Published in final edited form as:

Pain. 2011 September ; 152(9): 1976–1987. doi:10.1016/j.pain.2011.03.038.

Self-medication of a cannabinoid CB₂ agonist in an animal model of neuropathic pain

Tannia Gutierrez^{1,2}, Jonathon D. Crystal^{1,2,*}, Alexander M. Zvonok³, Alexandros Makriyannis³, and Andrea G. Hohmann^{1,2,*}

¹Neuroscience and Behavior Program, Department of Psychology, University of Georgia, Athens, GA 30602-3013, USA

²Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN 47405-2204

³Center for Drug Discovery, Northeastern University, Boston, MA, USA

Abstract

Drug self-administration methods were used to test the hypothesis that rats would self-medicate with a cannabinoid CB₂ agonist to attenuate a neuropathic pain state. Self-medication of the CB₂ agonist (*R,S*)-AM1241, but not vehicle, attenuated mechanical hypersensitivity produced by spared nerve injury. Switching rats from (*R,S*)-AM1241 to vehicle self-administration also decreased lever responding in an extinction paradigm. (*R,S*)-AM1241 self-administration did not alter paw withdrawal thresholds in sham-operated or naive animals. The percentage of active lever responding was similar in naive groups self-administering vehicle or (*R,S*)-AM1241. The CB₂ antagonist SR144528 blocked both anti-allodynic effects of (*R,S*)-AM1241 self-medication and the percentage of active lever responding in neuropathic (but not naive) rats. Neuropathic and sham groups exhibited similar percentages of active lever responding for (*R,S*)-AM1241 on a fixed ratio 1 (FR1) schedule. However, neuropathic animals worked harder than shams to obtain (*R,S*)-AM1241 when the schedule of reinforcement was increased (to FR6). (*R,S*)-AM1241 self-medication on FR1, FR3 or FR6 schedules attenuated nerve injury-induced mechanical allodynia. (*R,S*)-AM1241 (900 µg i.v.) failed to produce motor ataxia observed following administration of the mixed CB₁/CB₂ agonist WIN55,212-2 (0.5 mg/kg i.v.). Our results suggest that cannabinoid CB₂ agonists may be exploited to treat neuropathic pain with limited drug abuse liability and central nervous system (CNS) side-effects. These studies validate the use of drug self-administration methods for identifying nonpsychotropic analgesics possessing limited abuse potential. These methods offer potential to elucidate novel analgesics that suppress spontaneous neuropathic pain that is not measured by traditional assessments of evoked pain.

© 2011 International Association for the Study of Pain. Published by Elsevier B.V. All rights reserved.

*Corresponding Authors: *Andrea G. Hohmann, Department of Psychological and Brain Sciences, Indiana University Bloomington, Bloomington, Indiana 47405, Tel: (812) 856-0672, Fax: (812) 856-7187, hohmanna@indiana.edu, *Jonathon D. Crystal, Department of Psychological and Brain Sciences, Indiana University Bloomington, Bloomington, Indiana 47405, Tel: (812) 856-2246, Fax: (812) 856-7187, jcrystal@indiana.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest A.M. serves as a consultant for MAK Scientific.

Keywords

allodynia; hyperalgesia; dependence; drug abuse; analgesic; drug self-administration

INTRODUCTION

Neuropathic pain is caused by injury to the peripheral or central nervous system (CNS) [3]. Treatments for neuropathic pain include anticonvulsants, antidepressants, opioids and adjuvants such as nonsteroidal anti-inflammatory drugs (NSAIDs) [20,51]. However, adverse side-effects limit use [51]. Anticonvulsants and tricyclic antidepressants produce sedation, fatigue, weight gain and ataxia. Newer antidepressants produce nausea and anorexia [20]. NSAIDs have gastrointestinal and cardiovascular risks [20]. Opioids produce nausea, drowsiness, constipation, respiratory depression [20,51], tolerance [2], hyperalgesia [2,14,32] and dependence [5]. Identification of improved treatments for neuropathic pain thus remains a significant clinical need. The clinical challenge is compounded by the fact that animal models of neuropathy typically assess evoked, rather than spontaneous, pain [but see, [30,39,49,50] and inadequately reflect the human condition. Analgesics screened for efficacy in traditional models may or may not translate into efficacious pharmacotherapies in humans. Therefore, novel approaches for identifying safe and effective analgesics with limited abuse liability are necessary.

Cannabinoids share the same target as the psychoactive ingredient in marijuana. Cannabinoids suppress neuropathic nociception through CB₁ and CB₂ mechanisms [6,13,24,27,29,31,47,54]. CB₁ is predominantly located within the CNS [57]. Here, activation is linked to hypoactivity, hypothermia, catalepsy, antinociception [38] and rewarding properties typical of addictive substances [15,16,37,43]. CB₂ is expressed predominantly, but not exclusively [4,7,52], in immune cells and occurs in brain at low levels [33,52]. CB₂ is upregulated in dorsal root ganglia [4] and spinal cord [4,55,56] following injury. CB₂ activation is not associated with CNS side-effects [22,36] linked to CB₁ (see [19]). However, abuse potential of CB₂ agonists is unknown.

CB₂ agonists are antinociceptive in animal models of pathological pain [8,12,19]. (*R,S*)-AM1241, a well-characterized CB₂ agonist, produces antinociception [36,46] and suppresses inflammatory [21,25,41,42,44], neuropathic [27,45] and cancer [18,34] pain. Antinociceptive effects of (*R,S*)-AM1241 are blocked by CB₂ but not CB₁ antagonists, are preserved in CB₁^{-/-} mice and are absent in CB₂^{-/-} mice [27]. Antinociceptive effects of CB₂ agonists, including (*R,S*)-AM1241, are not dependent upon opioid receptor activation [[26,46] but see [28]]. (*R,S*)-AM1241 suppresses spinal neuronal sensitization and windup and inflammation-evoked Fos protein expression in spinal cord regions implicated in nociceptive processing [41,42]. However, it is unknown whether animals will self-medicate with a nonpsychotropic analgesic to alleviate spontaneous chronic pain.

We used a drug self-administration approach to ask whether rats with a spared nerve injury (SNI) [11] would self-medicate with a CB₂ agonist to attenuate a neuropathic pain state. A two lever drug self-administration paradigm was used; responses on the active, but not the inactive, lever elicited an intravenous infusion. (*R,S*)-AM1241 self-administration was evaluated in neuropathic, sham-operated and naive rats. Paw withdrawal thresholds were measured before and after drug self-administration sessions to assess the impact of (*R,S*)-AM1241 self-administration on neuropathic pain behavior. (*R,S*)-AM1241 self-medication was also evaluated in an extinction paradigm. Effects of changing the schedule of reinforcement on (*R,S*)-AM1241 self-administration behavior was compared in neuropathic and sham-operated rats. Pharmacological specificity was evaluated using the CB₂ antagonist

SR144528. Our results suggest that the present approach offers potential to separate analgesic efficacy and drug abuse liability.

METHODS

Subjects

One hundred and nine male Sprague-Dawley rats weighing 275–300g at the beginning of the experiment (Harlan, Indianapolis, IN) were used. Animals were housed in a temperature controlled room with lights kept on a reverse 12-h light schedule. All procedures were approved by the institutional animal care and use committee and followed the guidelines for the treatment of animals of the International Association for the Study of Pain [58].

Drugs and Chemicals

(*R,S*)-AM1241 was synthesized (by AZ) in the Makriyannis laboratory. SR144528 was provided by NIDA. A 1.5% heparin saline solution was used to flush jugular catheters daily and dissolve drugs. Drugs were dissolved in 7.5% ethanol, 7.5% tween 80, 85% heparinized saline solution for intravenous (i.v.) drug self-administration. SR14428 was dissolved in vehicle containing a 1:1:18 ratio of emulphor, ethanol and saline for systemic (i.p.) administration.

Spared Nerve Injury

Spared nerve injury was performed as described previously [11]. An incision was performed directly on the biceps femoris muscle and the sciatic nerve and its three terminal branches, the sural, common peroneal and tibial nerves, were isolated. The tibial and the common peroneal nerve were ligated with 4.0 silk thread and cut (with 1–4 mm excised). The sural nerve was left intact [11]. In sham rats, the sciatic nerve and its terminal branches were exposed but all nerves were left intact. The overlying skin and muscle was sutured closed in layers in both sham and neuropathic groups. Nerves were not exposed in otherwise naive groups.

Jugular Catheterization

All rats were surgically implanted with an indwelling jugular catheter. Rats were anesthetized with halothane and catheters were prepared as described previously [1]. A catheter containing a bent cannula and silastic tubing was encased with dental cement. The cannula was passed under 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 patency. Rats were allowed to recover 5–7 days after surgery.

General Experimental Methods

Baseline mechanical withdrawal thresholds in the left (injured) and right (intact) paws were assessed in all rats one day prior to surgical manipulations. Thresholds were reassessed in all rats five to seven days after surgical manipulations to confirm that: i) spared nerve injury suppressed mechanical withdrawal thresholds relative to baseline (pre-surgery) levels, ii) sham surgery did not alter mechanical withdrawal thresholds relative to baseline, and iii) implantation of jugular catheters alone did not alter mechanical withdrawal thresholds in either paw relative to baseline. Spared nerve injury rats that did not exhibit mechanical hypersensitivity post-surgery were not used in the study. Mechanical withdrawal thresholds were assessed in all rats before (pre-session) and after (post-session) each drug self-administration session except where noted. Rats received 50 min drug self-administration sessions in which a lever press on the active lever resulted in a drug infusion on a minimum

of four test days. A lever press on the inactive lever did not deliver a drug infusion. Immediately after the drug self-administration session, rats were removed from the operant chambers, and placed on a wire mesh platform for assessment of post-drug self-administration mechanical withdrawal thresholds. Weights were monitored daily in all rats.

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 to reduce the animal's weight by 20% to facilitate the training procedure. Food restriction was terminated after the training procedure was completed; rats were returned to ad lib food and supplemental food (standard rat chow) was additionally made available to them on the floor of the home cage. Rats were individually placed in operant chambers and tested once per day. The first pre-training session consisted of one 30 min session in which one food pellet was delivered every 60 seconds. The number of head insertions into the food trough was recorded during this 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 was exposed and the other lever was retracted. This pattern was repeated until the rat reached a criterion of 60 pellets earned within the training session.

Drug self-administration apparatus

The operant chambers employed for drug self-administration were equipped with two levers (referred to as left and right levers). In an initial study, separate groups of rats were tested with either the left or the right lever designated as the active lever. Lever presses on the active lever elicited an intravenous drug infusion, whereas lever presses on the inactive lever failed to elicit an infusion. No difference in operant behavior was found regardless of whether the right or left lever was designated as the active lever (see results). Therefore, for subsequent studies employing a unilateral injury, the left lever was designated as the active lever and the right lever was designated as the inactive lever. In all cases, when the rat pressed the active lever, a Harvard infusion pump (Holliston, MA) was switched on for approximately 6 seconds, based on the rat's body weight, and resulted in one drug infusion. Three doses of (*R,S*)-AM1241 were tested in pilot studies (60, 30, 15 µg/kg/infusion), the middle dose was selected for subsequent experiments because this dose resulted in maximal elevation of the mechanical paw withdrawal threshold, and carry over effects on subsequent drug self-administration sessions were absent at this dose. The pump delivered approximately 6–8 µl of (*R,S*)-AM1241 (1.5 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 the active lever did not result in another drug infusion. Pressing the inactive lever produced no drug infusion.

Assessment of Mechanical Allodynia

Mechanical allodynia was assessed using a digital electrovonfrey anesthesiometer (IITC model Alemo 2290-4, Woodland Hills, CA) equipped with a rigid tip (0.8 mm diameter). 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 mid-plantar region of the hind paw through the floor of the mesh platform. Mechanical withdrawal thresholds to punctate mechanical stimulation were measured in duplicate for the left (manipulated, ipsilateral to spared nerve injury or sham surgery) and right (intact, contralateral to spared nerve injury or sham surgery) paw. Mechanical stimulation was terminated upon paw withdrawal. The electrovonfrey device 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 and after surgery. Mechanical withdrawal thresholds were additionally evaluated before and after (10–15 min post session) each drug self-administration session for four or five consecutive test days, except where noted. In a subset of studies performed in naive groups, measurements of paw withdrawal thresholds were restricted to initial and terminal sessions because earlier studies failed to detect differences in mechanical withdrawal thresholds across consecutive days following (*R,S*)-AM1241 or vehicle self-administration. Naive animals in which paw withdrawal thresholds were evaluated either daily or on the initial and terminal drug self-administration sessions only were combined for statistical analysis.

Assessment of vehicle or (*R,S*)-AM1241 self-administration

Effects of vehicle or (*R,S*)-AM1241 self-administration on mechanical withdrawal thresholds and lever pressing behavior was evaluated in separate groups of naive rats across 5 consecutive days. In some groups, the left lever was designated as the active lever and in other groups the right lever was designated as the active lever. Mechanical withdrawal thresholds were assessed in the left (intact) and right (intact) paws in duplicate before and after each experimental session as described previously. This study enabled us to confirm that observed drug self-administration behavior or changes in mechanical withdrawal thresholds could not be attributed to the placement of the jugular catheter or the location of the active lever. For the remainder of the studies (i.e. those employing unilateral injury), the left lever was designated as the active lever and right lever was designated as the inactive lever.

Assessment of (*R,S*)-AM1241 self-administration in naive animals in the presence and absence of the CB₂ antagonist SR144528

The impact of (*R,S*)-AM1241 self-administration and antagonist treatment on basal nociceptive thresholds and lever responding was evaluated in naive animals. Naive animals were allowed to lever press for (*R,S*)-AM1241 (1.5 µg/µl) in the presence or absence of the CB₂ antagonist SR144528 (1 mg/kg i.p. 30 min prior to the session) across five consecutive days. Mechanical withdrawal thresholds were assessed in each paw in duplicate before and after the initial (session 1) and terminal (session 5) sessions.

Assessment of vehicle self-administration on mechanical withdrawal thresholds in sham and neuropathic animals

Effects of vehicle self-administration on mechanical paw withdrawal thresholds and lever pressing behavior was evaluated in separate groups of sham and neuropathic rats across 4 consecutive days.

Extinction and reinstatement of (*R,S*)-AM1241 self-medication in neuropathic animals

To evaluate extinction of (*R,S*)-AM1241 self-medication, a single group of neuropathic animals were allowed to self-administer (*R,S*)-AM1241 (3–1.5 µg/µl) on 4 consecutive days followed by 6 days of access to vehicle, followed by return of access to (*R,S*)-AM1241 on 2 days. Not all animals completed a full 4 days of reinstatement due to technical problems related to catheter occlusion.

Pharmacological specificity

Separate groups of neuropathic rats were allowed to self-administer (*R,S*)-AM1241 in the presence or absence of the CB₂ antagonist SR144528 (1 mg/kg i.p.). The antagonist was administered 30 min prior to each of 4 consecutive drug self-administration sessions.

Effects of changing the schedule of reinforcement on (R,S)-AM1241 self-administration and mechanical withdrawal thresholds in sham and neuropathic animals

The schedule of reinforcement was increased from FR1 to FR3 to FR6 and finally to FR9 in neuropathic and sham-operated rats allowed to self-administer (R,S)-AM1241. Animals were required to press the lever 1, 3, 6 or 9 times on FR1, FR3, FR6 or FR9 schedules, respectively, to obtain the same single drug infusion. After establishing stable responding on an FR1 schedule, rats were evaluated for 3 sessions on each schedule of reinforcement for a total of 12 consecutive sessions. Lever responding on 3 consecutive sessions was averaged for each ratio to produce one data point per fixed ratio for each rat. Mechanical withdrawal thresholds were analyzed identically.

Assessment of Motor Ataxia

Effects of i.v. administration of the CB₂-selective agonist (R,S)-AM1241, the mixed CB₁/CB₂ agonist WIN55,212-2, and vehicle 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 [13]. 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 i.v. administration of the maximally self-administered dose of (R,S)-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 conditions. 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. ANOVA and planned comparison t-tests were additionally used to assess statistical significance of experimental differences between drug self-administration groups for the entire 50 min drug self-administration interval. The number of active and inactive lever presses was recorded in all studies and compared within and across groups using repeated measures ANOVA. The percentage of responding on the active lever was calculated over the entire 50 min drug self-administration session and used for data presentation using the formula: active lever presses / (active + inactive lever presses) × 100.

Changes in lever responding were quantified to determine the impact of switching neuropathic animals from (R,S)-AM1241 to vehicle self-administration (i.e. extinction). Total lever responses were determined for the (R, S)-AM1241 (sessions 1–4) and vehicle (sessions 5–10) self-administration phase for each animal. A paired t-test was used to compare responding in the (R,S)-AM1241 and vehicle self-administration phases.

To calculate the impact of changing the schedule of reinforcement on (R,S)-AM1241 self-administration behavior, the percentage of active lever presses was calculated for each session and averaged across all sessions of the same schedule for each animal. The slope of the regression line fit through the mean active lever responding on the increasing FR schedules (i.e. performance on FR1, FR3 and FR6, representing all schedules prior to the breaking point) was determined for each rat. Differences between sham and neuropathic groups in the slopes of the regression lines were determined by a planned comparison t-test (one-tailed). Mechanical withdrawal thresholds for each FR schedule were similarly averaged to produce one corresponding threshold data point for each schedule for each rat, and averaged across groups.

In all studies, post hoc comparisons were performed 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 [17]. $P < 0.05$ was considered significant.

RESULTS

Control Conditions

In all studies employing naive groups, mechanical withdrawal thresholds in the left (intact) and right (intact) paws did not differ before or after jugular catheterization (data not shown). Moreover, no difference in the number of active lever presses, inactive lever presses or the percentage of active lever responding was found within or between each naive group regardless of whether the right or left lever was designated as the active lever (data not shown). Similarly, drug self-administration behavior (i.e. number of active lever presses, number of inactive lever presses, percentage of active lever responding) in naive animals allowed to self-administer (*R,S*)-AM1241 did not differ regardless of whether the left or right lever was designated as the active lever (data not shown). Therefore, naive groups self-administering vehicle or (*R,S*)-AM1241, respectively, were pooled to form a single naive vehicle and a single naive (*R,S*)-AM1241 group for further statistical comparisons.

The number of active and inactive lever presses did not differ within the naive group self-administering either vehicle or (*R,S*)-AM1241 (data not shown). The percentage of active lever responding did not differ in naive groups self-administering vehicle or (*R,S*)-AM1241 (Fig. 1A). Because no difference in mechanical withdrawal thresholds or lever pressing behavior was found regardless of which lever was designated as the active lever, in subsequent studies employing unilateral nerve injury or sham surgery, only the left lever was designated as the active lever. Therefore, the percentage of active lever responding is presented and used for data analysis in the following studies.

Effects of the CB₂ antagonist SR144528 on (*R,S*)-AM1241 drug self-administration and mechanical withdrawal thresholds in naive rats

Mechanical withdrawal thresholds were assessed on the initial and terminal drug self-administration sessions in naive groups allowed to self-administer (*R,S*)-AM1241 in the presence and absence of the CB₂-selective antagonist SR144528. Pre- and post-self-administration mechanical withdrawal thresholds in either the left (intact) or right (intact) paw did not differ in these naive groups (data not shown). Identical conclusions were reached in the subset of experiments in which mechanical withdrawal thresholds were assessed in naive animals on each daily drug self-administration session (data not shown). Thus, CB₂ agonist or antagonist treatments did not alter basal nociceptive thresholds to mechanical stimulation in the absence of nerve injury. Furthermore, SR144528 did not alter the percentage of active lever responding across all sessions in naive groups self-administering the CB₂-selective agonist (*R,S*)-AM1241 (Fig. 1B).

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

Spared nerve injury (SNI) lowered mechanical withdrawal thresholds in the left (injured) paw of the neuropathic group ($F_{1,12} = 3151.59$, $P < 0.0001$, Fig. 2a) prior to initiation of drug self-administration sessions. Mechanical withdrawal thresholds in the left (injured) paw of the neuropathic group were lower compared to baseline thresholds and all other groups ($F_{1,12} = 5783.02$, $P < 0.0001$, $P < 0.001$ for all comparisons, Fig. 2a). SNI or sham surgery did not change mechanical withdrawal thresholds in the right (intact) paw (Fig. 2b).

Mechanical allodynia was evident in the left (injured) paw of the neuropathic but not the sham group ($F_{1,36} = 47810.82$, $P < 0.0001$, Fig. 2c) in animals allowed to self-administer vehicle. Vehicle self-administration did not alter mechanical withdrawal thresholds in the left (injured) paw of either the neuropathic or the sham group ($F_{1,36} = 10731.40$, $P < 0.0001$, Fig. 2c,d). A transient but reliable difference in pre-session mechanical withdrawal thresholds was observed in the right (intact) paw between sham and neuropathic animals self-administering vehicle ($F_{1,36} = 25.83$, $P < 0.003$, Fig. 2e). This difference was restricted to session 3 ($P < 0.05$). A transient but reliable difference in post-session thresholds was also detected in the right (intact) paw of sham and neuropathic animals self-administering vehicle ($F_{1,36} = 24.42$, $P < 0.003$, Fig. 2f). This difference was restricted to session 1 ($P < 0.05$) and absent from the remainder of the sessions. The percentage of active lever responding was elevated in neuropathic animals self-administering vehicle compared to sham animals ($F_{1,12} = 11.12$, $P < 0.0060$, Fig. 3). However, vehicle self-administration did not affect mechanical withdrawal thresholds as described above.

Extinction and reinstatement of (R,S)-AM1241 self-administration behavior in neuropathic rats

In the neuropathic group evaluated for extinction of (R,S)-AM1241 self-administration, SNI lowered mechanical withdrawal thresholds in the left (injured) paw ($P < 0.0001$, Fig. 4a) but not in the right (intact) paw (Fig. 4b) prior to initiation of drug self-administration testing. (R,S)-AM1241 self-administration increased mechanical withdrawal thresholds in the left (injured) paw of the neuropathic group when animals self-administered (R,S)-AM1241, but not when the neuropathic animals were only allowed access to vehicle ($F_{1,110} = 171.31$, $P < 0.0001$, Fig. 4c). Anti-allodynic effects were specific to days when animals self-administered (R,S)-AM1241 ($F_{11,110} = 33.82$, $P < 0.0001$, Fig. 4c). Mechanical withdrawal thresholds increased when the neuropathic group self-administered (R,S)-AM1241 (sessions 1 – 4: $P < 0.0001$, session 11 – 12: $P < 0.0001$), but returned to baseline levels when the neuropathic group self-administered vehicle (sessions 5 to 10, Fig. 4c). Subsequent access to (R,S)-AM1241 (session 11 – 12) also reinstated the suppression of allodynia in the same animals (Fig. 4c). No change in mechanical withdrawal thresholds was observed in the right (intact) paw in the neuropathic group self-administering (R,S)-AM1241 or vehicle (Fig. 4d).

The switch from (R,S)-AM1241 to vehicle self-administration decreased the number of responses per session relative to the (R,S)-AM1241 (pre-extinction) baseline ($t(5) = 6.25$, $P < 0.01$, Fig. 5). The same conclusion was reached when the analysis was restricted to the number of active lever responses ($t(5) = 6.59$, $P < 0.01$, data not shown).

Pharmacological specificity

Prior to initiation of drug self-administration procedures, SNI produced mechanical allodynia in the left (injured) paw of neuropathic animals compared to baseline thresholds ($P < 0.001$ for all comparisons, Fig. 6a) without affecting mechanical withdrawal thresholds in the right (intact) paw (Fig. 6b). Moreover, prior to each daily self-administration session, mechanical allodynia did not differ in the left (injured) paw of neuropathic groups self-administering (R,S)-AM1241 in the presence or absence of the CB₂-selective antagonist SR144528 (Fig 6c). Pre-treatment with SR144528 blocked the anti-allodynic effects produced by (R,S)-AM1241 self-administration ($F_{1,36} = 56.24$, $P < 0.0001$, Fig. 6d). Self-administration of (R,S)-AM1241 also increased mechanical withdrawal thresholds compared to pre-session thresholds ($F_{1,14} = 90.82$, $P < 0.0001$, Fig. 6c–d). These anti-allodynic effects were observed across all (R,S)-AM1241 self-administration sessions ($F_{3,42} = 5.72$, $P < 0.002$, $P < 0.0008$ for all comparisons, Fig. 6c–d). Mechanical withdrawal thresholds did not differ in the right (intact) paw of either neuropathic group (Fig. 6e–f). The CB₂-selective antagonist SR144528 also decreased the percentage of active lever responding for (R,S)-

AM1241 in the neuropathic group across all experimental sessions ($F_{1,36} = 5.26$, $P < 0.04$, Fig. 7).

Effects of changing schedule of reinforcement on (R,S)-AM1241 self-administration in sham and neuropathic rats

SNI lowered mechanical withdrawal thresholds in the left (injured) paw ($F_{1,8} = 2162.97$, $P < 0.0001$, Fig. 8a) prior to initiation of drug self-administration sessions. Mechanical withdrawal thresholds were lower in the neuropathic group compared to baseline thresholds and all other groups ($F_{1,8} = 3350.16$, $P < 0.0001$, $P < 0.0001$ for all comparisons, Fig. 8a). Mechanical withdrawal thresholds in the right (intact) paw did not change in sham and neuropathic groups before and after surgical manipulations (Fig. 8b). Neuropathic and sham groups self-administering (R,S)-AM1241 from this study were initially tested on an FR1 schedule to document stable responding over 7 consecutive sessions (data not shown). The same neuropathic and sham groups self-administering (R,S)-AM1241 were further tested by increasing the schedule of reinforcement from FR1 to FR3 to FR6 and finally to FR9.

Mechanical withdrawal thresholds (averaged across 3 consecutive sessions for a given FR schedule) were compared in sham and neuropathic groups self-administering (R,S)-AM1241. Pre-session mechanical withdrawal thresholds remained low in the left (injured) paw of neuropathic animals compared to the sham-operated animals across all schedules ($F_{1,8} = 27328.19$, $P < 0.0001$, Fig. 8c). After each (R,S)-AM1241 self-administration session, mechanical withdrawal thresholds increased in the left (injured) paw of neuropathic animals compared to sham rats on schedules FR1, FR3 and FR6 ($F_{1,8} = 1085.29$, $P < 0.0001$, Fig. 8d). The magnitude of the anti-allodynic effect of (R,S)-AM1241 was also dependent upon the FR schedule ($F_{3,24} = 25.05$, $P < 0.0001$, $P < 0.0001$ for all comparisons, Fig. 8d). Post-session mechanical withdrawal thresholds in neuropathic animals self-administering (R,S)-AM1241 declined toward baseline levels when animals were switched to FR9 ($P < 0.0001$, Fig. 8d), suggesting that FR9 represented the breaking point for responding for (R,S)-AM1241. In neuropathic rats, self-administration of (R,S)-AM1241 increased post-session mechanical withdrawal thresholds in rats on FR1, FR3 and FR6 schedules relative to pre-session thresholds ($F_{1,6} = 328.38$, $P < 0.001$, Fig. 8c–d). The magnitude of this anti-allodynia was also dependent upon the FR schedule ($F_{3,18} = 18.31$, $P < 0.001$, $P < 0.0001$ for all comparisons, Fig. 8c–d). No change in mechanical withdrawal thresholds was observed in the right (intact) paw of neuropathic rats before or after (R,S)-AM1241 drug self-administration session on any FR schedule (Fig. 8e–f). Moreover, (R,S)-AM1241 self-administration did not alter mechanical withdrawal thresholds in sham rats in either paw on any FR schedule.

The neuropathic group self-administered (R,S)-AM1241 to a greater extent than the sham group on FR6, but not on the other schedules ($P < 0.05$, Fig. 9a). Percent active lever responding was similar in neuropathic and sham rats on the FR1, FR3 and FR9 schedules (Fig. 9a). Active lever responding notably declined for both groups on the FR9 schedule, indicating that the animals were less inclined to work for (R,S)-AM1241. The slope of the regression line fit to FR responding (i.e. fit to the percentage of active lever responding for (R,S)-AM1241 on the FR1, FR3 and FR6 schedules) was greater in the neuropathic group self-administering (R,S)-AM1241 compared to shams ($P < 0.05$, Fig. 9b). Thus, the neuropathic group was willing to work harder than the sham group to obtain the drug.

Assessment of motor ataxia

The non-selective CB₁/CB₂ agonist WIN55,212-2, decreased rotarod latencies relative to all other groups ($F_{4,25} = 6.347$, $P < 0.001$, $P < 0.01$ for all comparisons) whereas the CB₂-selective agonist (R,S)-AM1241 was without effect. (R,S)-AM1241 failed to alter rotarod

latencies in either naive or neuropathic groups relative to vehicle treatment. By contrast, WIN55,212-2 produced 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. 10). The latency to descend from the rotarod did not differ between groups on either training session prior to i.v. drug administration.

DISCUSSION

Neuropathic animals self-medicated with (*R,S*)-AM1241, a well-characterized CB₂ agonist, to attenuate a neuropathic pain state. CB₂ agonists suppress measures of evoked pain in behavioral [12,21,27,36,41,42,44,45,47] and electrophysiological [12,42,47] studies. However, the present study is the first to suggest that neuropathic animals self-administer a putative nonpsychotropic analgesic to attenuate a neuropathic pain state. CB₂ receptor blockade prevented both anti-allodynic effects of (*R,S*)-AM1241 self-medication as well as the preference for responding for (*R,S*)-AM1241 on the active lever. Thus, the present drug self-administration paradigm offers potential for identifying analgesics with limited abuse liability in intact animals that are nonetheless self-medicated in the presence of injury to attenuate neuropathic pain. In the presence of injury, a putative non-psychotropic analgesic may become rewarding for its negative reinforcing properties (i.e. ability to attenuate a neuropathic pain state). Operant assessments of therapeutic self-medication combined with assessment of pain thresholds may identify analgesics that suppress spontaneous neuropathic pain that is not measured in traditional assessments of evoked pain. Our study, by allowing animals to control self-medication of the analgesic, attempts to target the motivational aspects of pain relief that is difficult to assess in animal studies. Our data are consistent with results of conditioned place preference studies that target spontaneous pain relief by pairing an exogenously administered analgesic with contextual cues [30,49,50]. However, in these latter studies, animals do not control intake of the analgesic.

Drug self-administration paradigms are widely used to assess positive reinforcing properties of drugs in the absence of pain. However, few studies have attempted to use a drug self-administration paradigm in the context of assessing analgesic efficacy and its impact on pathological pain [9,10,35,39,40]. An initial study showed that arthritic rats self-administered less morphine compared to naive rats, suggesting that the rewarding aspects of morphine self-administration may be related to the expectation of alleviating pain caused by arthritis [35]. Arthritic rats exhibited some pain relief in the tail pressure test [35]. Although the naive group reliably self-administered more morphine compared to the arthritic group, rewarding and addictive properties of morphine in the arthritic group could not be discounted [35]. Rewarding, but not antinociceptive, properties of heroin self-administration [40] are attenuated by a delta antagonist [40]. A limitation of prior studies was that drug self-administration and antinociception were not assessed in the same animals and these studies did not employ a two lever paradigm. By contrast, in our study, we used the cannabinoid CB₂-agonist (*R,S*)-AM1241 to assess, in the same animals, both analgesic self-administration behavior and reversal of neuropathic pain. In our study, all animals were allowed to self-medicate and control drug intake to alleviate pain behavior. The failure of animals to persevere in responding for (*R,S*)-AM1241 during extinction also suggests that the self-administered analgesic possesses limited abuse liability.

A unique feature of our study is that we employed a two lever drug self-administration task wherein responding on the active, but not the inactive, lever results in a drug infusion. This experimental design better enables us to identify the intention of the animal to selectively self-administer the drug. Self-medication cannot be attributed to lever bias because animals were pretrained to lever press on two levers and showed no preference for responding for (*R,S*)-AM1241 or vehicle in the intact state. In our design, paw withdrawal thresholds were

assessed immediately after each self-medication session to evaluate the immediate efficacy of the compound in alleviating neuropathic pain. Moreover, we assessed paw withdrawal thresholds in both the injured and intact paws, allowing us to determine whether changes in paw withdrawal threshold elicited by analgesic self-medication were confined ipsilaterally to the site of injury or reflected more global antinociceptive effects. These methods also enabled us to verify that residual analgesia did not produce carryover effects that influenced self-administration behaviors or paw withdrawal thresholds on subsequent days.

It is unlikely that lever pressing for (*R,S*)-AM1241 reflects a nonspecific change in motor activity or food-seeking behavior. First, each operant chamber was equipped with two levers, but only one lever was designated as the active lever and produced an intravenous infusion. The observation that neuropathic animals respond preferentially on the active lever and are willing to work harder than shams to obtain the drug also rules out this alternative explanation. Second, animals are pretrained to lever press for food on two levers, and rats did not exhibit a lever bias prior to initiation of drug self-administration sessions. Third, food restriction was terminated after lever training was completed, and animals were given free access to food for the remainder of the experiment (i.e. prior to surgical procedures, during recovery and throughout all drug self-administration phases). Fourth, in an extinction paradigm, switching animals from (*R,S*)-AM1241 to vehicle self-administration produced a decline in lever responding and eliminated the anti-allodynic effect of (*R,S*)-AM1241 self-administration.

Selectivity of (*R,S*)-AM1241 self-medication is demonstrated in both the extinction study and in assessments of pharmacological specificity. Mechanical withdrawal thresholds in the left (injured) paw were elevated on days that neuropathic animals self-administered (*R,S*)-AM1241, but not on days that neuropathic animals self-administered vehicle. Mechanical withdrawal thresholds in the extinction group were not affected in the right (intact) paw regardless of whether neuropathic animals self-administered (*R,S*)-AM1241 or vehicle. The switch from (*R,S*)-AM1241 to vehicle self-administration also produced a decline in the percentage of lever responding. Identical conclusions were obtained whether responses on both levers or on the active lever only were assessed. This pattern of responding is strikingly distinct from that of classical abused drugs in which removal of the drug in extinction is associated with an increase in active lever responding [48,53]. A return of access to (*R,S*)-AM1241 reinstated the suppression of allodynia produced by (*R,S*)-AM1241 self-administration. However, problems with catheter patency prevented statistical evaluation of reinstatement of (*R,S*)-AM1241 self-medication on lever pressing behavior over the complete reinstatement phase. In all studies, paw withdrawal thresholds also returned to baseline before initiation of the next drug self-administration session, documenting the absence of carryover effects. Finally, the CB₂ antagonist SR144528 completely blocked both the percentage of active lever pressing for (*R,S*)-AM1241 and resulting anti-allodynic effects.

CB₁ receptors are expressed in areas implicated in drug reward [16,23]. CB₁ receptor activation produces tolerance and dependence, possibly involving interactions with opioid receptors in common neural substrates [37]. However, naive and neuropathic animals treated with the highest self-administered dose of (*R,S*)-AM1241 did not show CNS side-effects in the rota-rod test. (*R,S*)-AM1241 self-administration is thus, unlikely to nonselectively activate CB₁ receptors. First, both anti-allodynic effects of (*R,S*)-AM1241 self-medication and the percentage of active lever responding were blocked completely by a CB₂ antagonist. Second, the maximally self-administered dose of (*R,S*)-AM1241 failed to produce motor impairment, a cardinal sign of CB₁ receptor activation. Only neuropathic animals treated with the mixed CB₁/CB₂ agonist WIN55,212-2 demonstrated motor ataxia.

An unexpected observation of our studies was that sham animals showed a similar percentage of active lever responding as neuropathic animals on an FR1 schedule. However, (*R,S*)-AM1241 self-administration altered mechanical withdrawal thresholds in neuropathic animals only. It is important to emphasize that sham animals, although free of nerve injury, nonetheless sustained muscle damage (i.e. nerves were surgically exposed through an incision in the biceps femoris and the muscle and skin were sutured closed) and were not completely intact. More work is necessary to determine whether sham animals self-administer (*R,S*)-AM1241 to suppress pain resulting from incisional injury that responds to CB₂ agonists [31] but is not measured when mechanical allodynia is assessed on the plantar paw surface. Nonetheless, neuropathic animals worked harder than shams to obtain (*R,S*)-AM1241 when tested under identical conditions. A clear distinction in patterns of (*R,S*)-AM1241 self-administration emerged when the schedule of reinforcement was increased. Neuropathic animals worked harder to self-administer (*R,S*)-AM1241 compared to sham animals as demonstrated by the observation of both a greater percentage of active lever responding for (*R,S*)-AM1241 on an FR6 schedule and the greater slope of the regression line fit to FR schedule responding in the neuropathic group. Performance declined in both neuropathic and sham groups on an FR9 schedule, suggesting that the FR9 schedule represented the breaking point in responding for (*R,S*)-AM1241. It is possible that the 50 min drug self-administration session was not long enough for animals to learn that they were required to press the lever nine times to obtain a single i.v. infusion of (*R,S*)-AM1241. Nonetheless, (*R,S*)-AM1241 self-administration attenuated mechanical paw withdrawal thresholds in the left (injured) paw of the neuropathic group regardless of the schedule of reinforcement and did not alter mechanical paw withdrawal thresholds in the right (intact) paw of any group. More work is necessary to determine the sites of action implicated in this analgesic self-medication.

In conclusion, the present studies show that animals will self-medicate with a cannabinoid CB₂ agonist to attenuate a neuropathic pain state. Self-medication of the CB₂ agonist suppressed both nerve injury-induced mechanical hypersensitivity and the percentage of active lever responding for (*R,S*)-AM1241 through a CB₂-specific mechanism. The present findings also suggest that operant methods hold promise for identifying classes of nonpsychotropic analgesics that suppress spontaneous neuropathic pain but possess limited drug abuse liability in humans.

Acknowledgments

We thank Kenneth Maxwell for his technical support in this project. Supported by DA028200 (to AGH and JDC) and DA021644 (to AGH)

REFERENCES

1. Ahmed SH, Koob GF. Cocaine- but not food-seeking behavior is reinstated by stress after extinction. *Psychopharmacology (Berl)*. 1997; 132(3):289–295. [PubMed: 9292629]
2. Ballantyne JC, Shin NS. Efficacy of opioids for chronic pain: a review of the evidence. *Clin J Pain*. 2008; 24(6):469–478. [PubMed: 18574357]
3. Baron R. Neuropathic pain: a clinical perspective. *Handb Exp Pharmacol*. 2009; (194):3–30. [PubMed: 19655103]
4. Beltramo M, Bernardini N, Bertorelli R, Campanella M, Nicolussi E, Fredduzzi S, Reggiani A. CB₂ receptor-mediated antihyperalgesia: possible direct involvement of neural mechanisms. *Eur J Neurosci*. 2006; 23(6):1530–1538. [PubMed: 16553616]
5. Benyamin R, Trescot AM, Datta S, Buenaventura R, Adlaka R, Sehgal N, Glaser SE, Vallejo R. Opioid complications and side effects. *Pain Physician*. 2008; 11(2 Suppl):S105–S120. [PubMed: 18443635]

6. Bridges D, Ahmad K, Rice AS. The synthetic cannabinoid WIN55,212-2 attenuates hyperalgesia and allodynia in a rat model of neuropathic pain. *Br J Pharmacol.* 2001; 133(4):586–594. [PubMed: 11399676]
7. Buckley NE, McCoy KL, Mezey E, Bonner T, Zimmer A, Felder CC, Glass M. Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. *Eur J Pharmacol.* 2000; 396(2–3):141–149. [PubMed: 10822068]
8. Clayton N, Marshall FH, Bountra C, O'Shaughnessy CT. CB1 and CB2 cannabinoid receptors are implicated in inflammatory pain. *Pain.* 2002; 96(3):253–260. [PubMed: 11972997]
9. Colpaert FC, Meert T, De Witte P, Schmitt P. Further evidence validating adjuvant arthritis as an experimental model of chronic pain in the rat. *Life Sci.* 1982; 31(1):67–75. [PubMed: 7109855]
10. Colpaert FC, Tarayre JP, Alliaga M, Bruins Slot LA, Attal N, Koek W. Opiate self-administration as a measure of chronic nociceptive pain in arthritic rats. *Pain.* 2001; 91(1–2):33–45. [PubMed: 11240076]
11. Decosterd I, Woolf CJ. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain.* 2000; 87(2):149–158. [PubMed: 10924808]
12. Elmes SJ, Jhaveri MD, Smart D, Kendall DA, Chapman V. Cannabinoid CB2 receptor activation inhibits mechanically evoked responses of wide dynamic range dorsal horn neurons in naïve rats and in rat models of inflammatory and neuropathic pain. *Eur J Neurosci.* 2004; 20(9):2311–2320. [PubMed: 15525273]
13. Fox A, Kessingland A, Gentry C, McNair K, Patel S, Urban L, James I. The role of central and peripheral Cannabinoid1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. *Pain.* 2001; 92(1–2):91–100. [PubMed: 11323130]
14. Gardell LR, King T, Ossipov MH, Rice KC, Lai J, Vanderah TW, Porreca F. Opioid receptor-mediated hyperalgesia and antinociceptive tolerance induced by sustained opiate delivery. *Neurosci Lett.* 2006; 396(1):44–49. [PubMed: 16343768]
15. Gardner EL. Addictive potential of cannabinoids: the underlying neurobiology. *Chem Phys Lipids.* 2002; 121(1–2):267–290. [PubMed: 12505706]
16. Gardner EL. Endocannabinoid signaling system and brain reward: emphasis on dopamine. *Pharmacol Biochem Behav.* 2005; 81(2):263–284. [PubMed: 15936806]
17. Greenhouse SW, Geisser S. On methods in the analysis of profile data. *Psychometrika.* 1959; 24:95–112.
18. Guerrero AV, Quang P, Dekker N, Jordan RC, Schmidt BL. Peripheral cannabinoids attenuate carcinoma-induced nociception in mice. *Neurosci Lett.* 2008; 433(2):77–81. [PubMed: 18242856]
19. Guindon J, Hohmann AG. Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain. *Br J Pharmacol.* 2008; 153(2):319–334. [PubMed: 17994113]
20. Guindon J, Walczak JS, Beaulieu P. Recent advances in the pharmacological management of pain. *Drugs.* 2007; 67(15):2121–2133. [PubMed: 17927280]
21. Gutierrez T, Farthing JN, Zvonok AM, Makriyannis A, Hohmann AG. Activation of peripheral cannabinoid CB1 and CB2 receptors suppresses the maintenance of inflammatory nociception: a comparative analysis. *Br J Pharmacol.* 2007; 150(2):153–163. [PubMed: 17160008]
22. Hanus L, Breuer A, Tchilibon S, Shiloah S, Goldenberg D, Horowitz M, Pertwee RG, Ross RA, Mechoulam R, Fride E. HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. *Proc Natl Acad Sci U S A.* 1999; 96(25):14228–14233. [PubMed: 10588688]
23. Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci.* 1991; 11(2):563–583. [PubMed: 1992016]
24. Herzberg U, Eliav E, Bennett GJ, Kopin IJ. The analgesic effects of R(+)-WIN 55,212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. *Neurosci Lett.* 1997; 221(2–3):157–160. [PubMed: 9121688]
25. Hohmann AG, Farthing JN, Zvonok AM, Makriyannis A. Selective activation of cannabinoid CB2 receptors suppresses hyperalgesia evoked by intradermal capsaicin. *J Pharmacol Exp Ther.* 2004; 308(2):446–453. [PubMed: 14610224]
26. Hsieh GC, Pai M, Chandran P, Hooker BA, Zhu CZ, Salyers AK, Wensink EJ, Zhan C, Carroll WA, Dart MJ, Yao BB, Honore P, Meyer MD. Central and peripheral sites of action for CB(2)

- receptor mediated analgesic activity in chronic inflammatory and neuropathic pain models in rats. *Br J Pharmacol*. 2010
27. Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, Mata HP, Vanderah TW, Lai J, Porreca F, Makriyannis A, Malan TP Jr. Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc Natl Acad Sci U S A*. 2003; 100(18):10529–10533. [PubMed: 12917492]
 28. Ibrahim MM, Porreca F, Lai J, Albrecht PJ, Rice FL, Khodorova A, Davar G, Makriyannis A, Vanderah TW, Mata HP, Malan TP Jr. CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proc Natl Acad Sci U S A*. 2005; 102(8):3093–3098. [PubMed: 15705714]
 29. Jhaveri MD, Elmes SJ, Richardson D, Barrett DA, Kendall DA, Mason R, Chapman V. Evidence for a novel functional role of cannabinoid CB(2) receptors in the thalamus of neuropathic rats. *Eur J Neurosci*. 2008; 27(7):1722–1730. [PubMed: 18380669]
 30. King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, Fields HL, Porreca F. Unmasking the tonic-aversive state in neuropathic pain. *Nat Neurosci*. 2009; 12(11):1364–1366. [PubMed: 19783992]
 31. LaBuda CJ, Koblish M, Little PJ. Cannabinoid CB2 receptor agonist activity in the hindpaw incision model of postoperative pain. *Eur J Pharmacol*. 2005; 527(1–3):172–174. [PubMed: 16316653]
 32. Lian B, Vera-Portocarrero L, King T, Ossipov MH, Porreca F. Opioid-induced latent sensitization in a model of non-inflammatory viscerosomatic hypersensitivity. *Brain Res*. 2010; 1358:64–70. [PubMed: 20727859]
 33. Liu QR, Pan CH, Hishimoto A, Li CY, Xi ZX, Llorente-Berzal A, Viveros MP, Ishiguro H, Arinami T, Onaivi ES, Uhl GR. Species differences in cannabinoid receptor 2 (CNR2 gene): identification of novel human and rodent CB2 isoforms, differential tissue expression and regulation by cannabinoid receptor ligands. *Genes Brain Behav*. 2009; 8(5):519–530. [PubMed: 19496827]
 34. Lozano-Ondoua AN, Wright C, Vardanyan A, King T, Largent-Milnes TM, Nelson M, Jimenez-Andrade JM, Mantyh PW, Vanderah TW. A cannabinoid 2 receptor agonist attenuates bone cancer-induced pain and bone loss. *Life Sci*. 2010; 86(17–18):646–653. [PubMed: 20176037]
 35. Lyness WH, Smith FL, Heavner JE, Iacono CU, Garvin RD. Morphine self-administration in the rat during adjuvant-induced arthritis. *Life Sci*. 1989; 45(23):2217–2224. [PubMed: 2601574]
 36. Malan TP Jr, Ibrahim MM, Deng H, Liu Q, Mata HP, Vanderah T, Porreca F, Makriyannis A. CB2 cannabinoid receptor-mediated peripheral antinociception. *Pain*. 2001; 93(3):239–245. [PubMed: 11514083]
 37. Maldonado R. Study of cannabinoid dependence in animals. *Pharmacol Ther*. 2002; 95(2):153–164. [PubMed: 12182962]
 38. Martin BR, Compton DR, Thomas BF, Prescott WR, Little PJ, Razdan RK, Johnson MR, Melvin LS, Mechoulam R, Ward SJ. Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. *Pharmacol Biochem Behav*. 1991; 40(3):471–478. [PubMed: 1666911]
 39. Martin TJ, Kim SA, Buechler NL, Porreca F, Eisenach JC. Opioid self-administration in the nerve-injured rat: relevance of antiallodynic effects to drug consumption and effects of intrathecal analgesics. *Anesthesiology*. 2007; 106(2):312–322. [PubMed: 17264726]
 40. Martin TJ, Kim SA, Cannon DG, Sizemore GM, Bian D, Porreca F, Smith JE. Antagonism of delta(2)-opioid receptors by naltrindole-5'-isothiocyanate attenuates heroin self-administration but not antinociception in rats. *J Pharmacol Exp Ther*. 2000; 294(3):975–982. [PubMed: 10945849]
 41. Nackley AG, Makriyannis A, Hohmann AG. Selective activation of cannabinoid CB(2) receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience*. 2003; 119(3):747–757. [PubMed: 12809695]
 42. Nackley AG, Zvonok AM, Makriyannis A, Hohmann AG. Activation of cannabinoid CB2 receptors suppresses C-fiber responses and windup in spinal wide dynamic range neurons in the absence and presence of inflammation. *J Neurophysiol*. 2004; 92(6):3562–3574. [PubMed: 15317842]

43. Onaivi ES. An endocannabinoid hypothesis of drug reward and drug addiction. *Ann N Y Acad Sci.* 2008; 1139:412–421. [PubMed: 18991888]
44. Quartilho A, Mata HP, Ibrahim MM, Vanderah TW, Porreca F, Makriyannis A, Malan TP Jr. Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. *Anesthesiology.* 2003; 99(4):955–960. [PubMed: 14508331]
45. Rahn EJ, Makriyannis A, Hohmann AG. Activation of cannabinoid CB1 and CB2 receptors suppresses neuropathic nociception evoked by the chemotherapeutic agent vincristine in rats. *Br J Pharmacol.* 2007; 152(5):765–777. [PubMed: 17572696]
46. Rahn EJ, Zvonok AM, Makriyannis A, Hohmann AG. Antinociceptive Effects of Racemic AM1241 and Its Chirally Synthesized Enantiomers: Lack of Dependence upon Opioid Receptor Activation. *Aaps Journal.* 2010; 12(2):147–157. [PubMed: 20127295]
47. Sagar DR, Kelly S, Millns PJ, O'Shaughnessy CT, Kendall DA, Chapman V. Inhibitory effects of CB1 and CB2 receptor agonists on responses of DRG neurons and dorsal horn neurons in neuropathic rats. *Eur J Neurosci.* 2005; 22(2):371–379. [PubMed: 16045490]
48. Shalev U, Grimm JW, Shaham Y. Neurobiology of relapse to heroin and cocaine seeking: a review. *Pharmacol Rev.* 2002; 54(1):1–42. [PubMed: 11870259]
49. Sufka KJ. Conditioned place preference paradigm: a novel approach for analgesic drug assessment against chronic pain. *Pain.* 1994; 58(3):355–366. [PubMed: 7838585]
50. Sufka KJ, Roach JT. Stimulus properties and antinociceptive effects of selective bradykinin B1 and B2 receptor antagonists in rats. *Pain.* 1996; 66(1):99–103. [PubMed: 8857636]
51. Toth C, Au S. A prospective identification of neuropathic pain in specific chronic polyneuropathy syndromes and response to pharmacological therapy. *Pain.* 2008; 138(3):657–666. [PubMed: 18691815]
52. Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS, Marnett LJ, Di Marzo V, Pittman QJ, Patel KD, Sharkey KA. Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science.* 2005; 310(5746):329–332. [PubMed: 16224028]
53. Weissenborn R, Yackey M, Koob GF, Weiss F. Measures of cocaine-seeking behavior using a multiple schedule of food and drug self-administration in rats. *Drug Alcohol Depend.* 1995; 38(3):237–246. [PubMed: 7555624]
54. Whiteside GT, Lee GP, Valenzano KJ. The role of the cannabinoid CB2 receptor in pain transmission and therapeutic potential of small molecule CB2 receptor agonists. *Curr Med Chem.* 2007; 14(8):917–936. [PubMed: 17430144]
55. Wotherspoon G, Fox A, McIntyre P, Colley S, Bevan S, Winter J. Peripheral nerve injury induces cannabinoid receptor 2 protein expression in rat sensory neurons. *Neuroscience.* 2005; 135(1):235–245. [PubMed: 16084654]
56. Zhang J, Hoffert C, Vu HK, Groblewski T, Ahmad S, O'Donnell D. Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. *Eur J Neurosci.* 2003; 17(12):2750–2754. [PubMed: 12823482]
57. Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc Natl Acad Sci U S A.* 1999; 96(10):5780–5785. [PubMed: 10318961]
58. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain.* 1983; 16(2):109–110. [PubMed: 6877845]

Figure 1.

(A) Intravenous self-administration of either vehicle or the CB₂ agonist (R,S)-AM1241 (1.5 μg/μl) did not alter the percentage of active lever presses in naive groups. *n* = 8–12 per group. (B) Pre-treatment with the CB₂-selective antagonist SR144528 (1 mg/kg i.p.) did not reliably alter the percentage of active lever presses in naive groups allowed to intravenously self-administer the CB₂-agonist (R,S)-AM1241 (1.5 μg/μl). Data are expressed as percentage mean ± s.e.m. *n* = 5–7 per group.

Figure 2.

(A–B) Spared nerve injury produced mechanical allodynia in the left (injured) paw of the neuropathic group prior to initiation of drug self-administration sessions. Mechanical withdrawal thresholds in the right (intact) paw of neuropathic and sham groups were similar and did not differ from baseline thresholds. (C–D) Mechanical allodynia was not altered by intravenous self-administration of vehicle in neuropathic or sham groups. (E–F) Mechanical withdrawal thresholds remained unchanged in the right (intact) paw of sham and neuropathic groups allowed to self-administer vehicle. Data are expressed as mean $\pm$ s.e.m. $n = 7$ per group.

Figure 3.

The percentage of active lever presses differed between sham and neuropathic groups self-administering vehicle. Data are expressed as mean $\pm$ s.e.m. $**P < 0.05$, different from Neuro vehicle (ANOVA, followed by two tailed t-test). $n = 7$ per group.

Figure 4.

(A–B) Spared nerve injury produced mechanical allodynia in the left (injured) paw of the neuropathic group without affecting withdrawal thresholds in the right (intact) paw prior to initiation of drug self-administration sessions. (C–D) (R,S)-AM1241 (1.5 $\mu\text{g}/\mu\text{l}$) but not vehicle self-administration elevated mechanical withdrawal thresholds in the neuropathic group following each drug self-administration session. Data are expressed as mean $\pm$ s.e.m. *** $P < 0.0001$, different from post-session threshold (ANOVA, followed by two tailed t-test). $n = 6$.

Figure 5.

In neuropathic rats, the number of lever responses per session declined when animals were switched from (R,S)-AM1241 to vehicle self-administration, documenting extinction of responding. Data are expressed the number of responses per session, mean $\pm$ s.e.m. *** $P < 00.01$ (t-test, two tailed). $n = 6$.

Figure 6.

(A–B) Spared nerve injury produced mechanical allodynia in the left (injured) paw of the neuropathic groups without affecting mechanical withdrawal thresholds in the (right) intact paw prior to initiation of drug self-administration sessions. (C–D) Pre-treatment with the CB₂-selective antagonist SR144528 (1mg/kg i.p.) blocked the anti-allodynic effects produced by (R,S)-AM1241 self-administration. (E–F) Pre-treatment with SR144528 (1mg/kg i.p.) did not alter mechanical withdrawal thresholds in the right (intact) of neuropathic groups self-administering (R,S)-AM1241 (1.5 µg/µl) before or after each test session. Data are expressed as mean ± s.e.m. ** $P < 0.001$, different from Baseline (BL) ** $P < 0.0001$, different from post-session (ANOVA, two tailed t-test). $n = 6–8$ per group.

Figure 7.

Pretreatment with the CB₂-selective antagonist SR144528 (1 mg/kg i.p.) blocked the percentage of active lever responding in neuropathic groups self-administering (*R,S*)-AM1241 (1.5 μg/μl). Data are expressed as mean ± s.e.m. ***P* < 0.05, different from Neuro AM1241 (ANOVA). *n* = 6–8 per group.

Figure 8.

(A–B) Spared nerve injury produced mechanical allodynia in the left (injured) paw of the neuropathic group paw without affecting withdrawal thresholds in the right (intact) paw prior to initiation of drug self-administration sessions. (C) Pre-self-administration mechanical paw withdrawal thresholds were lower in the injured paw of neuropathic rats relative to shams across all schedules of reinforcement. (C–D) Intravenous self-administration of (R,S)-AM1241 (1.5 $\mu\text{g}/\mu\text{l}$) attenuated mechanical allodynia in the left (injured) paw of the neuropathic groups relative to pre-session thresholds. Paw withdrawal thresholds in the sham group were not altered by (R,S)-AM1241 self-administration. (E) Pre-self-administration and (F) post-self-administration paw withdrawal thresholds did not

differ in the right (intact) paw in sham and neuropathic groups self-administering (*R,S*)-AM1241(1.5 µg/µl) across all schedules of reinforcement. Data are expressed as mean ± s.e.m. *** $P < 0.001$ different from all other groups (ANOVA) followed by two tailed t-test). $n = 4-6$ per group.

Figure 9.

(**A**) Percent active lever presses increased in neuropathic relative to sham-operated groups self-administering (*R,S*)-AM1241 (1.5 $\mu\text{g}/\mu\text{l}$) when the schedule of reinforcement increased to FR6. (**B**) (*R,S*)-AM1241 (1.5 $\mu\text{g}/\mu\text{l}$) self-administration was greater in neuropathic compared to sham groups, as defined by the greater slope of the regression line fit to active lever responding. Data are expressed as mean $\pm$ s.e.m. * $P < 0.05$ different from Sham (**A**, ANOVA followed by two tailed t-tests), (**B**, two tailed t-test). $n = 4-6$ per group.

Figure 10.

Intravenous administration of the mixed CB₁/CB₂ agonist WIN55,212-2 (0.5 mg/kg, i.v.), but not the CB₂-selective agonist (R,S)-AM1241 (900 μg, i.v.), produced motor ataxia, defined by a decrease in rota-rod descent latency. Data are expressed as mean ± s.e.m. **P* < 0.01, ***P* < 0.001 different from all other groups (ANOVA followed by Fisher's PLSD *post hoc* test). *n* = 6 per group.