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Elucidating interplay between myrcene and cannabinoid receptor 1 receptors to produce antinociception in mouse models of neuropathic pain

Myra Alayoubi[a,b], Akeesha Rodrigues[b], Christine Wu[b], Ella Whitehouse[b], Jessica Nguyen[b], Ziva D. Cooper[b,c,d,e], Patrick R. O'Neill[b,c], Catherine M. Cahill[b,c,d,*]

Abstract

The need for nonaddictive and effective treatments for chronic pain are at an all-time high. Historical precedence, and now clinical evidence, supports the use of cannabis for alleviating chronic pain. A plethora of research on delta-9-tetrahydrocannabinol exists, yet cannabis is comprised of a multitude of constituents, some of which possess analgesic potential, that have not been systematically investigated, including the terpene myrcene. Myrcene attenuates pain hypersensitivity in preclinical models and is one of the most abundant terpenes found in cannabis. Despite these findings, it remains unclear how myrcene elicits these effects on nociceptive systems. The present study uses a male and female mouse model of neuropathic pain as well as in vitro experiments with HEK293T cells to explore these questions. We first demonstrate myrcene (1-200 mg/kg i.p.) dose-dependently increases mechanical nociceptive thresholds, where potency was greater in female compared with male pain mice. Testing canonical tetrad outcomes, mice were tested for hypolocomotion and hypothermia after myrcene administration. Myrcene did not alter locomotion or temperature, but female pain mice showed a conditioned place aversion to myrcene. A cannabinoid receptor 1 (CB1) antagonist inhibited myrcene's anti-allodynia. By contrast, in vitro cell culture experiments using a TRUPATH assay revealed myrcene does not directly activate CB1 receptors nor alter CB1 receptor activity elicited by CB1 agonist (CP 55,940) or endocannabinoids (anandamide or 2-arachidonoylglycerol). Understanding engagement of CB1 receptors in pain modulation and myrcene's mechanism of action warrants further study to understand the diversity of cannabis pharmacology and to further the frontier of pain research.

Keywords: Terpenes, Neuropathic pain, Cannabinoid 1 receptor

1. Introduction

There is an established intersection between the use of cannabis and chronic pain, a condition that affects approximately 1 in 5 US adults.[49] Pain relief is the most common reason for consumption of medical cannabis.[18,20,34] Although most cannabis-based products do not have approval from the US Food and Drug Administration (FDA), the National Academies of Sciences, Engineering, and Medicine concluded there is substantial evidence that cannabis is an effective treatment for chronic pain in adults.[27] Two compounds in cannabis that have been studied for their potential pain-relieving effects are delta-9 tetrahydrocannabinol (THC) and cannabidiol (CBD).

Although there is strong evidence that THC has pain relieving properties, it is not the only compound in cannabis that contributes to analgesia. The cannabis plant contains a vast array of chemical constituents, some of which have been shown to possess antinociception or antihyperalgesic effects in isolation in preclinical studies. There are over 550 compounds identified in cannabis, including 120 identified terpenes; compounds that contribute to distinct odor profiles of cannabis chemovars.[35] Terpenes are stored in the glandular trichomes (epidermal outgrowths) of cannabis.[22] They are volatile organic compounds that consist of hydrocarbons with isoprene units that link to one

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[a] *UCLA, Neuroscience Interdepartmental Graduate Program, University of California, Los Angeles, Los Angeles, CA, United States,* [b] *Shirley and Stefan Hatos Center for Neuropharmacology, Jane and Terry Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, Los Angeles, CA, United States,* [c] *Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, United States,* [d] *UCLA Center for Cannabis and Cannabinoids, Jane and Terry Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, Los Angeles, CA, United States,* [e] *Department of Anesthesiology and Perioperative Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, United States*

**Corresponding author. Address: Department of Psychiatry and Biobehavioral Sciences, Semel Institute for Neuroscience and Human Behavior, Shirley and Stefan Hatos Center for Neuropharmacology, MacDonald Research Laboratory Building, 675 Charles E Young Drive South, Office 2774, Los Angeles, CA 90095. Tel.: 310-206-0083. E-mail address: cmcahill@ucla.edu (C. M. Cahill).*

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another.[42] Myrcene is a monoterpene, meaning that is has 2 isoprene units, and is one of the 8 predominant terpenes identified in cannabis. Patients with pain report preferring cannabis chemovars with high concentrations of terpenes relative to other chemovars.[1] Nevertheless, there have been few studies examining the potential pain-relieving effects of this terpene.

Preclinical rodent studies have reported myrcene possesses antinociceptive effects in various nociceptive assays. Myrcene increased reaction time to the hot plate test compared with vehicle in male mice.[29,33] In a chronic constriction nerve injury mouse model, myrcene improved mechanical nociceptive thresholds (von Frey) 1 and 2 weeks postsurgery in male mice.[30] In an arthritis pain model, myrcene elevates von Frey thresholds in male rats.[24] To date, all experimentation used male rodents and few studies have examined the mechanisms underlying these findings.

In this study, we used in vivo and in vitro models to study the potential dose- and sex-dependent antinociceptive and anti-allodynic effects of myrcene in a model of neuropathic pain; we probed myrcene's mechanism of action by investigating engagement of the cannabinoid receptor 1 (CB1). We used both male and female mice, given the previously reported sex differences in cannabinoid-induced antinociception.[44,45] We used a conditioned place preference (CPP) test to assess learned associations of myrcene to produce positive and negative conditioned effects in sham and neuropathic pain mice. We also tested myrcene compared with a known CB1 agonist, CP 55,940, on endpoints known to be affected by CB1 agonists (ie, locomotion and temperature). To understand how myrcene modulates CB1 receptor activity and the activity of CB1 receptor agonists/partial agonists, we applied in vitro experiments using a TRUPATH assay to assess the potential for myrcene to modulate CB1 receptor activity.

2. Methods

2.1. Animals

The UCLA Office for the Protection for Animal Subjects at UCLA approved experimental protocols. We obtained C57BL/6J male and female mice from JAX Laboratories and housed 4 per cage in a temperature-controlled room with a reverse 12-hour light/dark cycle (lights off at 8:00 AM). Mice received enrichment of nesting material and a plastic dome. We used red lights during testing (the animals' dark cycle). Mice had free access to food and water and were acclimated to the housing conditions for 1 week. We handled mice for a minimum of 20 minutes per day for 3 days before experimentation and habituated mice to the behavioral rooms for 30 minutes before testing. Testing of males and females were conducted separately, and all apparatuses and materials cleaned thoroughly with Vimoba disinfectant and allowed to air-dry between testing of animals and at the end of experimentation.

2.2. Rigor

To the extent possible, experimenters handling mice were blind to surgery, sex, and drug treatment. We assigned mice to experimental conditions in a randomized block design so that the running of subjects counterbalanced factors such as time of day and drug treatment over experimental conditions. We also completed experiments in successive replications where replications were balanced with respect to experimental groups. All experimenters carrying out behavioral studies were female.

2.3. Surgery

2.3.1. Chronic constriction injury

Chronic constriction injury (CCI) is a method of loosely constricting the sciatic nerve to produce chronic neuropathic pain evidenced by the occurrence of mechanical allodynia assessed using von Frey testing.[47] During CCI surgery, we anesthetized animals with isoflurane (2% maintenance) and exposed the main branch of the sciatic nerve. We placed a 2-mm-long PE-20 polyethylene cuff around the entire sciatic nerve proximal to bifurcation, such that the nerve was firmly wrapped by the tubing. Mice received eye gel, supportive lactated ringers (2 mL s.c.), and acetaminophen (32 mg/kg, orally) before and daily for 72 hours postoperatively. Mice recovered for 1 week before further experimentation and handling. Sham surgeries consisted of similar exposure to isoflurane anesthesia and skin incision, but no muscle damage or manipulation of the nerve.

2.4. Behavioral outcomes

2.4.1. Mechanical hypersensitivity

To assess the time course of myrcene's effect on mechanical withdrawal thresholds, we tested separate cohorts of animals in pilot experiments using a 10-poke von Frey test. During this test, animals are stimulated with a 2-g filament 10 times and the number of positive withdrawal (flinch) responses are recorded. We repeated this measure every 30 minutes for 2.5 hours after administration of myrcene (100 mg/kg, i.p.). The maximal effect to attenuate mechanical withdrawal thresholds in the CCI mice revealed that the 30-minute time point produced a robust effect and this time point was then used for all subsequent experiments.

We use a handheld electronic von Frey (eVF), purchased from Ugo Basile, to assess mechanical withdrawal thresholds. The handheld unbending filament attaches to a detector that records the grams of force when applied to the plantar surface of the mouse ipsilateral hind paw.[9] Thus, less sensitivity to the filament results in higher grams of force and increased sensitivity to the filament results in less grams of force recorded. We tested each mouse 3 times and averaged the 3 values for each mouse. We tested all mice before subsequent testing rounds, as opposed to each mouse getting 3 consecutive tests to avoid sensitization/desensitization to the filament. Two weeks after CCI surgery, animals began a 3-day eVF protocol where animals are given no injections on the first day, then counterbalanced across vehicle and drug treatment on days 2 and 3. Taking into consideration a possible order effect of myrcene administration, we analyzed the effects of myrcene compared with vehicle when administered on day 2 and on day 3 and found no difference in the order of administration ($P > 0.05$). This experimental design allows for a within- and between-subject analysis of mechanical withdrawal thresholds of myrcene compared with vehicle. We gave separate cohorts of animals 1, 10, 100, or 200 mg/kg i.p. injections of myrcene. In a separate cohort, we pretreated mice with vehicle or CB1 antagonist (SR141716 1 mg/kg i.p.) 15 minutes before myrcene injection (or the vehicle).

2.4.2. Conditioned place preference

The conditioned place preference (CPP) assay is a Pavlovian conditioning test that measures the conditioned aversive effects of a drug in CCI animals or the conditioned positive (rewarding) effects of a drug in sham animals, without pain.[7,15] We used a two-chamber CPP apparatus where one chamber has striped

walls and mesh flooring, and the other chamber has circle walls and hard wire flooring that were used for contextual cues. Before surgery, we habituated and tested animals for chamber bias, then drug-assignment was balanced according to time spent in each chamber during this precondition test, allowing for an unbiased design. Seven days after sham or CCI surgery, we conditioned mice daily for 6 consecutive days using a counterbalanced design for chamber and treatment. We conditioned both sham and CCI mice to chambers with either myrcene (100 mg/kg i.p.) or vehicle, and another cohort conditioned to chambers gabapentin (50 mg/kg i.p.) or vehicle to serve as a positive control. After conditioning, we tested animals for "context dependence" 24 hours after the last conditioning session, and for "state dependence" the following day where all mice were injected with their conditioning drug. We video recorded mice during both the context- and the state-dependent testing to capture time spent in each chamber. We calculated a CPP score for both context and state dependence days: (time in drug paired chamber − time in vehicle paired chamber) − (time in drug assigned chamber at preconditioning − time in vehicle assigned chamber at preconditioning). During context-dependent testing, no injections were administered, to test the reinforcement of the drug treatments. During state-dependent testing, all animals received the drug injection, testing the extent to which they learn to associate the drug state with contextual cues in the chambers. We analyzed video recordings using AnyMaze software for time spent in each chamber during the 2 test days. This experimental design allows for a within-subject analysis of the time spent in the vehicle compared with the drug-paired chamber.

2.4.3. Locomotion

Cannabinoid receptor 1 receptor agonists produce the canonical tetrad effects, which include hypolocomotion and hypothermia. To test the effect of myrcene on these outcomes, we administered CCI male and female mice 100 mg/kg myrcene (i.p.) or vehicle and measured locomotor and hypothermic effects. We tested locomotion over the course of 3 days, 2 weeks after CCI surgery and recorded distance traveled (in meters) using AnyMaze software. We administered myrcene (100 mg/kg, i.p.) or vehicle on days 1 and 2 and CP 55,940 (1 mg/kg, i.p.) on day 3. We recorded animals for 4 hours in separate 235 × 235-mm plexiglass chambers on each day.

2.4.4. Temperature

We measured body core temperature over the course of 3 days of testing, 3 weeks after CCI surgery using a Kent Scientific thermometer and rectal probe. We administered myrcene (100 mg/kg, i.p.) or vehicle on days 1 and 2 and CP 55,940 (1 mg/kg, i.p.) on day 3. We recorded temperature before and every 30 minutes after administration of myrcene (100 mg/kg, i.p.) for 4 hours. Animals were returned to their home cage after each temperature recording.

2.5. Plasmids for TRUPATH experiments

For cell culture experiments we used TRUPATH plasmids, which enable G protein activation to be quantified as a change in BRET (Bioluminescence Resonance Energy Transfer) ratio. The plasmid for human CB1 was from the cDNA Resource Center, Bloomsburg University of Pennsylvania (#CNR0100001). TRUPATH plasmids were from Addgene: Triple GaoA (#196051) and Triple Gai1 (#196048). Triple GaoA expresses 3 G protein subunits: Alpha-oA-RLuc8, Beta3, and GFP2-Gamma8. Triple Gai1 expresses 3 G protein subunits: Alpha-i1-RLuc8, Beta3, and GFP2-Gamma9.

2.6. Cell culture and transfection

We obtained HEK293T cells from ATCC and maintained in culture media containing DMEM (ThermoFisher #11995065), 10% FBS (Genesee Scientific #25-550H), and penicillin–streptomycin (ThermoFisher #15140122). We maintained cells in an incubator at 37°C and 5% CO_2, and cells were verified to be mycoplasma negative by using the MycoAlert mycoplasma detection kit (Lonza, #LT07-218). We plated cells in 6-well plates at a density of 2 million cells per well, allowed to adhere for at least 2 hours, and transfected using PolyJet (SignaGen #SL100688). We used the following amounts of plasmids and PolyJet per well: 1.0 mg CB1 receptor and either 0.9 mg Triple GaoA (for experiments with CP 55,940) or 0.9 mg Triple Gai1 (for experiments with 2-AG and anandamide) and 2 mL PolyJet per well. After transfection, we returned the cells to the incubator overnight.

2.7. TRUPATH measurements

On the day after transfection, we harvested cells from 6-well plates using trypsin-EDTA (ThermoFisher #25300054) and plated in white-bottom 96-well plates at 62.5k cells per well and incubated overnight at 37°C and 5% CO_2. We performed TRUPATH measurements the following day using a GloMax Discover Microplate Reader (Promega). Before measurements, we replaced the cell culture media with 60 mL of BRET assay buffer containing HBSS (ThermoFisher #14175-095), supplemented with 20 mM HEPES (ThermoFisher #15630-080), and pH adjusted to 7.4. We performed luminescence measurements to collect emission from the RLuc8 donor, using a 495-nm short pass filter and the GFP2 acceptor using a 530-nm-long pass filter, with 1-second integration times. Coelenterazine 400a (NanoLight Technology #340) was prepared as a stock solution in NanoFuel solvent (NanoLight Technology, #399) at 1 mg/mL and stored in aliquots at −80°C. Immediately before use, we diluted it to 18.6 mg/mL in BRET assay buffer, of which we added 10 mL per well for a final concentration of 2.65 mg/mL. We recorded a baseline of 5 BRET measurements, followed by the addition of 30 mL of drug or vehicle, again followed by 10 additional BRET measurements. The BRET ratio is calculated as the acceptor divided by the donor emission signal. We computed delta BRET as the change in BRET relative to the mean of the 5 baseline BRET measurements. We performed each condition in triplicate, and the mean and SD are reported in the figures. We prepared drugs in DMSO, and a 1:100 intermediate dilution in BRET assay buffer was performed immediately before adding the drug to the 96-well plate. The final DMSO concentration after addition of drug or vehicle in all wells was 0.3% DMSO.

2.8. Drugs used in vivo

We purchased myrcene from True Terpenes, a manufacture that is GMP/ISO/FSSC22000 compliant and specifies purity and completes testing for heavy metals, pesticides, and residual solvents. Myrcene doses ranged from 1 to 200 mg/kg[38] and was dissolved in corn oil (used as vehicle). Gabapentin 50 mg/kg[26] was dissolved in saline, and saline used as vehicle. We administered a 1 mg/kg dose of SR141716,[41] a CB1 antagonist, that was obtained from RTI, NIDA drug supply and dissolved in corn oil. We administered a 1 mg/kg dose of CP 55,940,[14] a CB1 agonist, that was obtained from Sigma-Aldrich and dissolved in

corn oil. We administered drugs with weight based i.p. injections using an injection volume of 0.05 mL/10 g body weight.

2.9. Drugs used in vitro

We purchased 2-Arachidonoylglycerol (2-AG) (#A8973), arachidonylethanolamide (anandamide [AEA]) (#A0580) and CP 55,940 (#C1112) from Sigma-Aldrich. These drugs were dissolved in DMSO and diluted to 0.3% DMSO final concentration for experiments.

2.10. Statistical analyses

In vivo experiments: Data are presented as mean values with raw data points with SEM and sex presented separately. All behavioral data fit assumptions of the general linear model allowing us to use factorial ANOVA analyses. Data were analyzed in GraphPad PRISM using a 2-way ANOVA with either Sidak or Tukey multiple comparison tests or by a paired Student *t* test. Significance notation is expressed as follows: nonsignificant (ns), $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$.

In vitro experiments: A high BRET signal is recorded when the receptor is inactivated because of the proximity of the fluorescent molecular tags when the GPCR subunits are all associated. A low BRET signal indicates receptor activation, as the subunits dissociate and the fluorescent tags are less able to interact. Thus, a sudden decrease in BRET signal when a ligand is added indicates activation of the receptor.

3. Results

3.1. Myrcene dose-dependently attenuated mechanical allodynia

To determine the extent mechanical allodynia induced by chronic constriction of the sciatic nerve (CCI) may be attenuated by myrcene, we used separate cohorts of mice to test the effects of myrcene in both male and female mice. We tested CCI mice with vehicle or myrcene in a counterbalanced, crossover within subject design at 2 weeks postinjury. CCI mice showed a significant reduction in the mechanical withdrawal threshold to evoke a response compared with sham animals (**Fig. 1A**). Within subjects paired *t* tests were performed for male (**Fig. 1B**) and female (**Fig. 1C**) CCI mice for vehicle or myrcene (1, 10, 100, and 200 mg/kg i.p.) by assessing eVF withdrawal thresholds 30 minutes postinjection. There was no significant difference between eVF thresholds for males $t_{(7)} = 0.1163$, $P = 0.9107$, or for females $t_{(7)} = 0.6707$, $P = 0.5239$ when administered vehicle or 1 mg/kg (i.p.) myrcene compared with vehicle (**Fig. 1B, C**). There was a significant difference in eVF thresholds for females ($t_{(7)} = 2.503$, $P = 0.0408$*), but not males ($t_{(7)} = 2.080$, $P = 0.0760$), administered 10 mg/kg myrcene compared with vehicle (**Figs. 1B, C**). Myrcene at the 100 and 200 mg/kg doses significantly attenuated mechanical withdrawal thresholds in both CCI male (100 mg/kg: $t_{(7)} = 9.630$, $P < 0.0001$****, 200 mg/kg: $t_{(7)} = 4.730$, $P = 0.0021$**) and female (100 mg/kg: $t_{(7)} = 2.420$, $P = 0.0461$*, 200 mg/kg: $t_{(7)} = 5.541$, $P = 0.0009$***) mice compared with vehicle. None of the doses tested significantly modified withdrawal thresholds in sham animals (data not shown). The dose-dependent effects are presented in **Figure 1D** to directly assess potential sex differences in treatment effects. A 2-way ANOVA revealed a significant effect of dose ($F_{(3,56)} = 24.90$, $P < 0.0001$****), an effect of sex ($F_{(1,59)} = 9.784$, $P = 0.0028$**), and an interaction ($F_{(3,56)} = 3.683$, $P = 0.0172$*). A Sidak multiple comparison post hoc test revealed a difference at the 10 mg dose between the sexes.

3.2. Antinociceptive properties of myrcene were blocked by a cannabinoid receptor 1 receptor antagonist in chronic constriction injury mice

To determine engagement of CB1 receptor activation by myrcene, we pretreated male and female CCI mice with a CB1 antagonist (SR141716 1 mg/kg i.p.) before myrcene administration (100 mg/kg i.p.). Four treatment conditions were implemented with 15 minutes between the first and second injection (ie, vehicle + vehicle; vehicle + myrcene; SR141716 + myrcene; or SR141716 + vehicle), with mice tested 30 minutes after their last injection (**Fig. 2A**). We performed 1-way ANOVAs to evaluate treatment condition on eVF withdrawal thresholds in CCI male and female mice. Results indicated a significant effect of treatment $F_{(3, 25)} = 4.992$, $P = 0.0075$** in males, and in females $F_{(3, 28)} = 11.94$, $P < 0.0001$**** (**Figs. 2B and 2C**). Sidak multiple comparison post hoc tests were evaluated to understand how treatment conditions differ. As expected, the vehicle + myrcene condition had higher withdrawal thresholds than the vehicle + vehicle condition for males and for females. The SR141716 + myrcene group had reduced withdrawal thresholds than the vehicle + myrcene condition for males and for females, demonstrating that CB1 receptor activation mediated the effect of myrcene in the eVF mechanical test. In fact, the SR141716 + myrcene condition's thresholds were not significantly different from the vehicle + vehicle condition's thresholds for males or for females. As a check for an unaccounted effect of SR141716 on withdrawal thresholds, a SR141716 + vehicle condition was included, but thresholds did not differ from the vehicle + vehicle condition for males or for females.

3.3. Male, but not female, chronic constriction injury mice showed a preference to gabapentin in a place-preference state-dependent test

We conducted a CPP test among male and female CCI mice using a drug commonly used for treating neuropathic pain, gabapentin. The CPP test consists of 6 days of conditioning beginning 7 days after CCI surgery, with context-dependent and state-dependent testing on day 14 and day 15 postsurgery, respectively (timeline depicted in **Fig. 3A**). We performed a 2-way ANOVA to evaluate the effect of sex and treatment condition (50 mg/kg i.p. gabapentin or vehicle) on time spent in the chambers. For the state-dependent data, results indicate no significant main effect of sex $F_{(1, 28)} = 0.000$, $P > 0.999$, but there was a main effect of treatment $F_{(1, 28)} = 13.65$, $P = 0.0009$***, and a significant interaction between sex and treatment $F_{(1, 28)} = 5.773$, $P = 0.0231$* (**Fig. 3B**) in CCI mice. Post hoc multiple comparison tests revealed a significant effect of treatment in males ($P = 0.0002$***) but not females ($P = 0.3689$). These data were further analyzed by calculating the CPP scores. We performed 1-sample *t* tests in male and female CCI mice to examine if CPP scores for state-dependence was different from a theoretical value of 0 (no preference) when administered gabapentin. Results indicated no statistical significance for males $t_{(7)} = 1.744$, $P = 0.1246$ or for females $t_{(7)} = 0.6163$, $P = 0.5572$ (**Fig. 3C**). An unpaired *t* test also showed no difference between sexes ($t_{(14)} = 1.176$, $P = 0.2590$).

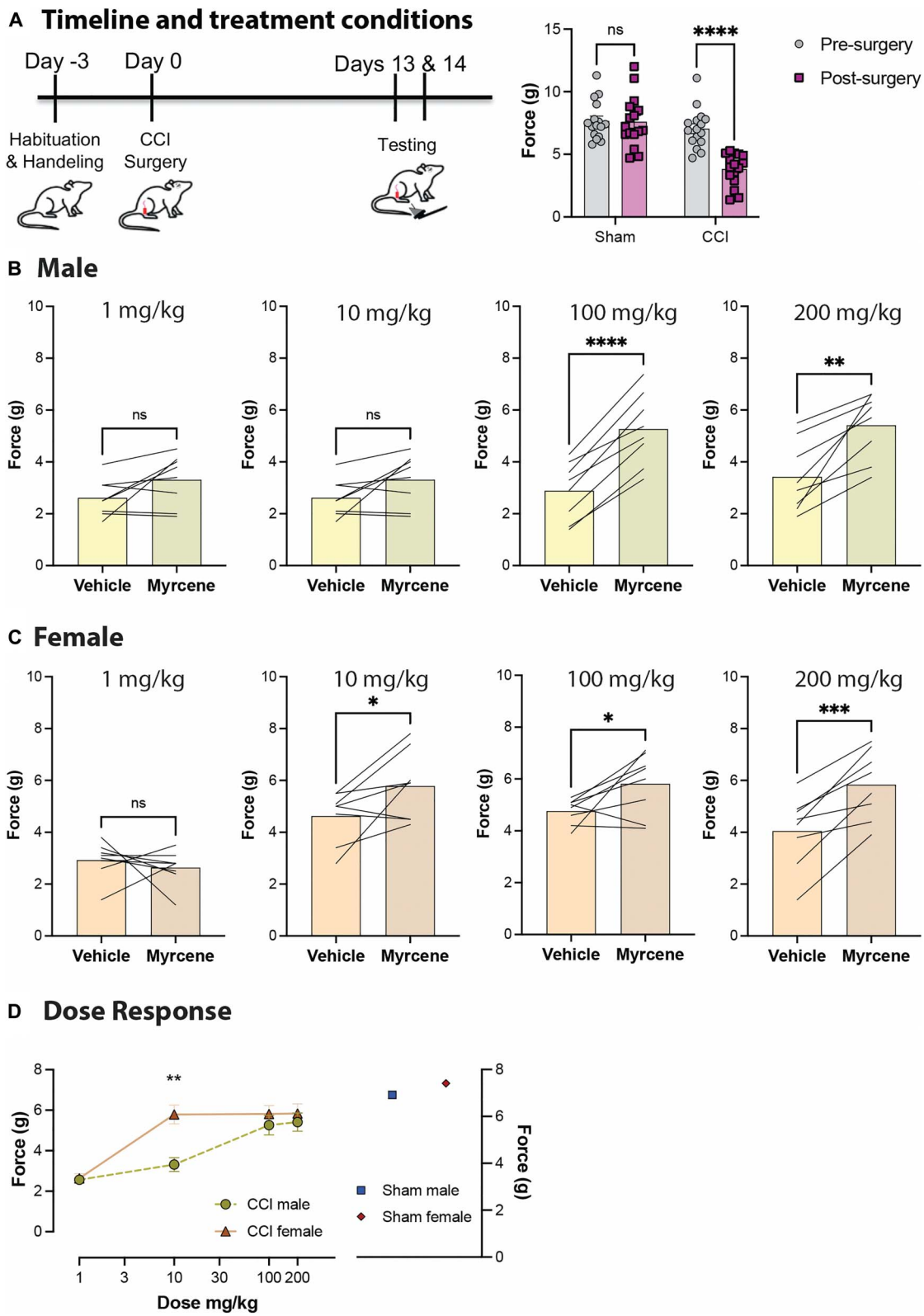

Figure 1. Myrcene dose-dependently attenuated mechanical allodynia. (A) Mice were habituated to the von Frey apparatus and handled for 3 days before sham or CCI surgeries. Mice recovered for approximately 2 weeks before undergoing a 2-day counterbalanced design where mice received either vehicle or myrcene. CCI mice displayed reduced mechanical withdrawal thresholds (force) postsurgery compared with presurgery, whereas sham mice did not change withdrawal thresholds. (B) Male mice had increased withdrawal thresholds given 100 and 200 mg/kg i.p. injection of myrcene compared with vehicle but not lower doses and (C) female mice had increased withdrawal thresholds given 10, 100, and 200 mg/kg myrcene compared with vehicle, but not 1 mg/kg dose. (D) The dose–response curve of myrcene is shifted left for female mice compared with male mice. Data are analyzed by within-subjects paired *t* tests (A–C) and 2-way repeated-measures ANOVA with Sidak multiple comparisons (D). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{***}P < 0.0001$. CCI, chronic constriction injury; ns, nonsignificant; g, grams.

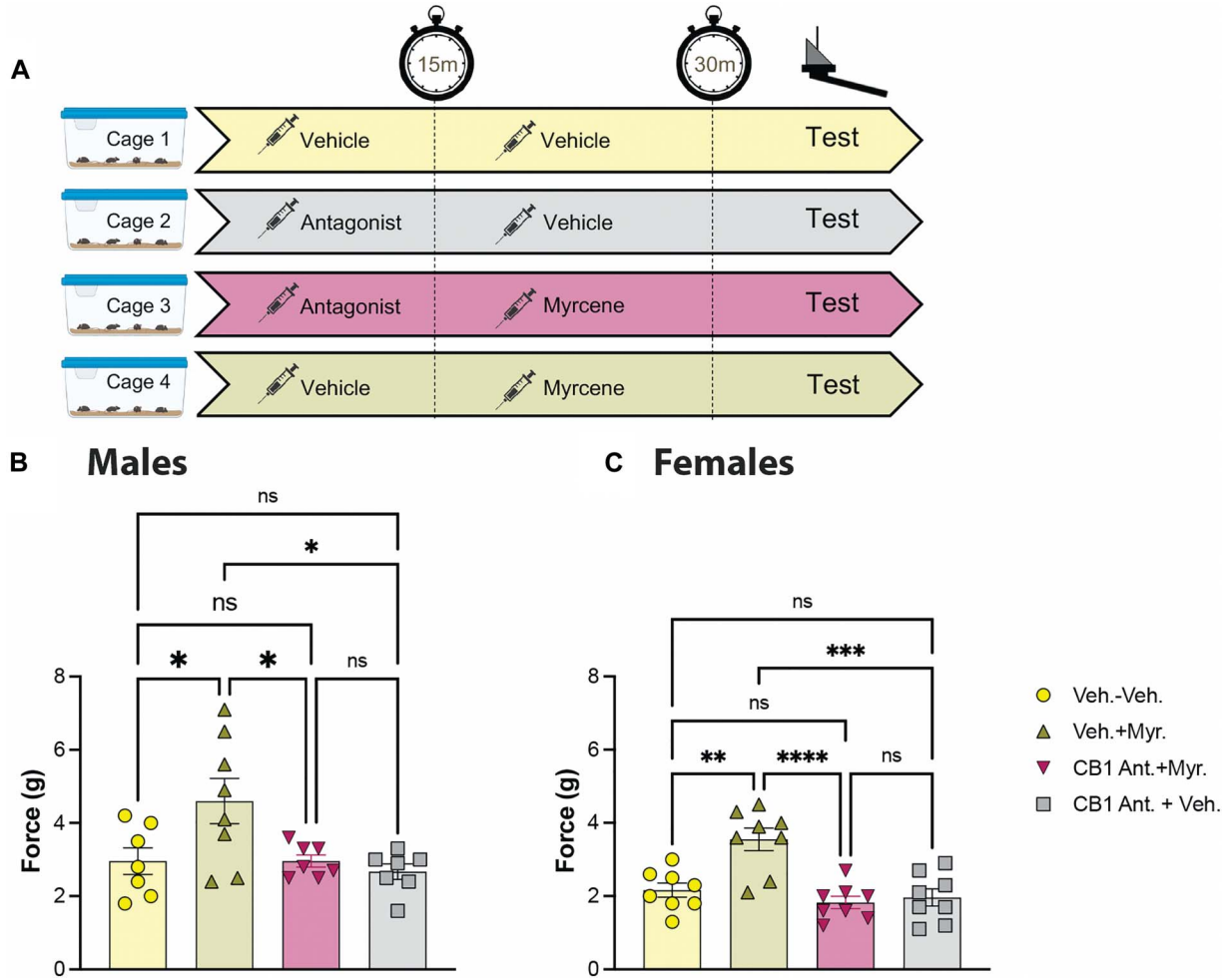

Figure 2. Antinociceptive properties of myrcene were blocked by a CB1 receptor antagonist in CCI mice. (A) CCI mice underwent a 2-day counterbalanced, crossover design where each day they received a pretreatment injection of vehicle (0.05 mL/10 g body weight) or a CB1 antagonist (SR141716, 1 mg/kg, i.p.) and 15 minutes later received an injection of myrcene (100 mg/kg i.p.), resulting in 4 possible drug conditions. Thirty minutes after the last injection, mice were tested on von Frey 3 times and data averaged across trials. (B–C) Male and female CCI mice had higher withdrawal thresholds when given the vehicle + myrcene drug condition compared with the vehicle + vehicle condition. SR141716 + vehicle condition did not change withdrawal thresholds compared with vehicle + vehicle condition. When myrcene was pretreated with SR141716 (SR141716 + myrcene), mice had reduced withdrawal thresholds similar to the vehicle + vehicle condition. Data were analyzed using 1-way ANOVAs with Sidak multiple comparisons. ns = nonsignificant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{***}P < 0.0001$. g, grams; Veh, vehicle; Myr, myrcene; CB1 Ant, cannabinoid 1 receptor antagonist.

3.4. Female, but not male, chronic constriction injury mice showed an aversion to myrcene in a place-preference state-dependent test

To determine the extent myrcene may produce positive or negative conditioned properties, a CPP test was conducted in separate cohorts of sham and CCI mice. We performed a 2-way ANOVA on CCI mice to evaluate the effect of sex on treatment condition (100 mg/kg i.p. myrcene or vehicle) on time spent in the chambers. Results from the state-dependent test are presented in **Figure 3D**. Results indicated no significant main effect of sex $F_{(1,\ 26)} = 0.000$, $P > 0.999$, but there was a main effect of treatment $F_{(1,\ 26)} = 9.245$, $P = 0.0053^{**}$, with no interaction between sex and treatment $F_{(1,\ 26)} = 1.592$, $P = 0.2182$ (**Fig. 3D**) in CCI mice. Post hoc multiple comparison tests revealed a significant effect of treatment in females ($P = 0.0067^{**}$), but not males ($P = 0.2043$), which spent more time in the vehicle-paired chamber compared with the myrcene-paired chamber. These data were further analyzed by calculating the CPP scores. We performed 1-sample *t* tests in male and female CCI mice to examine if CPP scores for state dependence were different from a theoretical value of 0 (no preference) when administered myrcene. Results indicated statistical significance for females $t_{(7)} = 7.232$, $P = 0.0004^{***}$, but not for males $t_{(7)} = 0.8169$, $P = 0.4409$ (**Fig. 3E**). To probe for potential rewarding effects in sham mice, a 2-way ANOVA was performed to evaluate the effect of sex on treatment (100 mg/kg i.p. myrcene or vehicle) on time spent in the chambers. Results indicated no significant main effect of sex $F_{(1,\ 26)} = 0.001153$, $P = 0.9732$, or a main effect of treatment $F_{(1,\ 26)} = 0.7870$, $P = 0.3831$, and no interaction between sex and treatment $F_{(1,\ 26)} = 0.1375$, $P = 0.7138$ (**Fig. 3F**) in sham mice. We performed 1-sample *t* tests on CPP scores in male and female sham mice, revealing no statistical significance for males $t_{(7)} = 0.5121$, $P = 0.6244$ or for females $t_{(7)} = 0.3789$, $P = 0.7178$ (**Fig. 3G**).

3.5. Myrcene did not exhibit locomotor or hypothermic effects

As CB1 receptor agonists are known to produce the canonical tetrad effects, which include hypolocomotion and hypothermia, CCI male and female mice were administered 100 mg/kg myrcene (i.p.) or vehicle and locomotor and hypothermic effects

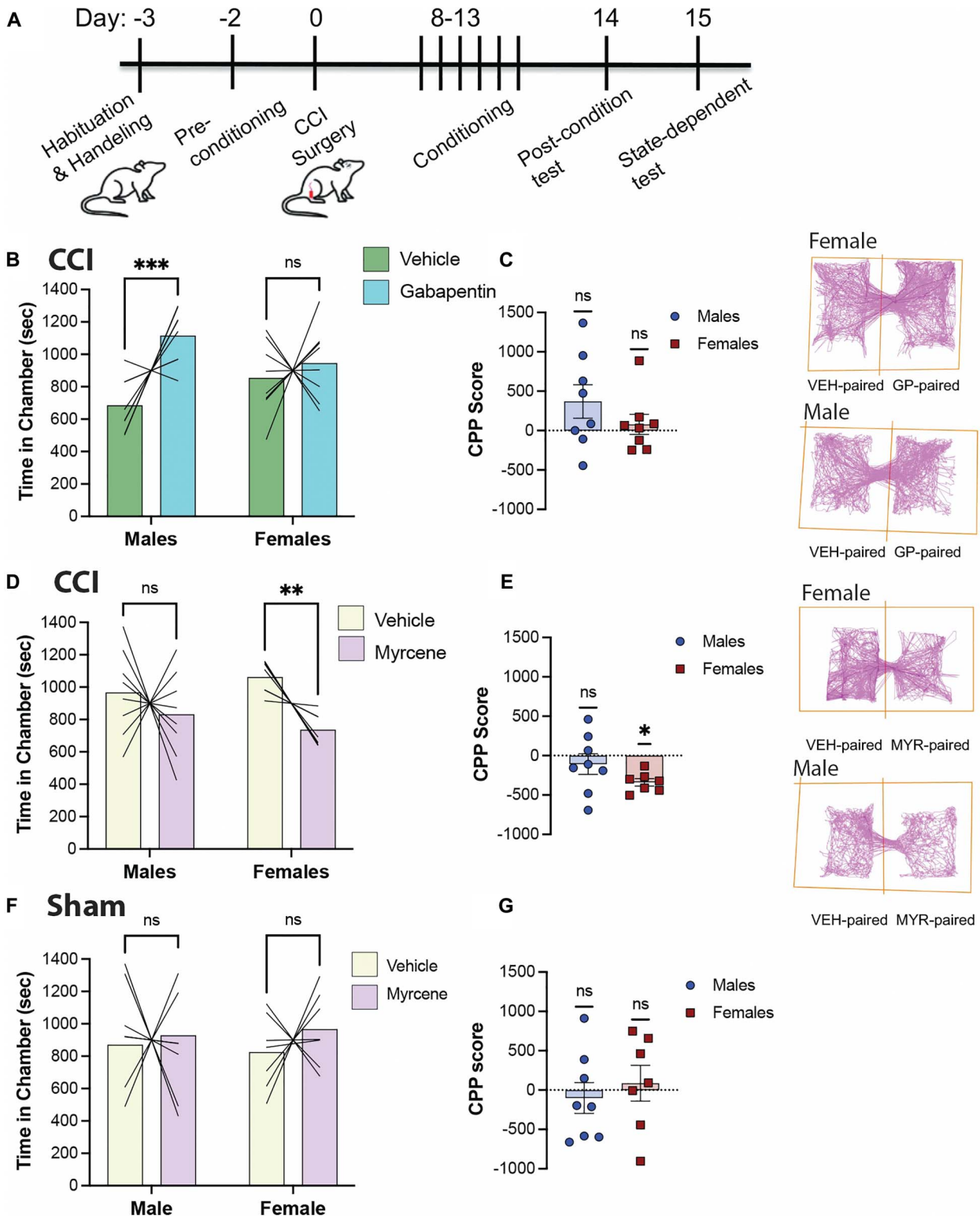

Figure 3. Male, but not female, CCI mice showed a preference to gabapentin in a place-preference state-dependent test. (A) Mice were habituated to the CPP apparatus and handled a day before preconditioning, which occurred 2 days before CCI surgery. Seven days after CCI surgery, mice underwent conditioning for 6 days followed by a postcondition and a state-dependent test (14 and 15 days postsurgery). (B) Male but not female CCI mice spent more time in the gabapentin (100 mg/kg i.p.)-paired chamber. (C) Neither male nor female CPP scores significantly differed from 0, and track plots of time spent in chambers are displayed in pink. (D) CCI male and female mice were tested on state-dependent day for time spent in vehicle-paired chamber compared with 100 mg/kg i.p. myrcene-paired chamber. Females but not males spent less time in the myrcene-paired chamber. (E) Neither male or female CPP scores differed significantly from 0, and track plots of time spent in chambers are displayed in pink. (F) There was no difference in time spent in the myrcene-paired chamber compared with vehicle for either male or female sham mice (G) or in CPP scores. Data were analyzed with 2-way ANOVAs for sex and treatment (B, D, F) and 1-sample *t* tests (C, E, G). ns = nonsignificant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{***}P < 0.0001$. CCI, chronic constriction injury; CPP, conditioned place preference; VEH, vehicle; GP, gabapentin; MYR, myrcene.

were assessed. To examine locomotion, a 2-way ANOVA was performed to evaluate the effect of sex and treatment condition (100 mg/kg myrcene, vehicle, or 1 mg/kg CP 55,940) on distance traveled 30 minutes postinjection. Results indicated no significant main effect of sex $F_{(1, 66)} = 2.034$, $P = 0.1585$, but a significant main effect of treatment $F_{(2, 66)} = 16.43$, $P < 0.0001$****. There was no interaction between sex and treatment $F_{(2, 66)} = 0.9397$, $P = 0.3959$ (**Fig. 4A**). Track plots and time course for males and females are displayed in **Figure 4A**.

To examine body temperature, a 2-way ANOVA was performed to evaluate the effect of sex and treatment condition (100 mg/kg myrcene, vehicle, or 1 mg/kg CP 55,940) on rectal temperature 30 minutes postinjection. Results indicate a significant main effect of sex $F_{(1, 68)} = 54.55$, $P < 0.0001$****, and treatment $F_{(2, 68)} = 75.44$, $P < 0.0001$****, and an interaction between sex and treatment $F_{(2, 68)} = 14.48$, $P < 0.0001$**** (**Fig. 4B**). Time course for males and females are also displayed in **Figure 4B**.

3.6. Myrcene did not activate cannabinoid receptor 1 receptors or modulate endocannabinoid binding in vitro

Because a CB1 antagonist block the anti-allodynic effects of myrcene in CCI mice, we wanted to understand the extent to which myrcene was producing this effect by directly activating CB1 receptors in vitro. We use the TRUPATH assay, in which GPCR-mediated activation of heterotrimeric G proteins is measured as a reduction in BRET between a luciferase tagged Ga subunit and a GFP2-tagged Gg subunit[28] (**Fig. 5A**). In HEK293T cells, the CB1 agonist CP 55,940 did not produce BRET changes unless the cells are transfected with CB1 plasmid. This allowed us to directly test if myrcene activated CB1 by measuring BRET responses to 10 mM in cells with or without transfected CB1. As depicted in **Figures 5B** 10 μM myrcene did not activate the Gi protein coupled to CB1 receptors, unlike CP 55,940 (3 μM). These results also show that myrcene did not activate CB1 or any endogenous Gi-coupled receptors in HEK293T cells. Furthermore, this high dose of myrcene (10 μM) did not modify the Gi-protein activation produced by CP 55,940 (**Fig. 5B**) nor 2 endocannabinoids, 2-AG (**Fig. 5C**) and AEA (**Fig. 5D**).

4. Discussion

Our study demonstrated that myrcene reduced mechanical hypersensitivity associated with a chronic constriction nerve injury that was blocked by a CB1 antagonist. These data showed that myrcene engaged the CB1 receptor to produce this anti-allodynic effect. Myrcene was effective in both male and female nerve injured mice, although there was a sex- and dose-dependent effect, where myrcene produced anti-allodynic effects at lower doses in female than male CCI mice. There was no effect of myrcene on mechanical thresholds in sham control mice of either sex. The anti-allodynic effects produced by myrcene are consistent with one aspect of the tetrad, behavioral and physiological outcomes that are reliably altered by CB1 agonists including hypolocomotion, hypothermia, catalepsy, and analgesia.[25] However, antinociceptive doses of myrcene did not elicit prototypical CB1 agonist effects of hypolocomotion or hypothermia. Other sex differences were observed where myrcene produced an aversion in the conditioned place preference test for females but not for males, possibly demonstrating aversive psychological properties despite the mitigation of mechanical hypersensitivity. This aversive effect is consistent with that produced by high doses of CB1 agonists.[4,10] The enhanced effects of myrcene in female mice is consistent with reports that females are more sensitive than males to the effects of THC.[43] It is possible that like THC, myrcene may possess beneficial and analgesic properties at low doses but may also elicit adverse effects at higher doses.

Supporting earlier studies, our findings demonstrate myrcene's anti-allodynic properties in male animals with pain, but we expanded this evidence by determining the therapeutic potential of myrcene in female rodents and inclusion of dose-dependent effects (1-100 mg/kg i.p.). Previous studies reported that male mice tested on hot plate for reaction time showed heat antinociception when given 10 and 20 mg/kg i.p. of myrcene.[33] In a model of chronic inflammation, male rats exhibited reduced inflammatory hyperalgesia when administered both 1 and 5 mg/kg s.c. myrcene.[24] These doses are much lower than what was effective in our experiments, which found the minimum effective dose to be 10 mg/kg i.p. in females and 100 mg/kg i.p. in males to reduce mechanical hypersensitivity. Another notable difference in experimental technique is time postinjection in which outcomes were measured. In rats given 1 and 5 mg/kg s.c., myrcene exhibited greatest effects on von Frey withdrawal thresholds at 60, 120, and 180 minutes postinjection, but not at 30 minutes postinjection.[24] In mice given 10 and 20 mg/kg i.p., myrcene showed peak effects on hot plate reaction time 45 to 60 minutes postinjection, but still an increase at 30 minutes postinjection.[33] Our study examined outcomes 30 minutes postinjection, which allowed for the effects of myrcene to be observed sooner after administration than was previously reported. Species and route of administration can affect drug metabolism and therefore the time for peak effects post administration and may play a role in the differences of results and should be considered along with the chosen outcome to be measured. Inconsistent with other outcomes of the tetrad (a set of tests used to capture CB1 receptor–mediated effects in rodents), myrcene did not elicit CB1 receptor agonist-like effects to produce either hypolocomotion or hypothermia. However, our data are consistent with manipulations of endogenous levels of endocannabinoids AEA and 2-AG, where they produced antinociception but were also devoid of hypothermia or hypolocomotion.[13] Our study adds important data to the literature, where myrcene produced dose-dependent anti-allodynic effects in both male and female neuropathic pain mice without modulating mechanical thresholds in sham animals and did not elicit other tetrad-like effects indicative of CB1 receptor agonist activity.

We found that pretreatment with a CB1 receptor antagonist blocked myrcene's ability to increase withdrawal thresholds in neuropathic mice that was more effective in female than male animals, consistent with sex differences in the endocannabinoid system in pain circuits.[2] Others similarly found that the thermal antihyperalgesic effects of myrcene were blocked by CB1 antagonist AM281 (75 μg s.c.) in rats.[24] In rats with sciatic nerve damage, CB1 expression was found to be increased in the spinal cord[21] and in the thalamus[40]; thus, the enhanced expression in neuropathic pain may explain the ability of myrcene to have nociceptive effects. Although the effects of myrcene were mediated through CB1 receptor engagement, our in vitro study did not support direct activation or modulation of CB1 receptor activity. Furthermore, myrcene was not able to modulate endocannabinoid signaling induced by 2-AG or anandamide. It should be noted that myrcene has previously been shown to modulate TRPV1 currents, opioid receptors, and alpha 2-adrenoceptors.[19] However, the doses to engage these

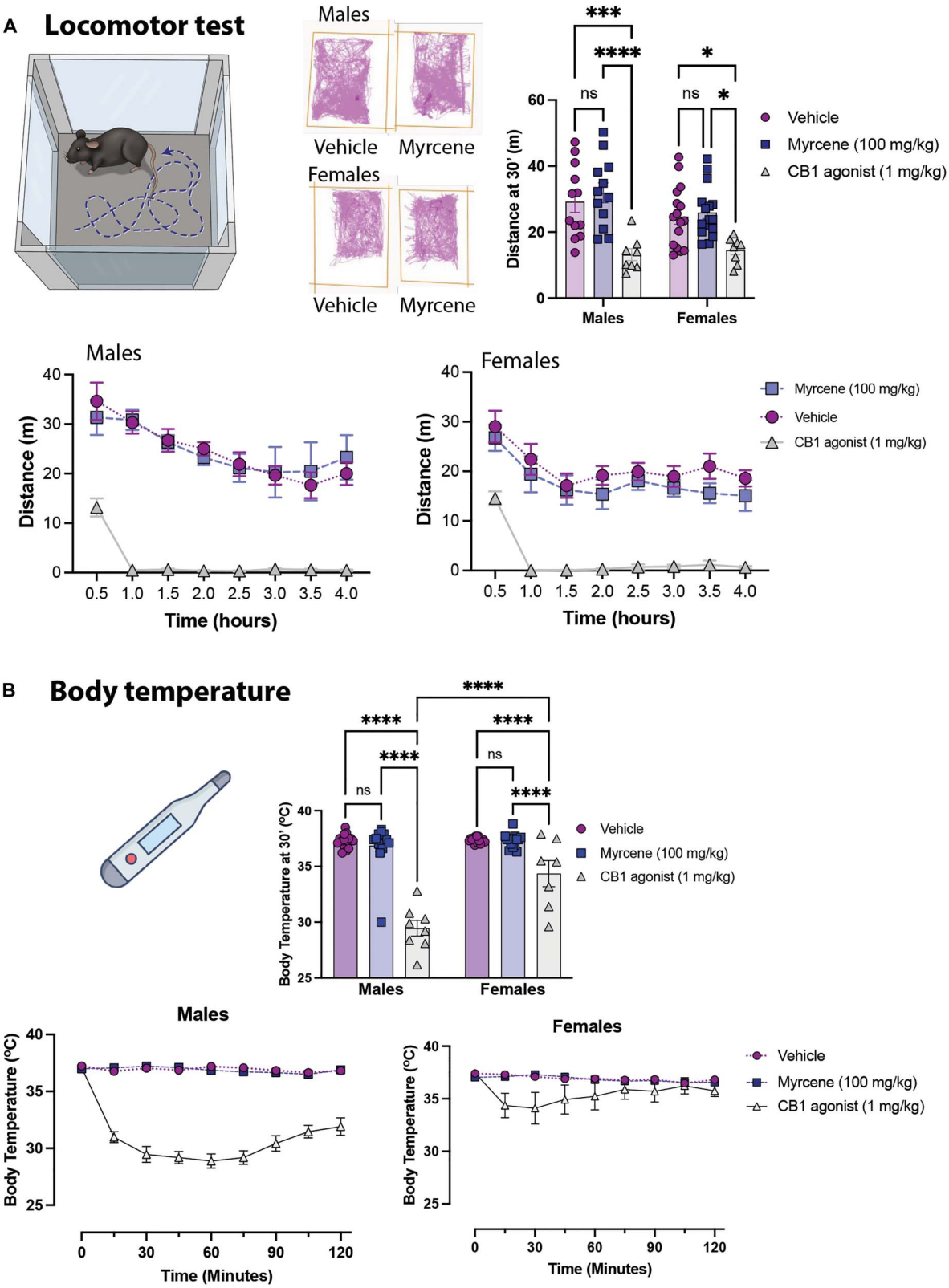

Figure 4. Myrcene did not exhibit locomotor or hypothermic effects. (A) CCI mice were recorded for 4 hours and locomotion recorded with track plots. There was no difference between distance traveled for vehicle-paired and 100 mg/kg i.p. myrcene-paired days for male or female mice, whereas the CB1 agonist CP 55,940 (1 mg/kg i.p.) produced a significant reduction in distance traveled. (B) CCI mice were administered vehicle or 100 mg/kg i.p. myrcene on a 2-day counterbalanced, crossover design test and body temperature recorded every 30 minutes after injection for 4 hours. There was no difference in temperature between vehicle and myrcene (100 mg/kg i.p.) for either male or female mice, whereas the CB1 agonist CP 55,940 (1 mg/kg i.p.) produced a significant reduction in temperature. Data were analyzed with 2-way ANOVAs for sex and treatment. ns = nonsignificant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ***$P < 0.0001$. m, meters; CB1, cannabinoid 1 receptor.

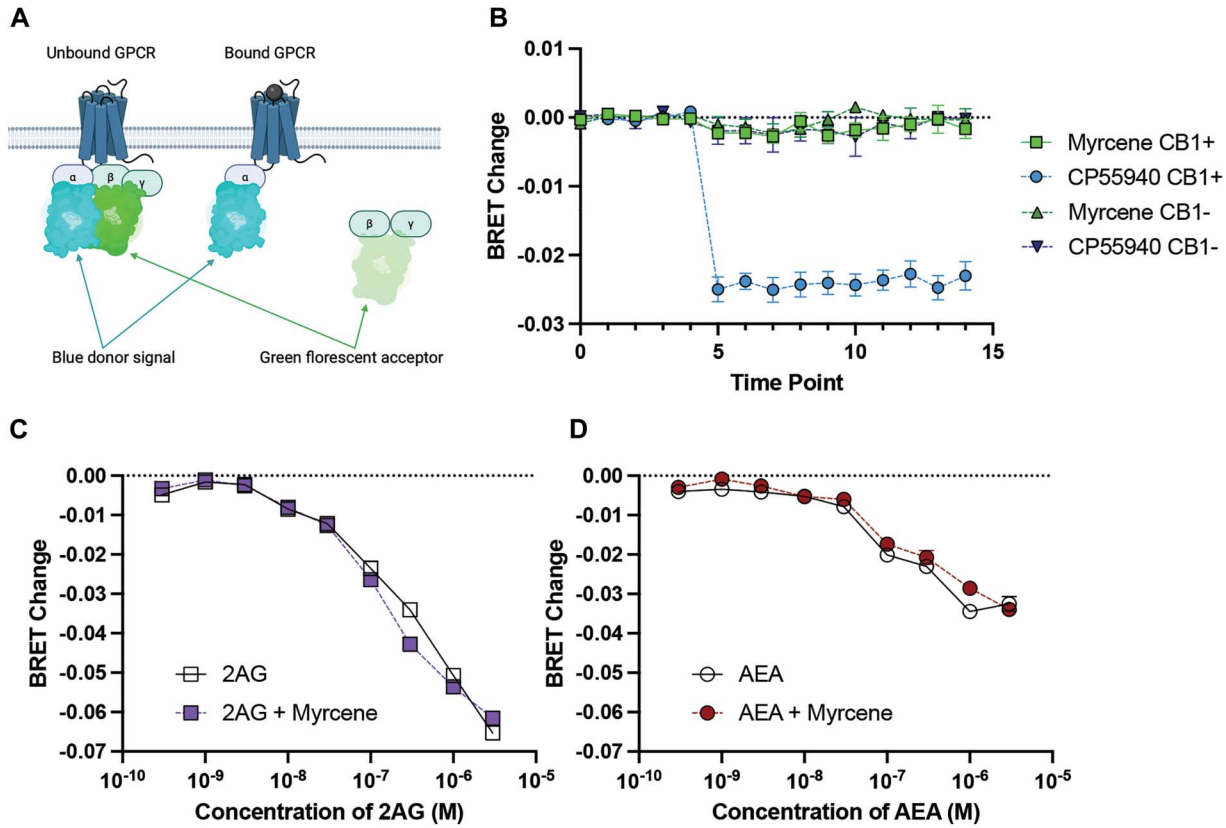

Figure 5. Myrcene did not activate CB1 receptors or modulate endocannabinoid binding in vitro. (A) TRUPATH assay measures change in BRET emission that results from the dissociation of the beta/gamma subunits that occurs when the G-protein is bound. This image was created using BioRender and adapted from Addgene. (B) A CB1 agonist, CP 55,940 (3 μM), produces a change in BRET emission that myrcene (10 μM) does not. When added to endocannabinoids (C) 2AG and (D) anandamide, myrcene does not change CB1 receptor activation. GPCR, G protein–coupled receptors; BRET, bioluminescence resonance energy transfer; CB1, cannabinoid 1 receptor; 2AG, 2-arachidonoylglycerol; M, moles; AEA, anandamide.

receptors was very high at millimolar concentrations. Although we cannot directly compare drug concentration in cells to drug dose in our study, it is unlikely that the dose we used engages these receptor systems. Some electrophysiology and cell culture experiments have shown that myrcene elicits TRPV1 currents by calcium mobilization and effects were blocked with a TRPV1 antagonist,[19] although another study reported that myrcene had a modulatory effect on HEK Flp-In T-Rex cells transfected with hTRPV1 channels.[16] In addition, the CB1 antagonist SR141716A can also act at vanilloid TRPV1 receptors, posing an alternative explanation and interpretation of our findings that SR141716 blocked myrcene's nociception.[32] Considering the CB1 receptor antagonist completely reversed myrcene's effects, the most parsimonious explanation of our findings is that myrcene increases binding of endocannabinoids 2-AG and AEA. These endocannabinoids subsequently activate CB1 receptors after myrcene administration to produce the anti-allodynic effects observed in the current study, which are blocked by SR141716. However, given earlier evidence that myrcene has activity at TRPV1, opioid, and adenosine receptors, an alternative explanation is that the SR blockade observed here may be due to CB1 receptor activation downstream of these targets, especially considering cooperativity of opioid and CB1 receptors in many functional outcomes including nociception[5,6,46] as is opioid and adenosine receptors.[3,23,37,48] Importantly, SR141716 has been shown to bind to mu opioid receptors and block morphine inhibition of cAMP production in CHO-hMOR cells, and in mice a 10 mg/kg injection can block morphine analgesia using tail-flick assay.[39] In addition, SR141716 was also shown to interact with TRPV1 receptors,[17,31] and because anandamide can interact with TRPV1,[8] we therefore cannot rule out the possibility that this ionotropic receptor is not involved in myrcene's effects. Finally, it is possible that myrcene could be acting at peripheral CB1 receptors to produce anti-allodynic effects. We have behavioral data that we infer the brain is modulating, but not out of the question, and ought to be explored with a CB1 peripheral antagonist pretreatment of myrcene.

In vitro heterologous systems have been used to study the effects of myrcene on cannabinoid receptors, supporting that myrcene does not bind directly to CB1 receptors. Our functional assay supports these data where myrcene did not alter the G-protein activation produced by CB1 agonists. Pretreatment of a CB1 agonist was used to analyze membrane potential measured by FLIPR blue membrane potential dye in mouse wild-type AtT20 FlpIn cells.[36] Myrcene did not alter the membrane potential or response to somatostatin (used as a positive control for desensitization). Myrcene also did not change the response to a CB1 agonist, CP 55,940. A panel of terpenes were tested in autaptic hippocampal neurons and dorsal root ganglion neurons for their effects on CB1 receptors. Patch clamp recordings showed that 1 μM myrcene had no effect on EPSC amplitudes nor alter the effects of depolarization-induced suppression of excitation.[11] Using HEK293 cells with hCB1 receptors, the effects of myrcene (10 μM) on CB1 agonist and CP 55,940 binding was recorded; CP 55,940 binding was not displaced with the addition of myrcene, demonstrating that myrcene does not directly bind to the CB1 receptor. In addition, myrcene did not alter cAMP

production induced by 5 μM forskolin.[12] These in vitro studies complement our findings and conclude myrcene is not acting directly on CB1 receptors. Taken together, there is strong evidence that myrcene has no direct action on CB1 receptors but does engage the endogenous cannabinoid system in vivo. Future studies will assess this relationship directly by assessment of endocannabinoid levels in vivo.

5. Conclusions

We established a dose–response curve for myrcene in male and female mice, demonstrating that the potency for the anti-allodynic effects is shifted to the left in females in a CCI mouse model of neuropathic chronic pain. Although myrcene increased mechanical thresholds in pain models, a translational concern that warrants further exploration is that myrcene was aversive in the conditioned place preference test, similar to higher doses of CB1 receptor agonists, including THC. Future research measuring real-time endocannabinoid dynamics will clarify the in vivo engagement of CB1 receptor activity, yet in vitro experiments support the lack of direct action on CB1 receptors. Such experiments should aim to refine myrcene's mechanism of action to produce anti-allodynia, including examination of how myrcene may alter endocannabinoid precursors (eg, DAGL and NAPE-PDL), enzymes that degrade endocannabinoid (eg, MAGL and FAAH), as well as lipid membrane properties. To better serve patients with unmet pain symptoms, which comprises a majority of pain patients, it is increasingly important to understand the mechanisms behind pain signaling to develop effective and nonopioid options for analgesics.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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References

[1] Baron EP, Lucas P, Eades J, Hogue O. Patterns of medicinal cannabis use, strain analysis, and substitution effect among patients with migraine, headache, arthritis, and chronic pain in a medicinal cannabis cohort. J Headache Pain 2018;19:1–28.
[2] Blanton HL, Barnes RC, McHann MC, Bilbrey JA, Wilkerson JL, Guindon J. Sex differences and the endocannabinoid system in pain. Pharmacol Biochem Behav 2021;202:173107.
[3] Cahill CM, White TD, Sawynok J. Spinal opioid receptors and adenosine release: neurochemical and behavioral characterization of opioid subtypes. J Pharmacol Exp Ther 1995;275:84–93.
[4] Cheer JF, Kendall DA, Marsden CA. Cannabinoid receptors and reward in the rat: a conditioned place preference study. Psychopharmacology (Berl) 2000;151:25–30.
[5] Christie MJ. Opioid and cannabinoid receptors: friends with benefits or just close friends? Br J Pharmacol 2006;148:385–6.
[6] Cichewicz DL. Synergistic interactions between cannabinoid and opioid analgesics. Life Sci 2004;74:1317–24.
[7] Cunningham CL, Gremel CM, Groblewski PA. Drug-induced conditioned place preference and aversion in mice. Nat Protoc 2006;1:1662–70.
[8] De Petrocellis L, Bisogno T, Maccarrone M, Davis JB, Finazzi-Agro A, Di Marzo V. The activity of anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. J Biol Chem 2001;276:12856–63.
[9] Deuis JR, Dvorakova LS, Vetter I. Methods used to evaluate pain behaviors in rodents. Front Mol Neurosci 2017;10:284.
[10] DeVuono MV, Wills KL, MacPherson DV, Hrelja KM, Parker LA. Effect of footshock stress on place conditioning produced by Δ(9)-tetrahydrocannabinol and the fatty acid amide hydrolase (FAAH) inhibitor, URB597, in Sprague-Dawley rats. Psychopharmacology (Berl) 2017;234:3229–40.
[11] Dvorakova M, Wilson S, Corey W, Billingsley J, Zimmowitch A, Tracey J, Straiker A, Mackie K. A critical evaluation of terpenoid signaling at cannabinoid CB1 receptors in a neuronal model. Molecules 2022;27:5655.
[12] Finlay DB, Sircombe KJ, Nimick M, Jones C, Glass M. Terpenoids from cannabis do not mediate an entourage effect by acting at cannabinoid receptors. Front Pharmacol 2020;11:359.
[13] Griebel G, Stemmelin J, Lopez-Grancha M, Fauchey V, Slowinski F, Pichat P, Dargazanli G, Abouabdellah A, Cohen C, Bergis OE. The selective reversible FAAH inhibitor, SSR411298, restores the development of maladaptive behaviors to acute and chronic stress in rodents. Scientific Rep 2018;8:2416.
[14] Hamamoto DT, Giridharagopalan S, Simone DA. Acute and chronic administration of the cannabinoid receptor agonist CP 55,940 attenuates tumor-evoked hyperalgesia. Eur J Pharmacol 2007;558:73–87.
[15] He Y, Tian X, Hu X, Porreca F, Wang ZJ. Negative reinforcement reveals non-evoked ongoing pain in mice with tissue or nerve injury. J Pain 2012;13:598–607.
[16] Heblinski M, Santiago M, Fletcher C, Stuart J, Connor M, McGregor IS, Arnold JC. Terpenoids commonly found in cannabis sativa do not modulate the actions of phytocannabinoids or endocannabinoids on TRPA1 and TRPV1 channels. Cannabis Cannabinoid Res 2020;5:305–17.
[17] Hu SS. Involvement of TRPV1 in the olfactory bulb in rimonabant-induced olfactory discrimination deficit. Chin J Physiol 2016;59:21–32.
[18] Ilgen MA, Bohnert K, Kleinberg F, Jannausch M, Bohnert ASB, Walton M, Blow FC. Characteristics of adults seeking medical marijuana certification. Drug Alcohol Dependence 2013;132:654–9.
[19] Jansen C, Shimoda LMN, Kawakami JK, Ang L, Bacani AJ, Baker JD, Badowski C, Speck M, Stokes AJ, Small-Howard AL, Turner H. Myrcene and terpene regulation of TRPV1. Channels (Austin) 2019;13:344–66.
[20] Light MK, Orens A, Lewandowski B, Pickton T. Market size and demand for marijuana in Colorado: marijuana policy group. Denver, CO: The Marijuana Policy Group (MPG); 2015.
[21] Lim G, Sung B, Ji RR, Mao J. Upregulation of spinal cannabinoid-1-receptors following nerve injury enhances the effects of Win 55,212-2 on neuropathic pain behaviors in rats. PAIN 2003;105:275–83.
[22] Livingston SJ, Quilichini TD, Booth JK, Wong DCJ, Rensing KH, Laflamme-Yonkman J, Castellarin SD, Bohlmann J, Page JE, Samuels AL. Cannabis glandular trichomes alter morphology and metabolite content during flower maturation. Plant J 2020;101:37–56.
[23] Matsui A, Jarvie BC, Robinson BG, Hentges ST, Williams JT. Separate GABA afferents to dopamine neurons mediate acute action of opioids, development of tolerance, and expression of withdrawal. Neuron 2014;82:1346–56.
[24] McDougall JJ, McKenna MK. Anti-inflammatory and analgesic properties of the cannabis terpene myrcene in rat adjuvant monoarthritis. Int J Mol Sci 2022;23:7891.
[25] Metna-Laurent M, Mondésir M, Grel A, Vallée M, Piazza PV. Cannabinoid-induced tetrad in mice. Curr Protoc Neurosci 2017;80:9.59.51–59.59.10.
[26] Miranda HF, Sierralta F, Aranda N, Poblete P, Noriega V, Prieto JC. Synergism between gabapentin-tramadol in experimental diabetic neuropathic pain. Fundam Clin Pharmacol 2018;32:581–8.
[27] National Academies of Sciences E, and Medicine. The health effects of cannabis and cannabinoids: the current state of evidence and recommendations for research. Washington (DC): National Academies Press, 2017.
[28] Olsen RHJ, DiBerto JF, English JG, Glaudin AM, Krumm BE, Slocum ST, Che T, Gavin AC, McCorvy JD, Roth BL, Strachan RT. TRUPATH, an open-source biosensor platform for interrogating the GPCR transducerome. Nat Chem Biol 2020;16:841–9.

[29] Paula-Freire LI, Andersen ML, Molska GR, Köhn DO, Carlini EL. Evaluation of the antinociceptive activity of Ocimum gratissimum L. (Lamiaceae) essential oil and its isolated active principles in mice. Phytother Res 2013;27:1220–4.
[30] Paula-Freire LIG, Molska GR, Andersen ML, de Araújo Carlini EL. Ocimum gratissimum essential oil and its isolated compounds (eugenol and myrcene) reduce neuropathic pain in mice. Planta Med 2016;82:211–6.
[31] Pegorini S, Zani A, Braida D, Guerini-Rocco C, Sala M. Vanilloid VR1 receptor is involved in rimonabant-induced neuroprotection. Br J Pharmacol 2006;147:552–9.
[32] Pertwee RG. Pharmacological actions of cannabinoids. Handb Exp Pharmacol 2005;168:1–51.
[33] Rao V, Menezes A, Viana G. Effect of myrcene on nociception in mice. J Pharm Pharmacol 1990;42:877–8.
[34] Reinarman C, Nunberg H, Lanthier F, Heddleston T. Who are medical marijuana patients? Population characteristics from nine California assessment clinics. J Psychoactive Drugs 2011;43:128–35.
[35] Rock EM, Parker LA. Constituents of cannabis sativa. Adv Exp Med Biol 2021;1264:1–13.
[36] Santiago M, Sachdev S, Arnold JC, McGregor IS, Connor M. Absence of entourage: terpenoids commonly found in cannabis sativa do not modulate the functional activity of δ9-THC at human CB1 and CB2 receptors. Cannabis Cannabinoid Res 2019;4:165–76.
[37] Sawynok J. Methylxanthines and pain. Handb Exp Pharmacol 2011;10: 311–29.
[38] Schwarz AM, Keresztes A, Bui T, Hecksel R, Peña A, Lent B, Gao Z-G, Gamez-Rivera M, Seekins CA, Chou K, Appel TL, Jacobson KA, Al-Obeidi FA, Streicher JM. Terpenes from Cannabis sativa induce antinociception in a mouse model of chronic neuropathic pain via activation of adenosine A2A receptors. PAIN 2024;165: e145–e161.
[39] Seely KA, Brents LK, Franks LN, Rajasekaran M, Zimmerman SM, Fantegrossi WE, Prather PL. AM-251 and rimonabant act as direct antagonists at mu-opioid receptors: implications for opioid/cannabinoid interaction studies. Neuropharmacol 2012;63:905–915.
[40] Siegling A, Hofmann HA, Denzer D, Mauler F, De Vry J. Cannabinoid CB(1) receptor upregulation in a rat model of chronic neuropathic pain. Eur J Pharmacol 2001;415:R5–7.
[41] Silva AAF, Barbosa-Souza E, Confessor-Carvalho C, Silva RRR, De Brito ACL, Cata-Preta EG, Silva Oliveira T, Berro LF, Oliveira-Lima AJ, Marinho EAV. Context-dependent effects of rimonabant on ethanol-induced conditioned place preference in female mice. Drug and Alcohol Dependence 2017;179:317–24.
[42] Sommano SR, Chittasupho C, Ruksiriwanich W, Jantrawut P. The cannabis terpenes. Molecules 2020;25:5792.
[43] Tseng AH, Craft RM. CB(1) receptor mediation of cannabinoid behavioral effects in male and female rats. Psychopharmacology (Berl) 2004;172: 25–30.
[44] Wakley AA, McBride AA, Vaughn LK, Craft RM. Cyclic ovarian hormone modulation of supraspinal Δ9-tetrahydrocannabinol-induced antinociception and cannabinoid receptor binding in the female rat. Pharmacol Biochem Behav 2014;124:269–77.
[45] Wakley AA, Wiley JL, Craft RM. Sex differences in antinociceptive tolerance to delta-9-tetrahydrocannabinol in the rat. Drug Alcohol Depend 2014;143:22–8.
[46] Welch SP. Interaction of the cannabinoid and opioid systems in the modulation of nociception. Int Rev Psychiatry 2009;21:143–51.
[47] Yalcin I, Megat S, Barthas F, Waltisperger E, Kremer M, Salvat E, Barrot M. The sciatic nerve cuffing model of neuropathic pain in mice. J Vis Exp 2014;89:51608.
[48] Yao L, McFarland K, Fan P, Jiang Z, Ueda T, Diamond I. Adenosine A2a blockade prevents synergy between mu-opiate and cannabinoid CB1 receptors and eliminates heroin-seeking behavior in addicted rats. Proc Natl Acad Sci U S A 2006;103:7877–82.
[49] Yong RJ, Mullins PM, Bhattacharyya N. Prevalence of chronic pain among adults in the United States. PAIN 2022;163:e328–e332.