid receptor plays a critical role in the beneficial effects of continuous shock.

Experiment 8

The work presented here was motivated by the hypothesis that the induction of antinociception plays an important role in the beneficial impact of continuous shock. The results of the previous experiment suggest that antinociception does indeed have a role in the immunizing effect of continuous shock. This effect is primarily mediated by the mu opioid receptor. In the current experiment, we examined if utilizing a mu opioid receptor agonist, [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO), could pharmacologically immunize against the learning deficit. The dose of DAMGO utilized has previously been shown to induce a comparable antinociception to that seen with continuous shock.

Procedure

Rats were fitted with an intrathecal cannula during spinal transection surgery. Twenty-four hours after surgery, both baseline and test tailflick scores were collected

prior to and after drug treatment, respectively. Rats received either saline or DAMGO at a dose of 10nmol/10 μl (n=8 for all groups). After thermal testing, all animals received intermittent leg shock and were instrumentally tested 24hrs later.

Results

Thermal

Mean baseline tailflick scores are presented in Figure 9. An ANOVA revealed no statistical difference in tail-flick latencies between groups prior to drug and shock treatment, all *F'*s<2.90, *p*>.05.

Treatment with intrathecal DAMGO caused an increase in tailflick latencies. This effect was not affected by subsequent administration of intermittent leg shock. An ANCOVA controlling for baseline scores revealed a significant main effect of Drug, F(1,27)=60.94, p<.05. No other term reached significance. *Post hoc* comparison of the group means revealed that groups that received intrathecal DAMGO, independent of leg shock treatment, had significantly higher tail-flick latencies than both the saline unshocked group and the saline-treated rats that received intermittent leg shock, *p*<.05.

Instrumental

Saline- treated rats treated with intermittent leg shock were unable to acquire the target response (Figure 9). Prior treatment with DAMGO blocked the induction of the intermittent shock-induced learning deficit. An ANOVA revealed a significant Drug X Shock interaction, *F*(1,28)=4.68, *p*<.05, and a significant Trials X Drug interaction, *F*(29, 812)=1.53, *p*<.05. A significant effect of Trials was also found, *F*(29, 812)=8.19,

p<.05. No other term reached statistical significance. Post hoc comparison of the group means revealed a significant difference between saline-treated rats that received intermittent leg shock and all other comparison groups, p<0.5.

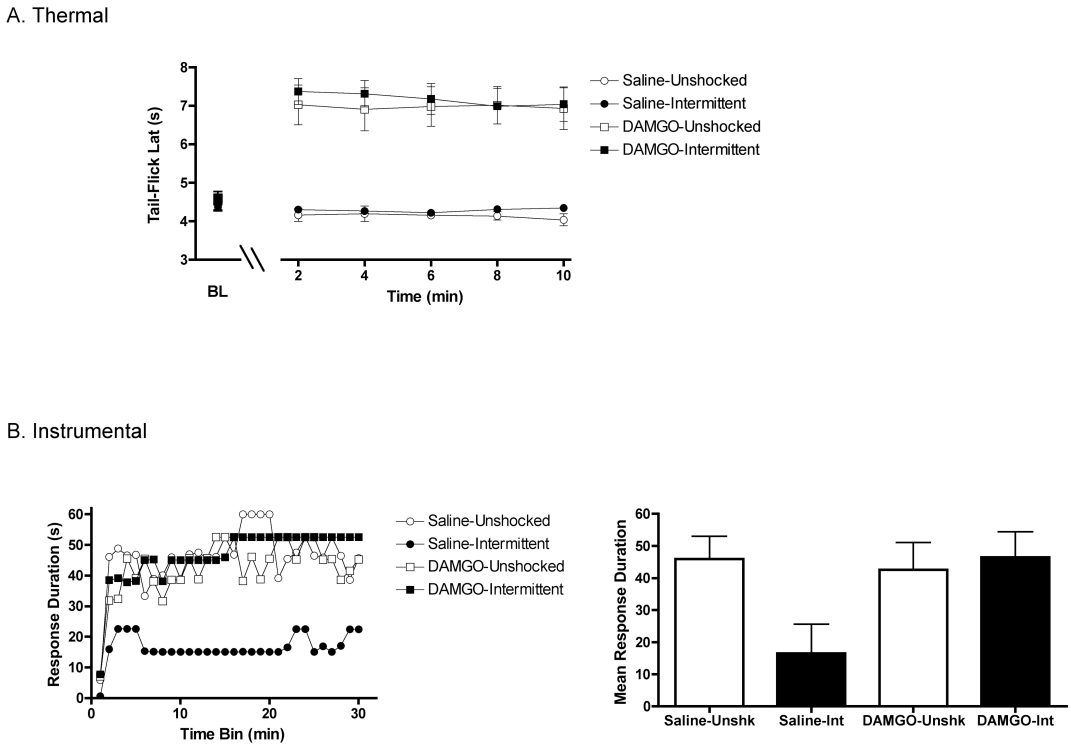

Figure 9. Intrathecal DAMGO induced an increase in tail-flick latencies. DAMGO-induced antinociception was not blocked by subsequent treatment with intermittent shock (A). Intermittent shock disrupted spinal learning. Saline and DAMGO treated animals were able to acquire the target response. Prior treatment with DAMGO blocked the induction of the intermittent shock-induced learning deficit (B).

Summary

Results revealed that pharmacologically activating the mu opioid receptor, at the level of the spinal cord, was sufficient to prevent the induction of the learning deficit. In

the previous experiment, we found that the mu opioid receptor plays an important role in the immunizing effect of continuous shock. Moreover, DAMGO produced a robust antinociception that was not reversed by subsequent intermittent shock treatment. These data, together, suggest that mu opioid receptor plays an important role in the beneficial impact of continuous shock, and leads to the induction of an antinociceptive effect that is not reversed by intermittent shock treatment.

Chapter Summary

Continuous shock induces a decrease in thermal responding. Pharmacological evidence suggests that the antinociceptive consequence of continuous shock is opioid mediated. The results of Experiment 7 revealed that selectively antagonizing the mu or kappa, and to a lesser extent the delta, opioid receptors blocked the induction of continuous shock-induced antinociception. However, only the mu opioid receptor antagonist, CTOP, was able to block the immunizing effect of continuous shock. Pharmacologically activating the mu opioid receptor at a dose sufficient to induce comparable antinociception to continuous shock, before administering intermittent shock treatment, prevented the induction of the learning deficit. Together, these results suggest that activation of the mu opioid receptor plays an important role in the beneficial impact of 0.5mA of continuous shock.

CHAPTER V

NEUROBIOLOGICAL PROFILE OF CONTINUOUS SHOCK

In this chapter, we further examined the neurobiological mechanisms mediating the protective effect of continuous stimulation through the use of both pharmacological manipulations and RT-PCR.

Experiment 9

Manipulations, such as training with controllable shock and exposure to fixed spaced stimulation, have been shown to have a protective effect against the learning deficit. The induction of antinociception after continuous shock differentiates this form of stimulation from the ones aforementioned. As a result, up to this point, we have examined what role antinociception and the release of endogenous opioids play in the protective effect of continuous shock. In the current experiment, we shifted our aim from identifying what is unique about continuous shock to finding a potential neurobiological parallel between these three different forms of stimulation. In particular, we examined the role of BDNF in mediating the immunizing effect of continuous stimulation. Evidence suggests that the protective effect of both fixed spaced shock and training with controllable shock depends on the release of endogenous BDNF. Prompted by these findings, we examined if disrupting BDNF action during continuous shock treatment interfered with both the induction of antinociception and the immunizing effect seen with continuous shock.

Procedure

This experiment did not utilize a full factorial design. The overall experimental design consisted of collecting baseline tail-flick latencies prior to drug treatment. After drug treatment, animals were exposed to one of two tailshock conditions, 0 or 0.5mA of continuous shock. Tailflick latencies were reassessed 30 min later. Rats then received either intermittent shock or remained unshocked. All animals were instrumentally tested 24 hours later. Again, because a full factorial design was not utilized for this experiment, the group conditions are as follow: Group 1 received saline vehicle, 0mA of continuous shock and 6 minutes of intermittent shock (Sal-Unshk-Int) (n=8); Group 2 was treated with saline, 0.5 mA of continuous shock and no intermittent leg shock (Sal-0.5Cont-Unshk) (n=8); Group 3 consisted of saline treated rats that received both 0.5 of continuous shock and intermittent shock (Sal-0.5Cont-Int); finally, Group 4, received the BDNF inhibitor TrkB-IgG (0.32 μg/μl), and both continuous and intermittent shock treatment (TrkB IgG-0.5Cont-Int).

Results

Thermal

Mean baseline tailflick scores are presented in Figure 10. An ANOVA revealed no statistical difference in tail-flick latencies between groups prior to drug and shock treatment, all F's<2.31, $p>.05$.

Continuous shock caused an increase in tailflick latencies, independent of both drug and leg shock treatment. Saline-treated rats that only received intermittent leg

shock did not show an increase in tailflick latencies. An ANCOVA controlling for baseline scores revealed a significant main effect of Condition, $F(3, 27)=8.82$, $p<.05$. The Trials X Condition interaction was also significant, $F(12, 108)=2.92$, $p<.05$. *Post hoc* comparison of the group means revealed that saline-treated rats that only received intermittent leg shock had lower tailflick latencies than all other comparison groups. Saline-treated rats that were treated with both continuous and intermittent shock had significantly higher tailflick latencies than saline-treated rats that only received continuous tailshock, $p<.05$.

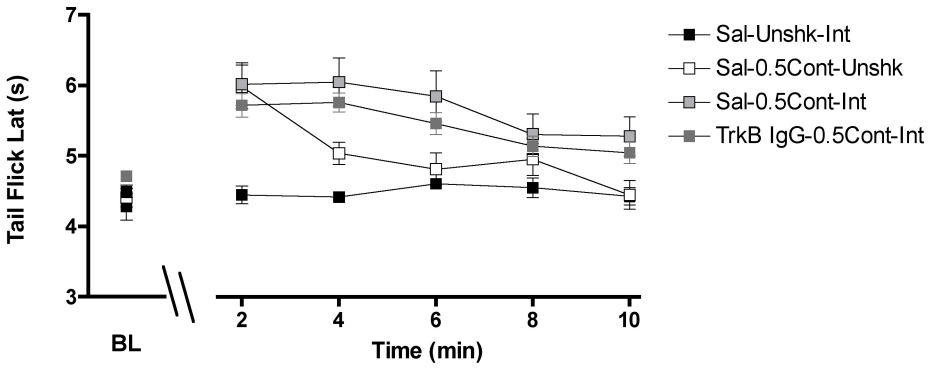

Figure 10. Saline-treated rats that received only intermittent shock (Sal-Unshk-Int) showed no changes in tail-flick latencies. Saline-treated animals that received only continuous shock (Sal-0.5Cont-Int) exhibited increased tail-flick latencies. Subsequent treatment with intermittent shock did not impact continuous shock-induced antinociception (Sal-0.5cont-Int). Intrathecal TrkB-IgG did not block continuous shock-induced changes in nociceptive responding (TrkB IgG-0.5Cont-Int).

Instrumental

Exposure to intermittent shock disrupted instrumental learning in saline treated rats (Figure 11), while saline-treated rats that received continuous electrical stimulation did not exhibit the learning deficit. Prior treatment with continuous electrical stimulation immunized against the detrimental effects of intermittent shock on learning. Delivery of TrkB-IgG prior to continuous tailshock attenuated the immunizing effect of continuous electrical stimulation on spinal plasticity. An ANOVA revealed a significant main effect of Group Condition, $F(3,28)=11.39$, $p<0.5$ and Trials $F(29, 812)=8.85$, $p<.05$. As well, there was a significant Trials X Group Condition interaction, $F(87,812)=2.25$, $p<.05$.

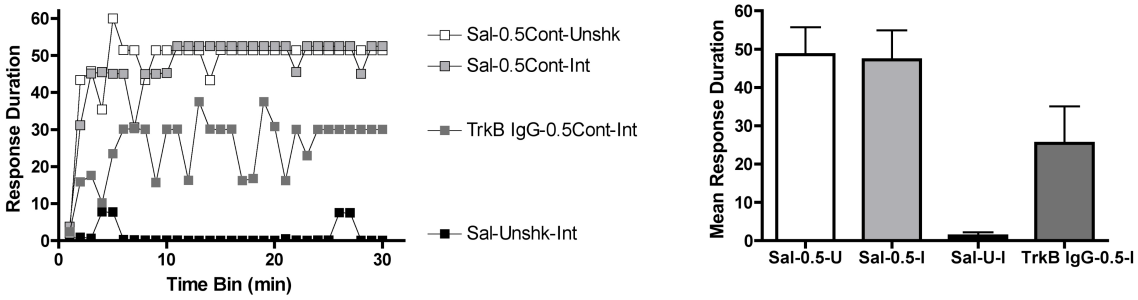

Figure 11. Intermittent shock disrupted spinal learning. Prior exposure to continuous stimulation prevented the induction of the learning deficit. Intrathecal administration of TrkB-IgG attenuated the immunizing effect of continuous shock.

Post hoc comparison of the group means showed that saline-treated rats that received no tailshock and 6 minutes of intermittent leg shock (Sal-Unshk-Int) had significantly lower respond durations than all other comparison groups. Rats that

received TrkB-IgG prior to both continuous tailshock and intermittent leg shock (TrkB-0.5cont-Int) had significantly lower response durations than saline-treated rats that received continuous tailshock in the first shock phase, independent of leg shock treatment (Sal-0.5Cont-Int and Sal-0.5Cont-Unshk), $p<.05$.

Summary

Previous work has shown that disrupting endogenous BDNF activity blocks the protective effect of controllable shock and fixed spaces stimulation. Similarly, we found that an intrathecal administration of TrB IgG attenuated the immunizing effect of continuous stimulation. Treatment with TrkB IgG did not, however, disrupt the antinociceptive consequence of 0.5mA of continuous shock. These data suggest that endogenous BDNF release plays a role in the protective effect of continuous shock, independent of changes in nociceptive processing.

Experiment 10

In order to further identify the neurobiological mechanisms implicated in the immunizing effects of 0.5mA of continuous shock, we conducted real-time RT-PCR. We assessed if 0.5mA of continuous shock caused an upregulation of BNDF and mu opioid receptor mRNA expression, which served to compliment our pharmacological data. As well, we examined what effect 0.5mA of continuous shock had on *c-fos*/*c-jun* mRNA expression in the spinal cord. Activation of *c-fos* and *c-jun* transcription is thought to play a role in the development of central sensitization. Exposure to uncontrollable

intermittent shock has been shown to cause a state akin to central sensitization. Consequently, we expected that intermittent shock would cause an increased expression of these early genes, and that prior treatment with 0.5mA of continuous would dampen intermittent shock-induced activation of *c-fos* and *c-jun*.

Procedure

Thermal baselines and tailflick latencies were collected prior to and after continuous shock (0 or 0.5mA) treatment. Rats were treated with either 0.5mA of continuous tailshock or remained unshocked. Immediately after thermal testing was completed, half the rats in each continuous tailshock condition received either intermittent leg shock or nothing (n=8 for all groups). 30 min after treatment, all subjects were sacrificed and tissue was collected. BDNF and mu opioid receptor mRNA levels, in addition to *c-fos* and *c-jun* expression levels, were assessed using real-time RT-PCR.

Results

Thermal

Mean baseline tailflick scores are presented in Figure 12. An ANOVA revealed no statistical difference in tail-flick latencies between groups prior to shock treatment, all F's<1.0, $p>.05$.

Continuous tailshock caused an increase in tailflick latencies. Continuous shock-induced increases in tailflick latencies were not altered by subsequent treatment with intermittent leg shock. An ANCOVA controlling for baseline tailflick latencies found a significant main effect of Tail Shock, $F(1,27)=71.67$, $p<.05$, and a significant Trials X

Tail Shock interaction, $F(4,108)= 14.27$, $p<.05$. *Post hoc* comparison of the group means revealed that rats that received continuous tailshock, independent of leg shock treatment, had significantly higher tail-flick latencies than both the unshocked control group and rats that received only intermittent leg shock, $p<.05$.

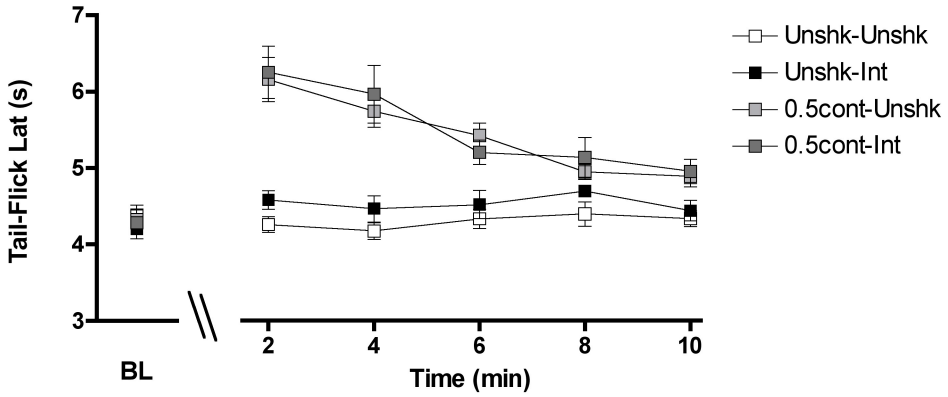

Figure 12. Continuous shock induced an increase in tail-flick latencies. Subsequent treatment with intermittent shock did not affect continuous shock-induced antinociception. No changes in tail-flick latencies were observed in intermittent shock and unshocked animals.

RT-PCR

C-Fos. Intermittent leg shock caused an increase in *c-fos* expression in the spinal cord (Figure 13). This effect was not evident in rats that received continuous electrical stimulation in the absence of intermittent leg shock. An ANOVA revealed a significant main effect of Tail Shock, $F(1, 28)=12.96$, $p<.05$. *Post hoc* comparison of the group means revealed a significant difference in spinal *c-fos* expression between rats that

received continuous tailshock but not leg shock, and rats that received only intermittent leg shock.

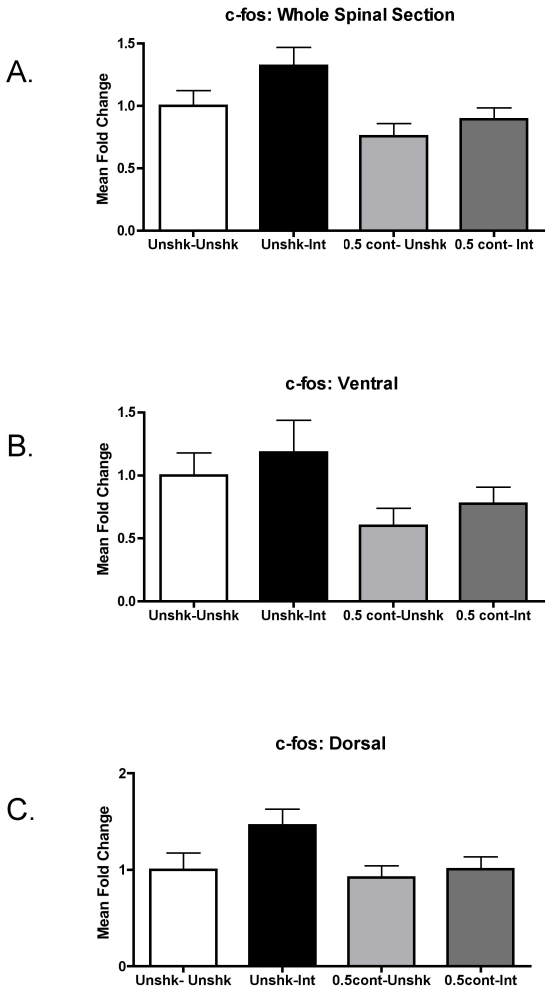

Figure 13. Intermittent shock caused an increase in *c-fos* expression in the spinal cord. Statistical analysis revealed that intermittent shocked rats had higher c-fos expression than rats that received only continuous shock (A). The same trend in *c-fos* expression that was observed in the whole spinal cord was found in the ventral horn of the spinal cord (B). No changes in *c-fos* expression were detected in the spinal ventral horn (C).

When the data were separated into anatomical sections, dorsal versus ventral, the same effect was observed in the ventral horn. *C-fos* expression was increased in rats that received intermittent leg shock. An ANOVA revealed a significant main effect of Tail Shock, $F(1,28)=4.18$, $p<0.5$. No other term reached significance. *Post hoc* comparisons showed a significant difference in *c-fos* expression in the ventral horn of animals that received only intermittent shock, as compared to the group that received only continuous tailshock, $p<0.5$ No statistical changes in *c-fos* expression were detected in the spinal dorsal horn as a result shock treatment, all *F'*s<3.24, $p>.05$.

C-Jun. Intermittent leg shock induced an increase in overall spinal *c-jun* expression, independent of continuous shock treatment (Figure 14). Conversely, treatment with continuous shock caused a decrease in *c-jun* expression. An ANOVA revealed a significant main effect of Leg Shock, $F(1,28)=5.39$, $p<.05$, and Tail Shock, $F(1,28)=13.32$, $p<.05$. A significant c-jun X Leg Shock interaction was also found, $F(1,28)=5.01$, $p<.05$. *Post hoc* comparison of the group means revealed significantly higher *c-jun* expression in rats that received only intermittent shock, as compared to unshocked control groups. Rats exposed to only continuous shock had significantly lower expression of *c-jun* than rats that received only intermittent shock. Subjects treated with both continuous and intermittent shock had significantly higher *c-jun* expression than rats that only received continuous shock, $p<.05$.

Analysis of the ventral section of the spinal cord revealed the same pattern of *c-jun* expression, resulting from shock treatment, as seen in the combined anatomical sections of the spinal cord. An ANOVA revealed a significant main effect of Leg Shock,

$F(1,28)=9.68$, $p<.05$, and Tail Shock, $F(1,28)=10.89$, $p<.05$. *Post hoc* comparison of the group means revealed that rats that received continuous shock alone had lower *c-jun* expression levels in the ventral horn than all other comparison groups.

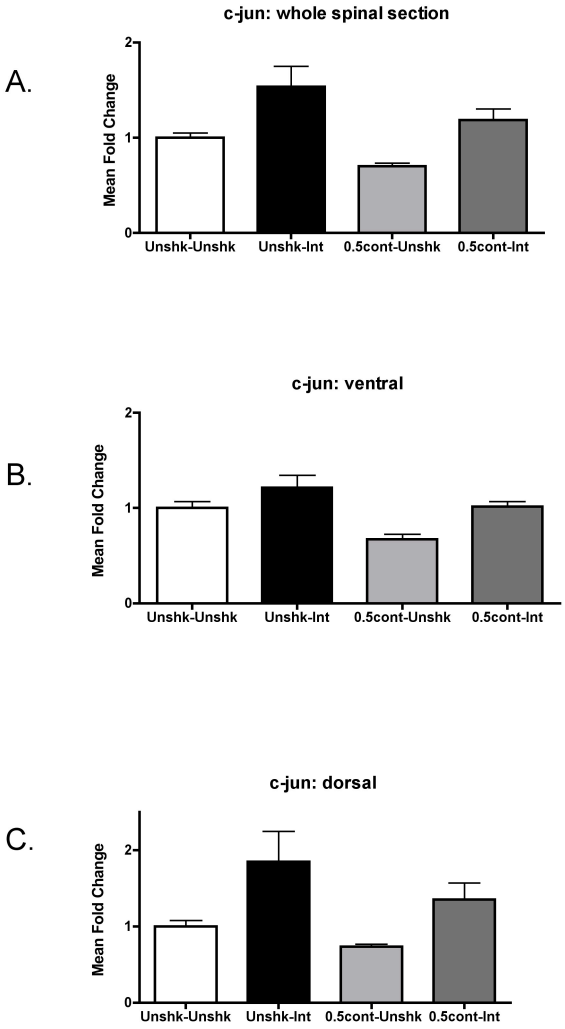

Figure 14. Intermittent shock caused an upregulation of *c-jun* expression in the spinal cord. Conversely, continuous shock caused a decrease in *c-jun* expression (A). The same pattern of results was detected in the ventral section of the spinal cord (B). In the spinal dorsal horn, intermittent shock was found to upregulate *c-jun* expression levels (C).

In the dorsal horn, while intermittent shock caused an increase in *c-jun* expression, there was no evidence that continuous shock caused a significant decrease in *c-jun* levels. An ANOVA revealed a significant main effect of Leg Shock, $F(1,28)=10.34$, $p<.05$. *Post hoc* comparison of the group means revealed that subjects that received intermittent shock, in the absence of continuous shock treatment, had significantly higher *c-jun* expression levels than both unshocked control groups and rats that received only continuous shock, $p<.05$.

*Mu Opioid Receptor.*Intermittent shock caused a decrease in mu opioid receptor expression in the spinal cord (Figure 15). Exposure to continuous shock prior to intermittent stimulation further decreased mu opioid receptor levels. An ANOVA revealed a significant main effect of Tail Shock, $F(1,28)=7.46$, $p<.05$, and Leg Shock, $F(1,28)=23.48$, $p<.05$. *Post hoc* comparison of the group means revealed that rats that were treated with only intermittent shock had significantly lower mu opioid receptor expression than the unshocked control group. Rats that received continuous tailshock prior to intermittent leg shock had significantly lower mu opioid receptor expression than both the unshocked group, and rats that received only continuous shock, $p<.05$.

Analysis of the ventral sections of the spinal cord revealed that intermittent leg shock decreased mu opioid receptor. An ANOVA revealed a significant main effect of Leg Shock, $F(1,28)=18.99$, $p<.05$. *Post hoc* comparison of the group means revealed the same pattern of results in the ventral horn as seen in the combined sections of the spinal cord. In the dorsal horn, intermittent shock caused a decrease in mu opioid receptor expression that was further decreased when continuous shock was given beforehand. An

ANOVA revealed a significant main effect of Tail Shock, $F(1,28)=5.83$, $p<.05$, and Leg Shock, $F(1,28)=11.41$, $p<.05$. *Post hoc* comparison of the group means revealed that rats that received continuous shock prior to intermittent shock had significantly lower mu opioid receptor expression than all other comparison groups, $p<.05$.

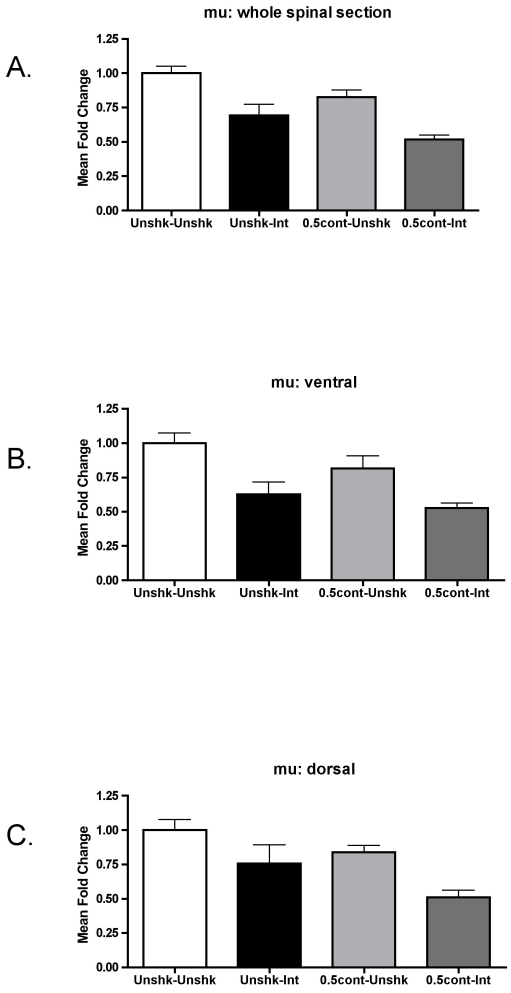

Figure 15. Intermittent shock caused a decrease in mu opioid receptor expression levels in the spinal cord. Rats treated with both continuous shock and intermittent shock showed a pronounced decrease in mu opioid receptor expression (A). The same pattern of results was observed in the ventral section of the spinal cord (B). In the spinal dorsal horn, combined treatment with continuous and intermittent shock led to a significant decrease in mu opioid receptor expression (C).

TrkB. Global changes of spinal TrkB were not detected as a consequence of shock treatment (all *F*'s<1.0, *p*>.05) (Figure 16); however, independent analysis of the ventral horn showed that intermittent shock caused a decrease in TrkB expression levels. An ANOVA revealed a main effect of Leg Shock, $F(1,28)=5.95$, $p<.05$. *Post hoc* comparison of the group means revealed that both groups that received intermittent

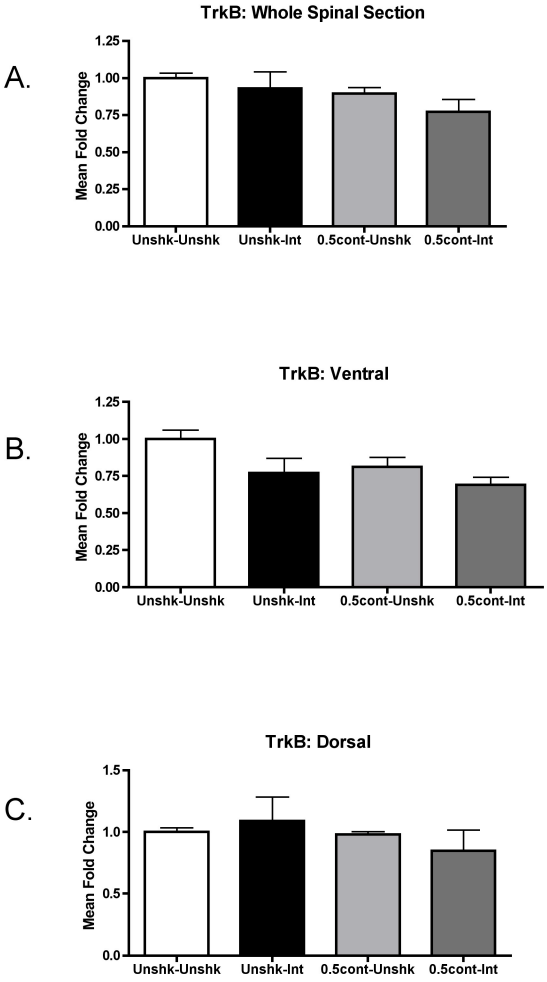

Figure 16. Global changes in TrkB were not detected as a consequence of shock (A). TrkB was downregulated as a consequence of intermittent shock in the spinal ventral horn (B). No changes in TrkB expression were detected in the spinal dorsal horn (C).

shock, independent of tailshock treatment, had lower TrkB expression levels than unshocked control groups, $p<.05$. No significant changes in TrKB expression levels were detected in the dorsal horn (all F's<1.0, $p>.05$).

Summary

As expected, continuous shock induced an antinociceptive response in spinally transected rats that was not attenuated by subsequent treatment with intermittent shock. More importantly, the results revealed that intermittent shock caused an increase in *c-fos* and *c-jun* expression in the spinal cord. Continuous shock, conversely, was not found to upregulate these early genes. Although not found to be statistically significant, the results indicated a trend towards continuous shock preventing the upregulation of c-fos and c-jun after intermittent shock treatment.

From pharmacological data, it was expected that 0.5mA of continuous shock would lead to an increase in mu opioid and BDNF receptor upregulation. However, the results revealed that shock, *per se*, leads to the downregulation of these receptors. Therefore, the combined effect of continuous shock and intermittent shock lead to the highest decrease in mu opioid receptor expression in both the ventral and dorsal horn of the spinal cord. Similarly, exposure to intermittent and continuous shock caused a statistically significant decrease in TrkB expression levels in the ventral, but not dorsal, horn of the spinal cord.

Chapter Summary

Further investigation into the underlying neurobiological mechanisms mediating the beneficial effects of continuous stimulation revealed that preventing endogenous BDNF activity attenuated the immunizing effect of continuous shock, while having no effect on antinociception. Interestingly, 0.5mA of continuous shock was also found to down-regulate the expression of early genes implicated in the development of central sensitization*, c-fos* and *c-jun*. Contrary to pharmacological data, however, the combined effect of intermittent and continuous shock treatment lead to a decreased expression of both the mu opioid and TrkB receptors. These results implicate alternative mechanisms that could function in conjunction with, or independent of, the opioid system in underscoring the immunizing effect of continuous stimulation. Further research, outside of this dissertation, will examine how these mechanisms interact.

CHAPTER VI

CONTINUOUS SHOCK AND RECOVERY OF FUNCTION

In this final chapter, we examined if continuous stimulation could be used as a therapeutic tool to promote locomotor recovery after SCI.

Experiment 11

Prompted by the finding that uncontrollable nociceptive input disrupts activity dependent modifications in the isolated spinal cord, we have previously examined the impact of uncontrollable stimulation on recovery of function after SCI. Using an animal model of SCI, we have shown that uncontrollable nociceptive stimulation disrupts locomotor, bladder, and sensory function, causes decreased weight gain, and exacerbates tissue loss after injury (Grau et al., 2004). In the current experiment we examined if 0.5mA of continuous shock could foster recovery of locomotor function after a contusion injury by hindering the effects of uncontrollable shock in the injured spinal cord.

Procedure

Subjects received a contusion injury using the MASCIS device developed by Gruner (1992) and Constantini and Young (1994). 24 hrs post-injury, rats were simultaneously treated with intermittent leg shock or nothing, and 0.5 mA of continuous shock or nothing (n=6 per group). Animals were allowed to recover for 21 days post-

injury. During this period, rats were assessed for changes in locomotor recovery using the BBB scale (Basso et al., 1995).

Results

Intermittent leg shock alone disrupted locomotor recovery after SCI (Figure 17). Continuous shock alone had the same negative consequence. The combined treatment of intermittent and continuous shock, however, did not hinder recovery after injury. An ANCOVA controlling for baseline locomotor scores revealed a significant effect of Days, $F(11, 209)=8.29$, $p<.05$. No other term reached significance. *Post hoc* comparison of the group means revealed that unshocked animals had significantly higher locomotor scores than both the group that received intermittent shock alone and continuous shock alone, $p<.05$.

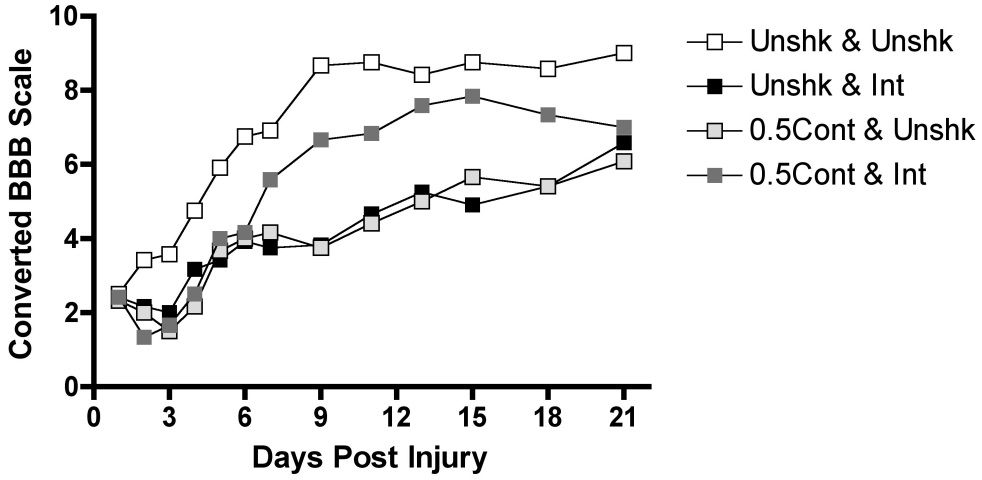

Figure 17. Intermittent and continuous shock, when presented alone, hindered locomotor recovery after SCI. When intermittent and continuous shock were co-administered, animals recovered at a comparable rate to unshocked rats.

Summary

As expected, uncontrollable stimulation disrupted locomotor recovery after SCI. Unfortunately, we found that 0.5mA of continuous shock also hindered locomotor recovery. The combined treatment of continuous and intermittent shock, however, did not have a cumulative negative effect. Rats treated with intermittent and continuous shock showed comparable recovery to unshocked animals.

Experiment 12

The results of the previous experiment lead us to question if 0.5mA of continuous shock could be used to immunize against the effects of uncontrollable stimulation on recovery of function. Also of interest was the confounding effect of continuous stimulation, which both impaired recovery when given alone and fostered recovery when given in combination with intermittent shock. The present experiment further explored these issues by evaluating whether continuous shock given prior to intermittent stimulation has a protective effect.

Procedure

Subjects received a contusion injury using the MASCIS device developed by Gruner (1992) and Constantini and Young (1994). Twenty-four hours post-injury, rats were treated with either intermittent shock or 0.5mA of continuous shock, or remained unshocked for an equal period of time (n=6 per group). Animals were allowed to

recovery for 21 days post-injury. During this period, rats were assessed for changes in locomotor recovery using the BBB scale (Basso et al., 1995).

Results

Shock treatment impacted BBB scores (our index of recovery) across days (Figure 18). An ANCOVA controlling for baseline scores revealed a significant effect of Days, $F(11, 154)=8.337$, $p<.05$ and a significant Days X Condition interaction, $F(22,154)=2.70$, $p<.05$. Trend analysis revealed a significant interaction of Days with linear contrast, $F(11, 154)=3.61$, $p<.05$. To further analyze the nature of this effect, BBB scores were compared after performance had stabilized (days 15-21) using an analysis of

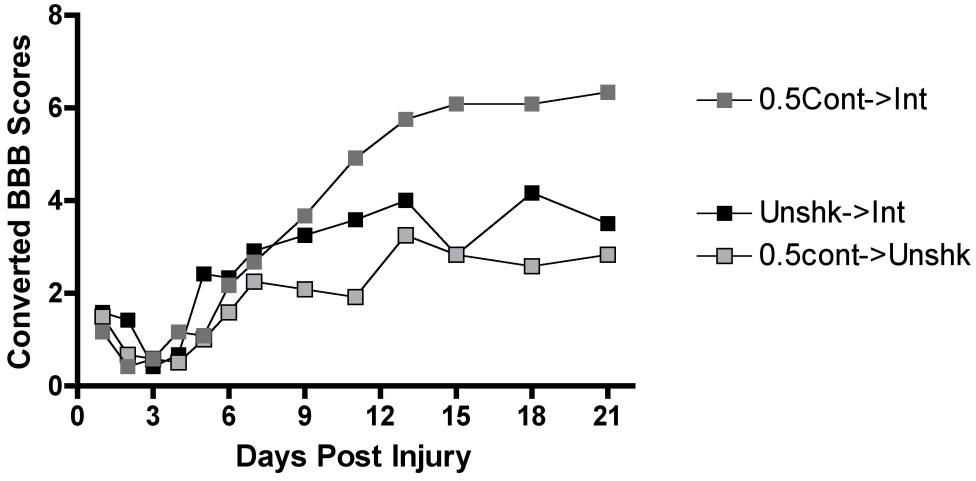

Figure 18. Continuous shock hindered recovery of locomotor function across days. This effect was not evident when continuous shock was given prior to intermittent shock.

covariance (with day 1 as the covariate). The ANCOVA revealed a significant effect of Days, $F(2,28)=3.44$, $p<.05$. *Post hoc* analysis of the means showed that rats that received continuous shock alone had significantly lower BBB scores on days 15-21 than animals that received both intermittent and continuous shock, $p<.05$.

Summary

Once again, continuous stimulation was found to hinder locomotor recovery after SCI. Of interest, when continuous shock was given prior to intermittent shock, locomotor recovery was not negatively influenced. These results replicate the findings of Experiment 11.

Chapter Summary

Previous work has shown that uncontrollable nociceptive input hinders locomotor recovery after SCI. Based on work collected in spinally transected animals, we expected that continuous stimulation would serve to harness recovery after SCI. Results collected for this chapter, in part, support this idea. Continuous shock was found to negate the effects of uncontrollable stimulation on locomotor recovery after SCI. On the other hand, continuous shock alone was shown to be detrimental to recovery. Thus, in the presence of nociceptive input, TENS-like stimulation may have a beneficial effect, but in its absence, cause harm.

CHAPTER VII

CONCLUSION: GENERAL DISCUSSION

The work presented here was motivated by the need to further elucidate the behavioral and underlying neurobiological mechanisms mediating the protective effect of continuous stimulation in the spinal cord. Previous work has shown that continuous shock has both a protective effect against the induction of the learning deficit and produces an antinociceptive response in the isolated spinal cord (Crown et al., 2002). Other manipulations known to protect spinal plasticity, such as controllable shock and fixed spaced stimulation, do not lead to the induction of antinociception. For this reason, we focused our attention on examining the relationship between the beneficial effects of continuous shock and antinociception.

The current results replicated the initial findings of Crown et al. (2002). Continuous shock at 1.5mA both led to reduced thermal responding (antinociception) and prevented the induction of the learning deficit (Experiments 1-2). Novel to this research was the inclusion of a lower intensity of continuous shock (0.5mA). Results revealed that 0.5mA of continuous shock induced a comparable antinociception to that seen with 1.5mA (Experiment 1). At this lower intensity, however, continuous shock was unable to protect the isolated spinal cord from the detrimental effect of intermittent stimulation (Experiment 2). Further examination revealed that co-administration of intermittent and continuous shock did not affect continuous shock-induced antinociception. This was true at both the higher (1.5mA) and lower (0.5mA) intensities of continuous shock (Experiment 3).

One possible explanation for the inability of 0.5mA of continuous shock to block the induction of the learning deficit (Experiment 2) is that, at a lower intensity, continuous stimulation engages a slower antinociceptive response than that observed with 1.5mA. This notion predicts that 1.5mA of continuous shock would be protective against intermittent shock when both shock treatments were simultaneously administered, but the protective effect of 0.5mA of continuous shock would not be evident unless the full antinociceptive consequence of this weaker shock intensity were allowed to fully emerge. Indeed, when 0.5mA of continuous shock was administered prior to intermittent shock, the lower intensity of continuous shock (0.5mA) was better able to prevent the induction of the learning deficit than 1.5mA (Experiment 4). Further analysis, however, called into question the link between antinociception and the protective effect of continuous shock.

Evidence revealed that the protective effect of continuous shock was present 3 hours after shock delivery (Experiment 5). We know from work collected here, and elsewhere, that continuous stimulation induces a transient antinociceptive response that starts to wane soon after shock termination. These results, thus, suggest that the antinociceptive consequence of continuous shock and its protective effect are independent of each other, as the beneficial effect of continuous shock outlasts the antinociception induced by of 0.5mA of continuous shock. Moreover, 0.5mA of continuous stimulation was able to block the expression of the learning deficit, when continuous shock was administered after intermittent shock treatment (Experiment 6). The finding that 0.5mA of continuous shock has a therapeutic effect against the learning

deficit contradicts our initial argument that the protective effect of 0.5mA of continuous stimulation is mediated by the induction of a slower-acting antinociceptive response.

In addition, the results of Experiment 7 revealed that blocking the induction of antinociception was not sufficient, *per se*, to prevent the immunizing effect of 0.5mA of continuous shock. As pharmacologically antagonizing the mu, kappa, or delta opioid receptors blocked continuous shock-induced antinociception; yet, only the mu opioid receptor antagonist, CTOP, reversed the immunizing effect of continuous stimulation (Experiment 7). Evidence, therefore, suggests that the mu opioid receptor plays an important role in the beneficial effects of continuous shock. Indeed, prior treatment with the mu opioid receptor agonist, DAMGO, prevented the induction of the learning deficit (Experiment 8).

Further investigation into the underlying neurobiological mechanisms mediating the beneficial impact of continuous shock revealed that (similar to what is seen with controllable shock and fixed spaced stimulation) preventing endogenous BDNF activity attenuated the immunizing effect of continuous shock, while having no effect on antinociception (Experiment 9). Interestingly, 0.5mA of continuous shock was also found to down-regulate the expression of early genes implicated in the development of central sensitization*, c-fos* and *c-jun*. Contrary to pharmacological data, however, the combined effect of intermittent and continuous shock treatment led to a decreased expression of both the mu opioid and TrkB receptors (Experiment 10).

From previous work we know that uncontrollable nociceptive input hinders locomotor recovery after SCI. Based on work collected in spinally transected animals,

we expected that continuous stimulation would foster recovery after SCI. Results revealed that, while continuous shock alone was detrimental, the combined treatment of intermittent and continuous shock (both concurrently and sequentially) had a protective effect (Experiments 11 and 12).

Spinal Plasticity and Nociceptive Processing

Behavioral studies examining the impact of intermittent shock have demonstrated that uncontrollable stimulation not only disrupts spinal plasticity, but also induces bilateral allodynia (Ferguson, Crown, & Grau). Numerous studies suggest that the induction of the learning deficit is related to the phenomenon of central sensitization. Central sensitization is induced by peripheral nociceptive input (resulting from injury or inflammation) and leads to an increase in mechanical reactivity (Coderre & Melzack, 1992; Campbell & Meyer, 2006; Ji et al., 2003). Pharmacological agents known to impact central sensitization have also been shown to interfere with the induction and maintenance of the learning deficit (Ferguson, Crown & Grau, 2006; Joynes et al., 2004b). Moreover, inflammatory agents, such as capsaicin and formalin, have been found to interfere with subsequent spinal learning (Ferguson, Crown & Gtrau., 2006; Hook, Huie, & Grau, 2008). Both neural and glial mediators have been implicated in the maladaptive consequences of uncontrollable stimulation. Many of these mediators also play an important role in the development of maladaptive pain following injury.

The use of controllable shock and fixed spaced stimulation both prevent and reverse the effects of uncontrollable stimulation in the isolated spinal cord (Crown &

Grau, 2001; Baumbauer et al., 2009a) Moreover, controllable shock has been shown to reverse the effects of capsaicin treatment on spinal learning (Hook, Huie, & Grau, 2008). New evidence suggests that fixed spaced stimulation, not only affords the isolated spinal cord protections from uncontrollable stimulation, but also leads to a decrease in mechanical reactivity (Baumbauer et al., 2008). As of today, it is still unclear how changes in nociceptive processing affect spinal plasticity. What we do know is that both behavioral and pharmacological manipulations known to inhibit the learning deficit, also counter behavioral evidence of allodynia, supporting the presumed link between the learning deficit and central sensitization.

Uncontrollable Stimulation: Opioid Release in the Spinal Cord

Initial inquiry into the underlying mechanisms implicated in the induction of the learning deficit brought to light the role of the opioid system. In particular, evidence suggests that the kappa opioid receptor plays an important role in the deficit produced by uncontrollable shock (Joynes et al., 2004a; Washburn et al., 2008). For instance, intrathecal administration of the kappa opioid receptor antagonist, norBNI, has been shown to block the expression, but not the induction, of the learning deficit (Joynes et al., 2004a). Furthermore, the kappa opioid receptor agonist GR89696 has been found to inhibit spinal learning in a dose-dependent manner (Washburn et al., 2004). How the kappa opioid system impacts spinal learning is unclear. One possible explanation is that uncontrollable shock leads to kappa-2 receptor activation, which in turn, impedes NMDAR-mediate plasticity. In keeping with this notion, there are data to suggest that

kappa-2 opioids inhibit NMDAR-mediated synaptic currents (Caudle, Chavkin, & Dubner, 1994). Blocking NMDAR activity has been shown to not only prevent the induction of the learning deficit, but also block spinal instrumental learning (Joynes, Janjua, & Grau, 2004). In this manner, engaging the kappa opioid system with uncontrollable shock could lead to a disruption in NMDAR-mediated function and disrupt spinal learning.

Alternatively, there is new data implicating the release of the cytokine tumor necrosis factor alpha (TNFα) in the deleterious effects of uncontrollable stimulation (Huie et al., 2009). TNFα has been found to play an important role in glial-neuronal communication, and numerous studies implicate TNFα as an important mediator of enhanced nociceptive processing after injury and inflammation (Gao et al., 2009; Youn, Wang, & Jeong, 2008). Of interest, kappa opioid activation with dynorphin has been shown to increase TNFα expression in the brain (Chao et al., 1995). If we revisit the idea that uncontrollable shock engages a central sensitization-like effect, the combined release of dynorphin and TNFα could lead to changes in nociceptive processing that effectively disrupt adaptive modifications at the level of the spinal cord. Independent of their known interaction, however, both dynorphin and TNFα have been implicated in the development of allodynia following inflammation (Gao et al., 2009; Laughlin et al., 1997; Vanderah et al,. 1996; Youn, Wang, & Jeong, 2008). These data, therefore, underscore an important parallel between the behavioral and neurobiological consequences of inflammation and uncontrollable stimulation at the level of the spinal cord.

Continuous Shock and Antinociception

If we were to envision allodynia and the induction of the learning deficit as being one side of the coin, in theory, changes in nociceptive processing that promote spinal plasticity would represent the opposite side of the coin. In the case of continuous shock, we have a peripheral manipulation that both induces antinociception and protects the spinal cord from the intermittent shock-induced learning deficit. As a result of this, we examined if there was a link between antinociception and the beneficial effects of continuous shock. Similar to the effects of controllable shock and fixed spaced stimulation, continuous shock prevented and reversed the effects of uncontrollable stimulation. Evidence, however, revealed that continuous shock induces a transient antinociceptive response that wanes soon after shock termination. Given that the antinociceptive consequence of continuous shock outlasted its protective effect, there is reason to believe that these two effects are unrelated.

The first piece of evidence that led us to question the sufficiency of antinociception in mediating the protective effect of continuous shock was the combined results of Experiment 2 and 3. Co-administration of intermittent and continuous shock did not impact antinociception, at either higher (1.5mA) or lower (0.5) intensities of continuous stimulation. However, only 1.5mA of continuous shock was able to block the induction of the learning deficit. Further assessment, however, revealed that if we permitted antinociception to fully emerge before administering intermittent shock, 0.5mA of continuous shock was able to prevent the induction of the learning deficit (Experiment 4). One possible explanation for this latter finding is that 0.5mA engages a slower

antinociceptive response than that observed with 1.5mA. On the other hand, we found that blocking the induction of antinociception was not sufficient to prevent the protective effect of continuous shock (Experiment 7). Indeed, pharmacologically antagonizing the kappa, delta, or mu opioid receptors blocked continuous shock-induced antinociception. However, only the mu opioid receptor antagonist, CTOP, blocked the protective effect of continuous shock; while, prior treatment with the mu agonist, DAMGO, prevented the induction of the learning deficit (Experiment 8). These results, thus, suggest that the mu opioid receptor plays an important role in the beneficial effects of continuous stimulation.

Inflammation and Mu Opiate-Mediated Antinociception

Electrophysiological evidence indicates that the development of peripheral inflammation leads to enhanced C-fiber evoked responses at the level of the spinal cord (Stanfa, Sullivan, & Dickenson, 1992). Interestingly, peripheral inflammation has been shown to enhance opioid-mediated analgesia (Hylden et al., 1991; Kayser & Guilbaud, 1987). This paradoxical modification is mediated by changes in C-fiber evoked responses in the dorsal horn (Stanfa, Sullivan, & Dickenson, 1992). Pharmacological activation of the mu, kappa, or delta opioid receptors, following carrageenan-induced inflammation, has been shown to produce a potentiated dose-related inhibition of C-fiber evoked activity. This effect is most pronounced in morphine-treated rats (Stanfa, Sullivan, & Dickenson, 1992). Mu opioid receptor (MOR) upregulation at the level of

the spinal cord is thought, at least in part, to mediate the enhanced potency of exogenous mu opiates after carrageenan-induced inflammation (Ji et al., 1995).

We know from work collected in our laboratory that prolonged C-fiber activity is both necessary and sufficient to induce the learning deficit (Ferguson, Crown, & Grau, 2008; Hook, Huie, & Grau, 2008). We also know that continuous shock leads to the activation of mu, kappa, and delta opioid receptors (Experiment 8). If we were to borrow from the experiments aforementioned, the case could be made that prior treatment with uncontrollable nociceptive input potentiates the inhibitory properties of continuous shock-induced opioid release in the spinal cord. Thus, explaining the results of Experiment 6. In which case, prior treatment with intermittent shock effectively potentiated continuous shock-induced inhibition of C-fiber activity, and attenuated the expression of the learning deficit. This explanation, however, does not account for the observed immunizing effect of continuous stimulation.

To address this issue, we return to a model of formalin-induced inflammation. Peripheral administration of formalin causes a biphasic excitatory response in dorsal horn neurons, which includes: an immediate acute peak of neuronal firing that is present 0-10 minutes post injection, and a second more prolonged tonic excitatory response that lasts 20-65 minutes after formalin treatment (Dickenson & Sullivan, 1987). Electrophysiological data indicate that prior intrathecal administration of the mu agonist, DAGO, completely inhibits both peaks of excitation (Dickenson & Sullivan, 1987). In keeping with this work, we would expect that any manipulation capable of engaging the mu opioid receptor would similarly function to silence c-fiber activity.

From work presented here, we know that 0.5mA of continuous shock leads to endogenous opioid release. More importantly, we know that the beneficial effects of continuous shock are mediated by the mu opioid receptor. If we return to the idea that uncontrollable stimulation engages a central sensitization-like phenomenon, using a manipulation that silences nociceptive signals at the level of the spinal cord should serve to prevent the detrimental effects of uncontrollable stimulation. We know from Dickenson & Sullivan's work (1987) that the mu opioid receptor is a potent inhibitor of c-fiber activity. Therefore, if the mu opioid receptor is activated as a result of continuous shock, it is expected that c-fiber activity would be inhibited, thereby disrupting the detrimental effects of subsequent intermittent shock treatment. Indeed, evidence indicates that prior treatment with continuous shock completely blocks the induction of the learning deficit (Experiment 4). Moreover, pharmacologically activating the mu opioid receptor was found to block the induction of the learning deficit (Experiment 8).

BDNF Mediates the Long-Lasting Consequences of Continuous Shock

Before we can proceed there is one important issue that needs to be addressed. From work presented here, it is clear that the mu opioid receptor plays an important role in the beneficial effects of continuous stimulation. It is unclear, however, for how long the mu opioid receptor is engaged following continuous shock administration. Behavioral data indicates that continuous shock leads to a transient antinociceptive response that starts to wane soon after shock termination. This finding would suggest that opioid release is short-lived once continuous shock is terminated. Moreover, the

results of Experiment 10 revealed that the mu opioid receptor is downregulated as a consequence of shock, *per se*, and that the combined treatments of continuous and intermittent shock lead to the greatest decrease in mu opioid receptor expression in the spinal cord. Thus, if continuous shock causes only a brief release of endogenous opioids, and the mu opioid receptor is downregulated as a consequence of shock, how can we account for the lasting beneficial effects of continuous stimulation?

One possible answer to this question has to do with the role of BDNF in spinal plasticity. Both controllable shock and fixed space stimulation have been shown to cause the release of endogenous BDNF (Baumbauer, Huie, Hughes, & Grau, 2009; Gomez-Pinilla et al., 2007). This is of particular importance, given that exogenous BDNF treatment has been found to have both a protective and therapeutic effect against the detrimental consequences of uncontrollable stimulation in the isolated spinal cord (Huie et al., 2006). The results of Experiment 9 revealed that preventing BDNF activity attenuated the immunizing effect of continuous stimulation, but it did not interfere with antinociception. Given the known beneficial effects of BDNF on spinal plasticity, one possible explanation for the long-lasting effects of continuous shock might be the release of endogenous BDNF.

Of interest, research suggests that exogenous BDNF leads to analgesia in the midbrain (Siuciak et al., 1995). This analgesic effect of BDNF has been found to be naloxone reversible (Siuciak et al., 1995). What's more, BDNF treatment has been found to decrease formalin-induced nociceptive reactivity in an opioid-dependent manner (Siuciak et al., 1995). This data provide us with the intriguing possibility that the

beneficial effects of continuous shock may be mediated by the interaction between the mu opioid receptor and the release of BDNF. We know from data collected here that both BDNF activity and the mu opioid receptor play an important role in the beneficial effects of continuous stimulation. One possible explanation for the long-lasting effects of continuous shock may be that this form of stimulation engages a BDNF-dependent process that fosters spinal plasticity through the mu opioid system. Further research is necessary to examine if, in fact, there is a link between the mu opioid system and BDNF activity in the isolated spinal cord, and to what extend these two systems interact to promote spinal plasticity.

Continuous Shock Downregulates Early Genes Associated with Pain

Peripheral injury and inflammation lead to an increase in cellular *fos* (*c-fos*) in both neuronal and non-neuronal cells (Doucet, Squinto, & Bazan, 1990). Transcriptional activation of this gene occurs rapidly and transiently minutes after stimulation, with mRNA accumulation reaching peak levels within 30 to 40 minutes (Harris, 1998). This early gene encodes for the nuclear protein Fos, which together with other nuclear proteins of the Jun family form the Fos-Jun complex. The Fos-Jun complex binds to the AP-1 DNA site where it regulates the downstream expression of target genes. Numerous studies have established the use of *c-fos* to assess spinal nociceptive responding. This approach originating with the work of Hunt et al. (1987), who showed that *c-fos* expression was upregulated in the superficial layers of the spinal dorsal horn after physiological stimulation of primary sensory neurons with both noxious heat and

chemical stimuli (Hunt, Pini, & Evan, 1987). Since then, follow up studies have strengthened the relationship between nociception and *c-fos*, which has led to *c-fos* expression being used as a functional marker to detect activity in spinal neurons in response to noxious stimulation (Harris, 1998).

The early gene *c-jun* has been also been shown to play an important role in the pathogenesis of pain. In an animal model of neuropathic pain, intrathecal administration with *c-jun* antisense oligodexeoxynucleotides (AS-ODN) has been found to reduce mechanical allodynia associated with chronic constriction injury (Son et al., 2007). Evidence indicates that *c-jun* is upregulated at the mRNA and protein levels in lumbar dorsal root ganglion (DRG) neurons following nerve injury (Jenkins & Hunt, 1991). In addition, axotomy has been shown to cause activation of c-Jun amino-terminal kinase (JNK) in the lumbar section of the spinal cord (Kenney & Kocsis, 1997). Of interest, evidence suggests that JNK activation occurs initially in small-sized C-fiber neurons within the DRG after spinal nerve ligation, contributing to the induction of neuropathic pain (Zhuang et al., 2006). However, the maintenance of neuropathic pain is thought to be mediated by activation of JNK in spinal astrocytes (Zhuang et al., 2006).

The results of Experiment 10 showed that continuous shock causes a downregulation of both of *c-fos* and *c-jun* expression. As well, we found that (while not statistically significant) there was a trend towards continuous shock preventing the upregulation of *c-fos* and *c-jun* after intermittent shock treatment. Manipulations known to induce analgesia, such as morphine, have been shown to decrease the expression of these early genes (Gogas et al., 1991). Of particular relevance, electroacupuncture has

also been found to decrease spinal *c-fos* expression in the rat spinal cord in response to noxious stimulation (Lee & Beitz, 1992). Moreover, this effect was been shown to be naloxone-reversible (Lee & Beitz, 1992). Similarly, the combined application of the NMDAR antagonist, AP5, and electroacupuncture reduces carrageen-induced behavioral hyperalgesia and spinal fos expression in the rat (Zhang et al., 2002). Treatment, with the NMDAR antagonist, MK-801, alone is a potent inhibitor of fos expression in the spinal cord after peripheral injury (Munglani et al., 1999). Together, these results support the findings of Experiment 10, and further implicate the role of the opioid system in the beneficial effects of continuous stimulation. Interestingly, these results also suggest that application of the NMDAR antagonist, MK-801, might potentiate the consequences of continuous shock. If this so, we expect that treatment with intrathecal MK-801 and continuous shock will significantly reverse the upregulation of *c-fos* and *c-jun* that is seen as a consequence of intermittent shock treatment. Future studies will examine this issue.

Continuous Shock: Pending Questions and Future Direction

We set out to examine if there was a link between the beneficial and antinociceptive consequences of continuous stimulation. Our findings led us to discount this possibility, as the protective effect of continuous shock appears to be independent of antinociception. In our investigation, however, we were able to further identify the behavioral potential of continuous stimulation. Similarly, we uncovered a number of neurobiological factors mediating the beneficial impact of this form of stimulation. In

spite of this, we were left with numerous unanswered questions pertaining to the underlying mechanisms responsible for the beneficial effects of continuous stimulation. In this section, we bring to light these questions and propose future studies that will attempt to address these issues.

In our initial studies, we identified an important difference between 0.5mA and 1.5mA of continuous shock. We found that 1.5mA of continuous shock was better able to protect spinal plasticity when administered simultaneously with intermittent shock. In contrast, 0.5mA of continuous shock was found to be more effective if given prior to intermittent shock. At the time, we suspected that this discrepancy was mediated by differences in the antinociceptive response initiated by these two intensities. As we have discounted the role of antinociception, we are left with the unanswered question of what is mechanistically different between 0.5 and 1.5mA of continuous shock. The simplest answer to this question has to do with how these two intensities relate to intermittent shock. If we envision intermittent shock as initiating a period of sensory overexcitation, a stimulus capable of "masking" this phase of sensory overdrive should negate the detrimental effects of uncontrollable stimulation. If this were true, then we would expect that a higher intensity (1.5mA) of continuous stimulation would be better able to "mask" this phase, than a lower intensity (0.5mA). Thus, explaining why 1.5mA of continuous shock was more effective at protecting spinal plasticity when both intermittent and continuous shock were co-administered.

This explanation, however, does not account for the ability of 0.5mA of continuous shock to both prevent and reverse the induction of the learning deficit. To

address this discrepancy, we look back at earlier work demonstrating that the antinociception initiated by mild and intense shock is different (Meagher et al., 1993). Evidence suggests that mild shock initiates an opioid-mediated antinociception, while intense shock engages a naltrexone-insensitive antinociception. These data, together with the current findings of this dissertation, suggest that 0.5mA of continuous shock engages an opioid-mediated antinociception. Now, we know that it is not the induction of antinociception, *per se*, that mediates the beneficial effect of 0.5mA of continuous shock, but we do know that the activation of the mu opioid receptor is critical for the beneficial effects of continuous stimulation. What's more, we know that pretreatment with the mu opioid receptor agonist DAMGO prevents the induction of the learning deficit.

It is unclear, however, through what mechanism the mu opioid receptor mediates the beneficial effects of continuous stimulation. One possibility that is supported by research conducted in the field of inflammation is that activating the mu opioid receptor leads to a silencing of c-fiber activity. Thus, if continuous shock engages the mu opioid receptor, we would expect that continuous stimulation would effectively silence c-fiber activity and prevent nociceptive overexcitation by uncontrollable stimulation. This notion is supported by the finding that 0.5mA of continuous shock prevented the induction of the learning deficit when continuous stimulation was given immediately before intermittent shock. Furthermore, continuous shock was found to downregulate *c-fos* expression levels in the spinal cord. This finding is in keeping with evidence

implicating the mu opioid system in the downregulation of *c-fos* expression after inflammation (Gogas et al., 1991).

On the other hand, we found that 0.5mA of continuous shock reversed the expression of the learning deficit. This finding is problematic, because it brings to question the simple idea that the beneficial effects of continuous shock are opioid-mediated. Previously we argues that the beneficial effects of 0.5mA of continuous shock was linked to the silencing of c-fiber activity. However, if intermittent shock was presented before continuous stimulation, we would expect that continuous shock would be unable to block the consequences of intermittent shock on spinal plasticity. Yet, this is not the case. One possible explanation for this finding is that activation of the mu opioid receptor, even after intermittent shock treatment, has the potential to negate the consequences of uncontrollable stimulation. This is an avenue that was not examined in this dissertation. One simple way of addressing this option is to administer a mu opioid agonist after intermittent shock treatment. If we find that pharmacologically activating the mu opioid receptor is therapeutic against the learning deficit, then we can further implicate the mu opioid receptor in the beneficial effects of continuous stimulation.

A second finding that counters the simple hypothesis that the opioid system mediates the beneficial effects of continuous shock is the results of experiment 5. Evidence revealed that the immunizing effect of 0.5mA of continuous shock was evident three hours after shock was terminated. While it may be possible to argue that the acute immunizing and therapeutic effects of continuous shock are mediated by the mu opioid receptor, it is highly unlikely that the same can be said about the long-lasting effects of

continuous stimulation. Particularly, as evidence suggests that continuous shock leads to a transient opioid release. Of interest, however, we found that disrupting BDNF activity attenuated the immunizing effect of continuous shock. We know from pharmacological data, and research conducted using both controllable shock and fixed spaced stimulation, that BDNF has a protective effect on spinal plasticity. Given the known beneficial effects of BDNF activity on spinal plasticity, one possible mechanism through which continuous shock could exert its long-lasting effects is through the release of endogenous BDNF.

Unfortunately, the results of Experiment 10 revealed a significant downregulation of the BDNF-binding TrkB receptor in the spinal ventral horn. A finding that, partially, discounts the hypothesis that BDNF activity mediates the long-lasting effects of continuous shock. Nevertheless, given that the TrkB receptor was not uniformly downregulated across the spinal cord, future studies will examine the role of BDNF and continuous stimulation. At which time, we will examine if pharmacologically disrupting BDNF activity blocks the long-lasting effects of 0.5mA of continuous shock. Moreover, we will examine if exogenous BDNF, similar to what is observed in the midbrain, can play a role in the induction of antinociception in the isolated spinal cord.

Clinical Application

Chronic pain, resulting from lower back injury and inflammatory disorders, is commonly treated with opiates. Unfortunately, there is a vast literature highlighting the potential for addiction and the development of tolerance to chronic opiate use. As an

alternative, peripheral stimulation is a commonly used tool to induce analgesia and treat inflammatory pain. There are a number of available treatments that make use of the body's own ability to release opioids in response to peripheral stimulation. Among these treatments are acupuncture and electrical stimulation applied at different frequencies in target areas of the body.

Numerous studies, from laboratory work to clinical trials, support the use of these techniques to treat pain (for review see Han, 2003 and Sluka & Walsh, 2003). For instance, transcutaneous electrical nerve stimulation (TENS) has been shown to reduce inflammatory pain in an animal model of arthritis (Sluka et al., 1999). This analgesic effect of TENS was prevented by blocking spinal opioid receptors (Sluka et al., 1999); thus, implicating the opioid system in the analgesic effects of TENS. Furthermore, TENS has been shown to decrease the release of the excitatory neurotransmitters glutamate and aspartate in animal models of inflammation (Sluka, Vance, & Lisi, 2005). In clinical practice, TENS is often used in combination with other treatment options, such as physical rehabilitation and anti-inflammatory agents. Evidence indicates that TENS can be used to treat arthritis, leading to improve joint function (Kumar & Redford, 1982). Similarly, postoperative use of TENS has been shown to improve recovery after thoracic surgery (Ali, Yaffe, & Seesle, 1981).

Here, we present an alternative form of peripheral stimulation that, similar to what is seen with TENS, engages the spinal opioid system and induces antinociception. Importantly, continuous shock-induced antinociception is not affected by intermittent shock treatment. Up to now, the case has been made that intermittent shock shares a

number of neurobiological and behavioral parallels with inflammation. In keeping with the known effects of TENS on inflammation, we have shown that continuous shock counters the consequences of uncontrollable stimulation in an opioid-dependent manner. Moreover, we have shown that continuous stimulation counters the negative impact of uncontrollable stimulation after SCI (Experiments 11 and 12).

Unfortunately, research presented here indicates that continuous shock alone is detrimental to locomotor recovery after injury (Experiments 11 and 12). While surprising, this finding is keeping with previous research collected in our laboratory. Treatment with intrathecal morphine has been shown to hinder functional recovery after SCI (Hook et al, 2009). We know from our studies using a spinal transection model that continuous shock leads to mu opioid activation. Thus, similar to the known effects of morphine, it is possible that continuous shock leads to a mu opioid receptor-dependent disruption in spinal function after injury. Contrary to this, however, we found that continuous shock was protective against the detrimental effects of intermittent stimulation. This was initially observed in Experiment 11 and later replicated in Experiment 12. While these observations are contradictory, they raise a number of questions. In particular, what is mediating the beneficial effect of continuous shock against uncontrollable stimulation after injury? And, how do we account for the negative effects of continuous shock in an animal model of spinal cord injury? Addressing these issues is important to understanding the potential therapeutic value of TENS and clarify whether TENS-like stimulation may have, under some circumstance, an adverse effect. Addressing these issues will require further study.

REFERENCES

Ainsworth, L., Budelier, K., Clinesmith, M., Fiedler, A., Landstrom, R., Leeper, B. J., et al. (2006). Transcutaneous electrical nerve stimulation (TENS) reduces chronic hyperalgesia induced by muscle inflammation. *Pain, 120*(1-2), 182-187.

Ali, J., Yaffe, C. S., & Serrette, C. (1981). The effect of transcutaneous electric nerve stimulation on postoperative pain and pulmonary function. *Surgery, 89*(4), 507-512.

Anderson, K. D. (2004). Targeting recovery: Priorities of the spinal cord-injured population. *J Neurotrauma, 21*(10), 1371-1383.

Basso, D. M., Beattie, M. S., & Bresnahan, J. C. (1995). A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma, 12*(1), 1-21.

Baumbauer, K. M., Hoy, K. C., Jr., Huie, J. R., Hughes, A. J., Woller, S. A., Puga, D. A., et al. (2008). Timing in the absence of supraspinal input I: variable, but not fixed, spaced stimulation of the sciatic nerve undermines spinally-mediated instrumental learning. *Neuroscience, 155*(4), 1030-1047.

Baumbauer, K. M., Huie, J. R., Hughes, A. J., & Grau, J. W. (2009a). Timing in the absence of supraspinal input II: regularly spaced stimulation induces a lasting alteration in spinal function that depends on the NMDA receptor, BDNF release, and protein synthesis. *J Neurosci, 29*(46), 14383-14393.

Baumbauer, K. M., Puga, D. A., Lee, K. H., Woller, S. A., Hughes, A. J., & Grau, J. W. (2009b). Regularly spaced low frequency electrical stimulation dampens tactile reactivity and attenuates capsaicin-induced allodynia. Program No. 79.20. 2009

Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2009. Online

Burchiel, K. J., Anderson, V. C., Brown, F. D., Fessler, R. G., Friedman, W. A., Pelofsky, S., et al. (1996). Prospective, multicenter study of spinal cord stimulation for relief of chronic back and extremity pain. *Spine (Phila Pa 1976), 21*(23), 2786-2794.

Campbell, J. N., & Meyer, R. A. (2006). Mechanisms of neuropathic pain. *Neuron, 52*(1), 77-92.

Caudle, R. M., Chavkin, C., & Dubner, R. (1994). Kappa 2 opioid receptors inhibit NMDA receptor-mediated synaptic currents in guinea pig CA3 pyramidal cells. *J Neurosci, 14*(9), 5580-5589.

Chao, C. C., Gekker, G., Sheng, W. S., Hu, S., Portoghese, P. S., & Peterson, P. K. (1995). Endogenous opioid peptides suppress cytokine-mediated upregulation of HIV-1 expression in the chronically infected promonocyte clone U1. *Adv Exp Med Biol, 373*, 65-72.

Coderre, T. J., & Melzack, R. (1992). The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. *J Neurosci, 12*(9), 3665-3670.

Constantini, S., & Young, W. (1994). The effects of methylprednisolone and the ganglioside GM1 on acute spinal cord injury in rats. *J Neurosurg, 80*(1), 97-111.

Crown, E. D., Ferguson, A. R., Joynes, R. L., & Grau, J. W. (2002). Instrumental learning within the spinal cord: IV. Induction and retention of the behavioral deficit observed after noncontingent shock. *Behav Neurosci, 116*(6), 1032-1051.

Crown, E. D., & Grau, J. W. (2001). Preserving and restoring behavioral potential within the spinal cord using an instrumental training paradigm. *J Neurophysiol, 86*(2), 845-855.

Crown, E. D., & Grau, J. W. (2005). Evidence that descending serotonergic systems protect spinal cord plasticity against the disruptive effect of uncontrollable stimulation. *Exp Neurol, 196*(1), 164-176.

Dickenson, A. H., & Sullivan, A. F. (1987). Subcutaneous formalin-induced activity of dorsal horn neurones in the rat: differential response to an intrathecal opiate administered pre or post formalin. *Pain, 30*(3), 349-360.

Donnelly, D. J., & Popovich, P. G. (2008). Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. *Exp Neurol, 209*(2), 378-388.

Doucet, J. P., Squinto, S. P., & Bazan, N. G. (1990). Fos-jun and the primary genomic response in the nervous system: Possible physiological role and pathophysiological significance. *Mol Neurobiol, 4*(1-2), 27-55.

Drugan, R. C., Ader, D. N., & Maier, S. F. (1985). Shock controllability and the nature of stress-induced analgesia. *Behav Neurosci, 99*(5), 791-801.

Ferguson, A. R., Crown, E. D., & Grau, J. W. (2006). Nociceptive plasticity inhibits adaptive learning in the spinal cord. *Neuroscience, 141*(1), 421-431.

Ferguson, A. R., Hook, M. A., Garcia, G., Bresnahan, J. C., Beattie, M. S., & Grau, J. W. (2004). A simple post hoc transformation that improves the metric properties of the BBB scale for rats with moderate to severe spinal cord injury. *J Neurotrauma, 21*(11), 1601-1613.

Gao, Y. J., Zhang, L., Samad, O. A., Suter, M. R., Yasuhiko, K., Xu, Z. Z., et al. (2009). JNK-induced MCP-1 production in spinal cord astrocytes contributes to central sensitization and neuropathic pain. *J Neurosci, 29*(13), 4096-4108.

Ghoname, E. S., Craig, W. F., White, P. F., Ahmed, H. E., Hamza, M. A., Gajraj, N. M., et al. (1999). The effect of stimulus frequency on the analgesic response to percutaneous electrical nerve stimulation in patients with chronic low back pain. *Anesth Analg, 88*(4), 841-846.

Gogas, K. R., Presley, R. W., Levine, J. D., & Basbaum, A. I. (1991). The antinociceptive action of supraspinal opioids results from an increase in descending inhibitory control: Correlation of nociceptive behavior and c-fos expression. *Neuroscience, 42*(3), 617-628.

Gomez-Pinilla, F., Huie, J. R., Ying, Z., Ferguson, A. R., Crown, E. D., Baumbauer, K. M., et al. (2007). BDNF and learning: Evidence that instrumental training promotes learning within the spinal cord by up-regulating BDNF expression. *Neuroscience, 148*(4), 893-906.

Grahn, R. E., Will, M. J., Hammack, S. E., Maswood, S., McQueen, M. B., Watkins, L. R., et al. (1999). Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor. *Brain Res, 826*(1), 35-43.

Grau, J. W., Barstow, D. G., & Joynes, R. L. (1998). Instrumental learning within the spinal cord: I. Behavioral properties. *Behav Neurosci, 112*(6), 1366-1386.

Grau, J. W., Crown, E. D., Ferguson, A. R., Washburn, S. N., Hook, M. A., & Miranda, R. C. (2006). Instrumental learning within the spinal cord: Underlying mechanisms and implications for recovery after injury. *Behav Cogn Neurosci Rev, 5*(4), 191-239.

Grau, J. W., Washburn, S. N., Hook, M. A., Ferguson, A. R., Crown, E. D., Garcia, G., et al. (2004). Uncontrollable stimulation undermines recovery after spinal cord injury. *J Neurotrauma, 21*(12), 1795-1817.

Gruner, J. A. (1992). A monitored contusion model of spinal cord injury in the rat. *J Neurotrauma, 9*(2), 123-126; discussion 126-128.

Han, J. S. (2003). Acupuncture: Neuropeptide release produced by electrical stimulation of different frequencies. *Trends Neurosci, 26*(1), 17-22.

Harris, J. A. (1998). Using c-fos as a neural marker of pain. *Brain Res Bull, 45*(1), 1-8.

Hook, M. A., Huie, J. R., & Grau, J. W. (2008). Peripheral inflammation undermines the plasticity of the isolated spinal cord. *Behav Neurosci, 122*(1), 233-249.

Huie, J.R., Grau, J., Beattie, M., Bresnahan, J., & Ferguson, A. (2009). Instrumental learning in the spinalized rat: TNF-alpha inhibits learning in the spinal cord. Program No. 79.21. 2009. *Neuroscience Meeting Planner*. Chicago, IL: Society for Neuroscience, 2009. Online.

Huie, J. R., Hoy, K. C., Miranda, R. A., & Grau, J. W. (2007). BDNF facilitates learning in the rat spinal cord. Program No. 404.12. 2007. *Neuroscience Meeting Planner*. Atlanta, GA: Society for Neuroscience, 2007. Online.

Hunt, S. P., Pini, A., & Evan, G. (1987). Induction of c-fos-like protein in spinal cord neurons following sensory stimulation. *Nature, 328*(6131), 632-634.

Hylden, J. L., Thomas, D. A., Iadarola, M. J., Nahin, R. L., & Dubner, R. (1991). Spinal opioid analgesic effects are enhanced in a model of unilateral inflammation/hyperalgesia: Possible involvement of noradrenergic mechanisms. *Eur J Pharmacol, 194*(2-3), 135-143.

Jackson, R. L., Maier, S. F., & Coon, D. J. (1979). Long-term analgesic effects of inescapable shock and learned helplessness. *Science, 206*(4414), 91-93.

Jenkins, R., & Hunt, S. P. (1991). Long-term increase in the levels of c-jun mRNA and jun protein-like immunoreactivity in motor and sensory neurons following axon damage. *Neurosci Lett, 129*(1), 107-110.

Ji, R. R., Kohno, T., Moore, K. A., & Woolf, C. J. (2003). Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends Neurosci, 26*(12), 696-705.

Ji, R. R., Zhang, Q., Law, P. Y., Low, H. H., Elde, R., & Hokfelt, T. (1995). Expression of mu-, delta-, and kappa-opioid receptor-like immunoreactivities in rat dorsal root ganglia after carrageenan-induced inflammation. *J Neurosci, 15*(12), 8156-8166.

Joynes, R. L., & Grau, J. W. (2004a). Instrumental learning within the spinal cord: III. Prior exposure to noncontingent shock induces a behavioral deficit that is blocked by an opioid antagonist. *Neurobiol Learn Mem, 82*(1), 35-51.

Joynes, R. L., Janjua, K., & Grau, J. W. (2004b). Instrumental learning within the spinal cord: VI. The NMDA receptor antagonist, AP5, disrupts the acquisition and maintenance of an acquired flexion response. *Behav Brain Res, 154*(2), 431-438.

Kayser, V., & Guilbaud, G. (1987). Local and remote modifications of nociceptive sensitivity during carrageenin-induced inflammation in the rat. *Pain, 28*(1), 99-107.

Kenney, A. M., & Kocsis, J. D. (1997). Timing of c-jun protein induction in lumbar dorsal root ganglia after sciatic nerve transection varies with lesion distance. *Brain Res, 751*(1), 90-95.

Kumar, V. N., & Redford, J. B. (1982). Transcutaneous nerve stimulation in rheumatoid arthritis. *Arch Phys Med Rehabil, 63*(12), 595-596.

Laughlin, T. M., Vanderah, T. W., Lashbrook, J., Nichols, M. L., Ossipov, M., Porreca, F., et al. (1997). Spinally administered dynorphin. A produces long-lasting allodynia: Involvement of NMDA but not opioid receptors. *Pain, 72*(1-2), 253-260.

Lee, J. H., & Beitz, A. J. (1992). Electroacupuncture modifies the expression of c-fos in the spinal cord induced by noxious stimulation. *Brain Res, 577*(1), 80-91.

Meagher, M. W., Chen, P. S., Salinas, J. A., & Grau, J. W. (1993). Activation of the opioid and nonopioid hypoalgesic systems at the level of the brainstem and

spinal cord: Does a coulometric relation predict the emergence or form of environmentally induced hypoalgesia? *Behav Neurosci, 107*(3), 493-505.

Munglani, R., Hudspith, M. J., Fleming, B., Harrisson, S., Smith, G., Bountra, C., et al. (1999). Effect of pre-emptive NMDA antagonist treatment on long-term Fos expression and hyperalgesia in a model of chronic neuropathic pain. *Brain Res, 822*(1-2), 210-219.

Siddall, P. J., & Loeser, J. D. (2001). Pain following spinal cord injury. *Spinal Cord, 39*(2), 63-73.

Siuciak, J. A., Wong, V., Pearsall, D., Wiegand, S. J., & Lindsay, R. M. (1995). BDNF produces analgesia in the formalin test and modifies neuropeptide levels in rat brain and spinal cord areas associated with nociception. *Eur J Neurosci, 7*(4), 663-670.

Sluka, K. A., Deacon, M., Stibal, A., Strissel, S., & Terpstra, A. (1999). Spinal blockade of opioid receptors prevents the analgesia produced by TENS in arthritic rats. *J Pharmacol Exp Ther, 289*(2), 840-846.

Sluka, K. A., Vance, C. G., & Lisi, T. L. (2005). High-frequency, but not low-frequency, transcutaneous electrical nerve stimulation reduces aspartate and glutamate release in the spinal cord dorsal horn. *J Neurochem, 95*(6), 1794-1801.

Sluka, K. A., & Walsh, D. (2003). Transcutaneous electrical nerve stimulation: Basic science mechanisms and clinical effectiveness. *J Pain, 4*(3), 109-121.

Son, S. J., Lee, K. M., Jeon, S. M., Park, E. S., Park, K. M., & Cho, H. J. (2007). Activation of transcription factor c-jun in dorsal root ganglia induces VIP and

NPY upregulation and contributes to the pathogenesis of neuropathic pain. *Exp Neurol, 204*(1), 467-472.

Stanfa, L. C., Sullivan, A. F., & Dickenson, A. H. (1992). Alterations in neuronal excitability and the potency of spinal mu, delta and kappa opioids after carrageenan-induced inflammation. *Pain, 50*(3), 345-354.

Stoll, G., Jander, S., & Schroeter, M. (2002). Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. *Adv Exp Med Biol, 513*, 87-113.

Vanderah, T. W., Laughlin, T., Lashbrook, J. M., Nichols, M. L., Wilcox, G. L., Ossipov, M. H., et al. (1996). Single intrathecal injections of dynorphin A or des-Tyr-dynorphins produce long-lasting allodynia in rats: Blockade by MK-801 but not naloxone. *Pain, 68*(2-3), 275-281.

Washburn, S. N., Maultsby, M. L., Puga, D. A., & Grau, J. W. (2008). Opioid regulation of spinal cord plasticity: Evidence the kappa-2 opioid receptor agonist GR89696 inhibits learning within the rat spinal cord. *Neurobiol Learn Mem, 89*(1), 1-16.

Watkins, L. R., Wiertelak, E. P., & Maier, S. F. (1992). Kappa opiate receptors mediate tail-shock induced antinociception at spinal levels. *Brain Res, 582*(1), 1-9.

Woolf, C. J., & Thompson, S. W. (1991). The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation: Implications for the treatment of post-injury pain hypersensitivity states. *Pain, 44*(3), 293-299.

Youn, D. H., Wang, H., & Jeong, S. J. (2008). Exogenous tumor necrosis factor-alpha rapidly alters synaptic and sensory transmission in the adult rat spinal cord dorsal horn. *J Neurosci Res, 86*(13), 2867-2875.

Zhang, Y. Q., Ji, G. C., Wu, G. C., & Zhao, Z. Q. (2002). Excitatory amino acid receptor antagonists and electroacupuncture synergetically inhibit carrageenan-induced behavioral hyperalgesia and spinal fos expression in rats. *Pain, 99*(3), 525-535.

Zhuang, Z. Y., Wen, Y. R., Zhang, D. R., Borsello, T., Bonny, C., Strichartz, G. R., et al. (2006). A peptide c-Jun N-terminal kinase (JNK) inhibitor blocks mechanical allodynia after spinal nerve ligation: respective roles of JNK activation in primary sensory neurons and spinal astrocytes for neuropathic pain development and maintenance. *J Neurosci, 26*(13), 3551-3560.

VITA

Denise Alejandra Puga
Department of Psychology
Texas A&M University
4235 TAMU
College Station, TX 77843-4235
C/O James Grau

Education: M.S. Texas A&M University, 2007
Major: Psychology

B.A. New Mexico State University, 2004
Major: Psychology

References:

Dr. James W. Grau Department of Psychology Texas A&M University College Station, TX 77843 j-grau@tamu.edu	Dr. Michelle Hook Department of Psychology Texas A&M University College Station, TX 77843 michelle-hook @tamu.edu	Dr. Rajesh C. G. Miranda Dept. of Neuroscience & Experimental Therapeutics Texas A&M University Health Science Center College Station, TX 77843 miranda@tamu.edu

Awards:
Texas A&M University Chapter of the Society for Neuroscience Travel Award
Texas A&M University Recovery of Function Program Travel Award

Selected Publications:

Hook, M.A., Washburn, S. N., Moreno, G. L., Woller, S. A., Puga, D. A. & Grau, J. (2010). An IL-1 receptor antagonist blocks a morphine-induced attenuation of locomotor recovery after spinal cord injury. *Brain, Behavior and Immunity*. In press.

Hook, M. A, Moreno, G. L., Woller, S. A., Puga, D. A., Hoy, K. C., Jr., Balden, R., & Grau, J. W. (2009). Intrathecal morphine attenuates recovery of functin after spinal cord injury. *Journal of Neurotrauma*. *26*, 741-752.

Washburn, S. W., Maultsby, M. L., Puga D. A. & Grau, J. W. (2008). Opioid regulation of spinal cord plasticity: evidence the kappa-2 opioid receptor agonist GR89696 Inhibits Learning within the rat spinal cord. *Neurobiology of Learning and Memory*, *89*, 1-16.