REVERSAL OF NEUROPATHIC PAIN WITH EXERCISE IS MEDIATED BY
ENDOGENOUS OPIOIDS

By
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DEDICATION

I dedicate my dissertation to the memory of my brother, Michael Redvers Stagg.
May your laughing, shining spirit live on in everything I do and accomplish in my life.
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ABBREVIATIONS

Aβ: A-beta
Aδ: A-delta
ALS: amyotrophic lateral sclerosis
ARTN: artemin
BDNF: brain derived neurotrophic factor
β-endorphin: beta-endorphin
BL: baseline
CAMP: cyclic adenosine monophosphate
CCC: chronic constrictive injury
CCK: cholecystokinin
CFL: complete Freund’s adjuvant
CGRP: calcitonin-gene related peptide
CNS: central nervous system
CSF: cerebral spinal fluid
CUS: chronic unpredictable stress
d: day (s)
δ: delta
DAMGO: D-Ala2,N-Me-Phe4-Gly5-ol- enkephalin
DPDPE: D-Pen2,D-Pen5- enkephalin
DRG: dorsal root ganglion
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked ImmunoSorbent Assay</td>
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<td>Fig.</td>
<td>Figure</td>
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<tr>
<td>GABA</td>
<td>gamma-aminobutyric acid</td>
</tr>
<tr>
<td>$G_{\text{ai/o}}$</td>
<td>G-protein coupled receptors</td>
</tr>
<tr>
<td>GDNF</td>
<td>Glial cell line-Derived Neurotrophic Factor</td>
</tr>
<tr>
<td>GFL</td>
<td>GDNF family of ligands</td>
</tr>
<tr>
<td>GFR$\alpha$</td>
<td>GDNF family receptor $\alpha$</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-protein coupled receptors</td>
</tr>
<tr>
<td>h</td>
<td>hour (s)</td>
</tr>
<tr>
<td>HCL</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>HSV</td>
<td>herpes simplex virus</td>
</tr>
<tr>
<td>IASP</td>
<td>International Association for the Study of Pain</td>
</tr>
<tr>
<td>i.c.v.</td>
<td>intracerebroventricular</td>
</tr>
<tr>
<td>i.p.:</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>i.pl.:</td>
<td>intraplantar</td>
</tr>
<tr>
<td>i.t.:</td>
<td>intrathecal</td>
</tr>
<tr>
<td>$\kappa$:</td>
<td>kappa</td>
</tr>
<tr>
<td>LC</td>
<td>locus coeruleus</td>
</tr>
<tr>
<td>min</td>
<td>minute (s)</td>
</tr>
<tr>
<td>mo</td>
<td>month (s)</td>
</tr>
<tr>
<td>MOR</td>
<td>(mu) $\mu$-opioid receptor</td>
</tr>
<tr>
<td>$\text{Na}_v1.3$:</td>
<td>voltage gated sodium channel subtype 1.3</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>Naᵥ₁.₈</td>
<td>voltage gated sodium channel subtype 1.8</td>
</tr>
<tr>
<td>NRTN</td>
<td>neurturin</td>
</tr>
<tr>
<td>NGF</td>
<td>nerve-growth factor</td>
</tr>
<tr>
<td>NPY</td>
<td>neuropeptide Y</td>
</tr>
<tr>
<td>NT₃</td>
<td>neurotrophin 3</td>
</tr>
<tr>
<td>NT₄/₅</td>
<td>neurotrophin 4/5</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamin</td>
</tr>
<tr>
<td>PAG</td>
<td>periaqueuctal gray</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>POMC</td>
<td>proopiomelanocortin</td>
</tr>
<tr>
<td>ProEnkA</td>
<td>pro-enkephalin A</td>
</tr>
<tr>
<td>PSL</td>
<td>partial sciatic nerve ligation</td>
</tr>
<tr>
<td>PSPN</td>
<td>persephin</td>
</tr>
<tr>
<td>rAAV</td>
<td>recombinant adeno-associated virus</td>
</tr>
<tr>
<td>RET</td>
<td>receptor tyrosine kinase</td>
</tr>
<tr>
<td>RVM</td>
<td>rostral ventromedial medulla</td>
</tr>
<tr>
<td>s</td>
<td>second (s)</td>
</tr>
<tr>
<td>s.c.</td>
<td>subcutaneous</td>
</tr>
<tr>
<td>SCI</td>
<td>spinal cord injury</td>
</tr>
<tr>
<td>SD</td>
<td>Sprague-Dawley</td>
</tr>
<tr>
<td>SHR</td>
<td>spontaneously hypertensive rats</td>
</tr>
<tr>
<td>SIA</td>
<td>stress-induced analgesia</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>SNL:</td>
<td>Spinal Nerve Ligation</td>
</tr>
<tr>
<td>Trk:</td>
<td>tyrosine kinase receptor</td>
</tr>
<tr>
<td>µ:</td>
<td>mu</td>
</tr>
<tr>
<td>µg:</td>
<td>microgram</td>
</tr>
<tr>
<td>VGSC:</td>
<td>voltage gated sodium channels</td>
</tr>
<tr>
<td>wk:</td>
<td>week (s)</td>
</tr>
<tr>
<td>WKY:</td>
<td>Wistar-Kyoto rats</td>
</tr>
<tr>
<td>yr:</td>
<td>year (s)</td>
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ABSTRACT

Exercise is often prescribed for patients with chronic pain, but there is little objective evidence supporting this recommendation. Therefore, we tested the effect of moderate aerobic exercise on the sensory hypersensitivity produced in an animal model of neuropathic pain. Male rats that underwent unilateral ligation of the L5 and L6 spinal nerves (SNL) were divided into exercise-trained or sedentary groups. Exercise training was performed using a treadmill, beginning 7 days after surgery, and continued 5 days a week for 5 weeks. Animals were exercised 30 min/day, at a speed of 14-16 m/min. Sensory testing was performed 23 hours after exercise training. Typical thermal and tactile hypersensitivity developed within 1 week after surgery. Treadmill training reversed thermal and tactile hypersensitivity in injured animals within 4 weeks, but had no effect on sham-operated or non-operated animals. One week after the cessation of exercise training, tactile hypersensitivity returned.

The effects of exercise training on SNL-induced sensory hypersensitivity were reversed by the opioid receptor antagonist naloxone. Naloxone or naloxone methiodide reversed the effects of exercise when administered intracerebroventricularly (i.c.v.). Immunohistochemistry revealed increased immunostaining for β-endorphin and met-enkephalin in the periaqueductal grey (PAG) and rostral ventromedial medulla (RVM) regions of exercise-trained animals compared to sedentary animals. An ELISA immunoassay revealed a 31% increase in PAG β-endorphin content in exercise-trained SNL animals. More BDNF was also present in the brain’s of exercise-trained animals.
compared to sedentary, specifically in the ventromedial hypothalamus, hippocampus, and outer rim of the PAG. Administering a BDNF sequestering agent reversed β-endorphin increases in the PAG of exercise-trained animals. Exercise-trained SNL animals treated with 25 µg BDNF sequestering agent (i.c.v.) had lower tactile thresholds compared to the exercise-trained vehicle group.

These results support the recommendation of moderate aerobic exercise for patients suffering from neuropathic pain, and suggest that exercise-induced pain reversal results from the upregulation of endogenous opioids in the brainstem. Additionally, increased BDNF with exercise training may play a role in exercise-induced reversal of neuropathic pain by increasing the expression of endogenous opioids, but this needs to be verified further.
INTRODUCTION

Pain Classification

The International Association for the Study of Pain defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merskey and Bogduk, 1994). Pain can be so complex that it is one of the most challenging human conditions to treat. Acute pain is the body’s way of protecting itself, warning of a potentially tissue-damaging event and possibly later signifying an injury and promoting immobility to allow the injury to heal. Pain is produced by a noxious stimulus that is capable of producing tissue damage. The noxious stimulus can be thermal (hot or cold), tactile (touch or pressure), or chemical in nature (Hunt et al., 2001). The noxious stimulus activates receptors in the periphery, resulting in propagation of a signal along nerve fibers, particularly Aβ and C-fibers (Ploner et al., 2002). Pain that is felt immediately is due to myelinated Aβ fibers that are fast-conducting and produce a sharp pain that causes withdrawal from the stimulus. The secondary longer-lasting and less intense pain that follows is a result of activation of unmyelinated c-fibers. The propagation of the action potential along the nerve fibers to the nerve terminal causes a release of excitatory transmitters in the spinal cord (glutamate, CGRP, substance P). The release activates second-order neurons of ascending pain pathways, the most important of which is the spinothalamic tract (Hunt et al., 2001) (Figure 1.1). The painful input is relayed by the thalamus to the cortex to undergo emotional and cognitive processing. Such acute pain normally resolves quickly. If pain
continues even after the original tissue injury has healed, it is classified as chronic.

Chronic pain is a term to describe the long lasting effects of certain pain conditions, and there are many different types of chronic pain. Each is unique in cause, biological mechanisms, and treatments. A few examples include back pain, fibromyalgia, visceral pain (i.e. pancreatitis), bone pain, neuropathic pain, rheumatoid arthritis, osteoarthritis, and headaches.

**Neuropathic Pain**

The focus of my work was neuropathic pain, which is a chronic pain condition that results from injury or disease of nerves. Neuropathic pain is defined by the International Association for the Study of Pain (IASP) as “Pain initiated or caused by a primary lesion or dysfunction in the nervous system.” (Merskey and Bogduk, 1994). Neuropathic pain is a collection of chronic pain syndromes that all have dysfunction of the nerves in common (Ossipov and Porreca, 2005). Examples of clinical conditions associated with neuropathic pain are listed in Table 1.1. Affected nerves normally serve the purpose of transmitting pain signals to the brain. Normally, once a noxious stimulus is removed, pain resolves. In neuropathic pain, injury or disease such as irritation of spinal nerves by herniated discs, or nerve infections such as shingles, or cancer infecting nerves cause a repeated cycle of painful impulses or sensitization to nerve stimuli long after the original cause of the pain has resolved. Neuropathic pain produces not only increased sensitivity to noxious stimuli (hyperalgesia), but non-noxious stimuli (such as light touch) also become painful (allodynia). Non-noxious stimuli activate fibers that
lead to excitatory neurotransmitter release that sends signals through the nucleus gracilis (where there is release of NPY) up to the somatosensory cortex through a pathway different from the spinothalamic tract (Hunt and Mantyh, 2001; Ossipov et al., 2002).

**Rodent Models of Neuropathic Pain**

There are many rodent models of neuropathic pain (over 40 reported) that have been and continue to be used to study the peripheral and central mechanisms of neuropathic pain and to develop potential therapies to treat these conditions. Some of the most common peripheral neuropathic pain animal models include chronic constriction injury (CCI) (Bennet and Xie, 1988), partial sciatic nerve ligation (PSL) (Seltzer et al., 1990), L5 and L6 spinal nerve ligation (SNL) (Kim and Chung, 1992), and selective spinal nerve ligation (Lee et al., 2000a). Spinal nerve ligation (SNL) is one of the more popular models to study peripheral neuropathic pain because it models human neuropathic pain. SNL animals develop thermal (noxious) and tactile (non-noxious) hypersensitivity to hindpaw stimulation, which serves to represent clinical pain responses to noxious stimuli and touch in humans with neuropathic pain. Animals show signs of hypersensitivity to sensory stimuli with an observable response. Allodynia and hyperalgesia are terms used to describe the type of pain. With the SNL model, the L5 and L6 spinal nerves are ligated adjacent to the dorsal root ganglion (Figure 1.2). This produces damage to only a subset of nerves forming the sciatic nerve.
Mechanisms of Neuropathic Pain

Studies of animal models of peripheral nerve injury have led to the discovery of many potential mechanisms that may lead to neuropathic pain. Increases in the expression of the neuropeptide dynorphin in the spinal cord, increased activity of voltage-gated sodium channels, and neuroplastic changes in supraspinal sites that facilitate a descending pain pathway to maintain a sensitized state are just a few examples of neuropathic pain mechanisms (Malan et al., 2000, Porreca et al., 2002, Ossipov and Porreca, 2005). Malan et al, 2000 found increased spinal dynorphin levels in spinal nerve ligated rats that corresponded with the onset of the behavioral signs of neuropathic pain, and intrathecal injections of dynorphin antiserum reversed the effects. These results clearly demonstrate a pathological role of spinal dynorphin in neuropathic pain.

Increased spontaneous and evoked discharges of peripheral nerves are characteristic of neuropathic pain, and they are related to increased activity of voltage-gated sodium channels (VGSCs) (Ossipov and Porreca, 2005). Both the increased expression of the voltage-gated sodium channel subtype NaV 1.3 and downregulation of the NaV1.8 subtype have been shown to contribute to neuropathic pain.

Also seen with neuropathic pain are neuroplastic changes in the rostral ventromedial medulla (RVM) that leads to descending facilitation of nociceptive input into the spinal cord and the persistence of the pain state (Porreca et al., 2002). Injections of lidocaine into the RVM reversed the behavioral signs of neuropathic pain and established its role in the descending facilitation pathway (Figure 1.3). The RVM contains ‘ON’ and ‘OFF’ cells that when activated contribute to facilitation and inhibition
of pain, respectively. Destroying RVM cells containing facilitatory ‘ON’ cells (i.e. treating SNL rats with saporin conjugated to mu-opioid-receptor agonist dermorphin) reverses neuropathic pain behaviors (Porreca et al., 2002).

**Therapies for Neuropathic Pain**

Many patients continue to suffer with neuropathic pain despite attempts at treatment. Therefore, neuropathic pain continues to be extensively researched in animal models and in clinical studies. Some of the more common neuropathic pain treatments include anticonvulsants like gabapentin, tricyclic antidepressants like amitriptyline, and opioids like morphine. The effectiveness of these treatments is limited because they don’t help in all cases of neuropathic pain. In some cases, they may reduce but not eliminate the pain, and many times they produce side effects that may limit dosage or outweigh any benefit of therapy. New approaches, such as cannabinoids, artemin, and benfotiamine, and 5HT receptor agonists are promising in pre-clinical studies (Ibrahim et al., 2003; Gardell et al., 2003; Obata et al., 2004; Ibrahim et al., 2005; Sanchez-Remirez et al., 2005), and will hopefully benefit neuropathic pain sufferers. It is almost certain that additional approaches will be needed, so research into pain mechanisms and therapies must continue.

**Opioids**

Opioids like morphine and fentanyl are commonly used to treat chronic pain. Morphine and other opioids produce their effects at peripheral nerves, in the spinal dorsal
horn, and supraspinally (Holden et al., 2005). Opioids, like morphine produce analgesia by binding to opioid receptors (μ, κ, and/or δ) and activating downstream pathways. Opioid receptors are G-protein coupled receptors (GPCR), and they are located throughout the peripheral and central nervous systems. Binding of the opioids to the opioid receptors modulate pain, but respiration, bowel movements, and other physiological functions are also affected, which can lead to unwanted side effects. Opioids act where opioid receptors are located, peripherally, in the spinal cord, and supraspinally. In the dorsal horn of the spinal cord, opioids bind to their receptors and inhibit release of excitatory transmitters (substance P, Glutamate, CGRP) from primary afferent neurons to prevent the activation of the second-order spinothalamic tract pathway. Decreased pre-synaptic calcium conductance, increased pre-synaptic potassium efflux, and increased post-synaptic potassium efflux prevent excitatory transmitter release and reduce the excitability of second-order neurons. Peripherally administered opioids act at opioid receptors on peripheral nerves to produce analgesia, and peripheral actions may contribute to the analgesia produced by systemic opioids. Supraspinal administration of opioids can produce significant modulation of pain, and the mechanism of action is very different from how opioids act in peripheral nerves or the spinal cord. The most well-established area for opioid-induced analgesia is in the brainstem, particularly in the periaqueductal gray (PAG) region and the rostral ventromedial medulla (RVM) (Figure 1.4). Central binding of opioids to the μ opioid receptors that are highly concentrated in the PAG reduces pain through a disinhibition mechanism. Activation of this pathway leads to inhibition of the inhibitory transmitter, gamma-aminobutyric acid
(GABA). Decreasing GABA release from these cells prevents the inhibitory action of GABA on the RVM. As a result, there is an increased release of the analgesic neurotransmitters serotonin and nor-epinephrine from neurons projecting from the RVM to the dorsal horn of the spinal cord.

**Opioids and Neuropathic Pain**

The effectiveness of opioids in treating neuropathic pain has been much debated. A considerable amount of research has shown spinal opioids to be less effective in animal models of neuropathic pain than in acute pain models. Systemic and intracerebroventricular (i.c.v.) administration of morphine reduce the behavioral signs of neuropathic pain in spinal nerve ligated (SNL) rats, while intrathecal morphine is ineffective (Bian et al., 1995; Ossipov et al., 1995; Lee et al., 1995). Sohn et al., 2000 also found opioids injected in microdoses into the PAG also produced attenuation of neuropathic pain in rats. The lack of efficacy of spinal morphine in the SNL model in rats has been attributed to a reduction of opioid receptors in the spinal cord (Porreca et al., 1998) and upregulation of an anti-opioid peptide, CCK in the dorsal root ganglion (DRG) neurons (Nichols et al., 1995). Dynorphin, however, is pronociceptive in models of chronic pain (Lai et al., 2001; Vanderah et al., 2001). Intrathecal administration of anti-dynorphin antiserum reverses thermal and tactile sensory hypersensitivity in injured animals, while not affecting sensory thresholds of non-injured animals (Malan et al., 2000). Zhao et al., 2004 found intrathecally administered morphine to be effective in reversing tactile hypersensitivity in SNL rats, suggesting that deficits of experimental
methodology may influence the results of this type of study. A comprehensive review of the literature indicates that the effectiveness of morphine in animal models of neuropathic pain depends on the route and dose of morphine, the animal model employed, and the assessments measures utilized. The consensus now is that opioids are effective in neuropathic pain, but relatively high doses may be necessary and combining them with other pain-relieving drugs may make them more effective in humans (Dickenson and Suzuki, 2005; Stillman, 2006). Furthermore, clinical trial studies show they are effective (Watson and Babul, 1998; Huse et al., 2001; Raja et al., 2002; Gimbel et al., 2003).

**Endogenous Opioids**

Endogenous opioids act on the same opioid receptors as exogenous opioids to produce antinociception. The three major classes of endogenous opioids include β-endorphin, the met- and leu-enkephalins, and the dynorphins. These opioid peptides are derived from different precursor molecules: β-endorphin from proopiomelanocortin (POMC), met- and leu-enkephalins from Pro-enkephalin A (ProENK A), and dynorphin from Prodynorphin (Basbaum and Fields, 1984). The enkephalins and dynorphins are distributed throughout the brain, but POMC-neurons are mainly in the hypothalamus and β-endorphin is then released into the circulation or projects to other areas of the brain through nerve fibers. There is a specific pathway of β-endorphin neurons originating from the hypothalamus and projecting to the PAG that is important for analgesia (Millan et al, 1980). The endogenous opioids act at central (e.g. PAG and RVM), spinal, and peripheral opioid receptors. The enkephalins and β-endorphin appear to play a more
significant role in pain reduction (especially centrally) than does dynorphin (Basbaum and Fields, 1984).

**Endogenous Opioids and Pain**

Endogenous opioids are thought to relieve pain through mechanisms similar to exogenous opioids. Stimulation of the PAG in a rat model of neuropathic pain was found to reduce signs of neuropathic pain (Lee et al., 2000b). The antiallodynic effects of PAG stimulation were completely reversed after naloxone administration, indicating that increased release of endogenous opioids were responsible for this effect. Activation of the endogenous opioid pathway and subsequent pain reduction has also been shown with drugs like cannabinoids that stimulate peripheral opioid release (Ibrahim et al., 2005), electroacupuncture (Han, 2004; Zhang et al, 2005), and exercise which will be discussed in detail later. Another study found that continuous release of endogenous opioid peptides produced antinociceptive effects. The hindpaws of SNL rats were inoculated with HSV-derived vectors bearing human proenkephalin A (ProENK A), which led to over-expression of enkephalin in lumbar DRG (Hao et al., 2003). They demonstrated a reversal of sensory hypersensitivity in these animals that returned with naloxone administration. A similar reversal was seen with morphine treatment.

There is some evidence to suggest that the effects of endogenous and exogenous opioids are mediated through different mechanisms. The ProENK A treated animals had increased enkephalin in lumbar DRG and a reversal of hypersensitivity that remained for 5 weeks, whereas the effects of repeated morphine administration were diminished within
a week. Also, the recombinant HSV vector treated SNL group had reduced c-fos-like immunoreactive neurons in the spinal cord that was not seen in the morphine treated SNL group (Hao et al., 2003). Smith et al., 1992 also suggest the mechanisms underlying analgesia are different between β-endorphin and morphine. β-endorphin and morphine were microinjected into the PAG region of rats. They found that pentobarbital anesthesia caused a 10-fold increase in the antinociceptive effects of β-endorphin, while the antinociceptive response of morphine was markedly reduced (Smith et al., 1992). Activation of G-proteins by endogenous and exogenous µ-opioid agonists has also been found to differ (Saidak et al., 2006). The µ-opioid receptor (MOR) couples to produce analgesia by decreasing cAMP, inhibiting calcium channel conductance, and stimulating potassium channel conductance. Activation of Gαi/o subunits was higher with endogenous opioids compared to exogenous. Morphine and fentanyl produced only 66% to 88% maximal activation, while the enkephalins produced 96% to 103% maximal activation (Saidak et al., 2006). While endogenous and exogenous opioids both produce analgesia, their mechanism of action may not be the same.

**Exercise**

Exercise has been found to be effective in preventing or treating many diseases. It is well established that exercise helps to prevent and treat obesity (Ross et al., 2000), Type 2 diabetes (Henriksen, 2002), heart disease and hypertension (Stampfer, 2000), and osteoarthritis (Sisto and Malanga, 2006). Exercise is also often recommended by physicians as a way to manage chronic pain, although there is a relative paucity of
evidence supporting this recommendation and an inadequate understanding of how exercise may lead to pain relief. Many physicians who recommend exercise do so in the belief that strengthening supporting muscles will improve pain symptoms.

The form of exercise used plays a critical role in its effectiveness for different conditions. Exercise is categorized into aerobic exercises including walking, running, or biking that result in increased cardiovascular endurance and anaerobic exercises, such as weight lifting and isometric training that increase muscle strength. Stretching is a third category of exercise that improves the range of motion of muscles and joints. A combination of all three forms of exercise is best for overall balanced health (USDHHS, 1996). Increased bone mass and lean muscle mass is best obtained by anaerobic exercises (Sisto and Malanga, 2006). Aerobic exercise is best for managing diabetes and obesity, improving cardiovascular health, and perhaps for reducing pain (Ross et al., 2000, Henriksen, 2002, Stampfer, 2000).

**Animal Models of Exercise**

Different forms of exercise have been used in animal studies. In the past, the predominant form of exercise used to study exercise-induced analgesia was swimming. Animals were forced to swim in glass tanks filled with cold or warm water for several minutes. Running exercise protocols with voluntary running wheels or motorized rodent treadmills have now replaced swimming in exercise-induced analgesia studies because they are less stressful and more natural forms of exercise for rodents. Voluntary running wheels consist of a solid-surface wheel that is usually attached to one side of the cage.
Animals have free access to the wheel and complete control over when, how much, and how intensely they run. An odometer is attached to the wheel to count the number of revolutions. Treadmill training is performed using a motorized treadmill with individual covered lanes for the rodents. Speed and incline are controlled by the operator. A wire surface at the back end of each lane provides a mild electrical shock to the animal to force them to continue to exercise. An exercise pre-test is normally performed to acclimate the animals to the treadmill conditions and remove animals that won’t exercise.

**Exercise and Stress**

The relationship between exercise and stress is complicated. Exercise is a physical stressor that challenges homeostasis. Acute forms of exercise that last for only a short duration cause an increased release of stress hormones like cortisol and catecholamines due to increased activation of the hypothalamo-pituitary-adrenal axis (Mastorakos and Pavlatou, 2005). This also leads to a stress-induced analgesic response, where there is an increased release of endogenous opioids and possibly activation of other non-opioid analgesic systems (e.g. endocannabanoids). A review of early human and animal studies found acute forms of aerobic exercise produced analgesia primarily through activation of the endogenous opioid system (Koltyn K, 2000). Stress-induced analgesia (SIA) has been extensively studied using the electric foot shock technique, and endogenous opioids and cannabinoids mediate the effects depending on the stimulus protocol (continuous vs. intermittent) (Rossier et al., 1977; Hohmann et al., 2005).
Chronic stress is known to produce many negative health consequences. Research suggests that the immune system becomes compromised with chronic stress, and this can increase susceptibility to inflammatory diseases such as allergies, autoimmune conditions, and cardiovascular disease (Miller et al., 2002). It’s also been associated with causing depression, obesity, and negatively affecting brain health. In contrast, while acute exercise promotes stress, regular exercise training actually leads to a state of physical conditioning that is associated with a reduction in pituitary-adrenal activation and other adaptive changes. This reduction in stress may have a variety of health benefits.

**Regular Exercise and Pain**

There is recent evidence from animal studies suggesting that regular exercise can serve as an effective pain management intervention. Running exercise performed regularly for several weeks was found to increase pain thresholds in otherwise untreated rats (Shyu et al., 1982; Smith and Yancey, 2003; Smith and Lyle, 2006; Mathes and Kanarek, 2006). Naloxone, an opioid receptor antagonist reversed the effects of exercise in the majority of cases. While many studies have shown these effects in otherwise untreated rats, there are few studies showing opioid-mediated exercise effects on pain in an injured animal model. Bement and Sluka, 2005 found that low intensity exercise reversed hypersensitivity in a chronic muscle pain model. Animals that showed tactile hypersensitivity following injections of acidic saline into the gastrocnemius muscle were exercised on a treadmill at a low intensity for 6 consecutive days. The exercise group
showed complete reversal of hypersensitivity compared to the sedentary control, and the effects were reversed with injection of naloxone. Hutchinson et al., 2004 found that long-term exercise also reversed hypersensitivity in a spinal cord injury (SCI) animal model. SCI animals have superficial tissue and vertebral bone removed (T8 lamina of thoracic region in this model) and a force is applied to the spinal cord to induce the injury. SCI results in motor deficits and incapacitating neuropathic pain. These researchers studied the effects of three different forms of exercise (treadmill training, swimming, and standing) for 7 weeks duration on sensory hypersensitivity in SCI animals. They found a complete reversal of tactile hypersensitivity and attenuation of the response to noxious pinch after 5 weeks of treadmill training. The other exercise regimes were also beneficial, but didn’t produce as complete reversal as did treadmill training. These findings led to the hypothesis that exercise reduces pain sensitivity.

**Exercise and Endogenous Opioids**

Exercise-induced elevations in endogenous opioid levels in both humans and animals, support the hypothesis that exercise produces an opioid-mediated reduction in pain sensitivity. Animals exercised on both rodent treadmills and voluntary running wheels were found to have significantly higher plasma β-endorphin levels compared to sedentary controls (Aravich et al, 1993; Debruille et al.1999; Su et al., 2005). Other forms of moderate aerobic exercise were also found to elevate plasma β-endorphin in rats (Peijie et al., 2003). Further, pain thresholds appear to be affected by diurnal rhythms in rodents. Animals tolerate higher pain thresholds at night compared to daytime, which
they attributed to increases in circulating endogenous opioids during the active nighttime periods (Frederickson et al., 1977; Wright et al., 1981; Rasmussen et al., 2003). Humans also had elevated plasma β-endorphin levels following long-distance running (Janal et al., 1984; Angelopoulos, 2001).

Other studies have reported elevations of endogenous opioids in the brains of exercised animals. Voluntary wheel running for 9 days led to increased levels of hippocampal met-enkephalin expression (Persson et al., 2004). There was also a trend toward increased β-endorphin expression that may have become statistically significant after a longer duration of training. Elevated anterior pituitary β-endorphin content was found in animals that were food restricted and performed 5 times more voluntary wheel running than control exercise animals that were not food restricted (Aravich et al., 1993). A two-fold increase in endogenous β-endorphin was observed in cerebrospinal fluid (CSF) of rats that underwent voluntary wheel running for 5-6 weeks (Hoffman et al., 1990). It remained elevated for more than 48 hours after the discontinuation of exercise (Hoffman et al., 1990). Elevated β-endorphin levels were also found in the hypothalamus of animals subjected to high intensity swimming for 6 weeks (Peijie et al., 2003). In contrast, a few studies found no difference in brain opioid peptide levels in exercised rats (McLachlan et al., 1994; Blake et al., 1984). This may be due to the regions tested or to the duration of exercise not being long enough.
Exercised animals also demonstrate signs of physical dependence on opioids and opioid-mediated reward due to exercise, which is further evidence for the role of endogenous opioids. Exogenous opioids can produce withdrawal in humans and animals if suddenly discontinued. Signs of opioid withdrawal in animals include diarrhea, wet dog shakes, aggressive behavior, postural changes, etc. Smith and Yancey, 2003 found administering an opioid antagonist in exercised animals produced withdrawal. They injected naloxone at the end of 6 weeks of exercise training or sedentary conditions. The exercise trained animals displayed significantly higher levels of precipitated withdrawal compared to the sedentary animals. Hoffman et al., 1987 found that when exercise was abruptly discontinued after several weeks, the animals showed increased aggression and other withdrawal-like behaviors in an open-field test. Sisti and Lewis, 2001 found that naloxone injected before wheel running significantly suppressed the amount of voluntary running performed by the animals, while morphine produced increased wheel running that was dependent on dose and time after injection. Additionally, exercise has been found to have an effect on oral consumption of morphine. Non-exercised rats consumed significantly more morphine than exercised rats (McLachlan et al., 1994), which suggests that exercise animals have higher levels of endogenous opioids so their need for exogenous opioids may be less.
**Exercise and Opioid Tolerance**

Chronically exercised animals have also been found to develop tolerance to exogenous administration of opioids. With opioid tolerance, more drug is needed to produce the same amount of pain relief. Opioid tolerance is thought to be caused by desensitization of opioid receptor signaling and loss of functional receptors in the cell surface. Researchers administering μ opioid receptor agonists found them to be less effective in exercised rats compared to sedentary (Smith and Yancey, 2003; Smith and Lyle, 2006; Mathes and Kanarek, 2006). They attribute this to increased circulating endogenous opioids in exercise animals, which results in a decreased sensitivity to morphine and other μ opioid receptor agonists (i.e. tolerance). Smith and Lyle, 2006 found the effects were reversible. When exercise was discontinued and the animals were subjected to sedentary conditions for several weeks, they no longer showed the same tolerance to opioid receptor agonists.

**Motorized Treadmill Exercise and Endogenous Opioids**

The literature suggests that there are certain parameters of exercise that affect the endogenous opioid response. It appears that intensity and duration of exercise are important factors in exercise-induced release of endogenous opioids. These can be more difficult to control with voluntary running wheels. Goldfarb and Jamurtas, 1997 reviewed past literature regarding the response of β-endorphin to exercise, and they concluded that sufficient intensity and duration of exercise are required to elicit the release of endogenous β-endorphin and produce antinociception. Similarly, Peijie et al.,
2003 found that high-intensity exercise produced the most significant increases in plasma and hypothalamic \( \beta \)-endorphin compared to lower-intensity exercise.

**Voluntary Wheel Running and Endogenous Opioids**

Intensity and duration of exercise are harder to control using voluntary wheel running and studies comparing the effects of levels of voluntary running activity on the endogenous opioid system have produced mixed results. Increased intensity of running on voluntary wheels can be difficult to achieve. Increasing the amount of time on the wheels is probably the simplest way to increase the amount of total running. Studies that gave animals access to the running wheels for 24 hours a day for several weeks as opposed to 12 hours/day for only a couple of days showed more significant effects on pain and endogenous opioid release (Shyu et al., 1982; Hoffman et al., 1990). Aravich et al., 1993 found that animals that were food-restricted performed more exercise and had significantly elevated plasma and anterior pituitary \( \beta \)-endorphin content compared to non-food-restricted exercise rats. The food-restricted animals exercised nearly five times more, which may have contributed to the endogenous opioid increases. A food-restricted non-exercised control also showed elevations in \( \beta \)-endorphin, but not nearly as high as the exercised food restricted group.

The spontaneous hypersensitive rat (SHR) is a breed of animals that has elevated blood pressure for studying hypertension and stroke, but they are also used in exercise studies because they tend to run 3 times more than Sprague-Dawley rats on voluntary running wheels (Lambert and Jonsdottir, 1998; Tong et al., 2001). Early researchers also
reported decreased pain sensitivities due to endogenous opioid system activation in these animals compared to wild type rats (Zamir et al., 1980; Zamir et al., 1981). Most of the voluntary wheel running studies that demonstrated elevated endogenous opioid levels with chronic exercise was done with spontaneously hypertensive rats (SHR) (Hoffman et al., 1987; Hoffman et al., 1990; Persson et al., 2004). These studies reported higher amounts of exercise performed in SHR rats, as well as greater elevations in endogenous opioids and, in some cases, more pain reduction compared to studies using different breeds of rats, such as Sprague Dawley or Long Evans rats (Aravich et al., 1993; Mathes and Kanarek, 2006). Shyu et al., 1982 used both SHR and normotensive Wistar-Kyoto rats to make direct comparisons, and found no differences in running patterns or pain sensitivity between the groups.

Breeding rodents specifically for increased running activity is another approach to determine if higher voluntary running levels affect the endogenous opioid system more significantly. Mice specifically bred for high voluntary wheel running did not demonstrate higher pain thresholds compared to the wild type running group even though they performed nearly 3 times the amount of exercise (Li et al., 2004). Both groups showed increased pain sensitivity in response to naloxone, which suggests an exercise-induced endogenous opioid effect. The fact that pain sensitivity wasn’t further reduced in the higher performing exercise group could be because the lower amount of running already reached the threshold for reducing pain.
Neurotrophic Factors

Neurotrophic factors are extracellular signaling molecules that are responsible for neuronal survival and differentiation during embryonic development and maintenance of specific functions during adulthood (Ibanez, 1998). The neurotrophins include nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and neurotrophin-4/5 (NT4/5). They bind to specific tyrosine protein kinase (trk) receptors; NGF primarily binds to trk A, BDNF and NT 4/5 to trk B, and NT3 to trk C (Figure 1.5). Neurotrophic factors are released by neurons and bind to their specific receptors on nearby neurons. A signal is then produced and transported to the nucleus of the receiving neuron where it results in the increased production of proteins associated with neuronal survival and function. Glial cell line-derived neurotrophic factor (GDNF) is another neurotrophic factor that promotes the survival of many types of neurons. It is most commonly known for its ability to support the survival of dopaminergic and motor neurons. GDNF is a part of the GDNF family of ligands (GFL), which consists of four neurotrophic factors: GDNF, neurturin (NRTN), artemin (ARTN) and persephin (PSPN). GDNF signaling occurs through a receptor complex consisting of a signaling receptor, RET (receptor tyrosine kinase) and a cell surface-bound co-receptor, GFRα (GDNF family receptor α) (Figure 1.5). Upon ligand activation, this complex promotes cell survival, neurite outgrowth, cell differentiation, cell migration and other processes.
Neurotrophic Factors and Disease

The interest in neurotrophic factors is due to their potential for treating different diseases. They demonstrate both trophic (survival promoting) and tropic (directing axon growth) properties that have been shown to be beneficial for treating neurodegenerative diseases like Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, and peripheral neuropathies. BDNF may be a therapeutic target for Alzheimer’s disease due to its ability to alter synaptic plasticity and cognition. Supportive evidence for the potential for BDNF comes from an analysis of the current drugs used to treat Alzheimer’s disease, and the fact that they appear to produce their effects by modulating BDNF (Fumagalli et al., 2006). GDNF has shown promise in treating Parkinson’s disease and peripheral neuropathic pain. The potential in treating Parkinson’s disease stems from its ability to promote survival of dopaminergic neurons. While animal studies have shown convincing evidence that GDNF can reverse the disease, clinical studies have been inconsistent in their findings (Barker, 2006). The effectiveness of GDNF in humans with Parkinson’s disease continues to be studied. Both BDNF and GDNF show promise for treating amyotrophic lateral sclerosis (ALS), a condition characterized by degeneration and death of motor neurons.

Neurotrophic Factors and Pain

Neurotrophic factors have also been implicated in pain. Several studies have shown administering BDNF through intracerebroventricular (i.c.v.) or midbrain infusions in otherwise untreated or formalin-treated male Sprague-Dawley rats produces analgesia.
(Siuciak et al., 1994; Siuciak et al., 1995; Frank et al., 1997; Cirulli et al., 2000). Suiciak et al., 1994 also showed analgesic effects of NT-3 midbrain infusions, but they were slight compared to the effects of BDNF. The analgesic response of i.c.v. injections of BDNF in naïve animals was found to be reversed with naloxone (Suiciak et al., 1995). BDNF infusion increased expression of proenkephalin, enkephalin, and β-endorphin in the brain (Nawa et al., 1994, Sauer et al., 1994; Siuciak et al., 1995). Siuciak et al., 1995 administered BDNF i.c.v. and found that it produced increased β-endorphin expression in the PAG and spinal dorsal horn of formalin treated rats, while reversing thermal hypersensitivity measured as tail-flick and paw-withdrawal responses. Their data suggest that BDNF modulates β-endorphinergic fibers in the PAG to produce analgesia. Also reported were increases in serotonergic activity in the brain and spinal cord of animals given i.c.v. and midbrain injections of BDNF (Suiciak et al., 1996). Both serotonergic and opioidergic mechanisms appear to play a role in the analgesic effects of supraspinally administered BDNF. Chronic infusions of BDNF produced consistent analgesia without the development of tolerance after 12 days (Frank et al., 1997). A decrease in trk B receptor proteins was also reported after 6 days of BDNF treatment, but not after 4 hours or 1 day of BDNF administration (Frank et al., 1997). Thus, the changes in trk B receptor levels don’t appear to be mediating the immediate effects of analgesia. Chronic pain has been found to reduce BDNF expression (Duric and McCarson, 2006). Chronic inflammatory pain rats received repeated injections of complete Freund’s adjuvant (CFA) over 21 days. They had continuous thermal and tactile hypersensitivity, and a significant decrease in BDNF mRNA levels in hippocampal CA1-CA3 subregions.
Increased spinal BDNF expression was found to reverse signs of neuropathic pain (Eaton et al., 2002). A recombinant adeno-associated virus (rAAV)-BDNF vector that causes overexpression of BDNF was injected into the spinal cord of animals that had undergone chronic constrictive injury (CCI) surgery. Increased expression of spinal BDNF was confirmed. A complete reversal of allodynia and hyperalgesia was also noted (Eaton et al., 2002). Reversal of peripheral neuropathic pain was also seen after lumbar transplant of neurons genetically modified to secrete BDNF (Cejas et al, 2000).

GDNF and artemin, a member of the GDNF family, reversed the behavioral signs of neuropathic pain (Gardell et al., 2003; Nagano et al., 2003; Wang et al., 2003). Spinal nerve-ligated (SNL) animals treated with GNDF and artemin showed complete reversal of thermal and tactile hypersensitivity. These effects were attributed to normalization of the morphological and neurochemical changes in this injured state (Gardell et al, 2003). Pre-treatment with GDNF prevented the behavioral and neurochemical changes associated with neuropathic pain in SNL animals (Wang et al., 2003).

While research has demonstrated pain-inhibiting effects of BDNF and GNDF, the neurotrophic factors have also been suggested to enhance pain. Nerve growth factor (NGF) appears to be particularly important in facilitating pain. NGF is needed for the development of nociceptors derived from sympathetic and small fiber sensory neurons (Apfel, 2000). It also stimulates pain-transmitting neuropeptide expression and is present in increased amounts in inflamed tissue (Apfel, 2000). Intraperitoneal (i.p.), intramuscular (i.m.), and intraplantar (i.pl.) injections of NGF have been repeatedly shown to produce inflammatory hyperalgesia (Lewin et al., 1993; Zhao et al., 2006).
Anti-NGF treatments were found to reverse hyperalgesia through a decreased release of substance P expressing neurons in the dorsal root ganglion (DRG) (Otten, 1984). BDNF has also been implicated in inflammatory pain. BDNF is found in primary sensory neurons in the DRG, and upregulation during inflammatory pain has been shown to produce central sensitization in the spinal dorsal horn (Mannion et al., 1999). Guo et al., 2006 also report that supraspinal BDNF signaling in an inflammatory mouse model may facilitate descending pain. They demonstrated that the CFA (complete Freund’s adjuvant) model of inflammation led to increased BDNF in the PAG that projected to the RVM. Microinjections of anti-BDNF antibody and BDNF into the RVM reversed and promoted inflammatory hyperalgesia, respectively. They found higher doses of BDNF injected into the RVM, above the physiological levels produced following inflammation, led to analgesia. They attributed the high dose BDNF affects to downregulation of trk B receptor proteins, but previous BDNF-induced analgesia studies have shown delayed trk B receptor downregulation that doesn’t explain the immediate analgesia (Frank et al., 1997). Miki et al, 2000 also found dose related biphasic effects of BDNF administered systemically on mechanical sensitivity. In contrast, supraspinal administration of high doses of anti-BDNF (i.c.v.) was found to have no effect on pain sensitivity in otherwise untreated animals (Cirulli et al., 2000). Further, while supraspinal BDNF signaling has been suggested to facilitate inflammatory pain, a similar connection has not been established with neuropathic pain. A study on BDNF knock-out mice found a significant attenuation of inflammatory pain in the BDNF knock-out groups, as well as a reversal of some of the biochemical events associated with inflammation (i.e. NR1 phosphorylation
(Zhao et al., 2006). Tactile hypersensitivity in SNL animals was unchanged between in the BDNF knockout group compared to wild-type mice.

**Neurotrophic Factors and Depression**

BDNF has been shown to be modulated with antidepressants and to have antidepressant effects when administered on its own. An upregulation of hippocampal BDNF was found in animals administered antidepressants for 21 days compared to control animals (Nibuya et al., 1995). There are several different animal models used to study depression. BDNF was found to reverse behavioral signs of depression in animals subjected to forced swimming and learned helplessness after exposure to inescapable shock (Siuciak et al., 1997; Hoshaw et al., 2005). The forced swimming test protocol consists of plunging the animals into a glass container filled with water and measuring the duration of immobility. Immobility is believed to be a correlate of depression. In the learned helplessness paradigm, rats are pre-exposed to inescapable shock and then monitored for escape behavior under escapable shock conditions. Animals show severe impairments and escape behavior, which equates to depression. Rats receiving BDNF administered i.c.v. or in the midbrain showed reduced immobility and increased swimming in the rat modified forced swimming test and increased number of escapes and decreased escape latency time in the learned helplessness test (Siuciak et al., 1997; Hoshaw et al., 2005). As stated earlier, antidepressants are also frequently used to treat chronic pain conditions. It has been suggested that BDNF may be responsible for the antinociceptive effects of antidepressants (Tsai et al., 2005).
Neurotrophic Factors and Exercise

Animal studies have found elevated neurotrophic factors in specific areas of the brain and spinal cord after chronic exercise training. The increased expression of neurotrophic factors with exercise has been found to enhance learning and prevent the progression and possibly reverse the signs of Alzheimer’s and Parkinson’s disease (Van Praag et al 1999, Smith et al., 2003; Tillerson et al., 2003; Adlard et al., 2005). Exercise has and continues to be studied for its benefits to brain health and function. Van Praag et al., 1999 found exercise increased the generation of new neurons in the hippocampus and produced improved performance in the water maze test of learning and memory (Van Praag et al., 1999). Elevated BDNF levels in the hippocampus were found in the exercised animals and were proposed to be responsible for the exercise-induced increase in new neurons (Farmer et al., 2004). Adlard et al., 2005 found exercise slowed the progression of Alzheimer’s disease in a transgenic mouse model of Alzheimer’s disease, and they showed that five months of exercise led to a significant decrease in amyloid (beta) plaques in the frontal cortex and hippocampus (Adlard et al., 2005). Alzheimer’s disease improvements appear to be mediated by normalizing BDNF levels in the brain. GDNF was found to be upregulated in the brains of exercising animals as behavioral movements normalized in an animal model of Parkinson’s disease (Tillerson et al., 2003; Smith and Zigmond, 2003). Ding et al., 2004 found GDNF reversed the damage to dopamine neurons produced by 6-hydroxydopamine (6-OHDA), which causes oxidative stress to dopamine neurons.
There is convincing published evidence regarding endogenous opioid-induced reversal of pain with exercise in otherwise untreated or in injured animals. Research also suggests that the neurotrophic factor BDNF may also play a role in the anti-nociceptive effects of exercise. Hutchinson et al., 2004 found exercise reversed neuropathic pain in an animal model of spinal cord injury. They attributed this hypersensitivity reversal to BDNF upregulation. Injured animals had a down-regulation of spinal levels of BDNF compared to control animals, and exercise normalized expression of BDNF in the spinal cord (Hutchinson et al., 2004) and increased expression of BDNF in the brain (Vaynman and Gomez-Pinilla, 2005). Increased brain levels of BDNF have been found to produce analgesia through opioidergic and serotonergic pathways, which may serve as a mechanism for exercise-induced pain reversal.

Exercise has also been shown in clinical studies and animal studies to be effective for reducing depression (Dunn et al., 2001; Brosse et al., 2002; Zheng et al., 2006). Zheng et al., 2006 studied exercise in the chronic unpredictable stress (CUS) model of depression. CUS consists of exposing animals to several relatively mild and unpredictable stressors (i.e. water deprivation, tail suspension, restraint, food deprivation, etc.). Voluntary running wheel exercise reversed the effects of CUS on mood and spacial performance (Zheng et al., 2006), which the researchers attributed to normalization of hippocampal BDNF levels that were significantly downregulated in these animals prior to exercise. These findings may show promise for exercise treatment in chronic pain because of its association with depression and antidepressant treatments.
Purpose of Dissertation

The purpose of this dissertation is to determine the effects of moderate aerobic exercise on neuropathic pain. Specifically, it tests the hypothesis that moderate aerobic exercise reverses neuropathic pain through increased expression of endogenous opioids. It also begins to test the hypothesis that exercise-induced increases in BDNF expression lead to increased endogenous opioid expression and decreased signs of neuropathic pain.
Figure 1.1 | The main ascending and descending spinal pathways.  

**a** | There are two primary ascending nociceptive pathways. These are the spinoparabrachial pathway (red), which originates from the superficial dorsal horn and feeds areas of the brain that are concerned with affect, and the spinothalamic pathway (blue), which probably distributes nociceptive information to areas of the cortex that are concerned with both discrimination and affect. Many more less prominent pathways could be added.  

**b** | The descending pathway highlighted originates from the amygdala and hypothalamus and terminates in the periaqueductal grey (PAG). Neurons project from here to the lower brainstem and control many of the antinociceptive and autonomic responses that follow noxious stimulation. (A, adrenergic nucleus; bc, brachium conjunctivum; cc, corpus callosum; Ce, central nucleus of the amygdala; Hip, hippocampus; ic, internal capsule; LC, locus coeruleus; PB, parabrachial area; Po, posterior group of thalamic nuclei; Py, pyramidal tract; RVM, rostroventral medulla; V, ventricle; VMH, ventral medial nucleus of the hypothalamus; VPL, ventral posteriolateral nucleus of the thalamus; VPM, ventral posteriomedial nucleus of the thalamus.) (Hunt and Mantyh, 2001)
Figure 1.2 Diagrammatic illustrations of surgical procedures for 3 different groups tested in the present study. The procedures for experimental groups 1,2 and 3 are shown in A, B, and C, respectively. Darkened area indicates the part of the nerve ligated. DRG, the dorsal root ganglion.
Experimental group 1 (ligations of the L5 and L6 spinal nerves)
Experimental group 2 (L5 spinal nerve ligation)
Experimental group 3 (partial sciatic nerve ligation)
(Kim and Chung, 92)
Figure 1.3
**Figure 1.3:** Supraspinal contributions to the maintenance of hyperalgesic inflammatory (a) and neuropathic (b) pain states. (a) Inflammatory pain. Increased nociceptive stimuli arising from tissue injury or inflammation elicit increased afferent input to the spinal dorsal horn, promoting enhanced activity of second-order neurons projecting in the spinothalamic tract (STT) and other ascending tracts with projections to the rostroventromedial medulla (RVM). Sustained nociceptive input enhances activity of cells mediating descending facilitation via the ventrolateral funiculus (VLF). This tonic descending facilitation enhanced further nociceptive inputs, thus increasing pain from the primary injury and also enhancing sensory inputs from adjacent regions (secondary hyperalgesia). Substances that might drive descending facilitation in the RVM include neurotensin (NT), cholecystokinin (CCK), excitatory amino acids (EAAs) and nitric oxide (NO). Manipulations that block enhanced pain include RVM microinjections of lidocaine or NT antagonists, spinal cord transection or hemisection, and lesions of the VLF. (b) Neuropathic pain. Enhanced neuronal activity, driven by ectopic discharge of injured or adjacent fibers following injury to peripheral nerves results in increased input to spinal dorsal horn (via Aβ fibers) and to the nucleus gracilis (NG) via the ascending dorsal column (DC). Such inputs to the NG are likely to be transmitted to other supraspinal sites, possibly to the thalamus via the medial lemniscus (ML). Inputs to supraspinal sites are likely to result ultimately in enhanced descending facilitation from the RVM. Such descending facilitation could be driven by CCK in the RVM and mediated by descending facilitatory cells that express mu-opioid receptors (MOR). Descending facilitation further enhances nociceptive inputs and manifests behaviorally as enhanced pain. Injections of lidocaine, CCK antagonists or dermorphin–saporin conjugate into the RVM to lesion MOR expressing cells, or lesions of the ipsilateral dorsolateral funiculus (DLF), block manifestations of enhanced pain. Similarly, lesions of the DC block manifestations of neuropathic pain. (Porreca et al., 2002)
Figure 1.4: Opioids are effective as analgesics when given in minute doses. They excite neurones in the periaqueductal grey matter and nucleus reticularis paragigantocellularis, which project to the nucleus raphe magnus. Descending pathways from the midbrain exert a strong inhibitory effect on pain transmission in the dorsal horn (mediated by 5-HT, enkephalins and noradrenaline). Opioids also inhibit pain transmission by acting directly on the dorsal horn, and by inhibiting excitation of peripheral nociceptive afferent neurones. (Rang, 2001)
Figure 1.5
Figure 1.5: Specificity and cross-talk in families of neurotrophic factors and receptors. Ligand receptor interactions in the neurotrophin (top), glial cell line-derived neurotrophic factor (GDNF) (center) and neurokine (bottom) families. In the neurotrophins NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4), specificity is determined by ligand binding to different members of the Trk receptor tyrosine kinase family. Cross-talk is also apparent in these interactions as more than one neurotrophin may bind to the same Trk receptor, and more than one Trk may bind to the same neurotrophin. Although all members of the neurotrophin family interact with p75NTR, this receptor is also capable of detecting differences between the different neurotrophins (see text for details). In the GDNF ligand family, GDNF and neurturin (NTN) use the same signaling receptor subunit, RET. Differential binding to members of the GFRα receptor family has been proposed as a mechanism of specificity[14, 15]; however, substantial cross-talk also exists between GDNF ligands and GFRα receptors[8, 16]. GFRα-3 (Refs [9, 17, 18, 19, 20]) and GFRα-4 (Ref. [21]) are two recently discovered members of the GFRα receptor family. Although GFRα-3 does not bind GDNF directly, it can associate with GDNF in the presence of RET (Ref. [9]). This association, however, does not result in efficient RET tyrosine phosphorylation, suggesting the existence of cognate GFRα-3 ligand(s) distinct from GDNF. The receptor binding specificities of persephin (PSP), the third member of the GDNF ligand family, have not been investigated in detail, although it appears not to interact with GFRα-1 or GFRα-2 (Ref. [22]). In the neurokine family, ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) utilize the same signaling receptor subunits, that is, LIFR-β and gp130. However, CNTF, but not LIF, can only activate these receptors via prior binding to CNTFRα (Ref. [23]). Solid lines, major interactions; broken lines, minor or lower-affinity interactions. (Ibanez, 1998).
**Table 1.1: List of clinical conditions that have shown to be associated with neuropathic pain.**

**Clinical conditions associated with neuropathic pain**

- **Central nervous system**
  - Stroke—cortical and subcortical
  - Traumatic spinal cord injury
  - Demyelination
  - Syringomyelia and syringobulbia
  - Neoplastic and other space-occupying lesions
  - Trigeminal and glossopharyngeal neuralgia
  - Migraine*
  - Fibromyalgia*

- **Peripheral nervous system**
  - Nerve compression/entrapment neuropathy
  - Traumatic nerve injury
  - Ischemic neuropathy
  - Peripheral polyneuropathy (hereditary, metabolic, toxic, inflammatory, paraneoplastic, nutritional, vasculitic, infectious)
  - Plexopathy (neoplastic, autoimmune, radiation-induced, traumatic)
  - Nerve root compression
  - Post-amputation stump and phantom limb pain
  - Postherpetic neuralgia
  - Cancer-related neuropathy (infiltrative, chemotherapy-related, radiation-induced, post-surgical)

*Possible or theoretical neuropathic pain condition


(Stillman, 2006)
The methods, results, and conclusions of this study are presented in the paper appended to this dissertation. The following is a summary of the most important findings in this document. The goal of this project is to study the effects of moderate aerobic exercise on neuropathic pain in an animal model. Neuropathic pain is a debilitating condition caused by damage or disease to the nerves that is chronic in nature. Current therapies don't consistently treat this condition. Potential new treatment strategies continue to be researched in centers around the world. Physicians frequently recommend exercise for patients suffering from chronic pain. Many books and articles also allude to the potential for exercise to result in an improvement of symptoms; however, there is little objective evidence for utility of exercise in treating patients with pain. In addition, there is little understanding of the mechanisms by which exercise may improve neuropathic pain symptoms. Finally, there is little research on the effects of exercise training on chronic neuropathic pain in an animal model, where the effects of patient expectations would not be an issue and where mechanisms of pain reduction can more accurately be studied. The hypothesis of this study is that moderate aerobic exercise will reverse neuropathic pain in an animal model.

The spinal nerve ligation (SNL) model of neuropathic pain was employed (Kim and Chung, 1992). Male Sprague-Dawley rats underwent either a SNL or sham surgery. Surgery was performed under general anesthesia with isoflurane, an inhaled anesthetic. An incision was made lateral to the lumbar spinal cord. In the SNL surgery, the L5 and
L6 spinal nerves were tightly tied with a silk suture between the spinal cord and entry into the sciatic nerve. For the sham surgery, the nerves were exposed but are not tied.

SNL rats were subjected to a 5-week period of light to moderate endurance exercise training. A 10-lane motorized treadmill housed in our laboratory at the University of Arizona, College of Medicine was used. Prior to the start of the experiment, an exercise pre-test was performed. Rats exercised two times a week for 10 min at 18 m/min at 0% grade (a total of four initial exercise bouts in this screening phase). Animals that were unable to complete these very moderate exercise bouts were removed from the study. By the end of this screening period, rats were assigned to the sedentary group or the exercise-trained group and then underwent SNL surgery. The exercise-trained animals performed a running protocol 5 days/week for 5 weeks on this apparatus. During the first two weeks of training, the duration and intensity of running was increased to 30 min/day at 8% grade, running at a speed of 14-16 m/min. This represents a moderate intensity of exercise for a SNL rat.

Measurement of hypersensitivity was determined using tactile withdrawal threshold (Chaplan et al., 1994) and thermal withdrawal latency (Hargreaves et al., 1988). Tactile withdrawal threshold measurements were performed by placing rats in elevated observation chambers (approximately 4" x 6" x 10") with wire-mesh floors. Graded pressure was applied to a localized area on the plantar surface of the hind paw by using Von Frey filaments (monofilaments which are calibrated to bend at known pressures: Neuroscience Methods Vol. 53 (1994) pp. 55-63). Increasing stimuli were administered until the animal withdrew the paw, which indicated a positive response. This was
repeated until three changes in behavior were observed. A maximum cut off for rats of 15 g was used. Thermal withdrawal latency measurements were assessed by placing rats in chambers on top of a glass surface with a temperature maintained at 30°C. A light heat stimulus was applied to the plantar surface of the hind paw. When the paw was withdrawn from the surface, the light automatically shuts off and the time the rat was able to withstand the heat was recorded. A maximum cut off 40 seconds for rats was used to prevent damage to the tissue.

Naloxone, naloxone methiodide, and BDNF antagonists were administered to study the mechanism(s) of exercise-induced reversal of neuropathic pain. At the end of the 5 week exercise and sedentary protocol, the opioid antagonists naloxone and naloxone methiodide (a charged form of naloxone that only acts peripherally) were injected systemically and in site-specific locations. Naloxone was dissolved in saline and both vehicle (saline) and naloxone were administered s.c. in the neck (1 mg/kg) in a volume of 0.3 ml or s.c. in the plantar surface of the hindpaw (1 to 10 µg) in a volume of 50 ul to rats 20 minutes before tactile testing. Naloxone methiodide was dissolved in saline and both vehicle (saline) and naloxone methiodide were administered in a volume of 0.3 ml s.c. in the neck or 5 µl i.t. or 2.5 µl i.c.v. 20 minutes before tactile testing. A brain derived neurotrophic factor (BDNF) sequestering agent (human recombinant TrkB-Fc) was administered during the third and fourth week of exercise training. TrkB-Fc contains the BDNF-binding domain of the TrkB receptor to sequester all endogenous BDNF. The BDNF antagonist was dissolved in PBS and both vehicle (PBS) and BDNF antagonist
were administered i.c.v. in a volume of 2.5 µl with increasing doses ranging from 8µg to 25µg. Tactile tasting was performed 5 days post injection.

Immunohistochemistry and immunoassay methods were utilized to localize and quantify the endogenous chemicals responsible for the exercise effects. Following exercise and sedentary conditions, SNL animals were anesthetized with ketamine/xylazine administered i.p. and brain tissue was collected for endogenous chemical analysis. For immunohistochemistry, animals were perfused with 4% paraformaldehyde. Brain tissue was collected, post-fixed in 10% buffered formalin, and then transferred to 20% sucrose in 0.1 M PBS. Frozen sections of brain tissue were prepared, stained with met-enkephalin and β-endorphin antibodies, and then analyzed under the microscope. Images were taken of the periaqueductal grey (PAG) and rostral ventromedial medulla (RVM), and densitometry of the images measured. For immunoassays, specific sections of un-perfused brain tissue were collected and analyzed for β-endorphin and BDNF. The PAG and RVM regions were removed, sonicated with 1.0 ml of cold 0.1 N HCL, centrifuged at 12,000 X g for 15 min at 5°C in a refrigerated microcentrifuge, and the supernatant collected and measured for β-endorphin content. The amygdala, hippocampus, hypothalamus, and thalamus were collected, sonicated with a protease inhibitor mixture, centrifuged at 12,000 X g for 15 min at 5°C in a refrigerated microcentrifuge, and the supernatant collected and measured for BDNF. An ELISA technique was used for quantification.

The groups were compared using anova, followed by pairwise comparisons using the t-test with a Bonferroni's correction. Significance was defined as p < 0.05.
The results demonstrated that moderate aerobic exercise effectively reverses neuropathic pain in SNL rats through increased endogenous opioids in the CNS. The behavioral signs of neuropathic pain in the SNL animal model were reversed after 5 weeks of exercise training. Naloxone injected systemically reversed the exercise-induced effects, demonstrating that endogenous opioids were responsible. Site specific injections of naloxone and naloxone methiodide indicated the effects were being mediated by the brain, since a positive effect was seen with i.c.v., but not local or spinal injections. Increased endogenous met-enkephalin and β-endorphin in the RVM and PAG of exercise-trained compared to sedentary animals suggests they act on central opioid pathways to reduce pain.

Brain derived neurotrophic factor (BDNF) also appears to play a role in exercise-induced neuropathic pain reversal, possibly by increasing the expression of the endogenous opioids in the PAG and RVM. Exercise-trained animals demonstrated higher brain levels of BDNF compared to sedentary, specifically in the ventromedial hypothalamus, hippocampus, and outer rim of the PAG. Injections of a BDNF sequestering agent reversed β-endorphin increases in the PAG of exercise-trained animals. Only a high dose of the BDNF sequestering agent diminished the effects of exercise training on tactile hypersensitivity.

The conclusion of this dissertation is that moderate aerobic exercise provides pain relief through an endogenous opioid mediated mechanism in the central nervous system. BDNF may play a role in the beneficial effects of exercise by increasing endogenous opioids, but this needs to be verified further. Patients with neuropathic pain may be able
to manage this chronic pain condition more effectively with the addition of daily moderate aerobic exercise.
REFERENCES


Brain-Derived Neurotrophic Factor Regulates Acute and Inflammatory but not Neuropathic Pain." Mol Cell Neurosci 31: 539-548.


APPENDIX A

REVERSAL OF NEUROPATHIC PAIN WITH MODERATE AEROBIC EXERCISE IS MEDIATED BY ENDOGENOUS OPIOIDS IN THE CNS

INTRODUCTION

Neuropathic pain is a chronic condition associated with an injury or disease that leads to malfunction of the nervous system. It affects 1-2% of the population (Merskey and Bogduk, 1994). While pain normally serves as a protective function to prevent further injury, neuropathic pain persists even after the injury has healed. The damage to the nervous system can occur peripherally or centrally. There are many different clinical conditions causing central or peripheral neuropathic pain. Some examples include diabetes, cancer, nerve compression, stroke, and trauma (Stillman, 2006). Neuropathic pain has considerable societal and financial implications. Those who suffer from it are unable to function normally in their daily lives, and it costs the economy billions of dollars in health care costs and lost productivity. Current neuropathic pain treatments include anticonvulsants like gabapentin, tricyclic antidepressants like amitriptyline, and opioids like morphine, but the effectiveness of these treatments is limited and the side effects sometimes outweigh any benefit of the medication. For this reason, neuropathic pain treatment strategies continue to be studied worldwide.

Exercise is often prescribed as a treatment or adjunctive therapy for neuropathic pain, due largely to clinical observations that it lessons pain. Many books and articles
suggest that exercise may be effective for chronic pain management, but there are few scientific studies of its efficacy. Extensive research has shown that short-term exercise increases the production of endogenous pain relieving chemicals, leading to transient analgesia in both humans and animals (Koltyn K, 2000). More recent animal studies have shown regular exercise produces long lasting analgesia in rats (Shyu et al., 1982; Smith and Yancey, 2003; Smith and Lyle, 2006; Mathes and Kanarek, 2006). There have even been some recent studies in injured animals to suggest that exercise can diminish measures of chronic pain (Hutchinson et al., 2004; Hoeger et al., 2005). However, there is still only limited research on exercise as a long term treatment for pain in animal models. In the present study, we hypothesized that moderate aerobic exercise would reverse neuropathic pain in a L5-L6 spinal nerve ligation (SNL) rodent model. We investigated the effect of aerobic exercise, consisting of treadmill training, on thermal and tactile hypersensitivities of spinal nerve-ligated rats.
METHODS

Animals

Approval was obtained from the University of Arizona Animal Care and Use Committee (IACUC). Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were 250-380 g throughout testing. They were allowed water and food ad libitum. They were maintained in a climate-controlled room on a reverse cycle - 12-h dark/light. With the reverse cycle conditions, the room was dark during the day (lights went out at 10am) and light at night (lights went on at 10pm). All animal procedures conformed to the guidelines for the Care and Use of Laboratory animals by the International Association for the Study of Pain (IASP) and the National Institutes of Health (NIH). Animals were handled twice daily in a stress-free environment. They were allowed to equilibrate to the surroundings for 7 days prior to starting procedures.

Spinal Nerve Ligation (SNL)

L5/L6 spinal nerve ligation (SNL) was performed as described by Kim and Chung, 1992 under isoflurane anesthetic. An incision was made lateral to the lumbar spine. The paraspinal muscles were separated from the vertebral processes located between L4 and S2. The left L6 transverse process was completely removed with a small ronguer, and the L4 to L6 spinal nerves were exposed. The left L5 and L6 spinal nerves were isolated and tightly ligated with 4-0 suture silk distal to the dorsal root ganglion (DRG). The incision was sutured closed, and animals were allowed to recover for 7 days prior to exercise. Sham-operated animals were prepared in an identical fashion except that
the spinal nerves were not ligated. All SNL rats were inspected for a deformity in the hindpaw on the injured side, where the foot was moderately everted with the toes held together. Animals demonstrating an inverted foot with markedly ventroflexed toes (sciatic nerve damage: Kim and Chung, 1992) or paralysis of hindpaw (damage to L4) were removed from the study.

**Exercise Training**

Prior to surgery, animals underwent an exercise pre-test on a rodent treadmill. The pre-test consisted of 18m/min for 10 minutes, 2 days a week for 2 weeks. The animals unable to complete the test were removed from the study.

Following surgery, animals were randomized to a sedentary or exercise trained group. Animals in the exercise-trained group ran in the late afternoon on a 10-lane motor-driven rodent treadmill at 8% grade. These animals ran 5 days/wk for 5 weeks. The training protocol consisted of gradually increasing the duration to 30 min/day at a speed of 14-16m/min over 14 days. An electric grid at the back of the treadmill was activated with a weak current. Animals were only allowed contact with the grid for 2 s at a time and not more than 3 times per session before being removed from the treadmill apparatus to minimize stress. Animals that were sedentary remained in their cages. To check for the effects of handling and environment, plexi-glass boxes were placed in the treadmill lanes. Animals in the sedentary group were placed in these boxes with the treadmill running. This exposed them to the same handling and environmental conditions as the exercised animals (e.g. noise, space, vibration from treadmill). Animals displaying
signs of stress (e.g. porphoryn rings around eyes, red nosees, curled toes on injured paw) during the course of the study were removed.

To test the effects of exercise frequency, high or low frequency exercise was conducted five or three times a week, respectively. Similarly, higher or lower intensity exercise was performed at a speed of 16m/min or 10m/min, respectively. To test the effect of timing on exercise in relation to surgery, exercise was performed three weeks after surgery in the delayed exercise condition. Diurnal differences were determined by measuring tactile thresholds of animals during the light cycle (their inactive period) and the dark cycle (when they are most active).

*Naturally sedentary animals*

The animals that were unable to complete the exercise pre-test were designated naturally sedentary. Thermal and tactile thresholds were determined for naïve, sham, and SNL naturally sedentary animals and compared to animals that completed the exercise pre-test to test for inherent differences in pain thresholds.

*Intracerebroventricular Cannulation*

Cannulas were inserted into the intracerebroventricular (i.c.v.) space of rats prior to the start of exercise. Under isoflurane anesthetic, the animals’ heads were shaved and cleaned before placing them in a stereotaxic head holder. The skull was exposed and a 22-guage guide cannula was directed to the right lateral ventricle (1.3 mm caudal to bregma, 1.5 mm lateral to the sagital suture, 3.5 mm vental to the dural surface). The
cannula was secured in place by small stainless steel screws and dental cement. Animals were allowed a five day recovery before the start of the exercise. Drugs were administered though a 28-gauge injection cannula inserted through the guide cannula. They were slowly administered in a total volume of 2.5 ul. Backflow was prevented by having the injection cannula 1mm longer than the guide cannula so that it protruded into the ventricular space.

**Intrathecal Injections**

Intrathecal (i.t.) injections were performed under isoflurane anesthesia. The lumbar region was shaved and cleaned. A 0.5 inch 30-gauge needle connected to a 25ul Hamilton syringe was passed through the L5/L6 interspace. Correct subarachnoid positioning of the tip of the needle was verified by a flicking motion of the tail or hindpaws. Lidocaine was administered i.t. (10-20 ul of a 4% solution) to a group of test animals, and temporary paralysis of the hindpaws was obtained to confirm the effectiveness of the injection technique.

**Drug Administration**

The opioid receptor antagonists, naloxone and naloxone methiodide, were administered after completion of five weeks of exercise training. Naloxone was dissolved in saline and administered subcutaneously (s.c.) in the neck in a volume of 0.3 ml or s.c. in the plantar surface of the hindpaw in a volume of 50 ul 20 minutes before tactile testing. Naloxone methiodide, a peripherally restricted opioid antagonist, was
dissolved in saline and administered in a volume of 0.3 ml s.c. in the neck, 5 ul i.t. or 2.5 ul i.c.v. 20 minutes before tactile testing. The opioid receptor agonist morphine was administered i.t. 30 minutes before behavioral testing.

Assessment of tactile sensitivity

Tactile sensitivity was assessed by measuring thresholds for withdrawal of the paw to normally non-noxious tactile stimuli. Rats were allowed to acclimate for 30 min within plexiglass enclosures with wire mesh bottoms. Paw withdrawal thresholds were determined in response to probing of the hind paw with a series of calibrated von Frey filaments, in logarithmically spaced increments, applied perpendicularly to the plantar surface of the paw. A maximal cutoff of 15 g was used because larger filaments lifted the paw even if the animal did not actively withdraw. Data were analyzed by the up-and-down method of Dixon, as described by Chaplan et al., 1994. Tactile sensitivity was measured at baseline, 1 week after surgery, and at weekly increments during exercise training. Where applicable, measurements were taken 23 hours after exercise. Measurements were confirmed by blinded observation. Tactile testing was also measured before and after drug administration. N=6 for all groups.

Assessment of thermal sensitivity

Thermal sensitivity was assessed measuring the latency to withdrawal of the paw from a noxious heat source, as described by Hargreaves et al., 1988. Rats were allowed to acclimate within plexiglass enclosures on a clear glass plate maintained at 30°C. A
radiant heat source was focused onto the plantar surface of the hind paw. Paw withdrawal latency was determined by a motion detector that shut off the heat stimulus and the timer upon withdrawal of the paw. A maximal cutoff of 40 s was used to prevent tissue damage. Thermal sensitivity was measured at baseline, 1 week after surgery, and at weekly increments during exercise training. Where applicable, measurements were conducted 23 hours after exercise. Measurements were confirmed by blinded observation. Thermal testing was also performed before and after drug administration. N=6 for all groups.

Immunohistochemistry

Rats were anesthetized with ketamine HCl/xylazine. The heart was surgically exposed and transcardially perfused with 0.01 M sodium phosphate-buffered saline (PBS; pH 7.4) until exudate ran clear, then for approximately 15 min with 10% buffered formalin (Fisherbrand). All harvested tissues were post-fixed in 10% buffered formalin, and transferred to 20% sucrose in 0.1 M PBS. Frozen sections of brain tissue were prepared. Slide-mounted serial sections of the periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM) were processed for immunohistochemistry. Tissues were pre-blocked with 10% normal goat serum in PBS (1 h, room temperature), followed by incubation with 2% normal goat serum/0.3% Triton X-100/PBS/primary antibody for 24 h at 4 °C. The secondary antibody was added for 2 h at room temperature. Primary antisera were: rabbit anti-β-endorphin (1:5000; ImmunoStar Incorporated, Hudson, WI), and rabbit anti-met-enkephalin (1:10000; Chemicon International, Temecula, CA). Secondary antiserum was Alexa Fluor 568 goat anti-rabbit IgG (1:1000; Invitrogen,
Carisbad, CA). Following PBS washes, sections were dried and sealed with fluorescent mounting medium (Vector Laboratories).

*Image analysis and quantification*

Fluorescence images of PAG and RVM brain sections were acquired with a Nikon E800 fluorescence microscope outfitted with 4×/NA 0.2, 10×/NA 0.45, 20×/NA 0.75 and 40×/NA 0.75 objectives, a filter set for Cy3 (excitation 540–580 nm/emission 560–620 nm) and a Hamamatsu C5810 color CCD camera and its proprietary Image Processor software (Hamamatsu Photonic System, Bridgewater, NJ). Digital images were produced using Adobe Photoshop 6.0 (Adobe System Inc., San Jose, CA). Quantitative measurements of 10 to 15 images with identical dimensions were taken per treatment using the densitometry program Scion Image 4.0.3.2 (Scion Corp., Frederick, MD). The results are reported with the actual amounts of immunoreactivity, and the % change from the sedentary control was calculated.

*β-endorphin Immunoassay*

Rats were anesthetized with ether and killed by decapitation following completion of the 5 weeks of exercise or sedentary conditions. Brain tissues were collected and sections were cut that contained the entire PAG or RVM regions. A punch technique was employed to remove both regions. The sections were placed on a flat surface, and tubes the diameter of the PAG and the RVM were attached to a drill and used to punch through the tissue. Regions of the RVM that were ipsilateral and contralateral spinal nerve ligation were collected. Tissues were immediately frozen on dry ice and stored at −70 °C.
until use. The PAG and RVM tissues were sonicated for 5 s each with 1.0 ml of cold 0.1 N HCL and centrifuged at 12,000 X g for 15 min at 5°C in a refrigerated microcentrifuge. The supernatant was collected and placed on ice. β-endorphin content in the supernatant was measured immediately using a commercially available enzyme immunoassay (Peninsula Laboratories Belmont, CA).

**Dynorphin Immunoassay**

Under ether anesthetic, rats were decapitated and the lumbar spinal cord tissue was collected as described by Malan et al., 2000. Spinal cord tissue was sonicated with 1.0 ml of acetic acid and centrifuged at 12,000 X g for 15 min at 5°C in a refrigerated microcentrifuge. The supernatant was used for dynorphin measurement using a commercial enzyme immunoassay with an antibody specific for dynorphin A<sub>1-17</sub> (Penninsula Laboratories, Belmont, CA).

**Statistical analysis**

Groups were compared using ANOVA, followed by pairwise comparison using Student’s t test with Bonferroni’s correction. Significance was defined as P < 0.05.
RESULTS

*Exercise training decreased thermal and tactile hypersensitivity*

Spinal nerve-ligated (SNL) and sham groups were subjected to five weeks of moderate aerobic exercise or sedentary conditions. There were no differences between groups in pre-treatment withdrawal thresholds or latencies. One week after surgery, SNL groups displayed thermal and tactile hypersensitivity compared to pretreatment values, while sham or non-operated animals did not. Treadmill training ameliorated thermal and tactile hypersensitivity in spinal nerve-ligated animals within 4 weeks (Fig. 2.1). Thermal withdrawal latency increased from 11.5 ± 0.6 s before exercise to 17.6 ± 1.0 s after 5 weeks of exercise. Tactile withdrawal thresholds increased from 2.6 ± 0.2 g to 12.0 ± 1.0 g after 5 weeks of exercise. Sham and non-operated animals were also subjected to exercise and sedentary conditions, but no significant (P < 0.05) differences in sensitivity were found with or without exercise in either group (Fig. 2.2).

While handling did not affect tactile hypersensitivity, it did have an effect on compliance with exercise training. Increased daily handling, exposing the animals to the treadmill more gradually, and exercising them during the dark cycle when they are normally most active reduced the number of animals not completing the exercise pre-test from 30% to less than 10%. In addition, among animals that successfully completed the exercise pre-test, the percentage of animals able to complete the full 5 weeks of exercise increased from 60% to 80%.
Thermal and tactile hypersensitivity returned within 1 week of cessation of exercise

When daily measurements of the thermal and tactile sensitivities of SNL animals were taken after discontinuing exercise training, tactile hypersensitivity returned within one week (Fig. 2.3). A significant (P < 0.05) decrease in tactile hypersensitivity occurred after 4 days, from 13.4 ± 0.7 g at day 0 to 10.2 ± 1.8 g at day 4. A complete return to sedentary levels occurred after 7 days. Thermal sensitivity could not be collected after five weeks. It returns to baseline after several weeks in the SNL model (the time-course varying with different environments, handling conditions, and animals), while tactile hypersensitivity seems to last indefinitely (Wang, 2006). For this reason, the remainder of the study mainly focused on the role of exercise in reversing tactile hypersensitivity.

Less frequent exercise also reduced sensory hypersensitivity

Animals were trained using either 5 days/wk or 3 days/wk of moderate aerobic exercise over 5 weeks. Training 3 days/wk for 5 weeks decreased thermal hypersensitivity from 9.6 ± 0.6 s to 21.0 ± 0.6 s and tactile hypersensitivity from 2.7 ± 0.1 g to 10.0 ± 1.7 g (Fig. 2.4a). There was no significant (P < 0.05) difference in tactile or thermal sensitivity between 3 days a week and 5 days a week of exercise.

More complete reversal of tactile hypersensitivity with higher intensity exercise

Higher and lower intensity exercise protocols were used to explore the relationship between exercise intensity and reversal of SNL-induced tactile hypersensitivity. SNL animals were exercised at speeds of 16m/min (higher intensity) or
10m/min (lower intensity) for 5 weeks. Running was required at the higher intensity and walking at the lower intensity. Speeds of more than 16 m/min were too stressful for the animals to complete and were not tested. The higher intensity group had a more complete reversal of tactile hypersensitivity than the lower intensity group (Fig. 2.4b). Tactile sensitivities increased from 3.5 ± 1.0 g to 12.3 ± 1.3 g after 5 weeks of high intensity exercise, while only from 2.1 ± 0.2 g to 4.9 ± 1.52 g after lower intensity exercise. The low intensity group also displayed considerably more variability in tactile response than did the higher intensity group.

No effect of delayed initiation of exercise post surgery

A delayed exercise protocol was conducted to assess whether the observed rate of reversal of sensory hypersensitivity was determined by the duration of exercise training or instead due in part to the timing of changes after surgery. In the delayed exercise group, exercise was initiated 4 weeks after SNL surgery instead of after 1 week. Four weeks was chosen because that is the time that an exercise-induced reversal of tactile hypersensitivity is nearly complete. Reversal of sensory hypersensitivity occurred 3 weeks after initiation of exercise, regardless of the interval after surgery (Fig. 2.4c). This suggests that the time course of the reversal of sensory hypersensitivity is determined by the length of exercise training.
Diurnal effects on tactile sensitivity of exercise SNL animals

Tactile measurements were taken on exercise-trained and sedentary SNL animals during the dark and light cycles to assess for diurnal effects. Tactile measurements in all other experiments were performed during the dark cycle, 23 hours after exercise. A significant (P < 0.05) difference was found in tactile sensitivity measurements during the light cycle and the dark cycle in SNL animals subjected to exercise (Fig. 2.4d). Tactile withdrawal thresholds decreased from 9.1 ± 1.5 g during the dark cycle to 7.1 ± 1.1 g during the light cycle in exercise-trained SNL animals. Tactile sensitivity in sedentary SNL animals was no different between the two conditions. Exercise-trained SNL animals had higher withdrawal thresholds than sedentary animals under both light and dark conditions.

Naturally sedentary animals had similar thermal and tactile sensitivities to sedentary

Animals unable to complete the exercise pre-test were categorized as naturally sedentary. To determine if naturally sedentary animals had different sensory sensitivities than animals that were able to complete the exercise pre-test, but were randomized to the sedentary group, they were subjected to sham and SNL surgeries and their thermal and tactile sensitivities measured. There was no significant (P < 0.05) difference in thermal and tactile sensitivities between the naturally sedentary animals and animals that completed the exercise pre-test, but did not undergo exercise training (Fig. 2.5).
Exercise did not change levels of spinal dynorphin, an endogenous neuropeptide that can contribute to neuropathic pain

Spinal dynorphin is increased after SNL, and contributes to sensory hypersensitivity after SNL (Ibrahim et al., 2004). We determined if exercise training reverses hypersensitivity in SNL animals by affecting spinal dynorphin content. Dynorphin content was not different in exercise animals compared to sedentary animals (Fig. 2.6). No difference was found with sham either, indicating that dynorphin content returns to baseline levels at this time point after SNL surgery.

Systemic administration of naloxone reversed exercise effects

During exercise, endogenous opioid levels increase in both humans and animals (Koltyn, 2000). We used the opioid receptor antagonist naloxone to evaluate the role of endogenous opioids in exercise-induced reversal of neuropathy-induced sensory hypersensitivity. Naloxone (1 mg/kg) was injected s.c. Tactile sensitivity was measured in sham, SNL sedentary, and SNL exercise animals before drug injection and after injection of vehicle (saline), naloxone, or naloxone methiodide. Naloxone decreased tactile withdrawal threshold in exercise-trained SNL animals from 13.9 ± 0.7 g to 3.6 ± 0.3 g (Fig. 2.7a). Naloxone effects peaked 20 min after injection, persisted after 40 min, but returned to baseline levels by 60 min (Fig. 2.7b). Naloxone methiodide, the peripherally acting form of naloxone, was also administered s.c. in the neck in a dose of 0.1 mg/kg. A dose of 0.1mg/kg was used because doses greater than 1mg/kg have been found to be high enough to enter the central nervous system so their affects are no longer
being mediated just in the peripheral nervous system (Shimizu et al., 2004). Naloxone methiodide (0.1 mg/kg) did not significantly (P < 0.05) affect tactile hypersensitivity in exercise-trained SNL animals. No effect was seen in sham or SNL sedentary animals with naloxone or naloxone methiodide. The effect of systemic administration of AM251, a CB1 cannabinoid receptor antagonist on exercise-induced inhibition of sensory hypersensitivity, was also tested. AM251 attenuated increased tactile hypersensitivity of exercise-trained animals. We chose to explore further the possibility of an endogenous opioid mediated mechanism of exercise-induced reversal of neuropathy-induced sensory hypersensitivity, but cannabinoids may also be involved.

To determine the anatomical location of exercise-induced endogenous opioid activity, site-specific injections of naloxone and naloxone methiodide were performed. Studies have shown peripheral acting opioids produce antinociception. Loperamide, an opioid agonist unable to cross the blood-brain barrier, produced analgesia in a model of bone cancer pain, inflammatory pain, and neuropathic pain (Mendez 2005; Sevostianova, 2005; Shinoda, 2007). CB2 cannabinoid receptor activation inhibited nociception in inflammatory and neuropathic pain models by stimulating peripheral release of endogenous opioids (Ibrahim et al., 2005). In this study, naloxone was administered subcutaneously in the hindpaw in exercise-trained SNL animals, and tactile sensitivity was measured to see if the effects were mediated peripherally. Higher doses (10 µg) produced tactile hypersensitivity in the hindpaws, both ipsilateral and contralateral to spinal nerve ligation. This suggested a systemic effect rather than a local (hindpaw) site of action of naloxone. No effect was observed with local hindpaw injection of lower (3
μg or 1 μg doses) doses of naloxone (Fig. 2.8a). No change in paw withdrawal threshold was seen in sham and sedentary SNL animals after hindpaw injection of 10 μg naloxone (Figure 2.8b).

Naloxone methiodide (10 μg) was administered i.t. to SNL exercise-trained animals and had no effect on tactile sensitivity of exercise-trained SNL animals (Fig. 2.9a). Intrathecal injection of vehicle (saline) had no effect. To verify the effectiveness of this dose of naloxone methiodide, we tested its ability to reverse the effects of exogenously administered morphine. In otherwise untreated animals, morphine (30 μg i.t.) increased paw withdrawal latency to radiant heat from 20.8 ± 1.0 g at baseline to 32.2 ± 2.6 g 30 min after drug administration (Fig. 2.9b). Pre-administration of naloxone methiodide 15 min before morphine injection prevented morphine-induced analgesia. Paw withdrawal latency was 24.4 ± 2.0 s (P < 0.05) (Fig. 2.9b).

Intracerebroventricular cannulas were inserted in SNL animals prior to exercise training. Following 5 weeks of exercise, naloxone methiodide was injected i.c.v. Paw withdrawal threshold decreased from 11.0 ± 2.0 g to 1.2 ± 0.1g in exercise-trained SNL animals after naloxone methiodide (2 μg) administration (Fig. 2.10a). Intracerebroventricular administration of vehicle (saline) produced no effect. The exercise-trained SNL animals injected i.c.v. with naloxone methiodide showed signs of opioid withdrawal (e.g. aggressive behavior, diarrhea). Naloxone methiodide (2 μg i.c.v.) did not change tactile sensitivity or produce behavioral effects or diarrhea in sham animals (Fig. 2.10b).
**Endogenous opioid levels increase in the PAG and RVM with exercise-trained SNL animals**

Immunohistochemistry was performed with β-endorphin and met-enkephalin antibodies to localize the region(s) in the brain where exercise-induced increases in endogenous opioid activity may be mediated. The periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) displayed increased immunoreactivity for β-endorphin and met-enkephalin in exercise-trained compared to sedentary SNL animals (Fig. 2.11, 2.12). PAG β-endorphin and met-enkephalin immunoreactivity in SNL animals increased by 304% and 114%, respectively with exercise (P < 0.05) (Fig. 2.11b). In the RVM, β-endorphin and met-enkephalin levels increased by 28% and 38%, respectively, with exercise training (Fig. 2.12b). No difference was observed in endogenous opioid content between sides of the RVM, ipsilateral and contralateral to spinal nerve ligation.

An immunoassay method was used to quantify the amount of β-endorphin in the PAG and RVM. β-endorphin content was higher in the PAG of exercise-trained (217.4 ± 17.0 pg/mg) compared to sedentary SNL animals (166.3 ± 19.0 pg/mg), a 31% increase (Fig. 2.13). β-endorphin content in the RVM was not in the detectable range of the assay.
DISCUSSION

These results indicate that regular aerobic exercise reverses the signs of neuropathic pain and suggest that exercise-induced reversal of neuropathic pain results from an upregulation of endogenous opioids in pain-inhibiting pathways in the brain. Thermal and tactile hypersensitivity produced in the spinal nerve ligation model of neuropathic pain was reversed with 5 weeks of moderate aerobic exercise, while sensory hypersensitivity returned within one week of cessation of exercise. Systemic and i.c.v. injections of naloxone reversed the effects of exercise on SNL-induced hypersensitivity, and levels of \( \beta \)-endorphin and met-enkephalin in the PAG and RVM were higher in exercise-trained than in sedentary animals. These findings support an endogenous opioid-mediated mechanism of exercise-induced pain reversal.

There is an extensive body of research in both humans and animals on the analgesic effects of exercise (reviewed in Koltyn, 2000). Most of the animal research has indicated that this analgesia is produced by activation of the endogenous opioid system. The majority of this research has been focused on swimming as the type of exercise, with short duration exercise, and analgesia measured immediately after exercise. While acute forms of exercise have been shown to increase the release of endogenous opioids, there is also an accompanying increase in stress hormones like cortisol and catecholamines due to the activation of the hypothalamo-pituitary-adrenal axis (Mastorakis and Pavlatou, 2005). Regular exercise actually leads to a state of physical conditioning that is associated with a reduction in the pituitary-adrenal activation and other modulators of stress. Shyu et al., 1982 were some of the earliest researchers to conduct a study on pain
thresholds in non-operated animals after several weeks of voluntary wheel running. They observed a decrease in pain sensitivity, as measured by squeak threshold. This increase in vocalization threshold was reversed to control levels with naloxone. Furthermore, the measurements were taken the day after exercise as opposed to other studies where measurements were taken immediately after exercise. More recently, other studies have shown the analgesic effects of regular exercise, with the effects being mediated by endogenous opioids (Smith and Yancey, 2003; Smith and Lyle, 2006; Mathes and Kanarek, 2006).

Most of the more recent exercise studies use running as the form of exercise, as opposed to swimming, since it is a more natural form of activity and less stressful for rodents. Running exercise studies with rodents include voluntary wheel running or forced treadmill running. Increased endogenous opioid release and analgesia have been observed with both chronic treadmill running studies and voluntary wheel running protocols (Boone et al., 1996; Debruille et al., 1999; Kapasi et al., 2001; Lee et al., 2003; Smith and Yancey, 2003; Li et al., 2004; Bement et al., 2005; Su et al., 2005; Mathes and Kanarek, 2006). While wheel running is certainly less stressful on the animals, as they are able to stop and start as desired, forced treadmill running is the only way to ensure sufficient intensity and duration necessary for endogenous opioid release (Goldfarb, 1997; Kotlyn, 2000). Interestingly, mice bred for higher voluntary wheel running demonstrated no significant increase in analgesia compared to their normal-running counterparts despite a 170% increase in exercise (Li et al, 2004). While this was a surprising finding, genetic breeding can produce compensatory changes, possibly in
endogenous opioid or opioid receptor expression that may explain the lack of difference between both running groups. It is also possible that the genetically breed animals may have done more exercise than the controls, but the intensity of the exercise may have been the same between the groups.

Our findings regarding exercise intensity, frequency, duration, and rate of return of sensory hypersensitivity after cessation of exercise are consistent with an endogenous opioid mediated mechanism of pain reversal. In the present study, animals subjected to higher intensity exercise for 5 days a week showed more complete reversal of tactile hypersensitivity compared to those undergoing less frequent and intense protocols of exercise. Similarly, Peijie et al., 2003 found that more intense exercise and a longer duration of exercise training produced higher levels of endogenous opioids. The present study also showed that it takes 4-5 weeks for exercise-induced reversal of sensory hypersensitivity to occur. This was true whether exercise training was initiated 1 week or 4 weeks after surgery. Other studies demonstrating exercised-induced increases in endogenous opioids and subsequent pain reduction were performed over 5 to 8 wks (Smith and Yancey, 2003; Smith and Lyle, 2006; Mathes and Kanarek, 2006). In our study, exercise-induced reversal of tactile hypersensitivity lasted 4 days after discontinuing exercise. Other studies have shown that endogenous opioid peptides have a rapid rate of turnover. Hoffman et al., 1990 found that 6 weeks of exercise produced elevated Cerebrospinal fluid (CSF) levels of β-endorphin that remained elevated 48 hours after cessation of exercise, but no other studies have reported endogenous opioid levels
remaining elevated beyond 48 hours. This would explain why pain returns within days of discontinuing exercise.

SNL exercise animals showed differences in tactile sensitivity that was not seen with sedentary SNL animals. Studies have shown diurnal variation in pain thresholds that is attributed to rhythmic changes in endorphin levels in the central nervous system (CNS) (Frederickson et al., 1977; Wright et al., 1981, Rasmussen et al., 2003). Exercise-trained SNL animals tolerated higher pain thresholds during the dark cycle compared to the light cycle, which is likely attributed to increased endogenous opioids during the active dark cycle. The sedentary SNL animals didn’t show any diurnal differences, perhaps due to lower levels of endogenous opioids. The data reported in this study also demonstrated that non-operated and sham-operated animals did not have the same increase in thermal and tactile sensitivity that was seen with the injured animals.

Exercise may be more effective in chronic or acute pain sufferers than those not suffering from pain. Kemppainen et al., 1998 found exercise-induced decreases in pain were greater for pilots with acute neck pain compared to those without. One possibility is that chronic pain causes a malfunction in the endogenous opioid system, and regular exercise leads to its normalization.

Handling and environment appear to be important when performing exercise studies, particularly treadmill training, which can be stressful to the animals. Handling and environmental factors strongly affected exercise compliance in this study. Under our handling conditions, the animals displayed no physical signs of stress or stress-induced vocalizations. Gentle daily handling and training the animals during the dark cycle when
they are most active, reduced the number of animals not completing the exercise pre-test and increased the number of animals able to complete the full 5 weeks of exercise. Other researchers also emphasize the importance of minimizing stress and of sufficient handling for successful exercise studies, particularly with injured animals (Hutchinson et al., 2004).

The pharmacological and biochemical data presented in this study suggest that exercise-induced reversal of neuropathic pain is due to an upregulation of endogenous opioids in the brainstem, PAG and RVM. The PAG and RVM are the two main areas of the brainstem that produce analgesia after the administration of opioids (Holden et al., 2005) and interactions between both structures produce the most effective analgesia. Supraspinal administration of morphine has been demonstrated to suppress neuropathic pain in animals (Lee et al., 1996; Bian et al., 1995), and microinjections of opioids into the PAG have been reported to attenuate behavioral signs of neuropathic pain in rats (Sohn et al., 2000). Further, increased endogenous opioid activity in the PAG induced by electrical stimulation of the PAG reversed tactile hypersensitivity in a neuropathic pain model (Lee et al., 2000b). The effects of electrical stimulation were blocked by naloxone, indicating an endogenous opioid mediated mechanism. Mathes and Kanarek, 2006 studied analgesia and tolerance in exercise-trained animals and found that the PAG plays a significant role in exercise-induced analgesia. Voluntary wheel-trained animals had increased pain thresholds compared to sedentary. The exercise-trained animals were less responsive to morphine, and this effect was mediated through the PAG. Mathes and Kanarek, 2006 attributed this finding to exercise-induced increases in endogenous
opioids producing analgesic tolerance. Smith and Yancey, 2003 similarly found exercise-induced analgesia and tolerance to systemic morphine. They also found that regular exercise led to physical dependence. Naloxone precipitated withdrawal signs in exercise-trained animals that were not observed in animals that remained sedentary. Consistent with these findings, the SNL exercise-trained animals in the present study also showed signs of withdrawal (diarrhea and aggressive behavior) after i.c.v. administration of naloxone. Non-operated and sham-operated animals in this study did not show increases in sensory thresholds with exercise-training, which may be due to the lower intensity running protocol used to accommodate the injured animals. For this reason, naloxone precipitated withdrawal was not expected, but this may need to be confirmed in future testing.

Our results demonstrate that exercise training reduces sensory hypersensitivity in an animal model of peripheral neuropathy and suggests that exercise may be beneficial in patients with clinical neuropathic pain. Even though there is considerable research on exercise-induced analgesia in animal models and numerous clinical publications recommending exercise for patients with chronic pain conditions, there is limited research on the effects of exercise in an animal model of pain. Hutchinson et al., 2004 found treadmill running completely reversed signs of neuropathic pain in animals with spinal cord injury (SCI). Another recent study showed that low intensity treadmill reversed mechanical hypersensitivity in animals with chronic muscle pain (Hoeger et al., 2005). The latter study attributed the pain reversal to an opioid-mediated mechanism since hypersensitivity returned with naloxone administration. Hutchinson et al., 2004
attributed the hypersensitivity with exercise in SCI animals to upregulation of brain derived neurotrophic factor (BDNF). BDNF administered in the brain or spinal cord has analgesic effects that can be reversed with naloxone (Nawa et al., 1994; Sauer et al., 1994; Suiciak et al., 1995), suggesting a link between BDNF and endogenous opioids. This potential link deserves further study.
Figure 2.1a: Exercise training reverses thermal hypersensitivity in spinal nerve-ligated rats. Withdrawal latency to focused radiant heat was measured in sham (filled circles), sedentary SNL (open triangles), and exercise-trained SNL (filled triangles) rats. Measurements were taken before SNL surgery, 1 week after SNL surgery, and weekly after exercise training was begun. They were made 23 hrs after the completion of exercise. Sedentary SNL animals were subjected to identical environmental and handling conditions but were not in contact with the treadmill. Sham animals remained sedentary during the course of the experiment. *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.1b: Exercise training reverses tactile hypersensitivity in spinal nerve-ligated rats. Withdrawal threshold to von Frey filaments was measured in sham (filled circles), sedentary SNL (open triangles), and exercise-trained SNL (filled triangles) rats. Measurements were taken before SNL surgery, 1 week after SNL surgery, and weekly after exercise training was begun. They were made 23 hrs after the completion of exercise. Sedentary SNL animals were subjected to identical environmental and handling conditions but were not in contact with the treadmill. Sham animals remained sedentary during the course of the experiment. *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.2a: Exercise training did not affect thermal sensitivities of sham-operated or non-operated animals. Thermal sensitivities were measured in non-operated rats subjected to sedentary (open circles) or exercise training (filled circles) conditions and sham-operated rats subjected to sedentary (open squares) or exercise (filled squares) conditions. Thermal measurements were taken 23 hours after exercise. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.2b: Exercise training did not affect tactile sensitivities of sham-operated or non-operated animals. Tactile sensitivities were measured in non-operated rats subjected to sedentary (open circles) or exercise (filled circles) conditions and sham-operated rats subjected to sedentary (open squares) or exercise (filled squares) conditions. Thermal measurements were taken 23 hours after exercise. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.3: Tactile hypersensitivity returns within one week after cessation of exercise training. *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.4a1: Less frequent exercise training also reverses thermal hypersensitivity in SNL rats. Thermal hypersensitivity was measured in SNL animals subjected to 5 weeks of exercise training for either 5 days a week (filled triangles) or 3 days a week (filled diamonds). Data shown as mean ± SEM. N=6 animals per group.
Figure 2.4a2: Less frequent exercise training also reverses tactile hypersensitivity in SNL rats. Tactile hypersensitivity was measured in SNL animals subjected to 5 weeks of exercise training for either 5 days a week (filled triangles) or 3 days a week (filled diamonds). Data shown as mean ± SEM. N=6 animals per group.
Figure 2.4b: Higher intensity of exercise training reverses tactile hypersensitivity more completely. Tactile sensitivity was measured in SNL animals subjected to either higher intensity (16m/min, filled triangles) or lower intensity (10m/min, filled squares) exercise training. *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.4c: Delaying the initiation of exercise training after surgery had no effect on the duration of exercise needed to reverse tactile hypersensitivity. Tactile hypersensitivity was measured in sedentary SNL (open triangles), exercise SNL (filled triangles), and delayed exercise SNL (filled squares) rats. In the exercise SNL group, exercise was initiated one week after surgery. In delayed exercise SNL, exercise was initiated 3 weeks after surgery. The exercise SNL and delayed exercise SNL both showed reversal of tactile hypersensitivity after 4 weeks of exercise, while the sedentary SNL animals continued to demonstrate tactile hypersensitivity. *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.4d: Tactile withdrawal thresholds of exercise-trained SNL animals were lower during the diurnal light cycle compared to the dark cycle. Tactile sensitivity was measured in SNL animals subjected to sedentary (black) and exercise (white) conditions. Tactile sensitivity in sedentary SNL animals remained unchanged. Exercise-trained SNL animals had higher paw withdrawal thresholds compared to the sedentary during the light and dark cycles. *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.5a: Naturally sedentary animals show no differences in thermal sensitivities compared to animals that successfully exercised, but were selected to be sedentary. Rats were subjected to an exercise pre-test. Thermal sensitivities were recorded in non-operated, sham-operated, and SNL-operated rats that completed the exercise pre-test (black bars) and naturally sedentary rats (white bars). Data shown as mean ± SEM. N=6 animals per group.
Figure 2.5b: Naturally sedentary animals show no differences in tactile sensitivities compared to animals that successfully exercised, but were selected to be sedentary. Rats were subjected to an exercise pre-test. Tactile sensitivities were recorded in non-operated, sham-operated, and SNL-operated rats that completed the exercise pre-test (black bars) and naturally sedentary rats (white bars). Data shown as mean ± SEM. N=6 animals per group.
Figure 2.6: Exercise did not change levels of spinal dynorphin, an endogenous neuropeptide that can contribute to neuropathic pain. Dynorphin content was measured in the spinal cords of sham (diagonal lines), sedentary SNL (white) and exercise-trained SNL (black) groups. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.7a: Systemic administration of naloxone reversed the effects of exercise on SNL-induced hypersensitivity. Tactile hypersensitivity was measured in sham, sedentary SNL, and exercise SNL animals at the completion of five weeks of exercise or sedentary conditions (black), after s.c. administration of vehicle (saline) (gray), naloxone (1mg/ml) (white), and naloxone methiodide (0.1mg/ml) (diagonal lines). The effects of exercise in the SNL group were revered by naloxone. Groups were compared using ANOVA followed by pairwise comparisons using Student’s t-test. *P<0.05 compared to SNL exercise and SNL exercise (vehicle). Data shown as mean ± SEM. N=6 animals per group.
Figure 2.7b: The effects of naloxone on tactile hypersensitivity were reversible. Exercise-trained SNL animals (filled triangles) were administered naloxone (1mg/kg s.c.). *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.8a: Opioid-mediated exercise training effects are not mediated in the hindpaw. Local hindpaw injections of low doses of naloxone ipsilateral to nerve injury did not reverse the effects of exercise on tactile sensitivity. Higher doses (10µg) produced tactile hypersensitivity when injected in the ipsilateral paw on the contralateral paw, indicating the effects were being mediated systemically. Groups were compared using ANOVA followed by pairwise comparisons using Student’s t-test. *P<0.05 compared to SNL exercise. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.8b: Opioid-mediated exercise training effects are not mediated in the hindpaw. Hindpaw injections of naloxone (10µg s.c.) in sham (black) and sedentary SNL (white) animals had no effect on tactile sensitivity. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.9a: Opioid-mediated exercise effects are not mediated in the spinal cord. Intrathecal injection of naloxone methiodide had no effect on exercise-induced reversal of tactile hypersensitivity. Tactile hypersensitivity was measured in SNL animals after 5 weeks of exercise training (black), and in exercise-trained SNL animals after i.t. injections of vehicle (saline) (white), naloxone methiodide (10µg) (diagonal lines). Data shown as mean ± SEM. N=6 animals per group.
Figure 2.9b: Intrathecal naloxone methiodide (10 µg) antagonizes opioid actions. Thermal sensitivity was measured in naïve animals at baseline (white), after 30 µg morphine i.t. (black), and after 10µg naloxone methiodide i.t. and 30 µg morphine i.t. (diagonal lines). Groups were compared using ANOVA followed by pairwise comparisons using Student’s t-test. *P<0.05 compared to naive. #P<0.05 compared to morphine. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.10a: The opioid-induced effects of exercise training are mediated at supraspinal sites. Tactile sensitivity was measured at the end of 5 weeks of exercise training (black), after i.c.v. injection of vehicle (saline) (white) and naloxone methiodide (2 µg i.c.v.) (diagonal lines). Groups were compared using ANOVA followed by pairwise comparisons using Student’s t-test. *P<0.05 compared to SNL exercise and SNL exercise (vehicle). Data shown as mean ± SEM. N=6 animals per group.
Figure 2.10b: Sham animals administered naloxone methiodide (2µg i.c.v.) showed no change in tactile sensitivity. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.11a: Exercise training increases endogenous opioid content in PAG. Immunohistochemistry was performed on PAG sections from sedentary and exercise-trained SNL animals stained with (A, B) β-endorphin antibodies and (C, D) met-enkephalin antibodies. Images were magnified 20x. N=3 animals per group.
Figure 2.11b: Exercise training increases endogenous opioid content in PAG. Densitometry was performed on 10-12 images from sedentary (white) and exercise trained (black) SNL animals using antibodies to β-endorphin and met-enkephalin. β-endorphin and met-enkephalin levels in SNL animals significantly increased by 304% and 114%, respectively with exercise. *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.12a: Exercise training increases endogenous opioid content in RVM. RMV sections were collected in SNL animals subjected to 5 weeks of exercise training and sedentary conditions and stained with antibodies to β-endorphin (A, B) and met-enkephalin (C, D). Images were magnified 40x. N=3 animals per group.
Figure 2.12b: Exercise training increases endogenous opioid content in RVM. Densitometry was performed on 12-15 images from sedentary (white) and exercise trained (black) SNL animals using antibodies to β-endorphin and met-enkephalin. β-endorphin and met-enkephalin levels increased by 28% and 38%, respectively, with exercise training. *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.
Figure 2.13: Exercise increases β-endorphin content in PAG. A punch technique was used to collect the entire PAG from SNL animals subjected to exercise and sedentary conditions. β-endorphin content was measured using an ELISA immunoassay. *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.
APPENDIX B

A POSSIBLE ROLE OF BRAIN-DERIVED NEUROTROPHIC FACTOR IN EXERCISE-INDUCED INCREASES IN ENDOGENOUS OPIOIDS AND SUBSEQUENT REVERSAL OF PERIPHERAL NEUROPATHIC PAIN

INTRODUCTION

Exercise has been shown to reduce acute and chronic pain in humans and animals (Koltyn K, 2000; Hoeger et al., 2005; Hutchinson et al., 2004). It is well established that short-term exercise raises pain thresholds through stress-induced increases in endogenous opioids and cannabinoids (Koltyn K, 2000). The findings that regular exercise can reduce chronic pain in animals is more recent, and the mechanism (s) through which exercise produces this effect are not yet understood. Regular exercise decreased pain sensitivity in a chronic muscle pain animal model after one week (Bement and Sluka, 2005), spinal cord injury animal model (SCI) after five weeks (Hutchinson et al., 2004), and in otherwise untreated rats after 5-8 weeks (Shyu et al., 1982; Smith and Yancey, 2003; Smith and Lyle, 2006; Mathes and Kanarek, 2006). Naloxone, an opioid receptor antagonist reversed the effects of exercise in most studies. Furthermore, animals exercised on either rodent treadmills or voluntary running wheels have been observed to have significantly higher plasma β-endorphin levels compared to sedentary controls (Aravich et al, 1993; Debruille et al.1999; Su et al., 2005). These findings suggest that
exercise-induced increases in opioid content may lead to inhibition of nociceptive systems and diminished pain sensitivity.

There is evidence to suggest that brain-derived neurotrophic factor (BDNF) or other neurotrophic factors may be responsible for the pain reversing effects of exercise. Animal studies have found elevated neurotrophic factors in the central nervous system after chronic exercise. Hutchinson et al., 2004 found that exercise reversed signs of neuropathic pain in an animal spinal cord injury (SCI) model. They attributed this to increased expression of BDNF in the spinal cord (Hutchinson et al., 2004) and brain (Vaynman and Gomez-Pinilla, 2005). Increased brain levels of BDNF have been suggested to produce analgesia through opioidergic and serotonergic pathways (Siuciak et al., 1994; Siuciak et al., 1995; Frank et al., 1997; Cirulli et al., 2000). Thus, exercise-induced increases in BDNF content leading to increased opioid levels may serve as a mechanism for exercise-induced pain reversal.

We hypothesized that increased BDNF in the brain resulting from regular moderate aerobic exercise leads to increased expression of endogenous opioids in the brainstem, resulting in reversal of neuropathic pain in rats with peripheral nerve injury. We measured the effects of exercise on brain BDNF content. We investigated the effects of endogenous brain BDNF on sensory hypersensitivity and B-endorphin midbrain levels in the periaqueductal gray (PAG) area of spinal nerve-ligated (SNL) rats subjected to exercise and sedentary conditions.
METHODS

Animals

Approval was obtained from the University of Arizona Animal Care and Use Committee. Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were allowed water and food *ad libitum*, and weighed 250-380 g throughout testing. Animals were maintained in a climate-controlled reverse cycle room on a 12-h dark/light cycle. Reverse cycle conditions consisted of housing animals in the dark during the day (lights off at 10 am) and in the light during the night (lights on at 10 am). All animal procedures conformed to the guidelines for the Care and Use of Laboratory animals by the International Association for the Study of Pain (IASP) and the National Institutes of Health (NIH). Animals were handled twice daily in a stress-free environment. All experiments were started after 7 days of equilibration to their new surroundings.

Spinal Nerve Ligation (SNL)

SNL surgery was performed as described by Kim and Chung (1992). Under isoflurane anesthesia, an incision was made lateral to the lumbar spine, and a small ronguer was used to remove the left L6 transverse process. The right L5 and L6 spinal nerves were isolated and tightly ligated distal to the dorsal root ganglion (DRG). The incision was closed, and animals were allowed to recover for 7 days. Sham-operated animals were prepared using an identical procedure without nerve ligation. All SNL rats were inspected for a deformity in the hindpaw on the injured side. The foot should be moderately everted with the toes held together (Kim and Chung, 1992). Animals
demonstrating an inverted foot with markedly ventroflexed toes (sciatic nerve damage: Kim and Chung, 1992) or paralysis of hindpaw (damage to L4) were removed from the study.

**Exercise Training**

Animals underwent an exercise pre-test on a rodent treadmill before surgery. The pre-test consisted of 4 sessions of running at 18m/min for 10 minutes. The sessions were performed 2 days a week over a period of 2 weeks. The animals unable to complete the test were removed from the study.

After surgery, animals were subjected to either sedentary or exercise training conditions. Exercise training consisted of running on a 10-lane motor-driven rodent treadmill at an 8% incline for 5 days/wk for up to 5 weeks. The training protocol was quickly increased to 30 min/day at a speed of 14-16m/min over 14 days. An electric grid at the back of the treadmill was activated with a weak current. Animals were only allowed contact with the grid for 2 s at a time and not more than 3 times per session before being removed to minimize stress. Sedentary animals remained in their cages.

**Intracerebroventricular Cannulation**

Animals were fitted with intracerebroventricular (i.c.v.) cannulas prior to the start of exercise. They were anesthetized with isoflurane, and the head was shaved, cleaned, and placed in a stereotaxic head holder. The skull was exposed and a 22-guage guide cannula was directed to the right lateral ventricle (1.3 mm caudal to bregma, 1.5 mm
lateral to the sagittal suture, 3.5 mm ventral to the dural surface). The cannula was secured in place by small stainless steel screws and dental cement. Animals were allowed a five day recovery before the start of exercise.

*Drug Administration*

Rats received i.c.v. injections of either vehicle (phosphate-buffered saline, PBS) or BDNF sequestering agent (human recombinant TrkB-Fc; R & D Systems, Minneapolis, MN) during weeks 3 and 4 of exercise. TrkB-Fc was dissolved in PBS and administered in doses of 8µg, 16µg, and 25 µg. The drugs were directed into the intraventricular space by inserting a 28-guage injection cannula through the guide cannula and slowly injecting 2.5 ul volume of fluid. Backflow was prevented by having the injection cannula 1mm longer than the guide cannula so it protruded into the ventricular space. The BDNF sequestering agent has an *in vivo* half-life of 7 days. Tactile testing was performed 5 days after drug administration.

*Assessment of tactile sensitivity*

Rats were allowed to acclimate for 30 min within Plexiglas® enclosures with wire mesh bottoms. Paw withdrawal thresholds were determined in response to probing of the hind paw with a series of calibrated von Frey filaments applied perpendicularly to the plantar surface of the paw. A maximal cutoff of 15 g was used because larger filaments lifted the paw even if the animal did not actively withdraw. Data were analyzed by the up-and-down method of Dixon, as described by Chaplan et al (1994). Tactile sensitivity
was measured before surgery (baseline), 1 week after surgery, and weekly during exercise. Measurements were taken 23 hours after exercise. N=6 for all groups.

**BDNF Immunoassay**

Spinal-nerve ligated rats subjected to exercise training or sedentary conditions were anesthetized with ether and killed by decapitation. Brains were collected, and sonicated for 10 s each with cold homogenization buffer. The buffer contained 100mM Tris/HCL, 2% bovine serum albumin (BSA), 1M NaCl, 4mM EDTA.Na₂, 2 % Triton X-100, 0.1% sodium azide, and protease inhibitors aprotonin (5 µg/ml), antipain (0.5 µg/ml), benzamidine (157 µg/ml), pepstatin A (0.1 µg/ml), and phenylmethyl-sulphonyl fluoride (17 µg/ml) (Sigma, St. Louis, MO). The sonicated solutions were centrifuged at 12,000 X g for 15 min at 5°C in a refrigerated microcentrifuge. The supernatant was collected and measured for BDNF content using a commercially available enzyme immunoassay (Chemicon International, Temecula, CA).

**β-endorphin Immunoassay**

After 5 weeks of exercise training, rats were anesthetized with ether and killed by decapitation. Whole brains were collected, and sections were cut that contained the entire PAG region. The sections were placed on a flat surface, and a punch technique was used to remove the PAG. Tubes the diameter of the PAG were attached to a drill and used to punch through the tissue. The PAG tissues were then sonicated for 5 s each with 1.0 ml of cold 0.1 N HCL and then centrifuged at 12,000 X g for 15 min at 5°C in a refrigerated
microcentrifuge. The supernatant was collected and placed on ice. β-endorphin content in the supernatant was measured immediately, using a commercially available enzyme immunoassay (Peninsula Laboratories Belmont, CA).

**Immunohistochemistry**

After five weeks of sedentary conditions or exercise training, rats were anesthetized with ketamine HCl/xylazine. They were perfused transcardially with 0.1 M PBS until the exudates ran clear, followed by 10% buffered formalin (Fisherbrand). All harvested tissues were post-fixed in 10% buffered formalin, and transferred to 20% sucrose in 0.1 M PBS. 20 µm slide-mounted serial frozen sections of the PAG, RVM, hippocampus, amygdala, thalamus, and hypothalamus were processed for immunohistochemistry. Tissues were pre-blocked with 10% normal goat serum in PBS (1 h, room temperature), followed by incubation with primary reagents (2% normal goat serum/0.3% Triton X-100/PBS/primary antibody) for 24 h at 4 °C, and secondary antibody was added before further incubation for 2 h at room temperature. The primary antibody was polyclonal rabbit IgG anti-BDNF (1:4000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Secondary antiserum was Alexa Fluor 568 goat anti-rabbit IgG (1:1000; Invitrogen, Carisbad, CA). Following PBS washes, sections were dried and sealed with fluorescent mounting medium (Vector Laboratories).
**Image analysis**

Fluorescence images of the PAG, RVM, hippocampus, amygdala, thalamus, and hypothalamus were acquired with a Nikon E800 fluorescence microscope outfitted with 4×/NA 0.2, 10×/NA 0.45, 20×/NA 0.75 and 40×/NA 0.75 objectives, a filter set for Cy3 (excitation 540–580 nm/emission 560–620 nm) and a Hamamatsu C5810 color CCD camera and its proprietary Image Processor software (Hamamatsu Photonic System, Bridgewater, NJ). Digital images were produced using Adobe Photoshop 6.0 (Adobe System Inc., San Jose, CA).

**Statistical analysis**

Groups were compared using Anova, followed by pairwise comparison using Student’s t test with Bonferroni’s correction. Significance was defined as P < 0.05.
RESULTS

_Treatment with a BDNF sequestering agent reversed the effects of exercise_

These experiments tested the role of brain derived neurotrophic factor (BDNF) in exercise-induced reversal of neuropathic pain resulting from peripheral nerve injury. Spinal nerve-ligated rats were subjected to either exercise training or sedentary conditions for 5 weeks, following a week recovery from surgery. Exercise-trained rats were treated with either vehicle or increasing doses of a BDNF sequestering agent (TrkB-Fc) during weeks 3 and 4 of the protocol. TrkB-Fc is a fusion protein that contains the BDNF-binding domain of the TrkB receptor. By acting like a false TrkB receptor, it sequesters and, thus, neutralizes endogenous BDNF. The week 3 and 4 time points for administration of BDNF sequestering agent were chosen because that is when tactile sensitivity begins to reverse in SNL animals subjected to exercise training. One week after surgery, SNL animals demonstrated tactile hypersensitivity on the side ipsilateral to surgery, compared to sham animals. Reversal of tactile hypersensitivity was observed after 5 weeks of exercise training was seen in the SNL vehicle group (P < 0.05) (Fig. 3.1). Exercise-trained SNL animals treated with 25 µg BDNF sequestering agent (i.c.v.) had lower tactile thresholds compared to the exercise-trained vehicle group (P < 0.05). There was no effect of 25 µg BDNF sequestering agent on sham-operated animals (Fig. 3.2). The tactile sensitivity of exercise-trained SNL animals administered lower doses of BDNF sequestering agent (8 µg and 16 µg) were not different from the vehicle group. BDNF sequestering agent did not affect SNL animals subjected to sedentary conditions.
**BDNF content increases in the brain of exercise-trained SNL animals**

An ELISA immunoassay technique was used to quantify BDNF levels in whole brains of exercise-trained and sedentary animals. Brain tissue from exercise-trained SNL rats had 18% greater BDNF content compared to sedentary (P < 0.05) (Fig. 3.3). BDNF content remained unchanged from wks 2 to wks 5 after SNL surgery in sedentary animals.

**Exercise training increases BDNF immunofluorescence in the PAG, hypothalamus, and hippocampus**

Immunohistochemistry for BDNF was performed on brain sections of sedentary and exercise-trained SNL rats to localize the region(s) in the brain where BDNF content increases after exercise training. The periaqueuctal gray (PAG), hippocampus, and hypothalamus displayed the most clear increases in BDNF content after exercise training, compared to sedentary conditions (Fig. 3.4). Little BDNF was detected in the RVM, thalamus, and amygdala (structures important in the modulation of pain).

**Sequestration of BDNF decreases B-endorphin content in exercise-trained animals**

β-endorphin content in the PAG of exercise trained and sedentary SNL rats were measured by immunoassay. Exercise trained SNL animals displayed a 67% increase in PAG β-endorphin content compared to sedentary SNL animals. Although, these results were not statistically significant, due to rather high variability, they were in previous studies. Treatment of exercise-trained SNL animals with a BDNF sequestering agent
resulted in lower content of β-endorphin in the PAG compared to the exercise trained vehicle control groups (P < 0.05) (Fig. 3.5). This decrease in PAG β-endorphin content in exercise trained SNL was observed with all doses of BDNF sequestering agent tested [sedentary SNL (691.8 ± 221.7 pg/mg); exercise-trained SNL (1153.7 ± 221.7 pg/mg); SNL exercise-trained plus BDNF sequestering agent 8 µg (208.6 ± 39.5 pg/mg), 16 µg (229.1 ± 34.2 pg/mg) or 25 µg (267.4 ± 44.4 pg/mg)].
DISCUSSION

We have shown that moderate aerobic exercise training reverses neuropathic pain through an endogenous opioid-mediated mechanism (chapter 3). Spinal nerve-ligated (SNL) rats that underwent 5 weeks of exercise training displayed a reversal of tactile and thermal hypersensitivity that was not observed with sedentary SNL rats. The effects were blocked with naloxone methiodide administered i.c.v., and β-endorphin and met-enkephalin contents were elevated in the PAG and RVM of exercise-trained SNL rats compared to sedentary SNL rats. These results were replicated in the present study. Furthermore, exercise animals had increased BDNF levels that may play a role in maintaining elevated endogenous opioids and reversing neuropathic pain.

Prior research has found elevated content of selected neurotrophic factors in specific areas of the brain and spinal cord after exercise training. Increased expression of neurotrophic factors with exercise training enhances learning, and prevents and improves the relevant signs in models of Alzheimer’s disease and Parkinson’s disease (Van Praag et al 1999, Smith et al., 2003; Tillerson et al., 2003; Adlard et al., 2005). Elevated BDNF levels in the hippocampus have been associated with improved learning and reversal of signs of Alzheimer’s disease (Van Praag et al 1999, Adlard et al., 2005). Reversing damage to dopaminergic neurons in Parkinson’s disease is attributed to increased GDNF expression (Smith et al., 2003; Tillerson et al., 2003).

In the present study, SNL animals that underwent exercise training had increased brain BDNF contents compared to sedentary SNL animals. The differences were present, but not large, possibly because analysis was performed on whole brain and not on
specific regions that have been previously found to have considerably higher relative
increases after exercise than were measured here. After finding a quantitative increase,
we performed immunohistochemistry to determine the region(s) where exercise led to
increased BDNF content. BDNF immunoreactivity in the PAG, hippocampus, and
hypothalamus increased with exercise. We previously showed that met-enkephalin and
β-endorphin content in PAG increases with exercise training (chapter 3). Here, we found
that BDNF immunoreactivity increased in PAG after exercise training. Interestingly,
BDNF immunoreactivity was increased in the outer portion of the PAG and not directly
surrounding the aqueduct where endogenous opioids are localized. The outer PAG BDNF
peptides may be projecting to the center of the PAG to modulate the endogenous opioids.
Alternatively, projections to PAG may be coming from the hippocampus and
ventromedial hypothalamus, where increased BDNF immunoreactivity was also found.
Increases in hippocampal BDNF with exercise is suggested by existing literature
(Vaynman and Gomez-Pinilla, 2005). Further, rats receiving repeated injections of
complete Freund’s adjuvant over 21 days to induce chronic inflammatory pain, developed
thermal and tactile hypersensitivity, and there was a significant decrease in BDNF
mRNA levels in hippocampal CA1-CA3 subregions (Duric and McCarson, 2006). One
possible explanation for this result is chronic pain leads to reduced hippocampal BDNF
levels, and this possibly affects the endogenous opioid system, contributing to sensory
hypersensitivity.

There is convincing published literature on endogenous opioid-induced increase
in pain thresholds with exercise in otherwise untreated or in injured animals. Further,
research suggests that neurotrophic factors may also play a role in the anti-nociceptive effects of exercise. Hutchinson et al., 2004 found that repeated exercise reversed neuropathic pain in an animal model of spinal cord injury. They attributed this hypersensitivity reversal to BDNF upregulation. Injured animals displayed a down-regulation of spinal levels of BDNF compared to control animals. In addition, exercise normalized expression of BDNF in spinal cord (Hutchinson et al., 2004) and increased expression of BDNF in the brain (Vaynman and Gomez-Pinilla, 2005). Increased spinal BDNF expression has been found to reverse pain in a model of neuropathic pain (Eaton et al., 2002; Cejas et al, 2000).

Treatment with exogenous BDNF has been found to produce analgesia. Intracerebroventricular (i.c.v.) or midbrain infusions of BDNF increased pain thresholds in otherwise untreated or in formalin-treated rats (Siuciak et al., 1994; Siuciak et al., 1995; Frank et al., 1997; Cirulli et al., 2000). The effects were reversed with naloxone (Suiciak et al., 1995). Other studies have found increased CNS expression of proenkephalin, enkephalin and β-endorphin during supraspinal BDNF infusions (Nawa et al., 1994, Sauer et al., 1994; Siuciak et al., 1995). Increased serotonergic activity was also found in the brain and spinal cord of animals given i.c.v. and midbrain injections of BDNF (Suiciak et al., 1996). Thus, both serotonergic and opiateergic mechanisms may play a role in the analgesic effects of supraspinally administered exogenous BDNF.

In the present study, administration of a BDNF sequestering agent to exercise-trained SNL animals reversed the antinociceptive effects of exercise training at least at the highest dose used (25 µg, i.c.v.). BDNF sequestration also reduced PAG β-endorphin
content compared to vehicle treated exercise-trained or sedentary SNL animals. Interestingly, β-endorphin was decreased even at the lowest dose of BDNF sequestering agent, suggesting different dose responsiveness, behavioral and neurochemical effects. However, we caution that behavioral and neurochemical measurements were made in different experiments, and that these results are preliminary and experiments studying the dose responsiveness of the effects of BDNF sequestering agent must be repeated.

While research has demonstrated pain-inhibiting effects of BDNF, it has also been shown to contribute to pain under some circumstances. It has been implicated in inflammatory pain. BDNF is distributed in primary sensory neurons in the dorsal root ganglion (DRG), and upregulation during peripheral inflammation has been shown to produce central sensitization in the spinal dorsal horn (Mannion et al., 1999). Guo et al., 2006 also reported that supraspinal BDNF signaling in a inflammatory pain model may facilitate descending pain. They demonstrated that hindpaw CFA (complete Freund’s adjuvant) injections led to increased BDNF content in RVM, and microinjections of BDNF neutralizers (anti-BDNF antibody or TrkB-Fc sequestering agent) and BDNF into the RVM reversed and promoted inflammatory hyperalgesia, respectively. They found that higher doses of BDNF injected into the RVM, above the physiological levels produced following inflammation, led to analgesia. They attribute the high dose BDNF affects to downregulation of trk B receptor proteins, but previous BDNF-induced analgesia studies have shown delayed trk B receptor downregulation that doesn’t explain the immediate analgesia (Frank et al., 1997).
Results in knockout mice suggest that the effects of BDNF signaling may differ in inflammatory models and neuropathic pain models. BDNF-deficient mice subjected to NGF, carrageenan, and formalin models of inflammatory pain had a significant attenuation of inflammatory pain compared to wild-type mice, but tactile hypersensitivity in SNL animals remained unchanged between wild-type and BDNF deficient groups (Zhao et al., 2006).

The results of this study suggest that exercise training may increase brain BDNF content, lending to increased expression of endogenous opiates. This increase may be responsible for the reversal of signs of neuropathic pain observed with exercise training.
Figure 3.1: Sequestration of BDNF blocked exercise-induced reversal of nerve injury-induced sensory hypersensitivity. Withdrawal threshold to von Frey filaments was measured in sedentary SNL rats (open triangles), exercise SNL rats treated with vehicle (filled triangles) and BDNF sequestering agent in amounts of 8µg (circles), 16 µg (diamonds), or 25 µg (squares) during weeks 3 and 4 of the protocol. Exercise training significantly reversed tactile hypersensitivity in SNL animals treated with vehicle (PBS). Exercise-trained SNL animals treated with 25 µg BDNF sequestering agent (i.c.v.) had lower tactile thresholds compared to the exercise-trained vehicle group. The tactile sensitivity of exercise-trained SNL animals administered lower doses of BDNF sequestering agent (8 µg and 16 µg) were not different from the vehicle group. *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.
Figure 3.2 Sequestration of BDNF had no effect sham-operated animals. Tactile sensitivity was measured in sham animals before (black) and 1 week after administering 25 µg BDNF sequestering agent (white). There was no effect of 25 µg BDNF sequestering agent on tactile sensitivities of sham animals. Data shown as mean ± SEM. N=6 animals per group.
Figure 3.3: Exercise training increased brain BDNF content. BDNF was measured in whole brain tissue from sedentary SNL wk2 (black), sedentary SNL wk5 (white), and exercise SNL (diagonal lines). Brain tissue from exercised SNL rats had significantly higher levels of BDNF compared to sedentary SNL animals. There was an 18% increase. There was no significant difference between 2 wks and 5 wks after SNL surgery in animals subjected to sedentary conditions. Sham animals had lower BDNF levels compared to exercise and sedentary SNL animals. *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.
Figure 3.4: Exercise training increases endogenous BDNF content in the brain. Images A, C, and E were taken from sedentary SNL animals, and images B, D, and F were taken from exercise SNL animals. Immunohistochemistry was performed on sedentary and exercise-trained SNL rats were stained with BDNF antibodies. Exercise training increased BDNF immunofluorescence in the outer PAG, ventrolateral hypothalamus, and hippocampus. Little BDNF was detected in the RVM, thalamus, and amygdala.
Figure 3.5: Sequestration of BDNF decreased brain β-endorphin content. β-endorphin content was measured in the PAG of sedentary SNL (black), Exercise SNL (white), and Exercise SNL animals treated with BDNF sequestering agent in amounts of 8 µg (diagonal lines), 16 µg (checkered lines), or 25 µg (vertical lines) during weeks 3 and 4 of the protocol. Treatment with the BDNF sequestering agent in SNL exercise animals produced lower amounts of β-endorphin in the PAG compared to the exercise control and sedentary SNL groups. There was no difference in PAG β-endorphin contents in exercise SNL animals treated with 8 µg, 16 µg, or 25 µg of BDNF sequestering agent. *P<0.05: Student’s t-test. Data shown as mean ± SEM. N=6 animals per group.