SELF-ADMINISTRATION OF A CANNABINOID CB_2 AGONIST IN AN ANIMAL MODEL OF NEUROPATHIC PAIN

by

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(Under the Direction of ANDREA G. HOHMANN)

ABSTRACT

We evaluated the impact of neuropathic pain on the propensity of rats to self-administer the cannabinoid CB_2 agonist AM1241. CB_2 is prevalent outside the central nervous system (CNS) and is induced in the CNS by traumatic nerve injury. A unilateral spared nerve injury was performed to induce neuropathic pain. Control rats were subjected to a sham surgery and naive animals were intact. Animals were surgically implanted with an indwelling jugular catheter to allow intravenous drug self-administration. Mechanical withdrawal thresholds were evaluated in the left and right paws before and after surgical procedures and before and after each drug self-administration session. AM1241 self-administration, but not vehicle, increased mechanical withdrawal thresholds in the left (injured) paw in neuropathic rats. Changes in mechanical withdrawal thresholds were absent following AM1241 self-administration in naive and sham-operated groups. Self-administration, as defined by preferential responding on the active but not the inactive lever, was observed in neuropathic and sham-operated groups receiving AM1241 (day 1). No difference was observed in the number of active and inactive lever presses in rats self-administering vehicle. The CB_2 antagonist SR144528 blocked the AM1241-induced suppression of nerve injury-induced tactile allodynia and attenuated active lever responding. The CB_1 antagonist SR141716 induced hypersensitivity in the right (intact) paw in neuropathic and naive groups self-administering vehicle or AM1241. SR141716 induced hypersensitivity in paw withdrawal thresholds in the left (intact) paw in naive groups self-administering vehicle. SR141716 attenuated active and inactive lever presses in naive and neuropathic groups self-administering vehicle. Morphine self-administration elevated paw withdrawal thresholds in neuropathic rats, but not in naive or sham-operated groups. By day 2, naive and neuropathic groups self-administering morphine responded preferentially on the active and not the inactive lever. The maximally self-administered dose of AM1241 failed to induce motor deficits in naive or neuropathic groups in the rotarod test. Our data demonstrate that self-administration of AM1241 suppresses nerve injury-induced tactile allodynia. The observation that naive animals self-administered morphine but not AM1241 is consistent with the hypothesis that activation of CB_2 is not inherently reinforcing and raises the possibility that CB_2 agonists may represent a class of analgesics with low abuse liability.

INDEX WORDS: Spared Nerve Injury, Neuropathic Pain, Intravenous, Self-administration

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DEDICATION

I would like to dedicate my dissertation to my family and dearest friends. Without the moral support of my family, I would not have made it through the difficult times in which I thought finishing my doctorate would not be possible. To my dearest and closest friends, I thank them for pushing me and being there when I needed some time out to distress. I thank my friends for reminding me to take some time for myself so that I could re-energize. To my dog, Loba, I thank for having a cuddly furry friend to pet and hug.

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CHAPTER 1: LITERATURE REVIEW

Historical Background and Significance

The medicinal and recreational properties of *Cannibis Sativa*, also known as marijuana or hemp have been known for many years. Asian cultures cultivated cannabis ten thousand years ago (Grispoon 1993; Azad and Rammes 2005). Indian, Chinese, Middle Eastern, South African as well as South American cultures used cannabis to treat different maladies such as malaria, constipation, rheumatic pains and absentmindedness (Grispoon 1993). However, it was not until the 19th century that western culture, specifically the United States, prescribed cannabis to treat pain, glaucoma, spasms and diminished appetite (Grispoon 1993; Azad and Rammes 2005). Cannabis use declined by the late 19th century because the potency of cannabis was variable and the development of synthetic drugs offered more stable drug delivery, but consequently, synthetic drugs had more adverse side effects than cannabis (Grispoon 1993). Increased recreational use of marijuana and the concern of the general public over the psychoactive effects associated with marijuana, led to its classification as a schedule 1 drug and outlawed marijuana from public use with the exception of research aimed at studying the medicinal properties associated with marijuana (Grispoon 1993).

There are 460 identified chemical compounds in the marijuana plant. Approximately, 60 of these chemical compounds are considered cannabinoids. Cannabinoids include chemical compounds derived from the marijuana plant which bind to cannabinoid receptors. Cannabinoids are implicated in pain modulation and suppress nociceptive processing at the level of the central nervous system (CNS) (Welch and Stevens 1992; Hohmann et al. 1995; Hohmann et al. 1998; Martin et al. 1998; Hohmann et al. 1999) as well as the periphery (Jaggar et al. 1998; Richardson et al. 1998b; Hohmann 2002; Nackley et al. 2003b; Nackley et al. 2004). The major active ingredient of the marijuana plant, Δ^9-tetrahydrocannabinol (Δ^9-THC) was isolated in 1964 (Gaoni and Mechoulam 1964). Preliminary evidence for the potential involvement of a cannabinoid receptor mediating cannabinoid drug action was provided by the

discovery that Δ^9-THC inhibited adenylate cyclase (Howlett and Fleming 1984; Howlett et al. 1986). It was later discovered that inhibition of adenylate cyclase involved the presence of a guanine nucleotide-binding protein complex, G_i (Howlett and Fleming 1984; Howlett et al. 1986). The identification of a cannabinoid receptor in the rat brain provided further evidence that the effects of cannabinoids were mediated through a G-protein coupled receptor mechanism (Devane et al. 1988). The anatomical distribution of cannabinoid receptors in the central nervous system (Herkenham et al. 1991; Tsou et al. 1998) and in the immune system (Lynn and Herkenham 1994), has provided the basis for the profound pharmacological effects produced by cannabinoids. Finally, cannabinoid receptors have been identified in key regions implicated in pain such as the periaqueductal gray (PAG) and spinal cord (Herkenham et al. 1991; Tsou et al. 1998).

Identification of Endogenous Cannabinoid Ligands

Several putative endogenous cannabinoid ligands have been identified in the brain. The two most studied endogenous ligands are arachidonylethanolamide (anandamide) and 2-arachidonoylgycerol (2-AG). Anandamide was isolated in the porcine brain (Devane et al. 1992) and binds to both cannabinoid CB_1 (Devane et al. 1992; Felder et al. 1993; Vogel et al. 1993) and CB_2 receptors (Munro et al. 1993). Anandamide preferentially binds to CB_1 (K_i = 89 vs. 371 nM) (Zygmunt et al. 1999; Smart et al. 2000; Gauldie et al. 2001). 2-AG, a naturally-occurring 2-monoacylglycerol was first isolated from the canine gut. 2-AG inhibits adenylate cyclase production (Mechoulam et al. 1995), and activates both CB_1 and CB_2 receptors (Mechoulam et al. 1995; Sugiura et al. 1995). 2-AG is present in the brain 170 times greater than anandamide (Stella et al. 1997). Recent work suggests that endogenous 2-AG suppresses pain at the level of the PAG (Hohmann et al. 2005). Effects of synthetically derived CB_1 and CB_2 receptor agonists on pain processing and pain behavior have been evaluated to elucidate functions of the endocannabinoid system.

CB_1 receptor subtype

CB_1 is expressed in the central nervous system (CNS) (Matsuda et al. 1990; Munro et al. 1993; Zimmer et al. 1999), is synthesized neuronally in cells of the dorsal root ganglia (Hohmann and Herkenham 1999a; b; Ahluwalia et al. 2002; Bridges et al. 2003) and is transported to peripheral terminals (Hohmann and Herkenham 1999a) where it may contribute to peripherally-mediated antihyperalgesic effects. CB_1 is abundantly expressed in the basal ganglia, hippocampal formation and olfactory bulbs (Herkenham et al. 1991; Tsou et al. 1998) and is also expressed in the periaqueductal gray (PAG) and spinal cord (Herkenham et al. 1991). CB_1 is negatively coupled to adenylyl cyclase, via Gi/o (Howlett et al. 1986; Felder et al. 1995). The CB_1 receptor subtype is negatively coupled to N and P/Q-type calcium channels and positively coupled to inward rectifying potassium (Felder et al. 1995; Mackie et al. 1995) and potassium A channels (Deadwyler et al. 1995).

CB_2 receptor subtype

CB_2 is expressed predominantly, but not exclusively (Azad and Rammes 2005; Van Sickle et al. 2005), outside the central nervous system (Munro et al. 1993; Zimmer et al. 1999; Buckley et al. 2000) and is most prevalent in cells of the immune system (Lynn and Herkenham 1994). CB_2 is expressed in immune tissues 10-100 fold higher than CB_1 (Galiegue et al. 1995). Immune tissues that highly express CB_2 include the marginal zone of the spleen, tonsil, monocytes, and B and T cells (Munro et al. 1993; Galiegue et al. 1995; Schatz et al. 1997). Unlike CB_1 receptor activation, CB_2 receptor activation does not regulate calcium conductance (Felder et al. 1995). Furthermore, CB_2 receptor protein has recently been identified in microglial cultures of rat spinal cord derived from neonatal rats (Beltramo et al. 2006), suggesting the existence of additional nonneuronal substrates capable of mediating antihyperalgesic actions. CB_2 mRNA has also been localized in the brainstem and cortex (Van Sickle et al. 2005), suggesting that elevated levels of endocannabinoids may engage central CB_2 receptors to alter neuronal physiology.

CB_1 and CB_2-selective agonists and antagonists (cannabinoid) receptor pharmacology

The development of subtype-selective cannabinoid agonists and antagonists has provided pharmacological tools required to assess the role of CB_1 and CB_2 in nociception. The CB_1-selective agonist ACEA binds to the CB_1 receptor with high affinity (K_i =1.4 ± 0.3 nM) and binds to the CB_2 receptor with low affinity (K_i = 3.1 ± 1.0 μM) (Hillard et al. 1999). The non-selective CB_1/CB_2 agonist WIN55-212-2 binds to cannabinoid receptors in both rat brain (K_i = 9.94 ± 1.04 nM) and spleen (K_i = 16.2 ± 5.5 nM) (Rinaldi-Carmona et al. 1994). The CB_1/CB_2-selective agonist CP55,940 binds to cannabinoid receptors in rat brain (K_i = 1.37 ± 0.43 nM) and spleen (Ki = 1.37 ± 0.38 nM) with high affinity (Rinaldi-Carmona et al. 1994). The prototypic cannabinoid agonist Δ^9-tetrahydrocannabinol (Δ^9-THC) binds to receptors in rat brain (Ki = 1.98 ± 0.36 nM) and spleen (Ki = 3.90 ± 0.95 nM) (Rinaldi-Carmona et al. 1994). The CB_2-selective agonist AM1241 exhibits a 90-340-fold selectivity for CB_2 over CB_1 in vitro (Malan et al. 2001). The CB_1 antagonist/inverse agonist SR141716A binds to cannabinoid receptors in rat brain with high affinity (K_i = 2 nM) and displays low affinity for rat spleen or cloned human CB_2 receptors (Ki > 1000 nM) (Rinaldi-Carmona et al. 1995; Showalter et al. 1996). By contrast, the CB_2 antagonist SR144528 shows high affinity for rat spleen and cloned human CB_2 receptors (K_i = 0.6 nM) but has a 700-fold lower affinity (K_i = 400 nM) for rat brain or cloned human CB_1 receptors (Rinaldi-Carmona et al. 1998).

CB_1-mediated antihyperalgesic effects

The antinociceptive effects of CB_1 are well established. Local hindpaw administration of anandamide suppresses thermal hyperalgesia in the carrageenan model of inflammation and inhibits capsaicin induced plasma extravasation via a CB_1 mechanism (Richardson et al. 1998b). Similarly, intrathecal administration of anandamide blocks carrageenan induced thermal hyperalgesia (Richardson et al. 1998a). Administration of the non-selective CB_1/CB_2 agonist WIN55,212-2 to the nucleus reticularis gigantocelluraris (GiA) increased tail flick withdrawal latencies in control animals and decreased responses in the formalin test (Monhemius et al.

2001). In animals with partial sciatic nerve ligation, administration of the CB_1 antagonist SR141716A increased pain related responses due to injection of formalin, indicating the involvement of CB_1 (Monhemius et al. 2001). In an animal model of cutaneous heat injury, the antihyperalgesic effects induced by WIN55,212-2 are attenuated by the CB_1 antagonist AM251, again indicating a role for CB_1 in antinociception (Johanek and Simone 2004). Intrathecal administration of WIN55,212-2 attenuated thermal and mechanical hyperalgesia induced by intraplantar injection of capsaicin through a CB_1 mechanism (Johanek et al. 2001). Local administration of WIN55,212-2 also suppresses carrageenan-evoked Fos protein expression and mechanical and thermal hyperalgesia through both CB_1 and CB_2-specific mechanisms (Nackley et al. 2003b). Electrophysiological studies indicate that C-fiber-mediated responses and windup are suppressed in spinal wide dynamic range neurons through activation of CB_1 (Strangman and Walker 1999; Drew et al. 2000; Kelly and Chapman 2001) receptor mechanisms.

CB_2-medidated antihyperalgesic effects

The mechanisms underlying the antihyperalgesic actions of CB_2 are not clearly understood. Intraplantar and systemic administration of the CB_2-selective agonist AM1241 increases paw withdrawal latencies to thermal stimulation of the paw in naive rats by a CB_2-mediated mechanism (Malan et al. 2001). Intraplantar administration of the CB_2 antagonist AM630 blocked the effects produced by AM1241 (Malan et al. 2001). Activation of CB_2 receptors suppresses the development of inflammation-evoked mechanical and thermal hyperalgesia (Clayton et al. 2002; Nackley et al. 2003a; Quartilho et al. 2003; Hohmann et al. 2004) and Fos protein expression, a marker of neuronal activity (Nackley et al. 2003a). Activation of CB_2 receptors also suppresses the maintenance of inflammatory pain (Quartilho et al. 2003; Elmes et al. 2005; Gutierrez et al. 2007). Systemic administration of the CB_2 agonist JWH-133 also suppresses inflammation-evoked decreases in hindpaw weight bearing and oedema after the establishment of inflammatory pain (Elmes et al. 2005). Administration of

anandamide inhibits innocuous and noxious mechanically-evoked responses of spinal neurons through a CB_2 mechanism since co-administration of anandamide with the CB_2 antagonist SR144528 blocked the inhibitory effects produced by anandamide on neuronal responses (Sokal et al. 2003). In addition, C-fiber-mediated responses and windup are suppressed in spinal wide dynamic range neurons through activation CB_2-mediated receptor mechanisms (Nackley et al. 2004). CB_2 agonists also suppress capsaicin-evoked release of calcitonin gene-related peptide in rat spinal cord *in vitro* (Beltramo et al. 2006), suggesting a likely neuronal mechanism of action. CB_2 receptor protein was recently identified in microglial cultures of rat spinal cord derived from neonatal rats (Beltramo et al. 2006), suggesting the existence of additional nonneuronal substrates capable of mediating antihyperalgesic actions. Activation of CB_2 receptors on nonneuronal cells has also been postulated to suppress the release of inflammatory mediators which excite nociceptors (Mazzari et al. 1996). Furthermore, activation of CB_2 receptors on skin keratinocytes stimulates production of β-endorphin to induce antinociception through activation of μ-opioid receptors (Ibrahim et al. 2005). Finally, expression of CB_2 is also markedly upregulated in dorsal root ganglia and spinal cord following sciatic nerve injury (Zhang et al. 2003; Wotherspoon et al. 2005; Beltramo et al. 2006), whereas expression levels remain near the threshold for detection in naive animals. Thus, several distinct mechanisms may contribute to antihyperalgesic actions of CB_2 agonists.

Neuropathic Pain

Neuropathic pain is caused by multiple etiological factors, involves multiple mechanisms, and is present across many diseases (Decosterd and Woolf 2000). The most pronounced feature of neuropathic pain is nerve injury (Decosterd and Woolf 2000). Neuropathic pain is spontaneous (without a cause), may produce burning pain, and produces hypersensitivity to different stimuli (Decosterd and Woolf 2000). Tactile allodynia (hypersensitivity to innocuous mechanical stimulation) and pin prick hyperalgesia is often expressed (Decosterd and Woolf

2000). Animal models of pain with partial nerve denervation can be utilized to better understand the symptoms and mechanisms associated with neuropathic pain.

Cannabinoids attenuate neuropathic pain in animal models. Most animal models of neuropathic pain involve some type of nerve injury. Administration of the non-selective CB_1/CB_2 agonist WIN55,212-2 suppresses thermal, mechanical hyperalgesia and allodynia in neuropathic rats through a CB_1-specific mechanism (Herzberg et al. 1997). Fox and colleagues, evaluated the effects of WIN55,212-2 on hyperalgesia in neuropathic pain induced by partial ligation of the sciatic nerve (Fox et al. 2001). WIN55,212-2 was effective at reversing mechanical hyperalgesia through different routes of administration whereas thermal hyperalgesia was reversed by subcutaneous administration of WIN55,212-2 (Fox et al. 2001). The antihyperalgesic effects of intraplantar administration of WIN55,212-2 were blocked by subcutaneous administration but not intrathecal administration of the CB_1 antagonist SR141716A (Fox et al. 2001). However, no CB_2 antagonist was administered to test the possible involvement of CB_2 receptors.

The CB_2-selective agonist AM1241, administered systemically, reversed tactile allodynia and thermal hyperalgesia in rats with spinal ligation of the L5/L6 nerves (Ibrahim et al. 2003). AM1241 effects were blocked by the CB_2 antagonist AM630 but not the CB_1 antagonist AM251 suggesting that the effects were CB_2 mediated (Ibrahim et al. 2003). Systemic administration of WIN55,212-2 also reverses cold and mechanical allodynia and thermal hyperalgesia in an animal model of neuropathic pain (Bridges et al. 2001). However, a high dose of WIN55,212-2 was needed to reverse mechanical hyperalgesia. Effects were blocked by a CB_1 antagonist but not a CB_2 antagonist (Bridges et al. 2001). Intraplantar administration of the CB_2-selective agonist JWH-133 suppresses innocuous and noxious mechanically evoked responses of WDR neurons in neuropathic rats (Elmes et al. 2004). The CB_2 antagonist SR144528 attenuated the inhibitory effects produced by JWH-133 on mechanically evoked responses of WDR neurons in neuropathic rats indicating the possibility that CB_2 receptors may be present on primary afferent

fibres (Elmes et al. 2004). In another study, the CB_2 agonist JWH-133 and the CB_1 agonist ACEA attenuated capsaicin-evoked responses in neuropathic and sham-operated rats; effects which were inhibited by the CB_2 antagonist SR144528 and the CB_1 antagonist SR141716A, respectively (Sagar et al. 2005). Similarly, spinal administration of JWH-133 or ACEA attenuated mechanically-evoked responses of dorsal horn neurons in neuropathic rats (Sagar et al. 2005). Therefore, cannabinoids may suppress some of the symptoms associated with neuropathic pain through peripheral and central mechanisms.

CHAPTER 2: INTRODUCTION

Neuropathic pain (normally due to nerve injury) closely mimics human clinical pain induced by partial nerve injury (Woolf and Mannion 1999; Decosterd and Woolf 2000; Woolf 2004). Neuropathic pain is associated with spontaneous (without a cause) pain and hypersensitivity to normally innocuous stimuli (Decosterd and Woolf 2000). Currently, there are no effective pharmacological interventions that alleviate the symptoms associated with neuropathic pain, making the discovery of alternative analgesics an urgent medical need. Patients with neuropathic pain do not typically respond to non-steroidal anti-inflammatory drugs or to opiates (Woolf and Mannion 1999). Opiates seem to lack potent analgesic efficacy in neuropathic pain states making their use in treating neuropathic pain questionable (Przewlocki and Przewlocka 2001). For example, morphine is beneficial in some but not in all patients with neuropathic pain (Przewlocki and Przewlocka 2001). In addition, it has been shown that the efficacy of intrathecal morphine is reduced in rats with nerve injury presumably because of a loss of presynaptic opioid receptors (Ossipov et al. 1995). Therefore, the cannabinoid system may offer a potential therapeutic alternative for the treatment of neuropathic pain since previous studies have demonstrated that cannabinoids are implicated in suppressing neuropathic pain.

Clinical studies suggest that cannabinoids or cannabis-derived plant extracts, offer promise for decreasing symptoms associated with neuropathic pain (Wade et al. 2003; Burstein et al. 2004; Svendsen et al. 2004; Corey 2005; Rog et al. 2005). Sublingual administration of whole plant extracts of Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), or a combination of CBD:THC improve neurogenic symptoms in patients with multiple sclerosis, spinal cord injury, brachial plexus damage, and in a patient with phantom limb pain. Pain reduction was assessed by the visual analogue scale (VAS) (Wade et al. 2003). Similarly, oromucosal THC:CBD decreased pain intensity in MS patients experiencing central pain (Rog et al. 2005). Central pain in this study was defined as being caused by a primary lesion to the CNS, where pain is described as nonparoxysmal with burning and pricking qualities (Rog et al. 2005). Oral

administration of the synthetic cannabinoid dronabinol (Δ^9-tetrahydrocannabinol), also shows a modest reduction in spontaneous pain intensity in patients with multiple sclerosis (Svendsen et al. 2004). The synthetic cannabinoid CT-3 has also shown promising results in decreasing pain in patients with neuropathic pain (Karst et al. 2003). In these clinical studies, the adverse side effects associated with cannabis derived plant extracts or cannabinoids were minimal and tolerable by patients at the drug doses administered (Wade et al. 2003; Svendsen et al. 2004; Rog et al. 2005). Together, these clinical studies indicate that the cannabinoid system is effective in suppressing neuropathic pain.

Two cannabinoid receptors subtypes, CB_1 and CB_2, have been implicated in suppressing pain. Cannabinoid agonists suppress pain related behavior in different animal models (Calignano et al. 1998; Martin et al. 1998; Hanus et al. 1999; Ko and Woods 1999; Malan et al. 2001). Peripheral administration of exogenous anandamide, a putative ligand for cannabinoid receptors, suppresses the development and maintenance of carrageenan-evoked thermal hyperalgesia through a CB_1 mechanism (Richardson et al. 1998b). CB_1 is primarily expressed in the central nervous system (CNS) (Matsuda et al. 1990; Munro et al. 1993; Zimmer et al. 1999), is synthesized in cells of the dorsal root ganglia (Hohmann and Herkenham 1999a; b; Ahluwalia et al. 2002; Bridges et al. 2003) and is transported to peripheral terminals (Hohmann and Herkenham 1999a) where it may contribute to peripherally-mediated antihyperalgesic effects. Consistent with this receptor distribution, electrophysiological studies indicate that C-fiber mediated responses and windup are suppressed in spinal wide dynamic range neurons through activation of CB_1 (Drew et al. 2000; Kelly et al. 2003). Although CB_1 activation has both antinociceptive and antihyperalgesic effects, CB_1 receptor activation is also associated with CNS side-effects such as catalepsy, hypothermia and hypoactivity that constrain therapeutic dosing (Zimmer et al. 1999).

In contrast to CB_1, which is readily associated with psychoactivity induced by cannabinoid actions at CNS receptors (Matsuda et al. 1990; Munro et al. 1993; Zimmer et al.

1999), CB_2 is expressed predominantly, but not exclusively (Van Sickle et al. 2005; Beltramo et al. 2006), outside the central nervous system (CNS). CB_2 is most prevalent in cells of the immune system (Lynn and Herkenham 1994) and unlike CB_1, activation of CB_2 is devoid of CNS side-effects (Hanus et al. 1999; Malan et al. 2001; Gutierrez in press). AM1241, a CB_2-selective agonist, when applied peripherally and systemically, induces a CB_2-mediated antinociception in rats (Malan et al. 2001). Furthermore, activation of CB_2 receptors suppresses the development of inflammation-evoked mechanical and thermal hyperalgesia (Clayton et al. 2002; Nackley et al. 2003a; Quartilho et al. 2003; Hohmann et al. 2004) and Fos protein expression, a marker of neuronal activity (Nackley et al. 2003a). Systemic administration of the CB_2 agonist JWH-133 also suppresses inflammation-evoked decreases in weight bearing and peripheral edema (Elmes et al. 2005).

The antihyperalgesic mechanism of CB_2 remains poorly understood. CB_2 receptors were initially detected in cultures of neonatal dorsal root ganglion cells (Ross et al. 2001). Activation of CB_2 on nonneuronal cells also suppresses the release of inflammatory mediators implicated in nociception (Mazzari et al. 1996) and stimulates production of β-endorphin on skin kerationcytes to induce antinociception (Ibrahim et al. 2005). New evidence indicates that CB_2 is not expressed exclusively on immune cells. CB_2 is markedly upregulated in dorsal root ganglia and spinal cord following sciatic nerve injury (Zhang et al. 2003; Wotherspoon et al. 2005; Beltramo et al. 2006), whereas expression levels remain near threshold for detection in naive animals. Therefore, it is possible that CB_2 receptors may be activated by elevated levels of endocannabinoids that also act at CB_1 receptors (Van Sickle et al. 2005). CB_2 is expressed in the spinal cord of neuropathic rats but not in rats with inflammation, indicating a role for CB_2 that may be exclusive to neuropathic pain states (Zhang et al. 2003). AM1241, a CB_2-selective agonist, reverses tactile and thermal hypersensitivity in rats with spinal nerve ligation (Ibrahim et al. 2003). In another study, peripheral application of a CB_2 agonist suppressed innocuous and noxious mechanically-evoked responses of wide dynamic range (WDR) neurons in control rats

as well as in inflamed and neuropathic rats (Elmes et al. 2004). Spinal administration of the CB_2 agonist, JWH-133 also attenuates mechanically evoked responses in neuropathic but not in sham operated rats (Sagar et al. 2005). These effects were CB_2-mediated since they were blocked by the CB_2 antagonist SR145528 (Sagar et al. 2005). JWH-133 also attenuated capsaicin-evoked calcium responses in DRG neurons of neuropathic and sham rats (Sagar et al. 2005). CB_2 agonists also suppress calcitonin gene-related-peptide (CGRP) in rat spinal cord *in vitro* (Beltramo et al. 2006), suggesting a neuronal mechanism of action. Finally, CB_2 receptor protein has been identified in microglial cultures of neonatal rat spinal cord (Beltramo et al. 2006), suggesting the existence of additional noneuronal substrates capable of mediating antihyperalgesic actions. Together these studies suggest that multiple sites of action may be implicated in the antihyperalgesic effects of CB_2 agonists.

Studies employing mixed CB_1/CB_2 agonist preferentially implicate a role for CB_1 in suppressing neuropathic nociception. Systemic administration of the non-selective CB_1/CB_2 agonist WIN55,212-2 also suppresses thermal, mechanical hyperalgesia and allodynia in neuropathic rats (Herzberg et al. 1997). These effects were blocked by a CB_1 antagonist, although a CB_2 antagonist was not assessed (Herzberg et al. 1997). Similarly, systemic administration of WIN55,212-2 dose dependently reverses cold, thermal, and tactile allodynia in animals with spinal nerve ligation (Bridges et al. 2001). Effects were blocked by a CB_1 but not a CB_2 antagonist (Bridges et al. 2001). Subcutaneous administration of WIN55,22-2 also reverses thermal and mechanical hyperalgesia in the spared nerve injury model of neuropathic pain (Fox et al. 2001). Mechanical hyperalgesia was blocked by subcutaneously administration of the CB_1 antagonist SR141716A. However, no CB_2 antagonist was tested to see if these effects also involved a CB_2 mechanism (Fox et al. 2001).

The present studies were conducted to evaluate whether animals with neuropathic pain will self-administer a cannabinoid CB_2 agonist to alleviate a chronic pain state induced by nerve injury. We compared the extent to which neuropathic animals would reliably self-administer the

CB_2-selective agonist AM1241 relative to control animals (naive and sham-operated rats), in order to decrease neuropathic pain. Tactile allodynia was assessed in all animals by measuring paw withdrawal thresholds prior to and after each drug self-administration session to determine if cannabinoid drug self-administration was sufficient to decrease some of the symptoms associated with neuropathic pain. Finally, we compared self-administration of a cannabinoid CB_2 agonist (AM1241) and a prototypical narcotic analgesic (morphine) in the absence and presence of nerve injury to assess whether self-administration of AM1241 was more efficacious at suppressing neuropathic pain relative to self-administration of morphine. We hypothesized that animals subjected to a spared nerve-injury would reliably self-administer AM1241 to suppress a neuropathic pain state. We also hypothesized that self-administration of AM1241 would be absent or minimal in the absence of spared nerve injury. We also evaluated whether doses of AM1241 that are self-administered by neuropathic or naïve animals would lack CNS side-effects (i.e. motor ataxia) associated with activation of CB_1. The present studies suggest that it may be possible to separate antihyperalgesic efficacy and abuse liability through selective activation of cannabinoid CB_2 receptors.

CHAPTER 3: MATERIALS AND METHODS

Subjects

One hundred and fourteen male Sprague-Dawley rats weighing 275-300g at the beginning of the experiment (Harlan, Indianapolis, IN) were used. Animals were housed in a temperature control room with lights kept in a reverse 12-h light schedule. All procedures were approved by the University of Georgia Animal Care and Use Committee and followed the guidelines for the treatment of animals of the International Association for the Study of Pain (Zimmermann 1983).

Drugs and Chemicals

AM1241, a potent CB_2-selective agonist, was synthesized (by Alexander Zvonok) in the laboratory of Dr. Alexandros Makriyannis. Morphine sulfate was purchased from Sigma. SR141716 and SR144528 were provided by NIDA. A 1.5% heparin saline solution was prepared to daily flush jugular catheters and to dissolve drugs. Drugs were dissolved in 7.5% ethanol, 7.5% tween 80, 85% heparinized saline solution for intravenous (i.v.) drug self-administration or were dissolved in vehicle containing emulphor, ethanol, saline (1:1:18) for systemic (i.p.) administration of SR141716 (1 mg/kg) or SR144528 (1 mg/kg).

Spared Nerve Injury

The spared nerve injury was performed as described previously (Decosterd and Woolf 2000). An incision was performed directly on the biceps femoris muscle and the sciatic nerve with its three terminal branches were isolated: the sural, common peroneal and tibial nerves. The tibial and the common peroneal nerve were ligated with 4.0 silk thread and cut 2-4 mm and the sural nerve was left intact (Decosterd and Woolf 2000). In sham rats, the sciatic nerve and terminal branches were exposed but all nerves were left intact. Jugular catheters were implanted in otherwise naive rats in which the nerves were not exposed.

Jugular Catheterization

All rats were surgically implanted with an indwelling jugular catheter regardless of experimental manipulation. Rats were anesthetized with halothane and catheters were prepared as described previously (Ahmed and Koob 1997). A catheter containing a bent cannula and silastic tubing was encased with dental cement. The cannula was passed through the skin of the back of the rat and 3.5 cm of the silastic tubing was inserted into the right external jugular vein. Catheters were flushed daily with 1.5% heparinized saline to maintain catheter potency. Rats were allowed to recover 4-5 days after jugular catheterization.

General Experimental Methods

Baseline mechanical withdrawal thresholds were assessed in all rats one day prior to surgical manipulations. Post-surgical thresholds were also assessed in all rats four to five days after surgical manipulations to confirm that spared nerve injury suppressed mechanical paw withdrawal thresholds relative to baseline (pre-surgery) levels and to verify that sham surgery did not alter mechanical withdrawal thresholds relative to baseline. Spared nerve injury rats that did not exhibit mechanical hyperalgesia, post-surgery, were not used in the study. Mechanical paw withdrawal thresholds were assessed in all rats before (pre-session) and after (post-session) each drug self-administration session. Rats received a 50 min drug self-administration session in which each lever press on the active lever resulted in a drug infusion on each of five consecutive days. Immediately after the drug self-administration session, rats were removed from the drug self-administration chambers, placed on a wire mesh platform.

Operant training procedure

Rats were pre-trained to lever press for food on two levers prior to surgical manipulations. Food was restricted to 15 g per day for 4-5 days prior to training and until the training procedure was completed. Rats were individually placed in the operant chambers and tested once per day. The first pre-training session consisted of one 30 min session in which one food pellet was automatically delivered every 60 seconds. The number of head insertions into

the food trough was recorded during this 30 min session. Rats were then trained to lever press in an operant box containing two levers (referred to as left and right levers). Each lever press resulted in one pellet reinforcement. Rats received 3-4 training sessions. During this training period, one lever was exposed and the other lever remained retracted. Once the exposed lever was pressed 10 times, the opposite lever would then be exposed and the other lever would retract. This pattern was repeated until the rat reach a criterion of 60 pellets earned within the training session.

Drug self-administration apparatus

The operant chambers were equipped with two levers. Lever presses on the left (active) lever elicited the intravenous infusion whereas lever presses on the right (inactive) lever failed to elicit the infusion. Separate group of animals were tested under the same parameters, except that the active and inactive levers were switched; lever presses on the right (active) lever elicited the intravenous infusion whereas lever presses on the left (inactive) lever failed to elicit an infusion. In all cases, when the rat pressed the active lever, a Harvard, infusion pump (Holliston, MA) was switched on approximately for 6 seconds based on the rat's body weight and resulted in one drug infusion. Three doses of AM1241 were tested in pilot studies (60, 30, 15 μg/kg/infusion); the middle dose was selected for all subsequent experiments because maximal self-administration of AM1241 was observed at this dose. The morphine dose was (100 μg/kg/infusion). The pump delivered approximately (6-8 μl) of AM1241 (1.5 μg/μl), morphine (5 μg/μl) or Vehicle. A light turned on when the rat pressed the active lever and remained on for the duration of the drug infusion. The drug infusion was followed by a 5-sec time out period, during which, pressing either the active or the inactive lever did not result in another drug infusion. Pressing the left (inactive) lever produced no result.

Drug self-administration training procedure

In week one, prior to any operant training, rats were handled daily and had free access to food and water. In week two, rats were food restricted (15g per day) to reduce the animal's body weight by 20% to facilitate lever training. In week three, the food restricted rats were trained to lever press for food. In week four, surgical manipulations were carried out and rats were no longer food restricted for the rest of experiment. Rats were then given approximately five days to recover from surgical procedures. Finally, rats were given an extra training session to lever press for food to ensure that surgical procedures did not disrupt the rats ability to accurately perform the lever pressing response. Rats were then switched to intravenous (i.v.) drug self-administration sessions. Weights were monitored daily in all rats.

Assessment of Tactile Allodynia

Tactile allodynia was assessed using a digital electrovonfrey anesthesiometer (IITC model Alemo 2290-4; Woodland Hills, CA) equipped with a rigid tip. Rats were placed underneath plastic cages and positioned on an elevated mesh platform. Rats were allowed to habituate to the testing apparatus for 15 min prior to testing. Stimulation was applied to the midplantar region of the hind paw through the floor of the mesh platform. Withdrawal thresholds to punctate mechanical stimulation were measured in duplicate for the left (ipsilateral to spared nerve injury or sham surgery) and the right (intact) paw. Mechanical stimulation was terminated upon paw withdrawal. The electrovonfrey was capable of applying a maximum force of 250g; consequently there was no upper threshold which imposed termination of any trial. Baseline responses to mechanical stimulation were evaluated before surgery and after surgery. Paw withdrawal thresholds were additionally evaluated before and after (10-15 min post session) each drug self-administration session.

Assessment of vehicle self-administration on mechanical withdrawal thresholds

Effects of vehicle self-administration on paw withdrawal thresholds and lever pressing behavior was evaluated in separate groups of naive, sham and neuropathic rats for 5

consecutive days. Mechanical paw withdrawal thresholds were assessed in each paw in duplicate before and after every experimental session as described previously.

Assessment of AM1241 self-administration on mechanical paw withdrawal thresholds

Effects of AM1241 (1.5 µg/µl) self-administration on paw withdrawal thresholds and lever pressing behavior was evaluated in separate groups of sham and neuropathic rats for 5 consecutive days. Pilot experiments verified that AM1241 self-administration was greater in neuropathic animals with the (1.5 µg/µl) dose relative to either the lower (0.75 µg/µl) or higher (3 µg/µl) doses. Mechanical paw withdrawal thresholds were assessed in each paw in duplicate before and after every experimental session as described previously.

Pharmacological Specificity: Assessment of AM1241 self-administration after systemic administration of SR141716 or SR144528

To determine pharmacological specificity of AM1241 self-administration and consequent changes in mechanical paw withdrawal thresholds, separate groups of neuropathic or naive rats received SR141716 (1 mg/kg i.p.) or SR144528 (1 mg/kg i.p.) 30 min prior to each drug self-administration session. Effects of AM1241 (1.5 µg/µl) self-administration on paw withdrawal thresholds and lever pressing behavior was evaluated in neuropathic and naive rats over 5 consecutive days. Mechanical paw withdrawal thresholds were assessed in each paw in duplicate before and after every experimental session as described previously.

Assessment of Vehicle self-administration after systemic administration of SR141716

Effects of SR141716 (1 mg/kg i.p.), administered 30 min prior to each drug self-administration session, on paw withdrawal thresholds and lever pressing behavior was evaluated in neuropathic and naive rats allowed to self-administer the vehicle. Testing was conducted over 5 consecutive days. Mechanical paw withdrawal thresholds were assessed in each paw in duplicate before and after every experimental session as described previously.

Assessment of Morphine self-administration on mechanical withdrawal thresholds

Effects of morphine (5 μg/μl) self-administration on paw withdrawal thresholds and lever pressing behavior was evaluated in neuropathic, sham and naive rats over 5 consecutive days. Mechanical paw withdrawal thresholds were assessed in each paw in duplicate before and after every experimental session as described previously.

Assessment of Motor Ataxia

Effects of intravenous administration of the CB_2-selective agonist AM1241 and a reference cannabinoid, the mixed CB_1/CB_2 agonist WIN55,212-2, on motor ataxia were evaluated in spared nerve injury and naive rats. Animals used in self-administration experiments received a one week washout period prior to rotarod assessments; during this interval no injections were administered with the exception of daily flushing of catheters with heparinized saline. Animals were required to walk on an accelerating rotating drum as described previously (Fox et al. 2001). Baseline responses were initially determined 24 h prior to the experimental manipulation. On the second day, baseline performance in the rota-rod was again assessed in all animals prior to intravenous drug self-administration of the maximally self-administered dose of AM1241 (900 μg, i.v.), a dose of WIN55,212-2 known to impair motor behavior (0.5 mg/kg, i.v.) or vehicle. Animals were randomly assigned to the drug condition. Latencies to descend from the rotarod were subsequently assessed 5, 15, 30, 45 and 60 min post drug administration.

Statistical Analysis

Behavioral data were analyzed parametrically using Analysis of Variance (ANOVA) for repeated measures, and ANOVA. ANOVA and planned comparison t-tests were used to assess the statistical significances of experimental differences between the drug self-administration groups for the entire 50 min drug self-administration interval. The number of active and inactive lever presses was compared for each drug self-administration group. Post hoc comparisons were performed for the behavioral and drug self-administration groups using Fisher's protected least significant difference (PLSD). The Greenhouse-Geisser was applied to all repeated factors

to avoid spurious significance due to lack of homogeneity of variance and covariance in repeated factors (Greenhouse and Geisser 1959). $P < 0.05$ was considered to be statistically significant.

CHAPTER 4: RESULTS

General effects of spared nerve injury

Spared nerve injury lowered mechanical paw withdrawal threshold in neuropathic groups relative to both naive and sham-operated groups ($F_{2,111} = 11138.213$, $P < 0.0002$; $P < 0.0002$ for all comparisons) (Fig. 1a). Spared nerve injury also decreased paw withdrawal thresholds in the left (injured) paw compared to pre-surgery thresholds ($F_{2,111} = 9552.843$, $P < 0.0002$) (Fig. 1a). By contrast, surgery did not alter paw withdrawal thresholds in either the left or right paws of naive or sham-operated groups (Fig.1a-b). Paw withdrawal thresholds in the right (intact) paw were similar between groups and were not affected by the surgical manipulations in any group (Fig 1b). A modest but reliable increase in paw withdrawal threshold was observed in both the left ($F_{2,111} = 5.175$, $P < 0.007$; $P < 0.03$ for each comparison) and right ($F_{2,111} = 6.972$, $P < 0.001$; $P < 0.01$) paws of naive rats relative to either sham or neuropathic groups (Fig. 1a-1b).

Effects of vehicle self-administration on mechanical withdrawal thresholds in naive, sham and neuropathic groups

Paw withdrawal thresholds observed in either the left (ipsilateral to spared nerve injury or sham surgery; Fig. 2a-b) or right (intact; Fig. 2c-d) paw after each drug self-administration session did not change from pre-session levels in naive, sham or neuropathic groups self-administering vehicle on any day. Paw withdrawal thresholds remained sensitized in neuropathic animals self-administering vehicle relative to naive or sham-operated groups both before ($F_{2,17} = 33124.892$, $P < 0.001$; $P < 0.001$ for each comparison) and after ($F_{2,17} = 23912.166$, $P < 0.001$; $P < 0.001$ for each comparison) each drug self-administration session (Fig. 2a-b). Paw withdrawal thresholds in either the left (ipsilateral to sham surgery) or right (contralateral to sham surgery) paws of sham-operated rats, assessed either pre- or post-session, were slightly higher relative to naive or neuropathic rats similarly self-administering vehicle ($P < 0.001$) (Fig. 2a-d).

Naive, sham-operated and neuropathic animals allowed to self-administer vehicle failed to respond differentially on either the active or inactive lever on test day 1 (Fig. 3). By day five, the last day on which self-administration data was collected, the number of active and inactive lever presses were minimal and non-significant in all groups self-administering vehicle. For all groups, lever press data is presented for day 1, the day of maximum change in threshold and lever press responses, following AM1241 administration.

Effects of AM1241 self-administration on mechanical withdrawal thresholds in sham and neuropathic groups

Paw withdrawal thresholds on the left (injured) paw were lower in neuropathic animals prior to each AM1241 self-administration session compared to the sham-operated group ($F_{1,15} = 55497.181$, $P < 0.0002$; $P < 0.0002$ for each comparison) (Fig. 4a). Self-administration of AM1241 increased paw withdrawal thresholds in the left (injured) paw in the neuropathic group ($F_{1,13} = 88.632$, $P < 0.001$; $P < 0.001$ for all comparisons), consistent with an AM1241-induced attenuation of neuropathic nociception (Fig. 4b). AM1241 self-administration increased paw withdrawal thresholds in the neuropathic group after every experimental session ($F_{4,52} = 4.931$, $P < 0.01$; $P < 0.001$ for all comparisons) (Fig. 4b). By contrast, changes in paw withdrawal thresholds were absent in sham-operated rats after each drug self-administration session (Fig. 4b). Paw withdrawal thresholds in the right (intact) paw did not differ before or after each AM1241 self-administration session in either sham or neuropathic groups (Fig. 4c-d). On the day associated with maximal change in paw withdrawal thresholds (day 1), both neuropathic and sham-operated groups responded preferentially on the active compared to the inactive lever ($F_{1,13} = 17.658$, $P < 0.01$), indicating that both groups reliably self-administered AM1241 (Fig. 5).

Pharmacological Specificity: Effects of the CB_1 antagonist SR141716 and the CB_2 antagonist SR144528 on AM1241 self-administration and paw withdrawal thresholds in neuropathic rats

Prior to each drug self-administration session, paw withdrawal thresholds in the left (injured) paw did not differ in neuropathic groups self-administering AM1241 in the presence or absence of either the CB_1 (SR141716) or CB_2 (SR144528) antagonist (Fig. 6a).

Treatment with the CB_2 antagonist, and to a lesser extent also the CB_1 antagonist, blocked the attenuation of mechanical hypersensitivity induced by AM1241 self-administration in the left (injured) paw ($F_{2,16} = 22.545$, $P < 0.0002$; $P < 0.001$ for all comparisons) (Fig. 6b). A decrease in pre-session paw withdrawal thresholds on the right (intact) paw emerged in neuropathic groups self-administering AM1241 that were pre-treated with CB_1 antagonist ($F_{2,16} = 5.421$, $P < 0.02$; $P < 0.04$ for all comparisons); this hypersensitivity was evident on day 5 relative to neuropathic groups self-administering AM1241 in the absence or presence of the CB_2 antagonist or not treated with the antagonist ($F_{8,64} = 4.905$, $P < 0.008$; $P < 0.004$ for all comparisons) (Fig. 6c). More strikingly, the CB_1 but not the CB_2 antagonist also lowered post-session paw withdrawal thresholds on the right (intact) paw in neuropathic animals self-administering AM1241 ($F_{2,16} = 512.591$, $P < 0.001$; $P < 0.003$ for all comparisons) (Fig. 6d). Hypersensitivity in the right (intact) paw emerged in a session dependent-fashion ($F_{8,64} = 10.399$, $P < 0.001$; $P < 0.03$ for all comparisons) following the third drug self-administration session. By day 5, the CB_2 antagonist induced a modest lowering of the paw withdrawal threshold in animals self-administering AM1241 ($P < 0.01$) (Fig. 6d).

The number of active and inactive lever presses and pharmacological specificity of AM1241 self-administration behavior was compared for the test session associated with the maximal elevation in paw withdrawal thresholds (day 1). Neuropathic animals self-administering AM1241 preferentially responded on the active, as opposed to the inactive lever ($P < 0.01$). The total number of active lever presses were greater in neuropathic animals self-administering AM1241 compared to neuropathic groups that were treated with either the CB_1 or the CB_2

antagonist ($F_{2,16} = 4.293$, $P < 0.03$; $P < 0.03$ for all comparisons). Systemic administration of either the CB_1 antagonist or the CB_2 antagonist suppressed responding on the active lever in neuropathic groups allowed to self-administer AM1241 ($P< 0.05$) (Fig. 7). There were no differences in the number of active or inactive lever presses on the terminal day of drug self-administration.

Effects of SR141716 on mechanical withdrawal thresholds and lever pressing behavior during self-administration of vehicle in naive and neuropathic rats

Pre-session paw withdrawal thresholds remained stable across experimental sessions in both the left (operated) and right (intact) paws of neuropathic and naive groups (allowed to self-administering vehicle) prior to pharmacological manipulations (8a,c). Pre-session paw withdrawal thresholds in the left (injured) paw were lower in neuropathic groups relative to naive groups prior to each vehicle self-administration session ($F_{3,26} = 13984.368$, $P < 0.0002$; $P < 0.0002$ for all comparisons; Fig. 8a), demonstrating that neuropathic groups exhibited robust tactile allodynia prior to testing. Paw withdrawal thresholds in the two naive groups were similar prior to each vehicle self-administration session (Fig. 8a). The CB_1 antagonist lowered post-session paw withdrawal thresholds in the left (intact) paw in the naive group (self-administering vehicle in the absence of AM1241) ($F_{3,26} = 1128.448$, $P < 0.0002$; $P < 0.0002$ for all comparisons) (Fig. 8b) without further suppressing paw withdrawal thresholds in the neuropathic group (Fig. 8b)

In the absence of AM1241, the CB_1 antagonist induced transient hypersensitivity to mechanical stimulation in naive animals in the left (intact) paw in a session-dependent manner ($F_{12,104} = 13.972$, $P < 0.0002$; $P < 0.01$ for all comparisons); this hypersensitivity was apparent immediately after, but not before, test sessions 2-5 ($P < 0.0002$) (Fig. 8a-b). The CB_1 antagonist also lowered the threshold for paw withdrawal in the right (intact) paw ($F_{3,26} = 214.088$, $P < 0.0002$) of both naive ($P < 0.001$ versus vehicle) and neuropathic groups ($P < 0.0002$ for all comparisons) (Fig. 8d). This SR141716-induced mechanical hypersensitivity was session-

dependent ($F_{12,104} = 22.631$, $P < 0.0002$), being evident immediately after, but not before, self-administration test sessions 2-5 (Fig. 8 c-d). The SR141716-induced mechanical hypersensitivity in the right (intact) paw was greater in the neuropathic group relative to the naive group ($P < 0.003$) over the same intervals (Fig. 8d).

In the absence of AM1241, the CB_1 antagonist decreased responding on both the active ($F_{3,26} = 7.241$, $P < 0.001$; $P < 0.02$ for all comparisons) and inactive ($F_{3,26} = 8.537$, $P < 0.001$; $P < 0.002$ for all comparisons) levers in both naïve and neuropathic groups allowed to lever press for vehicle (Fig. 9). The total number of active lever presses was greater than the total number of inactive lever presses ($F_{1,26} = 11.002$, $P < 0.003$) (Fig. 9). The CB_1 antagonist decreased responding on both the active and inactive levers ($P < 0.05$) (Fig. 9).

Pharmacological specificity: Effects of SR141716 and SR144528 on AM1241 self-administration and paw withdrawal thresholds in naive rats

Prior to each drug self-administration session, paw withdrawal thresholds in the left (intact) or right (intact) paws did not differ between naive groups self-administering AM1241 in the presence or absence of the CB_1 (SR141716) or CB_2 (SR144528) antagonists (Fig. 10a,c). The CB_2 antagonist produced a modest but negligible decrease in paw withdrawal thresholds in the left (intact) paw in naive animals self-administering AM1241 ($F_{2,19} = 86.947$, $P < 0.05$; $P < 0.02$ for all comparisons) (Fig. 10b). However, no such change in withdrawal thresholds was observed in the right (intact) paw following antagonist treatment (Fig. 10c-d).

SR144528 failed to alter responding on either the active or inactive lever in naive groups allowed to self-administer AM1241. By contrast, SR141716 decreased responding on the active lever in naive groups self-administering AM1241 ($P < 0.02$) (Fig. 11).

Assessment of Morphine self-administration on mechanical withdrawal thresholds in naive, sham and neuropathic groups

Pre-session paw withdrawal thresholds in the left (injured) paw were lower in neuropathic groups relative to naive and sham-operated groups similarly self-administering

morphine ($F_{2,23} = 49077.623$, $P < 0.0002$; $P < 0.0002$ for all comparisons) (Fig. 12a). Morphine self-administration increased paw withdrawal thresholds in the neuropathic group ($F_{2,23} = 123.783$, $P < 0.0002$; $P < 0.0002$ for all comparisons) (Fig. 10b). By contrast, paw withdrawal thresholds were not altered in naive and sham-operated rats following morphine self-administration (Fig. 12b). Morphine self-administration increased paw withdrawal thresholds in neuropathic groups compared to pre-session thresholds on all days ($F_{2,23} \geq 228.908$, $P < 0.0002$; $P < 0.0002$ for all comparisons) (Fig. 12a-b). The threshold for paw withdrawal in the right (intact) paw did not differ in any group before or after any morphine self-administration session (Fig. 12c-d).

Naive and neuropathic rats preferentially pressed the active lever as opposed to the inactive lever ($P < 0.05$ for each comparison) beginning on day 2. The total number of active lever presses was greater in naive animals self-administering morphine compared to sham or neuropathic groups ($P < 0.05$) (Fig. 13).

Assessment of motor behavior in spared nerve injury and naive animals after i.v. administration of AM1241,W IN55,212-2, or Vehicle

The latency to descend from the rotarod did not differ between groups on day 1 or day 2, prior to intravenous drug administration. The non-selective CB_1/CB_2 WIN55,212-2, but not the CB_2-selective agonist AM1241, decreased rotarod latencies relative to all other groups ($F_{4,25} = 6.347$, $P < 0.001$; $P < 0.01$ for all comparisons). AM1241 failed to alter rotarod latencies in either naive or neuropathic groups relative to vehicle treatment. By contrast, WIN55,212-2 induced a time-dependent motor ataxia in neuropathic animals ($F_{16,100} = 2.695$, $P < 0.01$), that lasted over 45 min ($P < 0.02$) (Fig. 14).

Average drug intake and number of infusions for all experimental groups on day 1 is shown on table (Table 1).

Table 1
Average drug infused and the number of infusions on day 1 of drug self-administration

Condition	Total µg	# of infusions
naive am1241	318.51 ± 48.05	28 ± 4.14
sham am1241	313.84 ± 54.89	32 ± 5.60
neuro am1241	669.02 ± 204.88	47 ± 9.30
naive am1241 + SR144528	227.01 ± 65.11	21 ± 6.27
naive am1241 +SR141716	110.91 ± 64.42	10 ± 5.87
neuro am +SR141716	184.79 ± 59.67	18 ± 5.50
neuro am + SR144528	247.80 ± 44.64	23 ± 4.31
naive vehicle + SR141716	0.00 ± 0.00	8 ± 3.76
neuro vehicle + SR141716	0.00 ± 0.00	10 ± 4.94
naive morphine	563.77 ± 122.03	17 ± 2.08
neuro morphine	457.30 ± 87.15	13 ± 2.61
sham morphine	438.62 ± 116.26	12 ± 3.24
naive vehicle	0.00 ± 0.00	34 ± 5.64
sham vehicle	0.00 ± 0.00	33 ± 3.56
neuro vehicle	0.00 ± 0.00	29 ± 6.83

Data are Mean ± SEM.

Figure 1. Spared nerve injury decreases mechanical paw withdrawal thresholds in neuropathic but not in naive and sham operated rats. The threshold for paw withdrawal in the (A) left and (B) right paw remained high prior to surgical manipulation in naive, sham and neuropathic animals. (A-B) A modest but reliable increase in the paw withdrawal threshold was observed in naive animals prior to surgery. Spared nerve injury decreased the paw withdrawal threshold response in the (A) left (injured) paw, but not in the (B) right (intact) paw without affecting the threshold response in naive and sham-operated animals. *$P < 0.05$, ***$P < 0.001$ different from all groups, (ANOVA, Fisher's PLSD post hoc test). Data are expressed as Mean ± SEM. N = 23-49 per group.

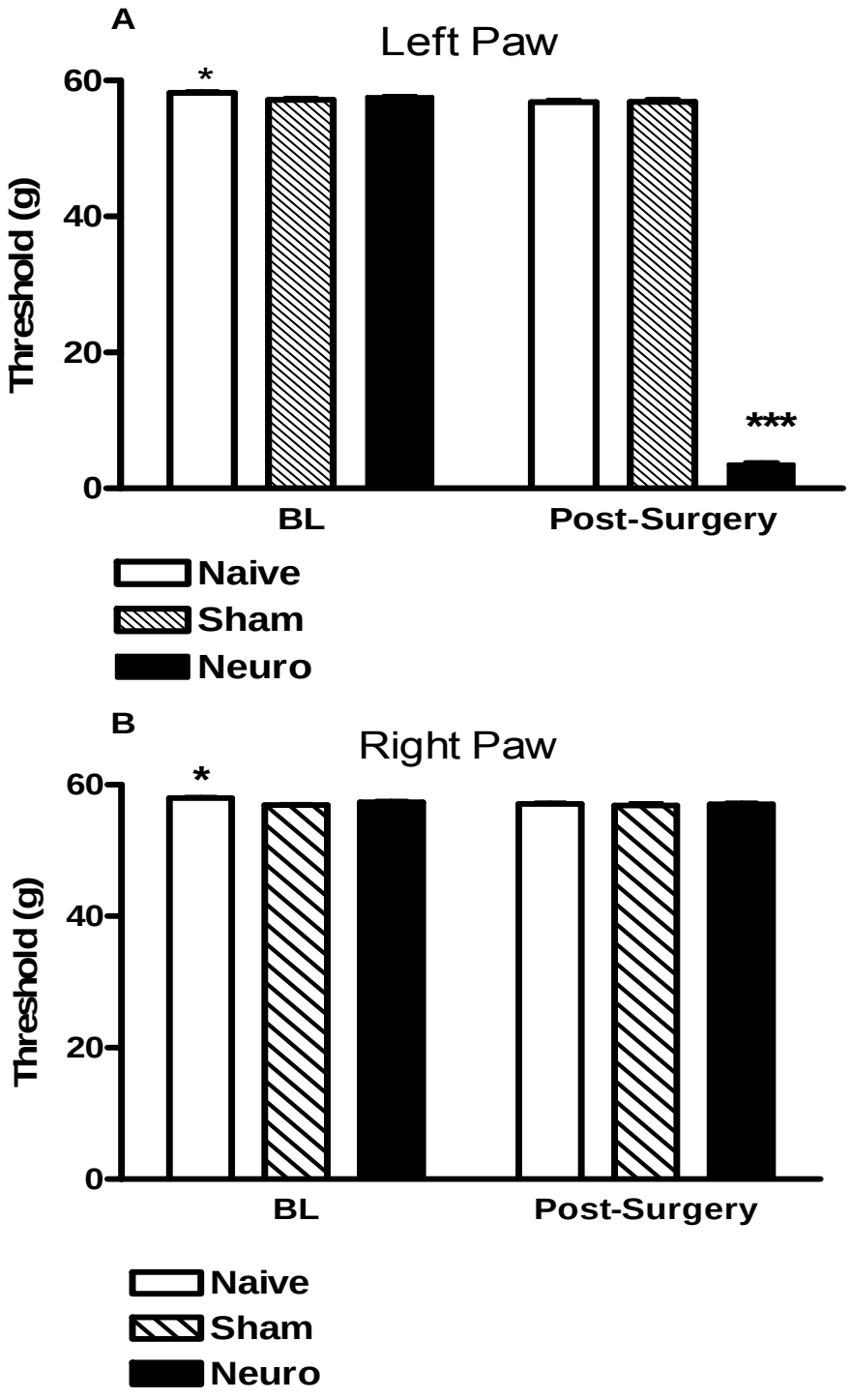

Figure 1. Unilateral spared nerve injury decreases the threshold for paw withdrawal in the injured but not the uninjured paw

Figure 2. Self-administration of vehicle does not alter paw withdrawal threshold in naive, sham and neuropathic groups self-administering vehicle. Paw withdrawal thresholds (A) prior to and (B) after each self-administration session of vehicle remained low in the left (injured) paw in neuropathic animals relative to naive and sham conditions. (A-B) Paw withdrawal thresholds remained high in all groups in the right (intact) paw prior to after each self-administration session of vehicle. (A-D) A modest but reliable difference in the pre – and post-threshold response on the left (affected) and right (intact) paw was observed between naive and sham-operated animals paw (ANOVA, Fisher's, PLSD post hoc test). Data are expressed as Mean ± SEM. *N* = 6-7 per group.

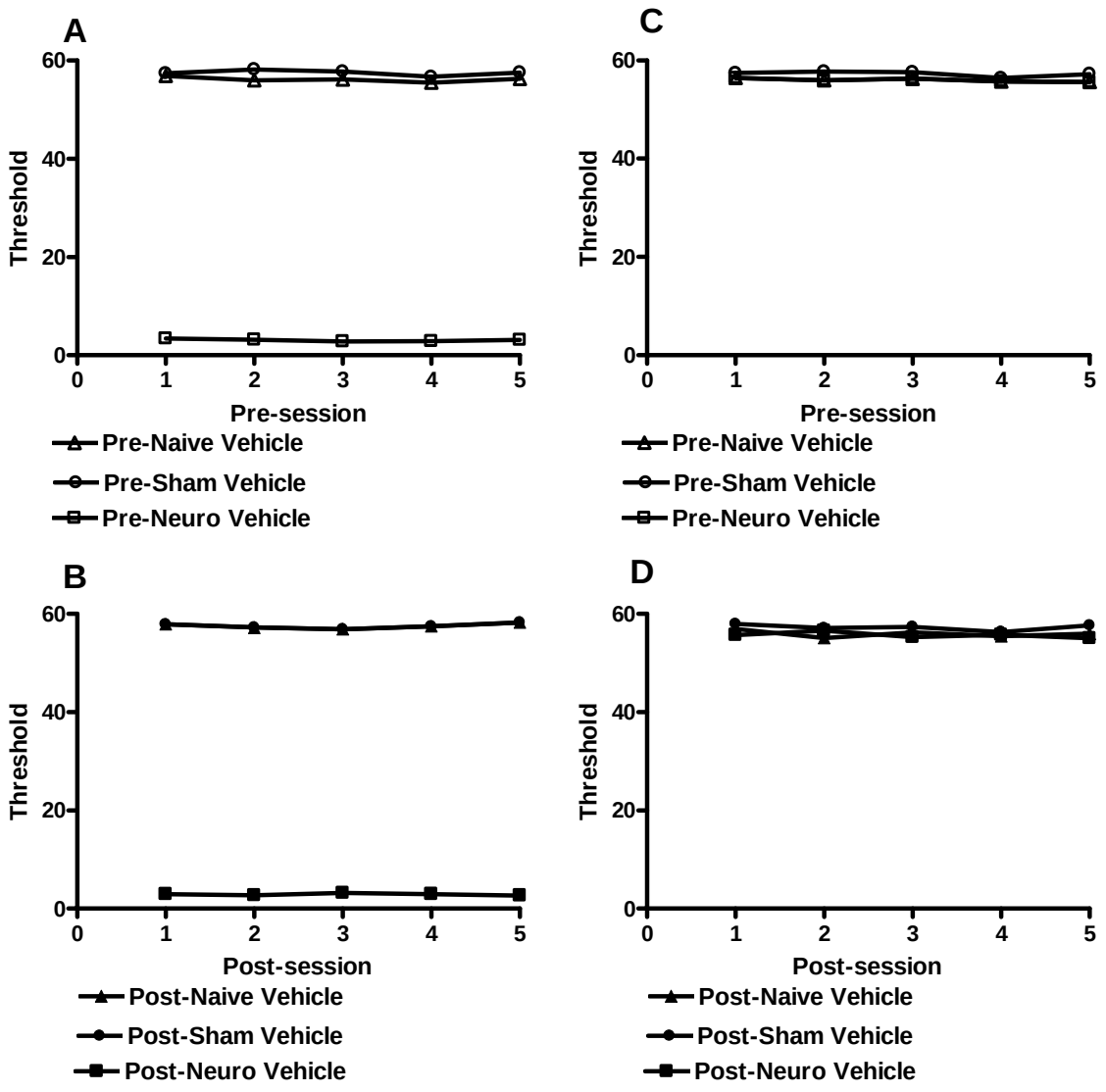

Figure 2. Self-administration of vehicle fails to alter mechanical withdrawal thresholds in the left (operated) or right (intact) paw of naive, sham and neuropathic animals self-administering vehicle

Figure 3. The number of active and inactive lever presses did not differ for naive, sham and neuropathic animals self-administering vehicle. Naive, sham and neuropathic animals self-administering vehicle did not exhibit a preference for either the active or inactive lever (ANOVA, Fisher's PLSD post hoc test). Data are expressed as Mean ± SEM. *N* = 6-7 per group.

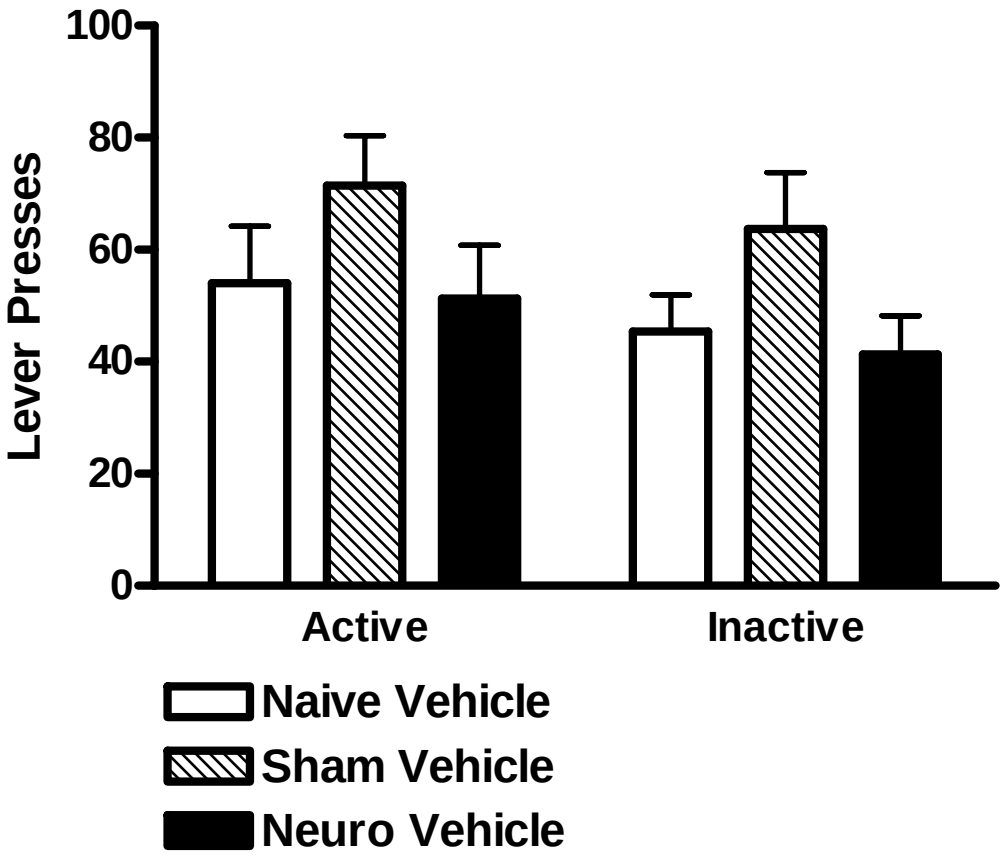

Figure 3. Active and inactive lever presses did not differ in naive, sham, and neuropathic animals self-administering vehicle

Figure 4. Self-administration of the cannabinoid CB_2 agonist AM1241 increases the threshold for paw withdrawal in neuropathic animals. (A) The threshold for paw withdrawal remained lower in the left (injured) paw of neuropathic relative to sham animals prior to each self-administration session. (B) AM1241 self-administration increased the threshold for paw withdrawal in the left (injured) paw in neuropathic animals. (C-D) AM1241 self-administration did not change the threshold for paw withdrawal prior to and after each self-administration session in the right (intact) paw in sham and neuropathic animals. ***$P < 0.001$, is different from sham animals, (ANOVA, Fisher's PLSD post hoc test). Data are expressed as Mean ± SEM. N = 7-8 per group.

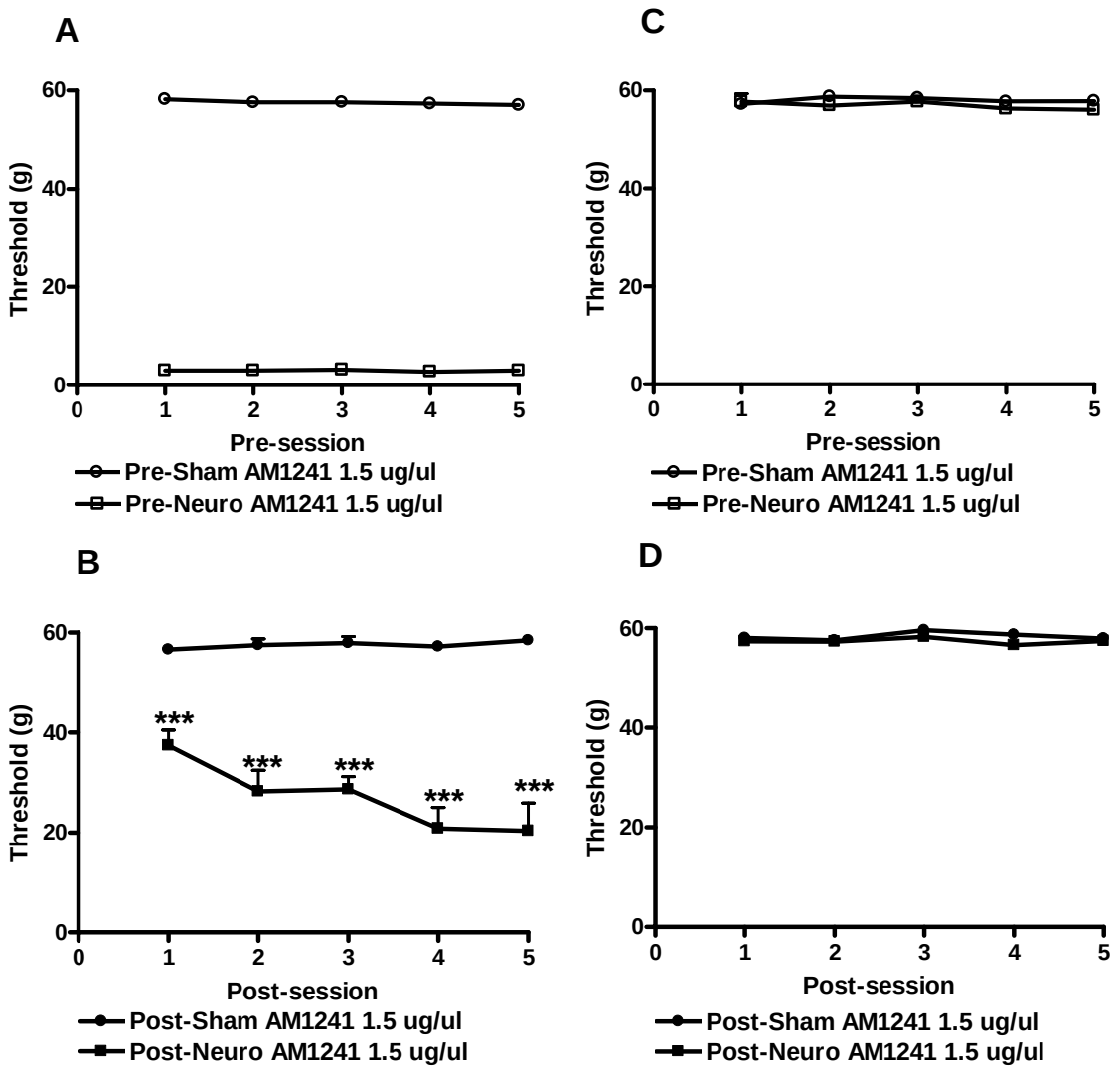

Figure 4. Self-administration of AM1241 increased the threshold for paw withdrawal in the left (injured) paw relative to pre-session levels in neuropathic animals

Figure 5. Sham-operated and neuropathic animals reliably self-administered AM1241. Sham-operated and neuropathic animals preferentially responded on the active lever as opposed to the inactive lever, $^{**}P < 0.01$ is different from inactive, (ANOVA, Fisher's PLSD post hoc test). Data are expressed as Mean ± SEM. N = 7-8 per group.

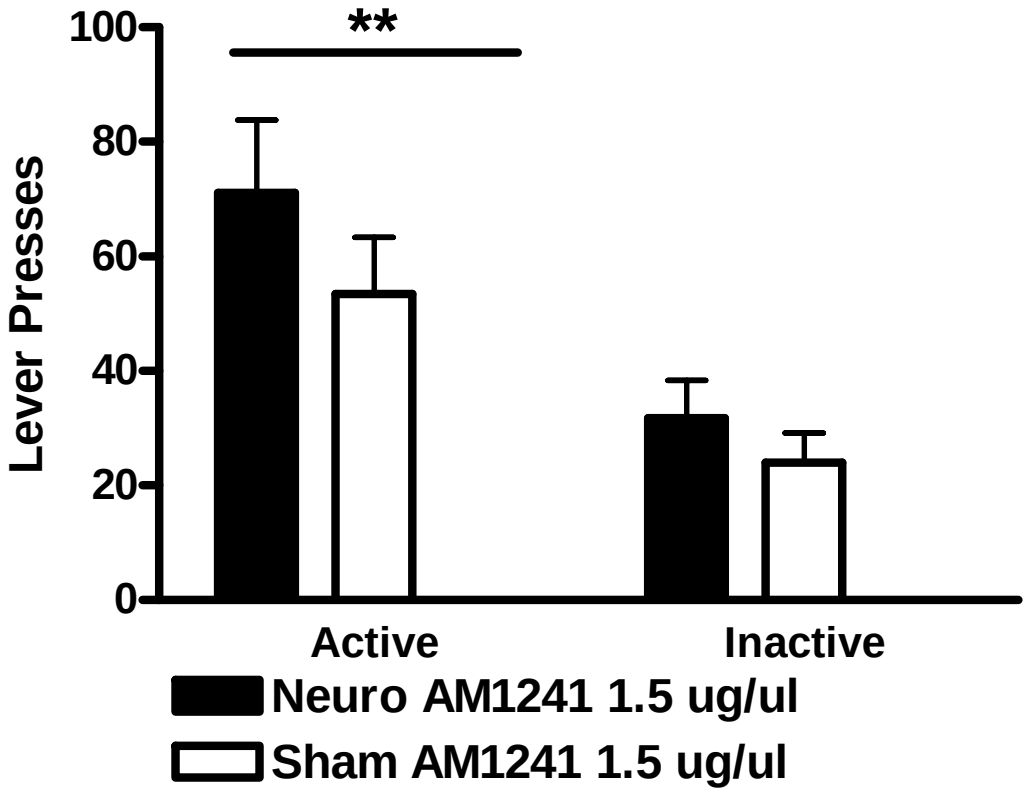

Figure 5. Neuropathic and sham animals permitted to self-administer AM1241 preferentially respond on the active but not the inactive lever

Figure 6. The CB_2 antagonist preferentially blocks the anti-allodynic effects produced by AM1241 in the left (injured) paw. The CB_1 antagonist produces hypersensitivity on the right (intact) paw. (A) The threshold for paw withdrawal remained low in all neuropathic groups in the left (injured) paw prior to AM1241 self-administration or antagonist treatment. (B) The CB_2 antagonist blocks the anti-allodynic effects produced by AM1241 self-administration in the left (injured) paw. The CB_1 antagonist partially attenuates the anti-allodynic effects produced by AM1241. (C) On day 5, neuropathic animals that were treated with CB_2 antagonist develop hypersensitivity in the right (intact) paw prior to self-administration of AM1241. (D) The CB_1 antagonist induces hypersensitivity in the right (intact) after AM1241 self-administration by day 3. CB_2 treatment induces hypersensitivity by day 5. $^{*}P < 0.05$,$^{**}P < 0.01$, is different from all others, $^{\#}P < 0.05$ is different from Post-Neuro AM1241, (ANOVA, Fisher's PLSD post hoc test). Data are expressed as Mean ± SEM. N = 5-8 per group.

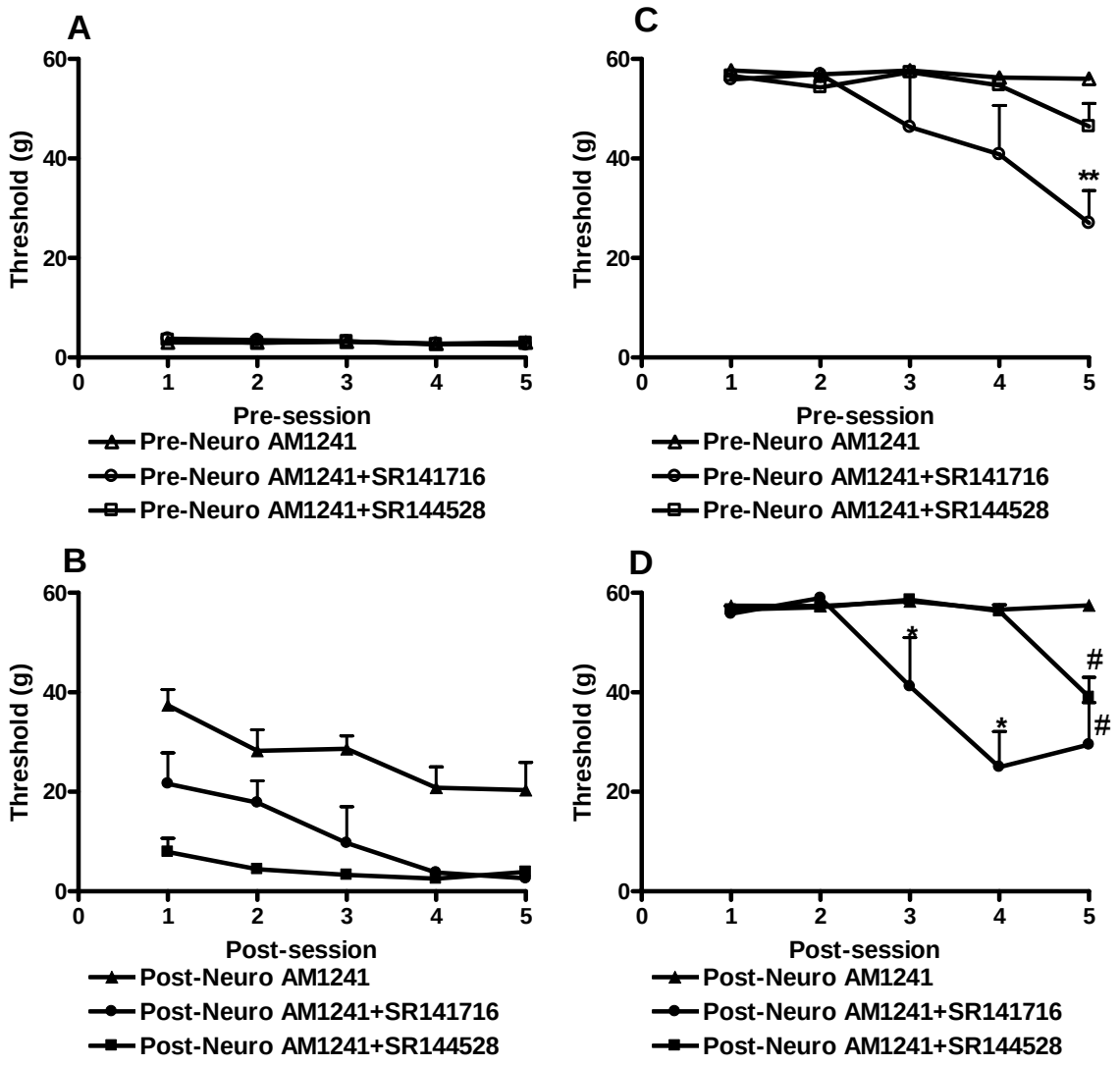

Figure 6. The CB_2 antagonist completely blocks the anti-allodynic effects produced by AM1241 self-administration in the left (injured) paw.

Figure 7. The CB_1 and the CB_2 antagonist attenuates AM1241 self-administration in neuropathic animals. The total number of active lever presses was greater in neuropathic animals self-administering AM1241 compared to inactive levers for all groups. The CB_1 and CB_2 antagonist attenuated active lever presses in neuropathic animals. A trend to suppression of responding on the inactive lever was also observed. $^{XX}P < 0.01$ is different from inactive lever presses, $^{*}P < 0.05$ is different from Neuro AM1241 active lever presses, (ANOVA, Fisher's PLSD post hoc test, one-way t-test). Data are expressed as Mean ± SEM. N = 5-8 per group.

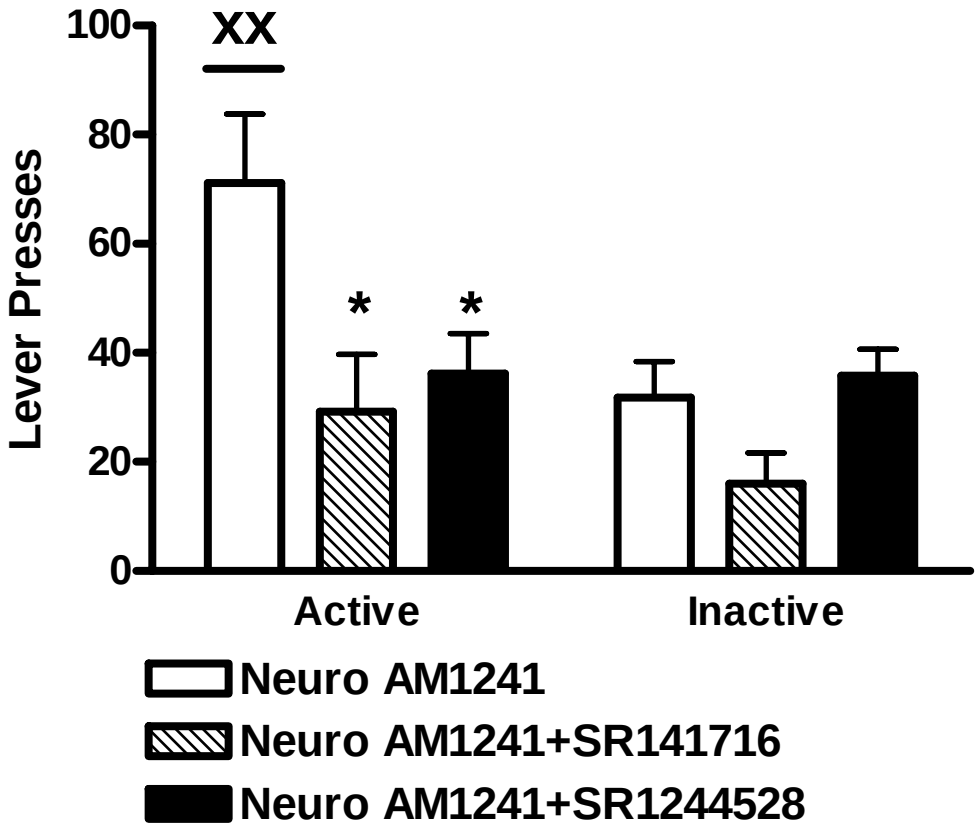

Figure 7. The CB_1 and CB_2 antagonist attenuate self-administration of AM1241 in neuropathic animals

Figure 8. The CB_1 antagonist SR141716 produces a transient hypersensitivity in naive and neuropathic animals permitted to self-administer vehicle. (A) The threshold for paw withdrawal in the left (injured) paw was lower in neuropathic relative to naïve groups prior to vehicle self-administration or treatment with the CB_1 antagonist. (C) The threshold for paw withdrawal in the right (intact) paw did not change in naive or neuropathic animals prior to vehicle self-administration or CB_1 antagonist treatment. (B and D) The CB_1 antagonist produced a transient hypersensitivity in naive groups self-administering vehicle in the left (injured) paw and right (intact) paw. (D) Similarly, hypersensitivity was observed in the right (intact) paw in neuropathic animals self-administering vehicle. ***$P < 0.001$, different from all others or different from Pos-naïve vehicle, **$P < 0.01$ different from post-naïve vehicle and post-neuro vehicle, (ANOVA, Fisher's PLSD post hoc test). Data are expressed as Mean ± SEM. N = 6-11 per group.

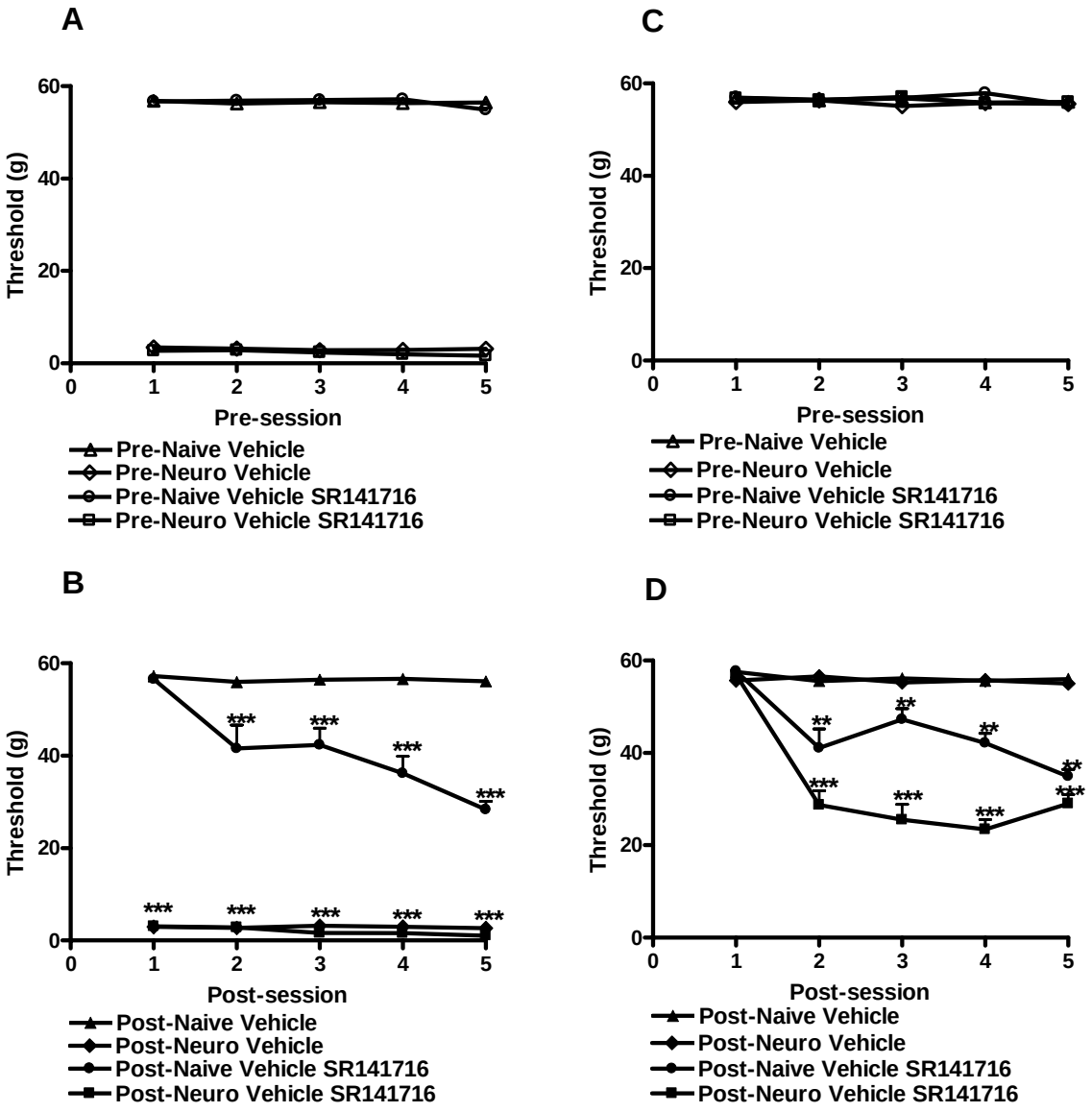

Figure 8. The CB_1 antagonist induces hypersensitivity in naive and neuropathic animals self-administering vehicle

Figure 9. The CB_1 antagonist attenuates responding on the active and inactive levers in rats permitted to self-administer vehicle. Treatment with the CB_1 antagonist attenuated responding on the active and inactive levers in naïve and neuropathic animals self-administering vehicle. The total number of active lever presses was greater in all the groups compared to the total number of inactive lever presses. (ANOVA, Fisher's PLSD post hoc test). Data are expressed as Mean ± SEM. *N* = 6-11 per group.

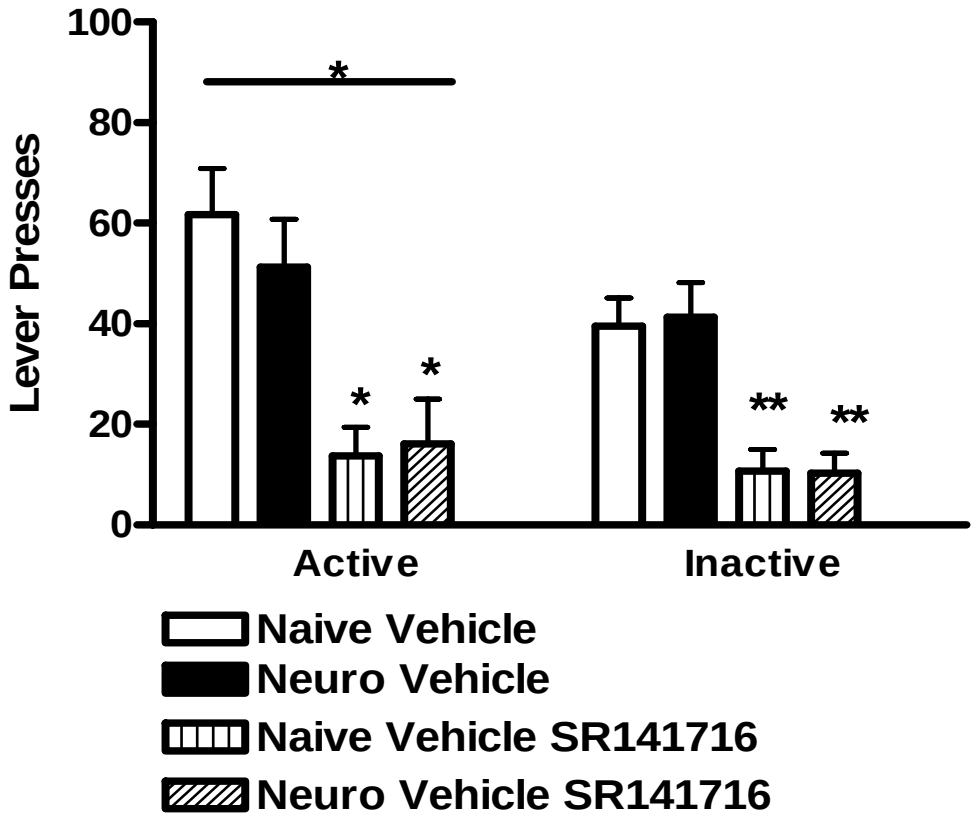

Figure 9. SR141716 attenuates responding on the active and inactive levers in naive and neuropathic animals permitted to self-administer vehicle

Figure 10. Treatment with either the CB_1 or CB_2 antagonist does alter paw withdrawal thresholds in naive animals in either the left (intact) or right (intact) paw prior to or after each AM1241 drug self-administration session. (A and C) No difference in paw withdrawal thresholds were found in the left (intact) paw or the right (intact) paw in naive groups prior to AM1241 self-administration. (B) A minimal decrease in the threshold response was observed in the left (intact) paw in naive animals self-administering AM1241 in the presence of the CB_2 antagonist compared to naive animals self-administering AM1241 in the absence of the CB_2 antagonist. (D) No difference in paw withdrawal thresholds were observed in the right (intact) paw after each self-administration session or treatment with either the CB_1 or CB_2 antagonist, (ANOVA, Fisher's PLSD post hoc test). Data are expressed as Mean ± SEM. *N* = 5-12 per group.

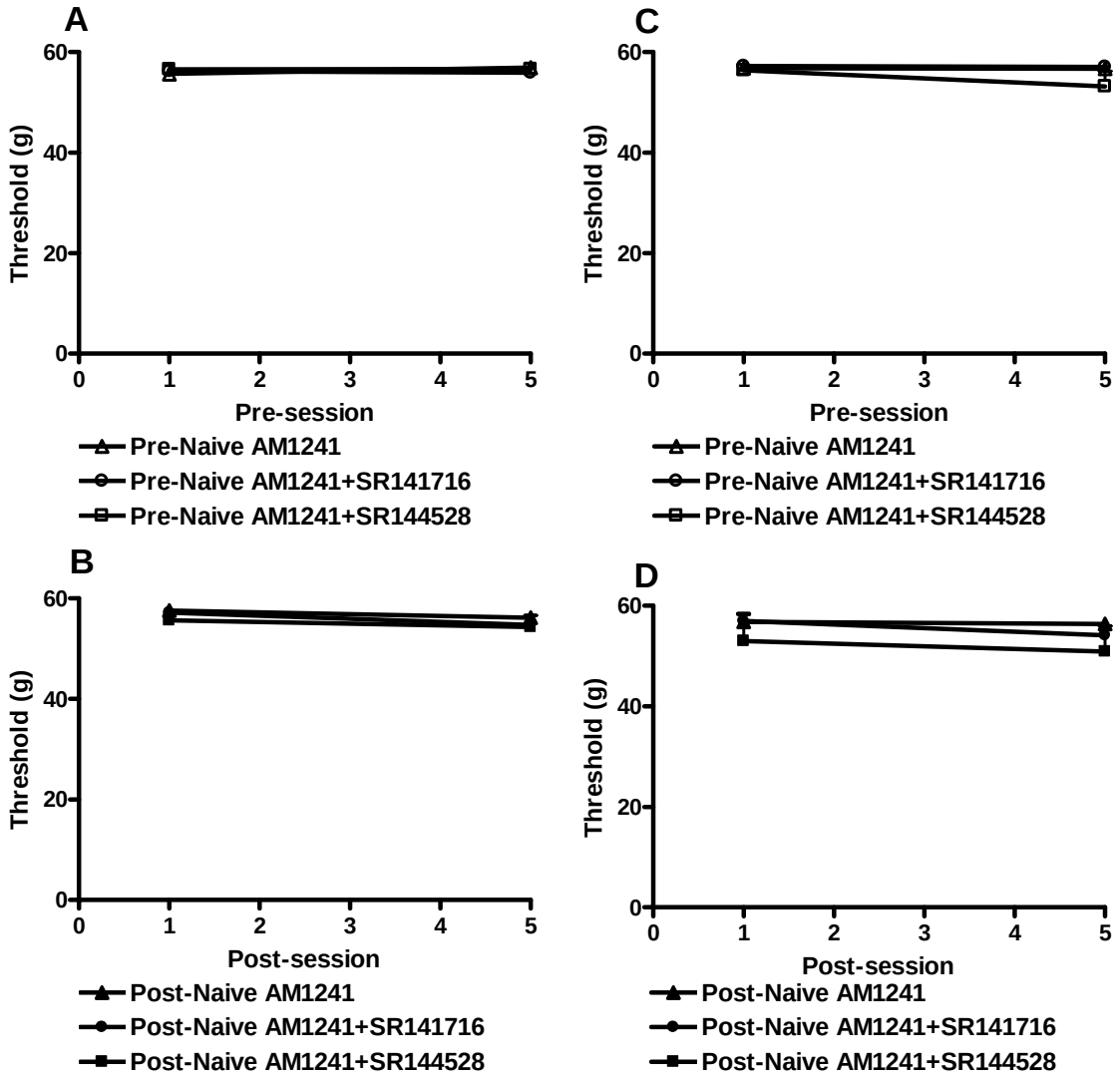

Figure 10. The CB_1 and CB_2 antagonist fail to alter mechanical paw withdrawal thresholds in naive animals permitted to self-administer AM1241

Figure 11. Treatment with the CB_1 antagonist attenuates responding on the active lever in naïve animals permitted to self-administer AM1241, $P < 0.05$, (ANOVA, Fisher's PLSD post hoc test, one-way t-test). Data are expressed as Mean ± SEM. N = 5-12 per group.

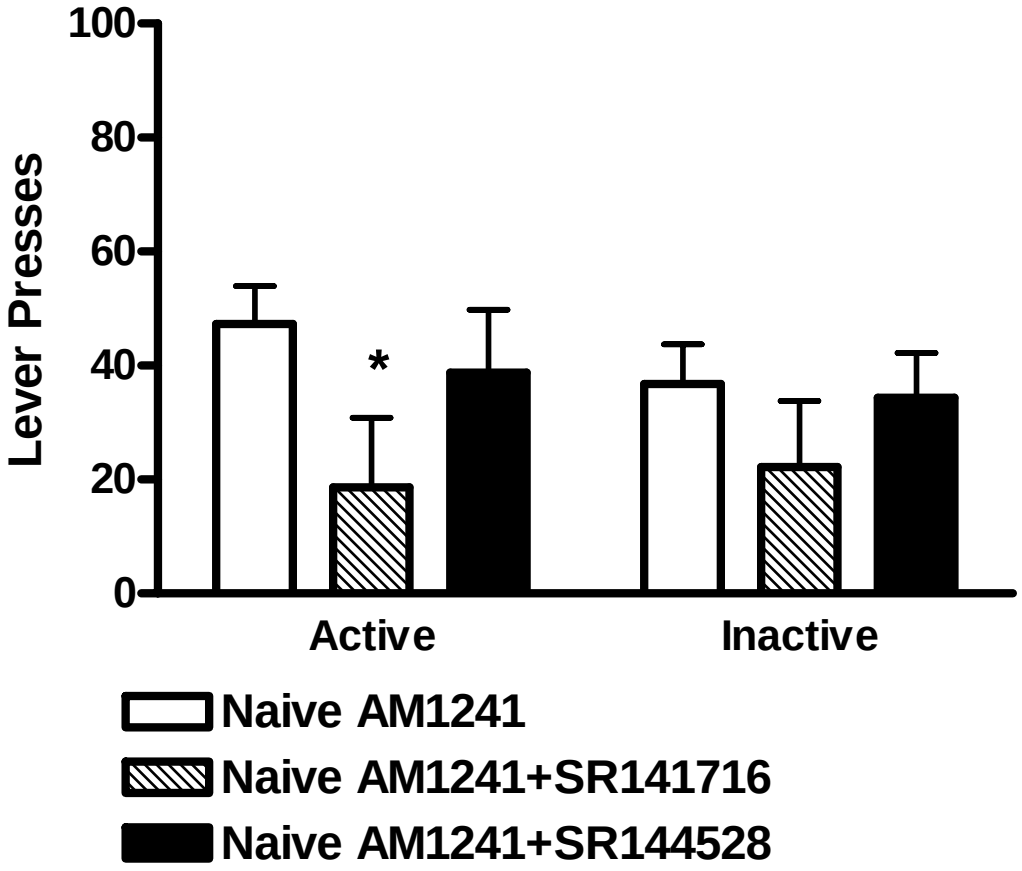

Figure 11. SR141716 attenuates active lever pressing in naive animals permitted to self-administer AM1241

Figure 12. Morphine self-administration increases the threshold for paw withdrawal in neuropathic animals. (A) Threshold responses in the left (injured) paw were low for neuropathic animals prior to each self-administration session of morphine. (B) Self-administration of morphine increased the paw withdrawal threshold in neuropathic animals. (C-D) No difference in paw withdrawal thresholds were observed in the right (intact) paw prior to and after each morphine session, (ANOVA, Fisher's PLSD post hoc test). Data are expressed as Mean ± SEM. *N* = 7-10 per group.

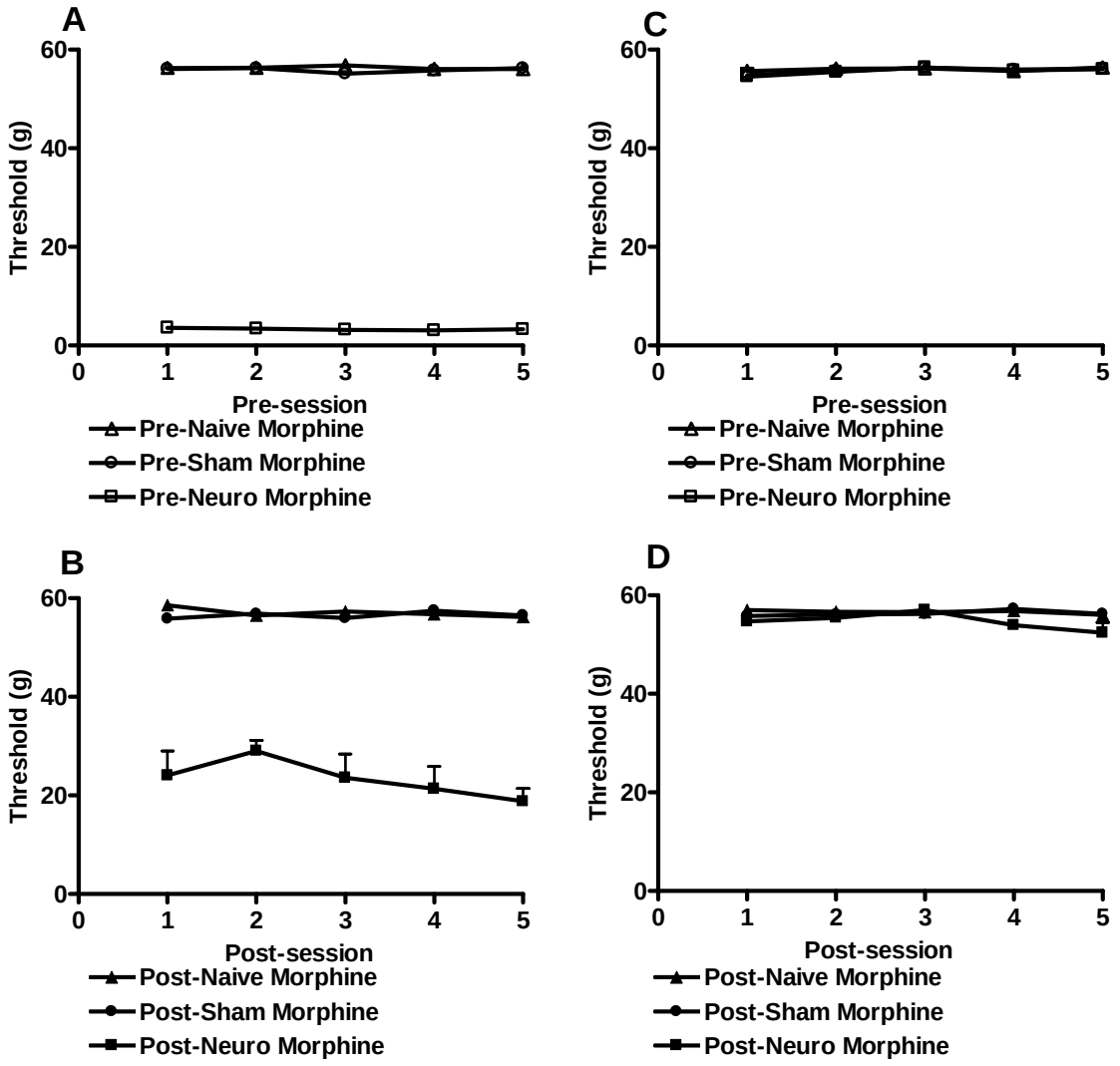

Figure 12. Morphine self-administration increases mechanical paw withdrawal thresholds in the left (injured) paw

Figure 13. Neuropathic and naive animals self-administering morphine preferentially responded on the active as opposed to the inactive lever, $^{x}P < 0.05$, is different from naive morphine inactive or neuro morphine inactive, $*P < 0.05$, is different from naive morphine active (by one-way t-test). Data are expressed as Mean ± SEM. N = 7-10 per group.

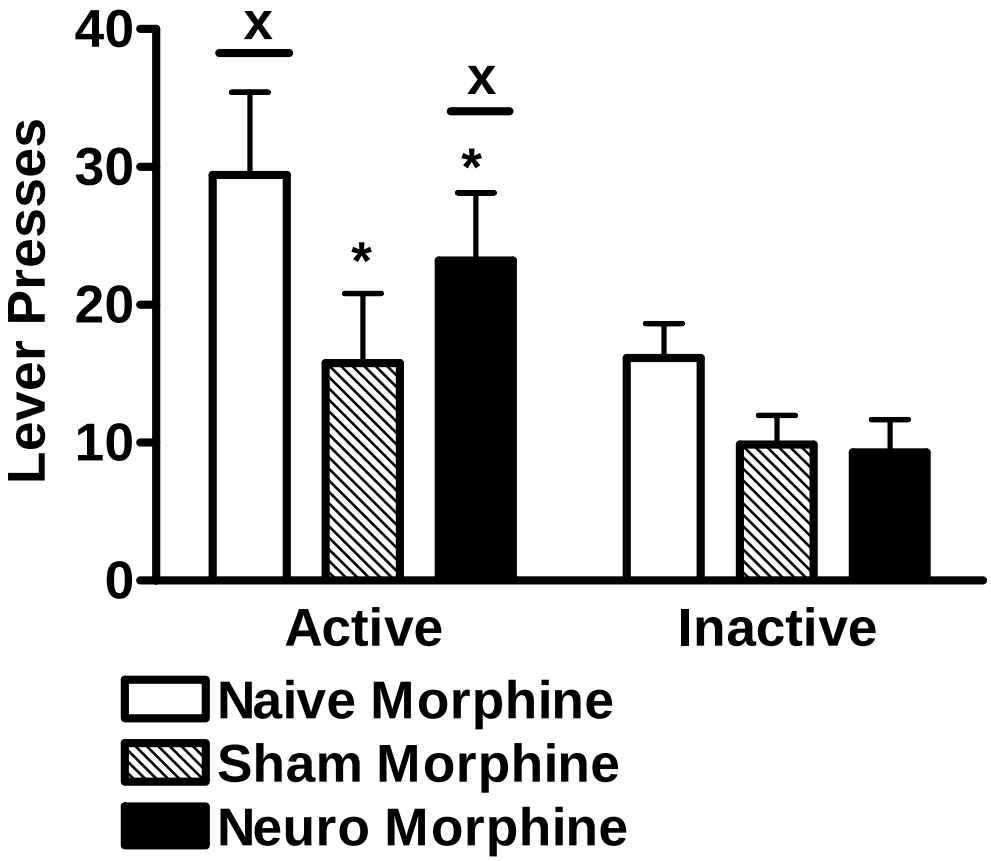

Figure 13. Naive and neuropathic animals self-administering morphine preferentially responded on the active as opposed to the inactive lever

Figure 14. The CB_2 antagonist does not impair motor activity in the rotarod test. The non-specific CB_1/CB_2 agonist WIN55,212-2 impairs motor activity in neuropathic animals. No motor deficit was observed in naïve or neuropathic animals that were treated with AM1241 or vehicle, (ANOVA, Fisher's PLSD post hoc test). Data are expressed as Mean ± SEM. *N* = 6 per group.

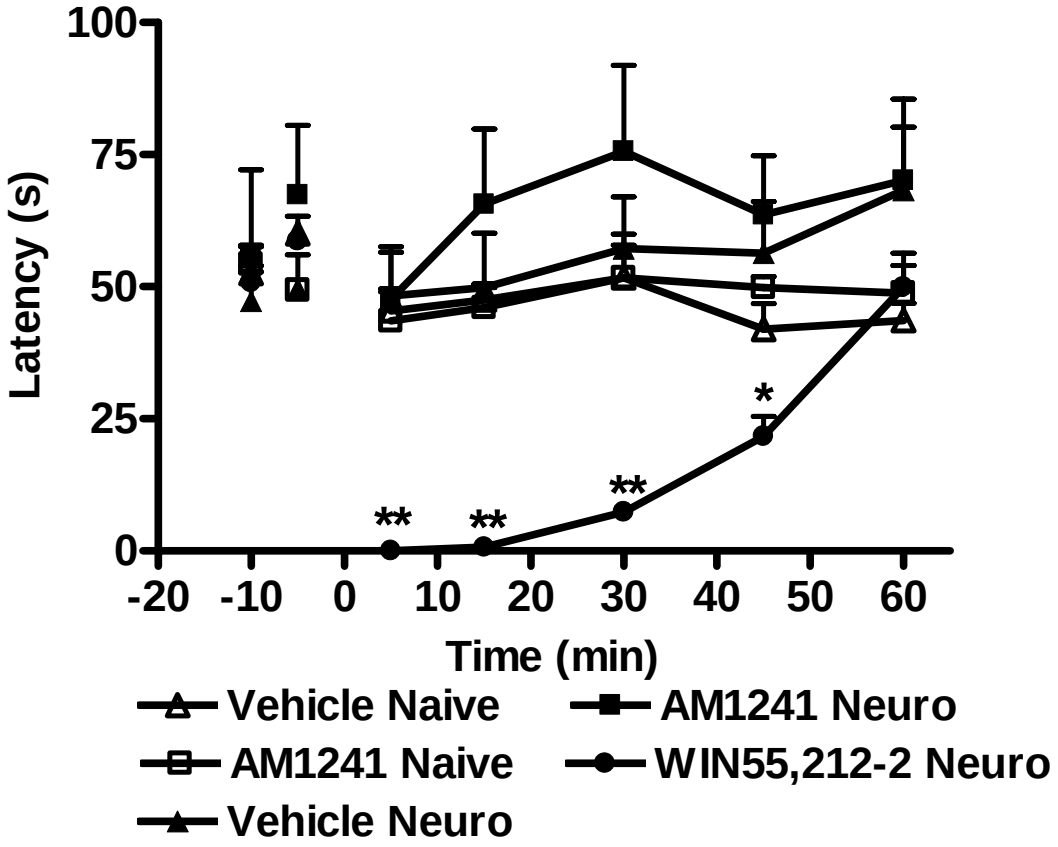

Figure 14. The non-selective CB_1/CB_2 agonist WIN55,212-2 but not the CB_2 selective agonist AM1241 decreases motor activity in the rotarod test

CHAPTER 5: DISCUSSION

Recent behavioral and electrophysiological studies have indicated a role of cannabinoids in suppressing neuropathic pain (Herzberg et al. 1997; Fox et al. 2001; Ibrahim et al. 2003; Elmes et al. 2004; Sagar et al. 2005). Most of these behavioral and electrophysiological studies have addressed whether local and systemic administration of cannabinoids suppress mechanical hypersensitivity in neuropathic rats through CB_1 and CB_2 mediated mechanisms (Fox et al. 2001; Malan et al. 2001; Ibrahim et al. 2003; Elmes et al. 2004). However, CB_1 activation is associated with centrally mediated side effects that limits its therapeutic use in treating acute and chronic pain states (Zimmer et al. 1999). By contrast, activation of CB_2 is not associated with the CNS side-effects (hypoactivity, hypothermia) associated with activation of CB_1. This observation likely reflects the paucity of CB_2 in naive CNS, although CB_2 may be upregulated in the spinal cord and dorsal root ganglia (Zhang et al. 2003; Beltramo et al. 2006). following challenges such as traumatic nerve injury. CB_2 , by contrast, is pronounced in peripheral tissues and the immune system (Hanus et al. 1999), and so it remains to be determined whether side-effects of CB_2 on the immune system could limit its therapeutic potential. More work is necessary to determine whether CB_2-mediated interventions provide better pharmacological therapies without the unwanted side effects.

Significant gaps exist in our understanding of cannabinoid CB_2-mediated therapeutic potential. No study has evaluated whether activation of CB_2 is associated with low abuse liability. Moreover, it is unclear whether animals will intravenously self-administer cannabinoids to decrease persistent pain behavior, and the impact of that pain on self-administration behavior remains poorly understood. Research on cannabinoid self-administration in animals has focused on the potential of cannabinoids that possess psychoactive effects to induce dependence. Secondly, these studies have assessed intravenous self-administration of cannabinoid agonists that activate CB_1, but CB_2 agonists have not been evaluated. Another problematic issue is that cannabinoid self-administration is a difficult behavior to be acquired by

rodents compared to other typical drugs of abuse. It has been shown that rats will self-administer the non-selective CB_1/CB_2 agonist WIN55,212-2 (12.5 μg/kg per injection) under stringent conditions and that pretreatment with the CB_1 selective antagonist SR141716 increases the rate of responding in rats self-administering WIN55,212-2 (Fattore et al. 2001).The CB_1 antagonist blocked the reinforcing effects produced by WIN55,212-2 self-administration (Fattore et al. 2001). Mice will also self-administer WIN55,212-2 but the rewarding effects and aversive effects due to self-administration of WIN55,212-2 rely heavily on the drug concentration used (Martellotta et al. 1998). Again pretreatment with the CB_1 antagonist SR141716A blocked WIN55,212-2 self-administration indicating that the effects were CB_1 mediated (Martellotta et al. 1998). However, whether or not a CB_2 antagonist would block self-administration of WIN55,212-2 was not assessed. Similarly, squirrel monkeys will intravenously self-administer anandamide and again SR141716 blocks anandamide self-administration (Justinova et al. 2003). However, all of these studies addressed the rewarding properties associated with cannabinoids in the absence of a chronic pain state.

In our studies we addressed whether animals with a spared nerve injury would reliably self-administer the CB_2 selective agonist AM1241, and whether this self-administration would suppress neuropathic pain behavior. We used Decostord and Woolf's s spared nerve injury model because this animal model of neuropathic pain produces robust tactile allodynia that lasts over 6 months (Decosterd and Woolf 2000). In our study, neuropathic and sham-operated rats reliably self-administered the CB_2 selective agonist AM1241, as demonstrated by a preference for responding on the active but not the inactive lever. Self-administration of AM1241 also decreased nerve injury-induced tactile allodynia after each drug self-administration session indicating that the CB_2 selective agonist decreased neuropathic pain behavior. By contrast, naive groups showed no preference for the active or inactive levers, demonstrating that they did not self-administer the CB_2 agonist.

Our data is consistent with the hypothesis that CB_2 mechanisms may be exploited to suppress neuropathic pain behavior with low abuse liability. Naive animals did not reliably self-administer AM1241 and no change was observed in paw withdrawal thresholds prior to and after each drug self-administration session of AM1241 in either the left (intact) or right (intact) paw. These data suggest that the CB_2 agonist may not be inherently reinforcing in the absence of nerve injury. Neuropathic and sham-operated animals showed AM1241 self-administration as demonstrated by their preference for responding on the active relative to the inactive lever. However, this preference was only moderate in intensity, further arguing against AM1241 possessing inherently addictive properties. Activation of CB_2 was evident because AM1241 self-administration increased paw withdrawal thresholds in neuropathic animals in the left (injured) paw but not in naive and sham operated groups. Our data are consistent with previous work implicating a role for CB_2 in the modulation of inflammatory and neuropathic pain behavior (Ibrahim et al. 2003; Nackley et al. 2003b; Quartilho et al. 2003; Elmes et al. 2004; Hohmann et al. 2004; Nackley et al. 2004; Elmes et al. 2005; Beltramo et al. 2006; Gutierrez et al. 2007). Quantification of endocannabinoid levels is necessary to determine whether the spared nerve injury increased endocannabinoid tone at CB_1 or CB_2.

An unexpected result of the present study was that sham-operated groups self-administered AM1241, as shown by a modest preference towards responding on the active as opposed to the inactive lever. Although this result was not anticipated, it is worth emphasizing that sham-operated animals, albeit free of nerve injury, are not completely intact. All animals were subjective to an invasive surgery involving surgical implantation of an indwelling jugular catheter that exited through an incision in the neck. More importantly, perhaps, sham-operated animals had a surgical incision performed on the left limb, and the muscle and overlying skin were closed in layers. Thus, residual tissue or muscle damage may have contributed to AM1241 self-administration in sham-operated rats, despite the fact that nerves were not severed. Changes in paw withdrawal thresholds were absent in sham-operated rats self-administering

AM1241 in either the left (sham-operated) or right (intact) paw. However, we did not assess mechanical thresholds at the site of the surgical incision, to determine if muscle hyperalgesia may have been present. Likewise, it has been shown that systemic administration of WIN55,212-2 reverses hyperalgesia due to carrageenan evoked deep tissue damage (Kehl et al. 2003).

Intravenous administration of the highest dose of AM1241 administered by our rats did not induced motor ataxia in naive or neuropathic animals. These data provide further evidence for the inability of the CB_2 agonists to induce pronounced CNS effects (Hanus et al. 1999; Malan et al. 2003) that are associated with activation of CB_1 (Zimmer et al. 1999). In our study, i.v. administration of WIN55,212-2 in neuropathic animals induced motor ataxia which is consistent with previous studies in which subcutaneous administration of the non-specific CB_1/CB_2 agonists (WIN55,212-2, CP-55,940 and HU-210) induce catalepsy, hypothermia and motor disruption in the rotarod test (Fox et al. 2001). These effects were all blocked by the CB_1 antagonist SR141716A indicating that the effects were attributed to CB_1 activation (Fox et al. 2001).

In our study naive, sham and neuropathic animals self-administering vehicle did not show a lever preference for the active or inactive lever. This observation verifies that in the absence of AM1241 there are no reinforcing effects associated with vehicle self-administration. Moreover, the data suggest that vehicle self-administration had no apparent pharmacological effect. All animals were pre-trained to use both levers equally; thus our experimental design precludes lever pressing as a nonspecific effect. Moreover, only animals that learned to press equally on both levers were included in the self-administration studies. This procedure is consistent with drug self-administration paradigms that use two levers or two nose poke holes to ensure that reinforcing effects are drug-mediated and not due to non-specific responses (Fattore et al. 2001; De Vries et al. 2003; Fattore et al. 2005). Moreover, naive, sham and neuropathic groups showed no preference for a particular lever in the absence of AM1241 or

morphine self-administration. Self-administration of vehicle did not alter the paw withdrawal threshold in naive, sham or neuropathic groups relative to pre-session levels in either paw. These data demonstrate that pharmacological activity at cannabinoid CB_2 or mu opioid receptors was required to alter paw withdrawal thresholds.

Self-administration of AM1241 suppressed tactile allodynia in neuropathic rats. Self-administration of AM1241 selectively increased paw withdrawal thresholds in the left (injured) paw but not in the right (intact) paw. These effects were CB_2-mediated because the CB_2 antagonist SR144528 completely blocked the antiallodynic effects of AM1241. Our data are consistent with results of electrophysiological studies demonstrating that CB_2 agonists suppress mechanically-evoked responses of dorsal horn neurons recorded in neuropathic rats. These electrophysiological effects were also mediated by a CB_2 mechanism because AM1241-induced suppressions of neuronal activity were blocked by CB_2-selective antagonists (Elmes et al. 2004; Nackley et al. 2004; Sagar et al. 2005). Our data are also consistent with recent work demonstrating that CB_2-selective agonists suppress the development (Nackley et al. 2003a; Quartilho et al. 2003; Hohmann et al. 2004) as well as the maintenance of thermal and mechanical hyperalgesia and allodynia in models of inflammatory nociception (Quartilho et al. 2003; Gutierrez et al. 2007).

The CB_2 antagonist blocked the anti-allodynic effects produced by AM1241 in the left (injured) paw of neuropathic animals. In our study, however, systemic administration of the CB_1 antagonist SR141716 also partially attenuated AM1241 self-administration in neuropathic rats. However, the CB_1 antagonist also attenuated responding on both the active and inactive levers when neuropathic and naive rats were permitted to self-administer the vehicle. Thus, apparent partial blockade of AM1241 self-administration by SR141716 in neuropathic groups can be attributed to a nonspecific decrease in lever-pressing behavior.

SR141716 transiently lowered paw withdrawal thresholds in the the right (intact) paw when naive and neuropathic groups were allowed to self-administer the vehicle; this

hypersensitivity was observed after but not before each drug self-administration session. Thus, effects of SR141716 on paw withdrawal thresholds in this study cannot be attributed to residual effects of SR141716 accumulation across testing sessions.

It is possible that hypersensitivity could not be measured on the left (injured) paw of neuropathic animals due to floor effects; paw withdrawal thresholds were already very low prior to each drug session, making it difficult to reliably detect further decreases in paw withdrawal thresholds induced by antagonist treatment. SR141716 was administered systemically in these studies because solubility limitations prevented intravenous self-administration of the antagonists with AM1241. SR141716-induced hypersensitivity may be attributed to antagonist-induced blockade of endocannabinoid tone. Our data correlates well with the finding that the CB_1 antagonist SR141716A produces hyperalgesia in the hotplate (Richardson et al. 1997) and formalin (Strangman and Walker 1999) tests. The CB_1 antagonist may be blocking the effects of endocannabinoids in the presence and absence of neuropathic pain. More work is necessary to determine if the endocannabinoid system tonically regulates mechanical withdrawal thresholds in naive or neuropathic groups. Our data is also consistent with the finding that anandamide, 2-AG and CB_1 receptor expression is up-regulated in dorsal root ganglia of spinal nerve ligated rats after the development of neuropathic pain. These findings may explain the hypersensitivity observed in our study following treatment with SR141716 (Mitrirattanakul et al. 2006). SR141716A administered alone also increases hyperalgesia and allodynia in rats subjected to chronic constriction injury of the sciatic nerve and produces side-effects; these observations provide further evidence for changes in endocannabinoid tone in neuropathic rats (Herzberg et al. 1997). In addition, 7 days after chronic constriction injury, anandamide and 2-AG levels are notably increased in the PAG, RVM and spinal cord and increases in anandamide are also observed in the dorsal raphe of neuropathic rats. Thus providing additional evidence for upregulation of endocannabinods at spinals and supraspinal sites in neuropathic rats (Petrosino et al. 2007). However, in naive animals self-administering AM1241, neither the CB_1 nor the CB_2

antagonist lowered paw withdrawal thresholds. Thus, it is possible that AM1241 was protective against hyperalgesic effects induced by SR141716 in neuropathic or naïve animals permitted to self-administer AM1241.

CB_1 is strongly implicated in neurobiological mechanisms that underly drug addiction with multiple drugs of abuse including the psychostimulants, opiates and central nervous system depressants. CB_1 receptors are readily found in the brain reward circuitry and seems to indirectly activate the dopamine system (Maldonado et al. 2006). CB_1 antagonists also block self-administration of morphine and heroin administration (Navarro et al. 2001) and impairs cocaine self-administration in CB_1 knock out mice (Soria et al. 2005). Thus, the observations that CB_1 antagonist attenuates lever pressing behavior was not unexpected.

We also demonstrated that both naïve groups and neuropathic groups self-administered morphine. Morphine self-administration in neuropathic groups was associated with preferential responding on the active but not the inactive lever and increases in paw withdrawal thresholds in the injured but not in the intact paw. Naive animals also showed preferential responding on the active as opposed to the inactive lever on the day of maximal change in paw withdrawal thresholds. By contrast, AM1241 self-administration was not observed in naive groups, whereas, morphine self-administration was observed in both naive and neuropathic groups. These observations reinforce the hypothesis that CB_2 agonists like AM1241 show low abuse potential relative to morphine. Qualitative evaluation by the experimenter also indicated that naive, sham and neuropathic groups allowed to self-administer morphine exhibited signs of withdrawal (e.g. trembling and chewing) with successive self-administration sessions. However, opioid withdrawal symptoms were not assessed in our study but could be quantified in future studies using antagonist-precipitated withdrawal. Our data compliments recent findings in which opioid self-administration decreases tactile allodynia in spinal nerve ligated rats (Martin et al. 2007). However, in this study animals were not trained to discriminate between levers

questioning whether lever pressing behavior which elicited opioid self-administration could be due to a non-specific drug effect.

In conclusion, our studies indicate that a drug self-administration paradigm may be used to study the anti-allodynic as well as the reinforcing effects that are associated with putative analgesics. We showed that neuropathic, but not naïve, animals will self-administer the CB_2-selective agonist AM1241 to decrease neuropathic pain behavior. Self-administration behavior was exhibited by preferential responding on the active, but not the inactive lever and resulted in attenuation of neuropathic pain behavior, as demonstrated by a self-administration-induced increase in paw withdrawal thresholds. In neuropathic groups, AM1241 self-administration behavior and changes in paw withdrawal thresholds were completely blocked by the CB_2 antagonist SR144528. Sham-operated groups also transiently self-administered AM1241 but their self-administration behavior was not as robust (when FR1 schedule was progressively increases to an FR6 schedule; data not shown) as neuropathic animals self-administering AM1241. However, AM1241 self-administration in sham-operated rats was not associated with changes in paw withdrawal thresholds. AM1241 self-administration also did not change paw withdrawal thresholds in naive animals. By contrast, both naïve and neuropathic animals self-administered morphine. Our findings suggest that AM1241 normalizes paw withdrawal thresholds when hyperalgesia and allodynia are present under pathological conditions, without inducing significant anesthesia or analgesia under normal conditions. Moreover, AM1241 does not appear to be inherently reinforcing in naive animals. Our data provides further evidence that therapeutic interventions targeting CB_2 may be used to suppress neuropathic pain behavior without CNS side effects. These observations raise the possibility that CB_2 agonists may represent a class of analgesics with low abuse potential.

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