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Dissociable effects of the cannabinoid receptor agonists Δ9-tetrahydrocannabinol and CP55940 on pain-stimulated versus pain-depressed behavior in rats

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THC, Δ9-tetrahydrocannabinol; ICSS, intracranial self-stimulation; NSAID, nonsteroidal anti-inflammatory drug; IP, Intraperitoneal; MCR, maximum control rate

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ABSTRACT

Cannabinoid receptor agonists produce reliable antinociception in most preclinical pain assays but have inconsistent analgesic efficacy in humans. This disparity suggests that conventional preclinical assays of nociception are not sufficient for prediction of cannabinoid effects related to clinical analgesia. To extend the range of preclinical cannabinoid assessment, this study compared effects of the marijuana constituent and low-efficacy cannabinoid agonist ∆9-tetrahydrocannabinol (THC) and the high-efficacy synthetic cannabinoid agonist CP55940 in assays of pain-stimulated and pain-depressed behavior. Intraperitoneal injection of dilute lactic acid (1.8% in 1 ml/kg) stimulated a stretching response or depressed intracranial self-stimulation (ICSS) in separate groups of male Sprague-Dawley rats. THC (0.1-10 mg/kg) and CP55940 (0.0032-0.32 mg/kg) dose-dependently blocked acid-stimulated stretching but only exacerbated acid-induced depression of ICSS at doses that also decreased control ICSS in the absence of a noxious stimulus. Repeated THC produced tolerance to sedative rate-decreasing effects of THC on control ICSS in the absence of the noxious stimulus but failed to unmask antinociception in the presence of the noxious stimulus. THC and CP55940 also failed to block pain-related depression of feeding in rats, although THC did attenuate satiation-related depression of feeding. In contrast to effects of the cannabinoid agonists, the clinically effective analgesic and nonsteroidal anti-inflammatory drug ketoprofen (1 mg/kg) blocked acid-stimulated stretching and acid-induced depression of both ICSS and feeding. The poor efficacy of THC and CP55940 to

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block acute pain-related depression of behavior in rats agrees with the poor efficacy of cannabinoids to treat acute pain in humans.

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INTRODUCTION

The marijuana plant has been used for centuries in an effort to treat pain, and cannabinoid receptor agonists such as the marijuana constituent Δ9-tetrahydrocannabinol (THC) and the synthetic compound CP55940 produce antinociception in nearly all preclinical assays of acute and chronic pain-related behavior (Rice, 2006; Karst et al., 2010). However, in contrast to the reliable antinociceptive efficacy of cannabinoids in preclinical studies, controlled clinical trials in humans have found that cannabinoids are ineffective against acute pain and have weak efficacy and a narrow therapeutic window for treatment of many types of chronic pain (Raft et al., 1977; Rice, 2006; Karst et al., 2010; Kraft, 2012). This discrepancy suggests that existing preclinical assays of antinociception in animals overestimate the analgesic potential of cannabinoids in humans and may be insufficient for preclinical behavioral assessment of candidate cannabinoid analgesics.

The goal of this study was to compare antinociceptive effects of THC and CP55940 in rats using assays of acute pain-stimulated and pain-depressed behavior that have been used previously to examine effects of opioid and non-opioid compounds (Pereira Do Carmo et al., 2009a; Negus et al., 2010a; Negus et al., 2010b; Negus et al., 2011). Pain-stimulated behaviors are defined as behaviors (e.g. withdrawal responses) that increase in rate or intensity following delivery of a noxious stimulus. In assays of pain-stimulated behavior, antinociception is indicated by decreases in the target behavior. However, decreases in pain-stimulated behavior can be produced either by a reduction in

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sensory sensitivity to the noxious stimulus (i.e. true analgesia) or by nonselective behavioral depressant effects (e.g. sedation, motor impairment) that limit the subject's ability to respond. Sedative drugs such as cannabinoid agonists are especially prone to produce false-positive antinociception in assays of pain-stimulated behavior (De Vry et al., 2004; Finn et al., 2004). In contrast, pain-depressed behaviors are defined as behaviors such as feeding, locomotion, or operant behavior that decrease in rate or intensity following delivery of a noxious stimulus. Assays of pain-depressed behavior have two attributes important to the assessment of candidate analgesics. First, antinociception is indicated by increases in the target behavior, and as a result, assays of pain-depressed behavior are not vulnerable to false-positive effects caused by nonselective behavioral depression (Negus et al., 2010a; Negus et al., 2010b; Negus et al., 2011). Second, assays of pain-depressed behavior may model pain-related functional impairment and/or depressed mood used to assess pain in both human and veterinary medicine (Cleeland and Ryan, 1994; National Research Council, 2003; Dworkin et al., 2005), and thus may provide insight into effects of candidate analgesics on these clinically relevant components of pain (Negus et al., 2006; Negus et al., 2010a). In view of these attributes, we have argued that assays of pain-depressed behavior may complement conventional assays of pain-stimulated behavior and increase the predictive validity of preclinical candidate analgesic assessment (Negus et al., 2006; Negus et al., 2010a). Given the poor efficacy of THC and other cannabinoid receptor agonists to treat acute pain in humans (Raft et al., 1977; Buggy et al., 2003; Naef et al., 2003; Beaulieu,

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2006; Kraft et al., 2008, Klooker et al., 2011) we predicted that THC and CP55940 would not produce antinociception in assays of acute pain-depressed behavior in rats despite the apparent efficacy of these drugs in standard assays of pain-stimulated behavior.

In the present study, intraperitoneal (IP) injection of dilute acid served as an acute noxious stimulus to stimulate stretching (a pain-stimulated behavior) and to depress intracranial self-stimulation (ICSS; a pain-depressed operant behavior in which subjects respond on a lever to receive pulses of electrical stimulation delivered via electrodes implanted in the brain's "reward pathway") (Pereira Do Carmo et al., 2009a; Negus et al., 2010a; Negus et al., 2010b; Negus et al., 2011). Initial experiments indicated that the cannabinoid agonists THC and CP55940 failed to produce antinociception in the assay of acid-depressed ICSS. Two follow-up studies were conducted to further evaluate conditions under which THC and/or CP55940 might be effective. First, previous studies with another drug class (delta opioid agonists) showed that expression of antinociception in the assay of acid-depressed ICSS could be obscured by rate-decreasing effects, but that repeated drug treatment could produce selective tolerance to rate-decreasing effects and unmask antinociception (Negus et al., 2012). Accordingly, THC effects in assays of acid-stimulated stretching and acid-depressed ICSS were evaluated during chronic THC administration to test the hypothesis that repeated THC might produce selective tolerance to rate-decreasing effects and unmask antinociception in the assay of acid-depressed ICSS. Second, effects of THC and CP55940 were evaluated in an assay of acid-

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induced depression of feeding (Stevenson et al., 2006). Feeding is reliably stimulated by THC and other cannabinoid agonists in the absence of pain (Williams et al., 1998; Miller et al., 2004; Jarbe and DiPatrizio, 2005; Farrimond et al., 2011), suggesting that cannabinoids might be more effective in blocking acid-induced depression of feeding than acid-induced depression of ICSS. The nonsteroidal anti-inflammatory drug (NSAID) and clinically effective analgesic ketoprofen (Flecknell, 2009; Sarzi-Puttini et al., 2010) was tested as a positive control in assays of acid-stimulated and acid-depressed behavior.

METHODS

Subjects

Seventy-nine male Sprague-Dawley rats (Harlan, Frederick, MD, USA) weighing approximately 300-320 g (age 10-11 weeks) at the time of surgery and/or delivery were individually housed and maintained on a 12 h light/dark cycle with lights on from 6:00 a.m. to 6:00 p.m. Rats had free access to food and water except during testing. Animal maintenance and research were in compliance with National Institutes of Health guidelines on care and use of animal subjects in research and adhered to guidelines of the Committee for Research (National Research Council, 2003) and Ethical Issues of the International Association for the Study of Pain (Zimmermann, 1983). All animal use protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

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Assay of lactic acid-stimulated stretching

Behavioral procedure. Twenty-six rats that failed to meet the criteria for ICSS within 4 weeks (see below) were used for studies of lactic acid-stimulated stretching as described previously (Pereira Do Carmo et al., 2009a; Negus et al., 2010b). During test sessions, rats were placed into an acrylic test chamber (31.0 x 20.1 x 20.0 cm) for a 30 min observation period that began immediately after injection of dilute lactic acid (1.8% in a volume of 1 ml/kg). A stretch was operationally defined as a contraction of the abdomen followed by a stretching of the hind limbs, and the number of stretches during the observation period was counted.

Studies with acute THC were conducted in four phases. First, a THC dose-effect curve was determined by administering THC (0.32-10 mg/kg or vehicle) 30 min prior to acid. Doses were delivered in a Latin-square dose order across rats and separated by at least one week. Second, the time course of effects produced by 3.2 mg/kg THC was determined by varying the interval between administration of THC and acid (10, 30, 100, 300 min, and 24 h). A dose of 3.2 mg/kg THC was chosen for time course studies because it was the lowest dose to significantly decrease acid-stimulated stretching during dose-effect testing. Each pretreatment time was tested in a different test session in randomized order, and test sessions were separated by at least one week. Third, to assess the role of cannabinoid 1 receptors in mediating THC effects, THC-induced antinociception was evaluated for its sensitivity to antagonism by the cannabinoid 1 receptor antagonist rimonabant. For these studies, rimonabant

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(0.01-1.0 mg/kg or vehicle) was administered 20 min prior to THC (3.2 mg/kg), and acid was administered 30 min after THC. All THC and rimonabant doses were delivered in a Latin-square dose order across rats and separated by at least one week.

Finally, to assess the potential for antinociceptive tolerance to repeated THC, acid-stimulated stretching was evaluated following chronic treatment with THC (3.2 mg/kg/day). Initially a vehicle test was conducted in which rats were administered THC vehicle prior to treatment with acid. Beginning one week later, THC (3.2 mg/kg) was administered once daily for 22 days. On days 1, 8, 15 and 22, acid (1.8% in 1 ml/kg) was administered 30 min after THC, and the stretching response was evaluated. Effects of 3.2 mg/kg THC on acid-stimulated stretching were redetermined one additional time two weeks after termination of chronic THC.

To provide a comparison for results with THC, two additional groups of rats were used to evaluate the high-efficacy cannabinoid 1 receptor agonist CP55940 (0.0032-0.1 mg/kg or vehicle) and the NSAID ketoprofen (1 mg/kg or saline). In both cases, the test drug was administered 30 min prior to acid, and tests were separated by one week.

Data Analysis. Drug effects on acid-stimulated stretching were evaluated by repeated measures one-way analysis of variance (ANOVA) or *t* test as appropriate. A significant ANOVA was followed by Newman Keul's or Dunnett's post hoc test, and the criterion for significance was set at $p < 0.05$.

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Assay of intracranial self-stimulation (ICSS)

Surgery. All rats were anesthetized with isoflurane (2.5-3% in oxygen; Webster Veterinary, Phoenix, AZ, USA) for implantation of stainless steel electrodes (Plastics One, Roanoke, VA, USA). One pole (the cathode) of each bipolar electrode was 0.25 mm in diameter and covered with polyamide insulation except at the flattened tip, whereas the other pole (the anode) was 0.125 mm in diameter and uninsulated. The cathode was implanted in the left medial forebrain bundle at the level of the lateral hypothalamus (2.8 mm posterior to bregma, 1.7 mm lateral from the midsagittal suture, and 8.8 mm below the skull). The anode was wrapped around one of the three skull screws to serve as the ground, and the skull screws and electrode assembly were secured to the skull with orthodontic resin. The animals were allowed to recover for at least 7 days prior to commencing ICSS training.

Apparatus. Experiments were conducted in sound-attenuating boxes that contained modular acrylic test chambers (29.2 x 30.5 x 24.1 cm) equipped with a response lever (4.5 cm wide, extended 2 cm through the center of one wall, 3 cm off the floor), stimulation lights (three lights colored red, yellow, and green, positioned 7.6 cm directly above the response lever), a 2 W house light, and an ICSS stimulator (Med Associates, St. Albans, VT, USA). Electrodes were connected to the stimulator via a swivel connector (Model SL2C, Plastics One, Roanoke, VA, USA). The stimulator was controlled by computer software that also controlled programming parameters and data collection (Med Associates, St. Albans, VT, USA).

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Behavioral procedure. After initial shaping of lever press responding, rats were trained under a continuous reinforcement schedule of brain stimulation using procedures similar to those described previously (Carlezon and Chartoff, 2007; Pereira Do Carmo et al., 2009b; Negus et al., 2010a). During sessions lasting 30-60 min, each lever press resulted in the delivery of a 0.5 s train of square wave cathodal pulses (0.1 ms pulse duration), and stimulation was accompanied by illumination of the stimulus lights over the lever. Responses during the 0.5 s stimulation period did not earn an additional stimulation. Initially, the frequency of stimulation was held constant at 158 Hz, and the stimulation intensity for each rat was adjusted gradually to the lowest value that would sustain a high rate of reinforcement (> 30 stimulations/min). This intensity (100-280 μA across rats) was then held constant for the remainder of the study, and frequency manipulations were introduced. Sessions involving frequency manipulations consisted of sequential 10 min components. During each component, a descending series of 10 current frequencies (158 to 56 Hz in 0.05 log increments) was presented, with each frequency available during sequential 1 min frequency trials. Each frequency trial began with a 10 s time out, during which responding had no scheduled consequences. During the last 5 s of this time out, five noncontingent “priming” stimulations were delivered at the frequency available during that trial, and the lever lights were illuminated during each stimulation. Noncontingent stimulations were separated by intervals of 0.5 s. This noncontingent stimulation was then followed by a 50 s “response” phase, during which responding produced electrical stimulation under the continuous

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reinforcement schedule. Training continued with presentation of three to six sequential components per day until rats reliably responded for only the first four to six frequency trials of all components for at least three consecutive days. In general, rats were implanted with electrodes and trained on ICSS procedures in groups of 10-12. The first six rats in each group to meet training criteria were then advanced to testing, while the remaining rats were assigned to assays of acid-stimulated stretching as described above.

Once training was completed, testing was initiated. The first component of each test session was considered to be an acclimation component, and data from this component were discarded. Data from the second and third "baseline" components were used to calculate baseline parameters of the frequency-rate curves for that session (see "Data Analysis"). Drugs were administered immediately after removing the subjects from the operant chamber after the third baseline component. Studies of THC effects on ICSS were conducted in four phases. In the first phase, the effects of acute THC on ICSS were studied in two separate groups of rats. In the first group of rats, THC effects on ICSS were studied in the absence of the noxious stimulus (control ICSS). Subjects were placed in their home cages after administration of THC (0.32-10 mg/kg or vehicle) and then transferred back to the operant chambers at designated times (30, 100, 180, 300 min) for two consecutive "test" components, totaling 20 min at each time point. In the second group of rats, THC effects on ICSS were studied in the presence of the noxious stimulus (acid-depressed ICSS). Subjects were administered THC (0.32-3.2 mg/kg or vehicle) 30 min prior to lactic acid (1.8% in

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a volume of 1 ml/kg), which was administered immediately before two consecutive “test” components. THC and acid doses were administered in Latin-square order and were separated by at least one week. The second phase examined the effects of 3.2 mg/kg THC administered 180 and 300 min before acid treatment. These times were selected because initial results indicated that treatment with 3.2 mg/kg THC significantly decreased acid-stimulated stretching after 180 and 300 min but did not significantly decrease ICSS at these pretreatment times in the absence of a noxious stimulus. For these experiments, subjects were placed in their home cages after THC administration and then injected with acid and transferred back to the operant chamber at the designated time (180 or 300 min) for two consecutive “test” components. Each pretreatment time/dose combination was tested in a different test session in randomized order, and test sessions were separated by at least one week. In the third phase, the ability of rimonabant to block THC effects on ICSS was investigated. In these experiments, rimonabant (1 mg/kg or vehicle) was administered 50 min prior to testing and 20 min before THC (3.2 mg/kg or vehicle). THC and rimonabant doses were administered in Latin-square order and were separated by at least one week. In phases 1-3, training and test sessions were conducted Monday-Friday for the duration of the experiment, with test sessions conducted on Thursdays or Fridays.

The final phase of studies with THC examined effects of chronic THC in two separate groups of rats. In the first group, the effects of chronic THC were studied in the absence of the acid noxious stimulus (control ICSS). For these

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experiments, subjects initially received chronic treatment of THC vehicle (1 ml/kg/day) for three weeks while being tested with THC (1-10 mg/kg or vehicle) once/week in a Latin-Square dose order. Following this treatment regimen, subjects were treated for 11 days with 1 mg/kg/day THC, 11 days with 3.2 mg/kg/day THC, and lastly 11 days with 10 mg/kg/day THC (i.e. 33 days of total THC treatment). On the last four days of treatment with each dose of chronic THC, subjects were tested with THC (1-10 mg/kg or vehicle) one dose/day in a Latin-Square dose order. Subjects that received test THC doses lower than the chronic THC dose for that day were administered the difference of the test and chronic doses at the end of the test session. In the second group of rats, the effects of chronic THC were studied in the presence of the noxious stimulus (acid-depressed ICSS). For these experiments, THC vehicle was initially administered prior to acid vehicle. One week later, THC vehicle was administered prior to acid. Beginning one week later, THC (3.2 mg/kg) was administered once daily for 22 days. On days 1, 8, 15, and 22, acid (1.8% in 1 ml/kg) was administered 30 min after THC, and ICSS was evaluated as described above. Effects of 3.2 mg/kg THC on acid-depressed ICSS were redetermined one additional time two weeks after termination of chronic THC. In both groups of rats, training and test sessions were conducted seven days/week.

In addition to these studies with THC, two additional groups of rats were tested with either CP55940 or ketoprofen. For these studies, one group was treated with CP55940 (0.01-0.32 mg/kg or vehicle) and the other with ketoprofen (1 mg/kg or saline) 30 min before treatment with 1.8% lactic acid or lactic acid

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vehicle (sterile water). Training and test sessions were conducted Monday-Friday for the duration of the experiment, with test sessions conducted on Thursdays or Fridays.

Data Analysis. The primary dependent variable in this ICSS procedure was the reinforcement rate in stimulations per minute during each frequency trial. To normalize these data, raw reinforcement rates from each trial in each rat were converted to percent maximum control rate (%MCR), with the MCR defined as the mean of the maximal rates observed during the second and third "baseline" components for that session in that rat. Thus, %MCR values for each trial were calculated as (Reinforcement Rate During a Frequency Trial ÷ Maximum Control Rate) x 100. For each test session, data from the second and third components were averaged to yield a baseline frequency-rate curve. Data from each test (two consecutive "test" components) were averaged for each test for each rat. Baseline and test curves were then averaged across rats to yield mean baseline and test curves for each manipulation. For statistical analysis, results were compared by repeated measures two-way ANOVA, with treatment and ICSS frequency as the two factors. A significant ANOVA was followed by Holm-Sidak post hoc test, and the criterion for significance was set at $p < 0.05$.

To provide an additional summary of ICSS performance, the total number of stimulations per component was calculated as the sum of stimulations delivered across all 10 frequency-trials of each component. Test data were then normalized to individual baseline data using the equation Percent Baseline Total Stimulations per Component = (Mean Total Stimulations per Test Component ÷

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Mean Total Stimulations per Baseline Component) x 100. Data were then averaged across rats in each experimental condition and compared by repeated measures one-way ANOVA or two-way ANOVA where appropriate. A significant one-way ANOVA was followed by Newman Keul's or Dunnett's post hoc test, a significant two-way ANOVA was followed by Holm-Sidak post hoc test, and the criterion for significance was set at $p < 0.05$.

Assay of lactic acid- and prefeeding-depressed feeding

Behavioral procedure. Sixteen rats were used for feeding studies. During test sessions, rats were placed into an acrylic test chamber (31 x 20.1 x 20 cm) within a sound- and light-attenuating cabinet for a 30 min feeding session. Rodent 45 mg purified food pellets (Product #F0021, Bio-Serv, Frenchtown, NJ) were delivered in pre-weighed glass petri dishes (60 x 15 mm) (Corning Life Sciences, Pittston, PA) securely taped to the bottom left corner of the test chamber. Spilled pellets/dust were collected at the end of the session and added back to the petri dish, which was then re-weighed. Percent Body Weight Food Consumed after each session was determined with the following equation: [(Pre-Session Dish Weight – Post-Session Dish Weight) ÷ [Daily Subject's Weight (g)] x 100]. Initial baseline feeding sessions were conducted for two weeks weeks until stable feeding baselines were achieved. Studies were conducted in four phases. First, pain-related depression of feeding was established by administering dilute lactic acid (0.56-1.8% or vehicle in a volume of 1 ml/kg) immediately before the test session. For these studies, drug vehicle was also

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administered 30 min before acid. Second, THC and ketoprofen effects on pain-related depression of feeding were determined by administering THC (0.32-3.2 mg/kg or vehicle) or ketoprofen (1 mg/kg or vehicle) 30 min prior to injection of lactic acid (1.8% or vehicle in a volume of 1 ml/kg), which was administered immediately before the test session. Third, satiation-related depression of feeding was established by exposing rats to a 60 min prefeeding session 30 min prior to the test session. Prefeeding sessions were similar to test sessions, except that they were conducted in separate chambers not housed in sound- and light-attenuated cabinets. Finally, THC and ketoprofen effects on satiation-related depression of feeding were determined by administering THC (0.1-1 mg/kg or vehicle) or ketoprofen (1 mg/kg or vehicle) immediately after a 60 min prefeeding session and 30 min prior to the test session. THC doses were delivered in a Latin-square dose order across rats and separated by at least one week. Ketoprofen was tested after THC. Rats were housed in their homecages with free access to food and water at all times except during feeding sessions.

In addition to these studies with THC and ketoprofen, an additional group of rats was tested with CP55940 under conditions of acid- and satiation-related depression of feeding. In this study, animals were treated with CP55940 (0.0032-0.032 mg/kg or vehicle) 30 min before the feeding session using procedures identical to those with THC and ketoprofen. In all feeding studies, rats were housed in their homecages with free access to food and water at all times except during feeding sessions. Training and test sessions were conducted Monday-Friday, with test sessions conducted on Wednesdays or Fridays.

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Data Analysis. Drug effects on acid- and satiation-related depression of feeding were evaluated by repeated measures one-way ANOVA. A significant ANOVA was followed by Newman Keul's or Dunnett's post hoc test, and the criterion for significance was set at $p < 0.05$.

Drugs

Lactic acid was purchased from Sigma Chemical Co. (St. Louis, MO). THC, CP55940 (see Lichtman and Martin, 1991 for structure information), and rimonabant were provided by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD). Ketoprofen was purchased from Spectrum Chemical Co. (New Brunswick, NJ). Lactic acid was prepared in sterile water. THC, CP55940, and rimonabant were prepared in a vehicle consisting of ethanol, emulphor EL-620 (Rhone-Poulenc; Princeton, NJ), and sterile saline in a ratio of 1:1:18, respectively. Ketoprofen was prepared in sterile saline except in feeding tests, in which it was prepared in the same vehicle as THC. All solutions were injected intraperitoneally in a volume of 1 ml/kg.

RESULTS

Effects of THC on acid-stimulated stretching. Figure 1 shows that THC produced dose-dependent, time-dependent, and rimonabant-reversible antinociception in the assay of acid-stimulated stretching. IP administration of acid (1.8% lactic acid in a volume of 1 ml/kg) stimulated approximately 30 stretches after administration of THC vehicle (*gray bars* in all panels). Figure 1a

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shows that stretching was significantly lower 30 min after administration of 3.2 and 10 mg/kg THC than after THC vehicle. Figure 1b shows that 3.2 mg/kg THC produced a significant reduction in acid-stimulated stretching from 10-300 min with recovery after 24 h. Figure 1c shows that rimonabant dose-dependently blocked the antinociceptive effect of 3.2 mg/kg THC, and significant antagonism was achieved at a dose of 1.0 mg/kg rimonabant.

Effects of THC on ICSS in the absence of a noxious stimulus. Figure 2 shows that THC produced a dose-dependent, time-dependent, and rimonabant-reversible decrease in ICSS in the absence a noxious stimulus. During each test session, a "baseline" frequency-rate curve was determined before testing to permit determination of the Maximum Control Rate (MCR) for that session. Over the course of the entire study this group of rats, the average MCR was 59.7 ± 2.6 stimulations/trial and the average baseline total stimulations was 266.6 ± 56.8. Reinforcement rates during each frequency trial of a session were then expressed as a percentage of that session's MCR, and the average baseline frequency-rate curve for studies with THC is shown in Figure 2a as a *gray line*. Rats generally did not respond at frequencies of 56-89 Hz, and reinforcement rates increased across a frequency range of 89-158 Hz. Maximum reinforcement rates were usually observed at the highest stimulation frequencies. When administered 30 min prior to an ICSS session, THC produced a dose-dependent rightward and downward shift in the ICSS frequency-rate curve (Fig. 2a). Low doses of 0.32 and 1 mg/kg THC had no effect on control ICSS in the absence of the acid noxious stimulus; however, treatment with 3.2 mg/kg THC

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significantly decreased reinforcement rates at a single frequency of 89 Hz, and treatment with 10 mg/kg THC significantly decreased reinforcement rates at frequencies of 89-158 Hz compared to treatment with THC vehicle. THC also produced a dose-dependent and time-dependent decrease in total stimulations (Fig. 2b). Low doses of 0.32 and 1 mg/kg THC had no effect on total stimulations at any time point, but 3.2 mg/kg THC significantly decreased total stimulations at a pretreatment time of 30 min, and 10 mg/kg THC significantly decreased total stimulations at pretreatment times of 30-180 min. THC-induced decreases in ICSS were also blocked by pretreatment with rimonabant (Fig. 2c). Rimonabant (1 mg/kg) administered 50 min before testing had no effect on ICSS alone, but significantly blocked decreases in ICSS induced by 30 min pretreatment with 3.2 mg/kg THC.

Effects of THC on acid-induced depression of ICSS. Figure 3 shows that the same noxious stimulus used in the stretching assay (IP injection of 1.8% lactic acid in 1 ml/kg) depressed ICSS. Treatment with acid vehicle had little effect on the frequency-rate curve; however, treatment with 1.8% lactic acid depressed ICSS, producing a significant rightward shift in the frequency-rate curve and a decrease in total stimulations delivered across all frequencies. Figure 4 shows that THC failed to produce antinociception in the assay of acid-depressed ICSS. Rather, when administered 30 min before acid treatment, THC produced a further, dose-dependent depression of ICSS. Low doses of 0.32 and 1 mg/kg THC significantly decreased ICSS at a single frequency of 141 Hz, and treatment with 3.2 mg/kg THC significantly decreased ICSS at frequencies of

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126, 141, and 158 Hz compared to treatment with THC vehicle (Fig. 4a). Additionally, treatment with THC (0.32-3.2 mg/kg) or its vehicle 30 min before acid administration significantly decreased total stimulations delivered across all frequencies, and there was a trend for treatment with THC to exacerbate acid-induced decreases in total stimulations, but this trend did not achieve statistical significance (Fig. 4b). A dose of 3.2 mg/kg THC also failed to block acid-induced depression of ICSS when it was administered 180 or 300 min before acid treatment (Fig. 4c). These were times at which this dose of THC significantly decreased acid-stimulated stretching (Fig. 1b) but did not significantly decrease ICSS in the absence of a noxious stimulus (Fig. 2b).

Effects of CP55940 and ketoprofen on acid-stimulated stretching and on ICSS in the absence or presence of acid. Figure 5a-b shows that CP55940, like THC, produced antinociception in the assay of acid-stimulated stretching but not in the assay of acid-depressed ICSS. However, unlike THC, CP55940 was approximately 10-fold more potent to produce antinociception in the assay of acid-stimulated stretching than to decrease control ICSS in the absence of the acid stimulus. In particular, doses of 0.01 and 0.032 mg/kg CP55940 produced significant antinociception in the assay of acid-stimulated stretching (Fig. 5a) while 10-fold higher doses of 0.1-0.32 mg/kg were required to decrease control ICSS (Fig. 5b, *open bars*). Despite this evidence for selective antinociception, CP55940 still failed to block acid-induced depression of ICSS. Rather, CP55940 only exacerbated acid-induced depression of ICSS (Fig. 5b, *closed bars*) at the same doses of 0.1 and 0.32 mg/kg that decreased control

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ICSS (Fig. 5a, *open bars*). Figure 5c-d shows that, in contrast to THC and CP55940, the NSAID ketoprofen produced antinociception in assays of both acid-stimulated stretching and acid-depressed ICSS. Thus, a dose of 1.0 mg/kg ketoprofen significantly reduced acid-stimulated stretching (Fig. 5c). The same dose of 1.0 mg/kg ketoprofen had no effect on control ICSS, but significantly blocked acid-induced depression of ICSS (Fig. 5d).

Effects of chronic THC treatment on acid-stimulated stretching and on ICSS in the absence or presence of acid. Chronic THC treatment produced a dose-dependent tolerance to THC-induced rate-decreasing effects on control ICSS. Specifically, complete tolerance was observed to the rate-decreasing effects of 3.2 mg/kg THC following chronic treatment with 3.2 and 10 mg/kg/day THC but not following 1 mg/kg/day THC (Fig. 6). In the assay of acid-stimulated stretching, chronic THC treatment (3.2 mg/kg/day) produced duration-dependent partial tolerance to THC-induced antinociceptive effects. Partial tolerance to THC-induced antinociceptive effects was observed on day 15 of chronic THC treatment (Fig. 7a). No greater tolerance was produced by an additional 7 days of treatment, and full THC antinociception recovered 2 weeks after termination of chronic THC. In contrast, chronic THC treatment (3.2 mg/kg/day) did not produce antinociception in the assay of acid-depressed ICSS (Fig. 7b) at treatment durations that produced significant antinociception in the assay of acid-stimulated stretching (Fig. 7a) and complete tolerance to THC-induced rate-decreasing effects on control ICSS (Fig. 6). Analysis of frequency-rate curves (*data not shown*) indicated that THC initially exacerbated acid-

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induced depression of ICSS on days 1 and 8 of chronic THC treatment, and that tolerance to this effect developed by days 15 and 22 of chronic THC treatment. No greater tolerance was produced by an additional 7 days of treatment, and THC exacerbation of acid-induced depression of ICSS recovered 2 weeks after termination of chronic THC.

Effects of THC, ketoprofen, and CP55940 on feeding depressed by acid or prefeeding. Lactic acid produced a concentration-dependent decrease in food consumption, and exposing rats to a 60 min prefeeding session before testing also significantly decreased food consumption by approximately the same extent as 1.8% lactic acid (Fig. 8a). Neither THC nor ketoprofen significantly altered food consumption in the absence of acid or prefeeding (Fig. 8b). Ketoprofen but not THC significantly blocked acid-induced depression of feeding (Fig. 8c), and in contrast, THC but not ketoprofen significantly attenuated prefeeding-induced depression of feeding (Fig. 8d). CP55940 did not produce significant effects on acid- (Fig. 8e) or prefeeding- (Fig. 8f) induced depression of feeding, although an intermediate dose of 0.01 mg/kg CP55940 did more than double mean food consumption after prefeeding. Higher CP55940 doses were not tested in the feeding assays because they significantly decreased both control and acid-depressed ICSS (Fig. 5b).

DISCUSSION

The purpose of this study was to assess effects of the cannabinoid receptor agonists THC and CP55940 in assays of pain-stimulated and pain-

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depressed behavior in rats. There were four main findings. First, in agreement with the large literature on antinociceptive effects of cannabinoid agonists in assays of pain-stimulated behavior (Rice, 2006; Karst et al., 2010), THC and CP55940 dose-dependently decreased acid-stimulated stretching. Second, THC and CP55940 also decreased control ICSS in the absence of the acid noxious stimulus; however, depression of control ICSS was shorter in duration (THC) or occurred at lower doses (CP55940) than depression of acid-stimulated stretching. Furthermore, chronic administration of THC produced complete tolerance to THC-induced depression of control ICSS and only produced partial tolerance to THC-induced antinociception in the assay of acid-stimulated stretching. These findings suggest that nonselective behavioral depression may have contributed to, but could not account entirely for, cannabinoid antinociception in the assay of acid-stimulated stretching. Third, despite evidence for antinociception in the assay of acid-stimulated stretching, both acute and chronic THC and acute CP55940 failed to produce antinociception in the assay of acid-depressed ICSS. Lastly, THC and CP55940 also failed to produce antinociception in the assay of acid-depressed feeding. Collectively, these findings demonstrate that although cannabinoid agonists are effective to produce antinociception in assays of pain-stimulated behavior, they are ineffective in assays of acute pain-depressed behavior. The effects of THC and CP55940 contrast with the antinociceptive efficacy of clinically effective analgesics such as ketoprofen (Negus et al., 2011, present study) and morphine (Negus et al., 2006; Pereira Do Carmo et al., 2009a) in these assays of pain-depressed behavior.

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Moreover, the lack of cannabinoid efficacy in these assays of acute pain-depressed behavior in rats agrees with the general lack of efficacy of cannabinoids in treating acute pain in humans (Raft et al., 1977; Rice, 2006; Karst et al., 2010; Kraft, 2012). Taken together, these results do not support the use of cannabinoid agonists to treat the behavioral depressant effects of acute pain and further suggest that preclinical assays of pain-depressed behavior may be useful during cannabinoid drug development for predicting clinical drug effects on pain in humans.

Cannabinoid agonist effects on pain-stimulated behavior. In assays of pain-stimulated behavior, delivery of a noxious stimulus increases the rate or intensity of the target behavior, and drug-induced antinociception is inferred from drug-induced decreases in the target behavior (Negus et al., 2010a). In the present study, acid stimulated a stretching response in rats, and THC and CP55940 produced antinociception insofar as they decreased acid-stimulated stretching. The antinociceptive effects of THC and CP55940 in the present study agree with a large literature showing that cannabinoid agonists produce antinociception in nearly all assays of pain-stimulated behavior (for recent reviews, see Rice, 2006; Karst et al., 2010). For example, previous studies in rodents have shown that cannabinoid agonists decreased stretching elicited by IP acid administration (Sofia et al., 1975; Anikwue et al., 2002; Booker et al., 2009), first and second phases of nociceptive behavior elicited by intraplantar formalin injection (Finn et al., 2004; Khodayar et al., 2006), tail-flick/paw-withdrawal responses elicited by noxious heat (Lichtman and Martin, 1991; De

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Vry et al., 2004; Wiley et al., 2007), and hypersensitive withdrawal responses elicited by thermal/mechanical stimuli in inflammatory or neuropathic pain models (Cheng and Hitchcock, 2007; Elikkottil et al., 2009; Sain et al., 2009). As in the present study, cannabinoid antinociception is often shown to be dose and/or time dependent, and sensitivity to rimonabant antagonism or genetic knockout of cannabinoid 1 receptors has been interpreted as evidence of cannabinoid 1 receptor mediation (Monory et al., 2007; Booker et al., 2009). It is generally appreciated that nonselective behavioral depression may confound measures of cannabinoid antinociception in assays of pain-stimulated behavior (De Vry et al., 2004; Finn et al., 2004), and in the present study, THC and CP55940 produced evidence of nonselective behavioral depression insofar as they decreased ICSS in the absence of pain. However, THC-induced depression of acid-stimulated stretching was longer lasting than THC-induced depression of control ICSS. Furthermore, chronic THC administration produced complete tolerance to THC-induced depression of control ICSS, while only partial tolerance developed to THC-induced depression of acid-stimulated stretching. Lastly, CP55940 produced antinociception in the assay of acid-stimulated stretching at doses that produced no effect on control ICSS. These findings provide evidence for a selective antinociceptive effect of cannabinoids in assays of pain-stimulated behavior. Similarly, other studies of pain-stimulated behavior on THC and CP55940 have found comparable dose selectivity for antinociception versus nonselective behavioral depression (Fox et al., 2001; Booker et al., 2009). Overall, the robust and reliable antinociceptive effects of cannabinoid agonists in

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preclinical assays of pain-stimulated behavior have encouraged development of cannabinoids as candidate analgesics.

Cannabinoid agonist effects on pain-depressed behavior. In assays of pain-depressed behavior, delivery of a noxious stimulus decreases the rate or intensity of the target behavior, and drug-induced antinociception is inferred from drug-induced increases in the target behavior (Negus et al., 2010a). In the present study, acid-induced depression of ICSS and of feeding served as assays of pain-depressed behavior, and in these assays, THC and CP55940 failed to produce antinociception. Rather, THC and CP55940 only exacerbated acid-induced depression of ICSS and were ineffective in the assay of acid-depressed-feeding. This lack of cannabinoid antinociception cannot be attributed to a lack of assay sensitivity. In the present study and in a previous study (Negus et al., 2011), the NSAID ketoprofen blocked acid-stimulated stretching and acid-induced depression of ICSS and feeding, and these data agree with the clinical efficacy of ketoprofen for treatment of acute pain in animals (Flecknell, 2009) and humans (Sarzi-Puttini et al., 2010). Similarly, the mu opioid receptor agonist and clinically effective analgesic morphine also blocked acid-stimulated stretching and acid-induced depression of ICSS in rats (Pereira Do Carmo et al., 2009a; Negus et al., 2010b) and acid-induced depression of feeding in mice (Stevenson et al., 2006). Both NSAID and mu-opioid analgesics have also been shown to block other examples of pain-depressed behavior including acid-induced depression of locomotion and wheel running in mice (Stevenson et al., 2009; Miller et al., 2011), laparotomy-induced depression of locomotion and food-

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maintained operant responding in rats (Martin et al., 2007), and depression of locomotion and wheel running induced by bilateral inflammation of the knee joints by complete Freund's adjuvant in rats (Matson et al., 2007; Cobos et al., 2012). Taken together, these results suggest that cannabinoid agonist effects on pain-depressed behavior are opposite to those produced by clinically effective NSAID and opioid analgesics.

Determinants of the poor efficacy of cannabinoids in assays of pain-depressed behavior remain to be understood. However, three points warrant mention. First, cannabinoids can produce general behavioral depressant effects manifested in this study as decreases in control ICSS, and such general behavioral depressant effects could obscure expression of antinociception in assays of pain-depressed behavior (Negus et al., 2010a). However, several findings argue against a major influence of this factor. For example, THC/CP55940 failed to block acid-induced depression of ICSS even at times/doses that did not decrease control ICSS but did block acid-stimulated stretching. Moreover, in contrast to previous results with a delta opioid receptor agonist (Negus et al., 2012), chronic administration of THC did not unmask antinociceptive effects of THC in the assay of acid-depressed ICSS, despite producing complete tolerance to THC-induced rate-decreasing effects on control ICSS and only partial tolerance to THC-induced antinociception in the assay of acid-stimulated stretching. Lastly, THC failed to block pain-related depression of feeding at a dose that did attenuate satiation-related depression of feeding in this study and that stimulated feeding by rats in other studies (Williams et al., 1998;

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Jarbe and DiPatrizio, 2005). Consequently, just as nonselective behavioral depression could not account entirely for the apparent *presence* of cannabinoid antinociception in the assay of pain-stimulated behavior, it also cannot account entirely for the *absence* of cannabinoid antinociception in the assays of pain-depressed behavior.

Second, the neural circuits that mediate acid-induced stimulation of stretching and depression of ICSS are incompletely mapped but may be dissociable (Willis, 2009). For example, noxious stimuli activate both serial and parallel spinal and supraspinal pathways, and different neural circuits have been associated with different components of pain (e.g. sensory vs. affective components of pain) (Price, 2002; Borsook and Becerra, 2011). Results of the present study suggest that THC and other cannabinoids may be more effective in modulating neural circuits that mediate acid-induced stimulation of stretching than those mediating acid-induced depression of ICSS.

Lastly, the present study evaluated effects of systemic cannabinoid administration on pain-related behaviors produced by an acute chemical noxious stimulus delivered to the abdominal cavity, and poor cannabinoid antinociception may be related to these or other experimental variables. Future research would be required to assess the degree to which other routes of administration (e.g. local treatment of inflamed tissue) or other cannabinoid agonists (e.g. agonists selective for cannabinoid 2 receptors) might produce antinociception in assays of other behaviors depressed by more chronic pain states, by other modalities of noxious stimuli, or by noxious stimulation of other parts of the body. However, the

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present results demonstrate the potential for diametrically opposite effects of cannabinoid receptor agonists on pain-stimulated and pain-depressed behaviors elicited by the same noxious stimulus. Moreover, these results distinguish these cannabinoids from clinically effective analgesics and suggest that cannabinoid antinociception in assays of acute pain-stimulated behavior cannot be attributed to a simple and selective blockade of sensitivity to noxious stimuli.

Abuse-related effects of cannabinoids. In control experiments for this study, cannabinoid effects on ICSS were evaluated in the absence of the acid noxious stimulus. Drug-induced facilitation of ICSS under these conditions is often interpreted as an abuse-related effect (Carlezon and Chartoff, 2007), but THC and CP55940 produced only decreases in ICSS. These results are consistent with previous studies showing only rate-decreasing effects of cannabinoids on ICSS (Vlachou et al., 2005, Vlachou et al., 2007), although other studies have found weak facilitation of ICSS by THC under certain conditions (Gardner et al., 1988; Panagis et al., 2008). Taken together, these findings suggest that THC and related cannabinoids often fail to produce abuse-related facilitation of ICSS under conditions that are sensitive to facilitation by other classes of abused drugs.

Implications for preclinical strategies of drug development. Preclinical assays of pain and analgesia play a critical role in analgesic drug development, but there is a growing appreciation that drug effects in conventional preclinical assays of pain-stimulated behavior are often poor predictors of clinical analgesic efficacy in humans (Blackburn-Munro, 2004; Negus et al., 2006;

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Whiteside et al., 2008; Mogil, 2009). Results with THC and CP55940 have illustrated this discordance insofar as cannabinoid agonists produce robust and reliable antinociception in most assays of acute pain-stimulated behavior but little or no analgesia against acute pain in humans (Rice, 2006; Karst et al., 2010; Kraft, 2012). For example, oral delivery of THC or other cannabinoids lacked analgesic efficacy or exacerbated pain in most well-controlled clinical studies of postoperative or acute experimental pain (Raft et al., 1977; Buggy et al., 2003; Naef et al., 2003; Beaulieu, 2006; Kraft et al., 2008, Klooker et al., 2011); for the lone exception, see (Campbell et al., 2001). Similarly, smoked marijuana at doses up to those producing untoward motor/cognitive/subjective effects produced little or no change in sensitivity to acute thermal, mechanical, or chemical noxious stimuli in clinical laboratory studies, and as with oral cannabinoids, pain ratings were sometimes worsened by smoked cannabis (Greenwald and Stitzer, 2000; Wallace et al., 2007). The present study suggests that preclinical assays of pain-depressed behavior may yield results that are more predictive of clinical cannabinoid effects than conventional assays of pain-stimulated behavior. As such, this study supports the utility of assays of pain-depressed behavior in development of cannabinoid analgesics. It will be of particular interest for future studies to assess the efficacy of cannabinoids in assays of chronic pain-depressed behavior, as nearly all clinical evidence for cannabinoid analgesia in humans has been under conditions of chronic pain (Rice, 2006; Karst et al., 2010; Kraft, 2012).

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Kwilasz and Negus

Conducted experiments: Kwilasz

Performed data analysis: Kwilasz and Negus

Wrote or contributed to writing of manuscript: Kwilasz and Negus

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FOOTNOTES

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FIGURE LEGENDS

Figure 1. Δ9-tetrahydrocannabinol (THC) produced dose-dependent, time-dependent, and rimonabant-reversible blockade of lactic acid-stimulated stretching. The left panel *(a)* shows effects of THC (0.1-10 mg/kg) or its vehicle administered 30 min before acid treatment. Abscissa: dose THC in milligrams per kilogram. Ordinates (all panels): number of stretches observed during a 30 min observation period. The center panel *(b)* shows effects of THC (3.2 mg/kg) administered 10 min-24 h before acid treatment. Effects of vehicle administered 30 min before acid treatment are included for comparison. Abscissa: time following THC or vehicle administration. The right panel *(c)* shows the effects of 50 min pretreatment with rimonabant (0.01-1.0 mg/kg) or its vehicle and 30 min pretreatment with THC (3.2 mg/kg) before acid treatment. Effects of rimonabant vehicle + THC vehicle + acid are included for comparison. Abscissa: dose rimonabant in milligrams per kilogram. One-way ANOVA indicated significant main effects of THC treatment in panel *a* [$F(5,25)=6.63$; $p<0.001$], time in panel *b* [$F(5,25)=7.65$; $p<0.001$], and rimonabant dose in panel *c* [$F(4,20)=11.21$; $p<0.001$]. *Asterisks* (*) indicate significantly different from vehicle + acid in all panels or *dollar signs* ($) indicate significantly different from 30 min pretreatment with THC (3.2 mg/kg) in panel *b* and from THC (3.2 mg/kg) + rimonabant vehicle in panel *c* as determined by Newman-Keuls post hoc test, $p < 0.05$. All *bars* show mean ± SEM in six rats.

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Figure 2. Δ9-tetrahydrocannabinol (THC) produced dose-dependent, time-dependent, and rimonabant-reversible depression of intracranial self-stimulation (ICSS) in the absence of a noxious stimulus. The left panel *(a)* shows ICSS frequency-rate curves determined 30 min after treatment with THC (0.32-10 mg/kg) or its vehicle. Abscissa: frequency of electrical brain stimulation in hertz (log scale). Ordinate: percent maximum control response rate (%MCR). The average baseline ICSS frequency-rate curve for the entire study in this group of rats is shown by the *gray line* for comparison, but these data were not included in statistical analysis. Two-way ANOVA indicated a significant main effect of THC treatment [$F(4,20)=11.78$; $p<0.001$], a significant main effect of frequency [$F(9,45)=19.31$; $p<0.001$], and a significant frequency × treatment interaction [$F(36,180)=4.77$; $p<0.001$]. *Filled symbols* indicate frequencies at which reinforcement rates after THC treatment were significantly lower than rates after THC vehicle treatment as determined by Holm-Sidak post hoc test, $p < 0.05$. All data show mean ± SEM in six rats. The center panel *(b)* shows the total number of stimulations per component expressed as a percent of baseline stimulations per component following treatment with THC (0.32-10.0 mg/kg) or its vehicle at various pretreatment times. Abscissa: time following THC or vehicle administration. Ordinate: percent baseline total number of stimulations per component. Two-way ANOVA indicated a significant main effect of THC treatment [$F(4,20)=12.35$; $p<0.001$] and a significant treatment × time interaction [$F(12,60)=5.93$; $p<0.001$]. *Filled symbols* indicate significantly lower than vehicle treatment at indicated time as determined by Holm-Sidak post hoc test, $p < 0.05$.

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All data show mean ± SEM in six rats. The right panel *(c)* shows the total number of stimulations per component expressed as a percent of baseline stimulations per component following 50 min pretreatment with rimonabant (1 mg/kg) or its vehicle and 30 min pretreatment with THC (3.2 mg/kg) or its vehicle. Abscissa: dose rimonabant in milligrams per kilogram. Ordinate: percent baseline total number of stimulations per component. One-way ANOVA indicated a significant main effect of treatment [$F(3,12)=17.04$; $p<0.001$]. The a*sterisk* (*) indicates significantly different from rimonabant vehicle + THC vehicle and *dollar signs* ($) indicate significantly different from rimonabant vehicle + THC (3.2 mg/kg) as determined by Newman-Keuls post hoc test, $p < 0.05$. All *bars* show mean ± SEM in five rats.

Figure 3. Lactic acid depresses intracranial self-stimulation (ICSS). The left panel *(a)* shows ICSS frequency-rate curves determined after treatment with THC vehicle 30 min before lactic acid vehicle or 1.8% lactic acid administration. Abscissa: frequency of electrical brain stimulation in hertz (log scale). Ordinate: percent maximum control response rate (%MCR). The average baseline ICSS frequency-rate curve for the entire study in this group of rats is shown by the *gray line* for comparison, but these data were not included in statistical analysis. Two-way ANOVA indicated a significant main effect of frequency [$F(9,36)=23.92$; $p<0.001$] and a significant frequency × treatment interaction [$F(9,36)=2.40$; $p=0.030$]. *Filled symbols* indicate frequencies at which reinforcement rates after acid treatment were significantly lower than rates after acid vehicle treatment as

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determined by Holm-Sidak post hoc test, $p < 0.05$. The right panel *(b)* shows the total number of stimulations per component expressed as a percent of baseline stimulations per component determined after treatment with THC vehicle 30 min before lactic acid vehicle or 1.8% lactic acid administration. Abscissa: Lactic acid concentration. Ordinate: percent baseline total number of stimulations per component. The *asterisk* (*) indicates 1.8% lactic acid significantly depressed ICSS compared to 0% lactic acid (i.e. lactic acid vehicle) as determined by paired *t* test [t(4)=6.95; p=0.002]. All *bars* show mean ± SEM in five rats.

Figure 4. Δ9-tetrahydrocannabinol (THC) exacerbates lactic acid-induced depression of intracranial self-stimulation (ICSS). The left panel *(a)* shows ICSS frequency-rate curves determined after treatment with THC (0.32-3.2 mg/kg) or its vehicle 30 min before acid administration. Abscissa: frequency of electrical brain stimulation in hertz (log scale). Ordinate: percent maximum control response rate (%MCR). The THC vehicle + acid vehicle frequency-rate curve is shown by the *gray line* for comparison, but these data were not included in statistical analysis. Two-way ANOVA indicated a significant main effect of THC treatment [F(3,12)=5.16; p=0.016], a significant main effect of frequency [F(9,36)=6.68; p<0.001], and a significant frequency × treatment interaction [F(27,108)=4.62; p<0.001]. Filled symbols indicate frequencies at which reinforcement rates after THC + acid treatment were significantly lower than after vehicle + acid treatment as determined by Holm-Sidak post hoc test, $p < 0.05$. All data show mean ± SEM in five rats. The center panel *(b)* shows the total number

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of stimulations per component expressed as a percent of baseline stimulations per component after treatment with THC (0.32-3.2 mg/kg) or its vehicle 30 min before acid administration. Abscissa: dose THC in milligrams per kilogram. Ordinate: percent baseline total number of stimulations per component. One-way ANOVA indicated a significant main effect of treatment [$F(4,16)=19.26$; $p<0.001$]. The *asterisks* (*) indicate treatment with THC vehicle + acid or THC + acid significantly depressed ICSS compared to treatment with THC vehicle + acid vehicle as determined by Newman Keul's post hoc test, $p < 0.05$. All bars show mean ± SEM in five rats. The right panel *(c)* shows the total number of stimulations per component expressed as a percent of baseline stimulations per component following treatment with THC vehicle 30 min before acid vehicle or acid administration, or THC (3.2 mg/kg) 30-300 min before acid administration. Abscissa: time following THC or vehicle administration. Ordinate: percent baseline total number of stimulations per component. One-way ANOVA indicated a significant main effect of treatment [$F(4,12)=14.43$; $p<0.001$]. The *asterisks* (*) indicate treatment with THC vehicle + acid or THC (3.2 mg/kg) + acid significantly depressed ICSS compared to treatment with THC vehicle + acid vehicle as determined by Newman Keul's post hoc test, $p < 0.05$. All bars show mean ± SEM in four rats.

Figure 5. Effects of CP55940 (top panels) and ketoprofen (bottom panels) on lactic acid-stimulated stretching and lactic acid-induced depression of intracranial self-stimulation (ICSS). Abscissae (all panels): drug dose in mg/kg. Left

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ordinates: number of stretches observed during 30 min observation periods. Right ordinates: percent baseline total number of stimulations per component. CP55940 dose-dependently blocked acid-stimulated stretching (panel a, [$F(4,16)=38.80$; $p<0.001$]) as indicated by one-way ANOVA. Two-way ANOVA on ICSS data in the presence and absence of acid treatment indicated a significant main effect of CP55940 dose [$F(4,16)=34.44$; $p<0.01$], a significant main effect of acid treatment ([$F(1,4)=15.69$; $p=0.017$], but no significant interaction (panel b). Ketoprofen blocked acid-induced stimulation of stretching (panel *c*, [$t(3)=4.43$; $p=0.021$]) as indicated by t-test. Two-way ANOVA on ICSS data in the presence and absence of acid treatment indicated a significant main effect of ketoprofen dose ([$F(1,3)=16.95$ $p=0.026$], a significant main effect of acid treatment ([$F(1,3)=13.71$ $p=0.034$], but no significant interaction (panel d). In panels *a* and *c*, *asterisks* (*) indicate significant difference from a “0” drug dose (i.e. vehicle) + lactic acid as determined by one-way ANOVA followed by Dunnett’s post hoc test (CP55940) or by t-test (ketoprofen), $p < 0.05$. In panels *b* and *d*, a*sterisks* (*) indicate significant difference from a “0” drug dose (i.e. vehicle) + lactic acid vehicle, *dollar signs* (*$*) indicate significant difference from a “0” drug dose + lactic acid, and *number signs* (#) indicate significant depression of ICSS by lactic acid as determined by two-way ANOVA followed by Holm-Sidak post hoc test, $p < 0.05$. All *bars* show mean ± SEM in five rats (CP55940) or four rats (ketoprofen).

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Figure 6. Chronic administration of THC produces tolerance to its rate-decreasing effects on intracranial self-stimulation (ICSS) in the absence of a noxious stimulus. Abscissa: THC challenge dose (mg/kg). Ordinate: percent baseline total number of stimulations per component. Two-way ANOVA indicated a significant main effect of chronic THC dose [$F(3,12)=16.27$; $p<0.001$], a significant main effect of THC challenge dose [$F(3,12)=55.33$; $p<0.001$], and a significant interaction [$F(9,36)=3.58$; $p=0.003$]. *Filled symbols* indicate chronic THC + THC challenge dose combinations after which reinforcement rates were significantly higher than rates after the same THC challenge administered during chronic vehicle, as determined by Holm-Sidak post hoc test, $p < 0.05$. All *bars* show mean ± SEM in five rats.

Figure 7. Chronic administration of THC produces partial tolerance to its antinociceptive effects in the assay of acid-stimulated stretching but does not unmask antinociceptive effects in the assay of acid-depressed intracranial self-stimulation (ICSS). Abscissae (all panels): Day of THC (3.2 mg/kg/day) administration. Left ordinate: number of stretches observed during 30 min observation periods. Right ordinate: percent baseline total number of stimulations per component. Effects of a 2-week washout period following chronic THC administration are also shown for comparison but were not included in the statistical analysis. The left panel (*a*) shows the effects of chronic administration of THC in the assay of acid-stimulated stretching. One-way ANOVA indicated a significant main of THC treatment duration (panel *a*, [$F(4,20)=9.41$; $p<0.001$]).

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Asterisks (*) indicate treatment with THC produced significant antinociception compared to treatment with THC vehicle on days 1, 8, 15, and 22 of chronic THC administration, and *dollar signs* ($) indicate significant tolerance to this antinociceptive effect on day 15 compared to day 1 of chronic THC administration as determined by Newman-Keul's post hoc test, $p < 0.05$. The right panel (*b*) shows the effects of chronic administration of THC in the assay of acid-depressed ICSS. One-way ANOVA indicated a significant main effect of treatment (panel *b*, [$F(5,20)=10.08$; $p<0.001$]). *Asterisks* (*) indicate treatment with acid significantly depressed ICSS compared to treatment with lactic acid vehicle as determined by Newman-Keul's post hoc test, $p < 0.05$. Chronic THC administration failed to alter THC effects on acid-induced depression of ICSS. All *bars* show mean ± SEM in six rats (stretching) or five rats (ICSS).

Figure 8. THC and ketoprofen effects on feeding depressed by acid or prefeeding. The upper left panel (*a*) shows the effects of lactic acid vehicle, lactic acid (0.56-1.8%), or a 60 min prefeeding session on feeding. Abscissa: percent acid concentration. Ordinates (all panels): percent body weight food consumed in grams during a 30 min feeding session. One-way ANOVA indicated a significant main effect of treatment [$F(4,20)=16.30$; $p<0.001$]. *Asterisks* (*) indicate lactic acid (1-1.8%) or a 60 min prefeeding session significantly decreased feeding as determined by Dunnett's post hoc test, $p < 0.05$. All *bars* show mean ± SEM in six rats. The upper right panel (*b*) shows effects of THC (0.32-3.2 mg/kg), ketoprofen (1 mg/kg), or vehicle administered 30 min before acid vehicle

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treatment on control feeding. Abscissa: drug dose in milligrams per kilogram. THC and ketoprofen did not significantly alter feeding in the absence of lactic acid or a 60 min prefeeding session. All *bars* show mean ± SEM in seven rats. The middle left panel (*c*) shows the effects of THC (0.32-1 mg/kg), ketoprofen (1 mg/kg), or vehicle administered 30 min before 1.8% lactic acid. Abscissa: drug dose in milligrams per kilogram. One-way ANOVA indicated a significant main effect of treatment [$F(4,24)=10.46$; $p<0.001$]. The *asterisk* (*) indicates ketoprofen significantly blocked acid-induced depression of feeding as determined by Dunnett's post hoc test, $p < 0.05$. All *bars* show mean ± SEM in seven rats. The middle right panel (*d*) shows the effects of THC (0.1-1 mg/kg), ketoprofen (1 mg/kg), or vehicle administered immediately after a 60 min prefeeding session and 30 min before the test session. Abscissa: drug dose in milligrams per kilogram. One-way ANOVA indicated a significant main effect of treatment [$F(4,28)=2.88$; $p=0.041$]. The *asterisk* (*) indicates THC significantly blocked prefeeding-induced depression of feeding as determined by Dunnett's post hoc test, $p < 0.05$. All *bars* show mean ± SEM in eight rats. The bottom left panel (*e*) shows the effects of CP55940 (0.0032-0.032 mg/kg or vehicle) administered 30 min before 1.8% lactic acid. One-way ANOVA indicated a significant main effect of acid treatment [$F(4,28)=19.64$; $p<0.001$]. *Asterisks* (*) indicate lactic acid (1.8%) significantly decreased feeding as determined by Newman-Keuls post hoc test, $p < 0.05$. All *means* ± *SEM* represent eight rats. The bottom right panel (*f*) shows the effects of CP55940 (0.0032-0.032 mg/kg or vehicle) administered immediately after a 60 min prefeeding session and 30 min before the test

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session. One-way ANOVA indicated a significant main effect of prefeeding treatment [$F(4,28)=4.25$; $p=0.008$]. *Asterisks* (*) indicate a 60 min prefeeding session significantly decreased feeding vs. CP55940 vehicle alone as determined by Newman-Keuls post hoc test, $p < 0.05$. CP55940 did not alter feeding under either condition tested. All means ± SEM represent eight rats.

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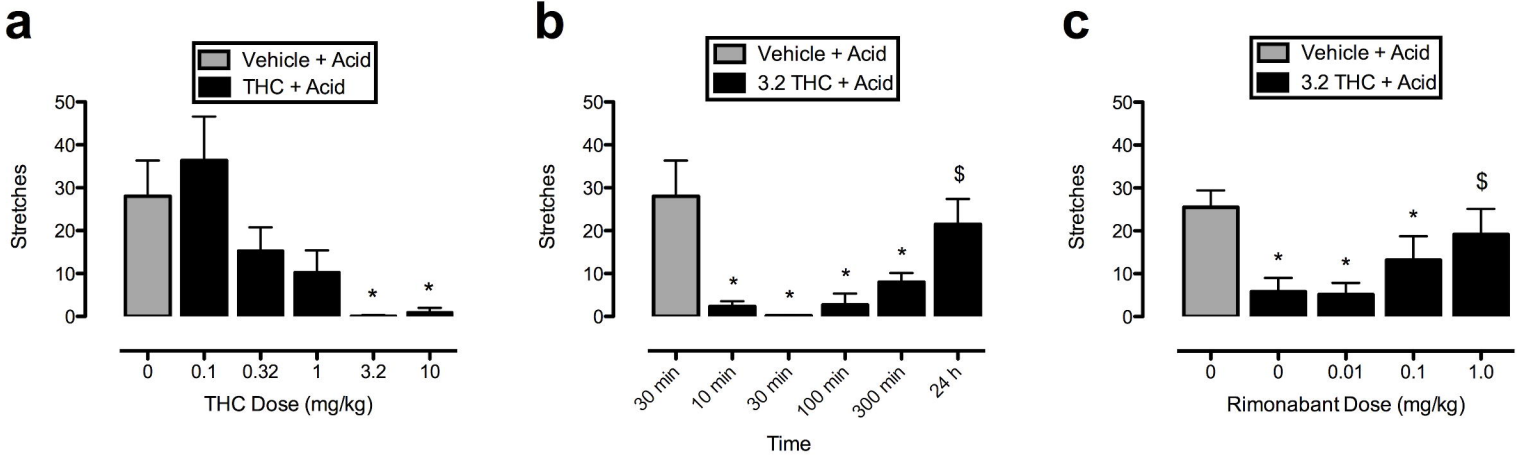
Figure 1


a
Vehicle + Acid
THC + Acid
Stretches
50
40
30
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10
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THC Dose (mg/kg)
b
Vehicle + Acid
3.2 THC + Acid
Stretches
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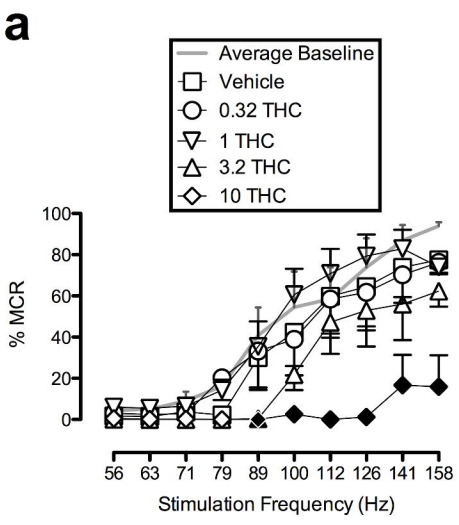
Rimonabant Dose (mg/kg)

Figure 2


a
Average Baseline
Vehicle
0.32 THC
1 THC
3.2 THC
10 THC
% MCR
100
80
60
40
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0
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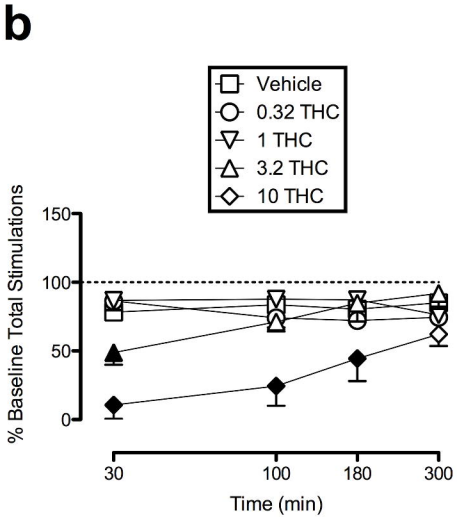
Stimulation Frequency (Hz)


b
Vehicle
0.32 THC
1 THC
3.2 THC
10 THC
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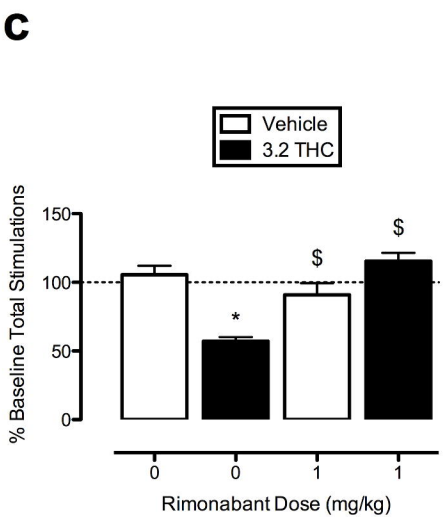
Time (min)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1
1
Rimonabant Dose (mg/kg)

April 19, 2024

Figure 3

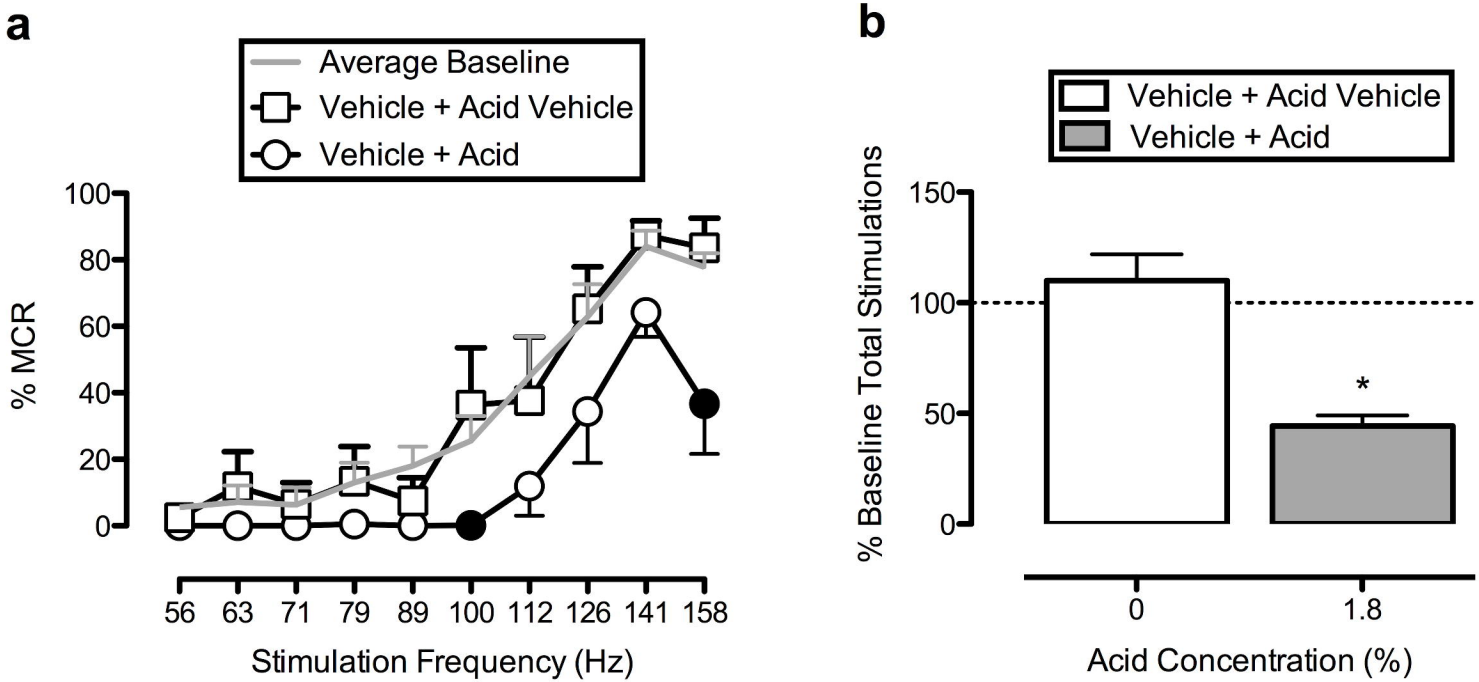


Figure 4

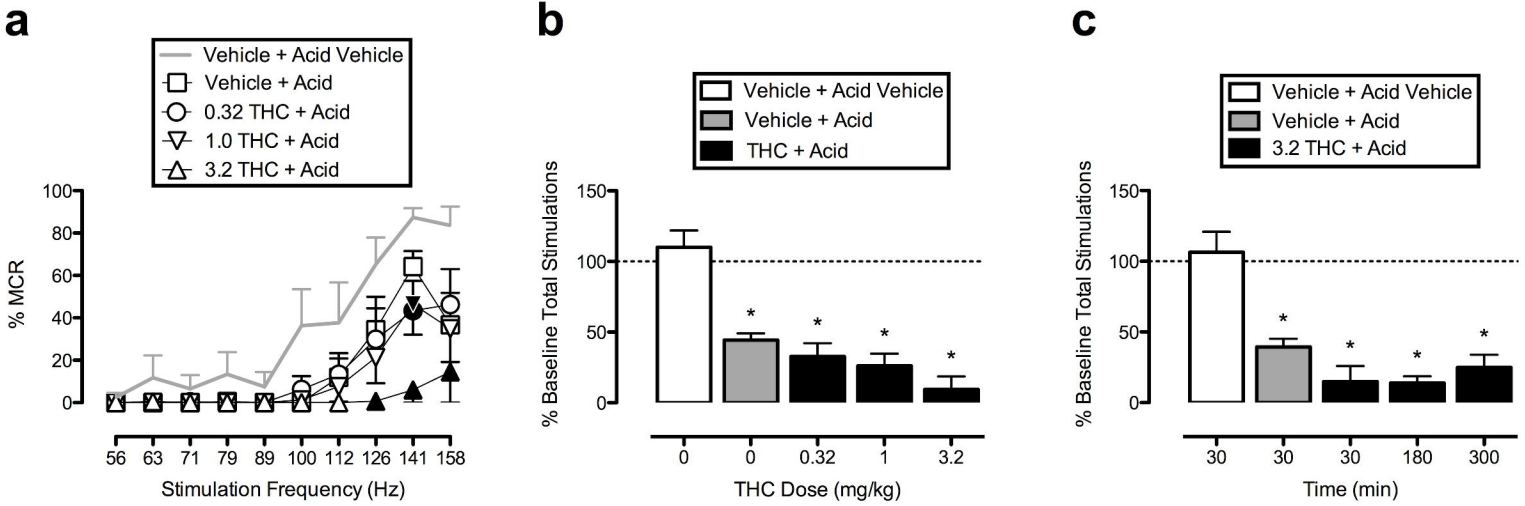

a
Vehicle + Acid Vehicle
Vehicle + Acid
0.32 THC + Acid
1.0 THC + Acid
3.2 THC + Acid
% MCR
100
80
60
40
20
0
56 63 71 79 89 100 112 126 141 158
Stimulation Frequency (Hz)
b
Vehicle + Acid Vehicle
Vehicle + Acid
THC + Acid
% Baseline Total Stimulations
150
100
50
0
0 0 0.32 1 3.2
THC Dose (mg/kg)
c
Vehicle + Acid Vehicle
Vehicle + Acid
3.2 THC + Acid
% Baseline Total Stimulations
150
100
50
0
30 30 30 180 300
Time (min)

Figure 5

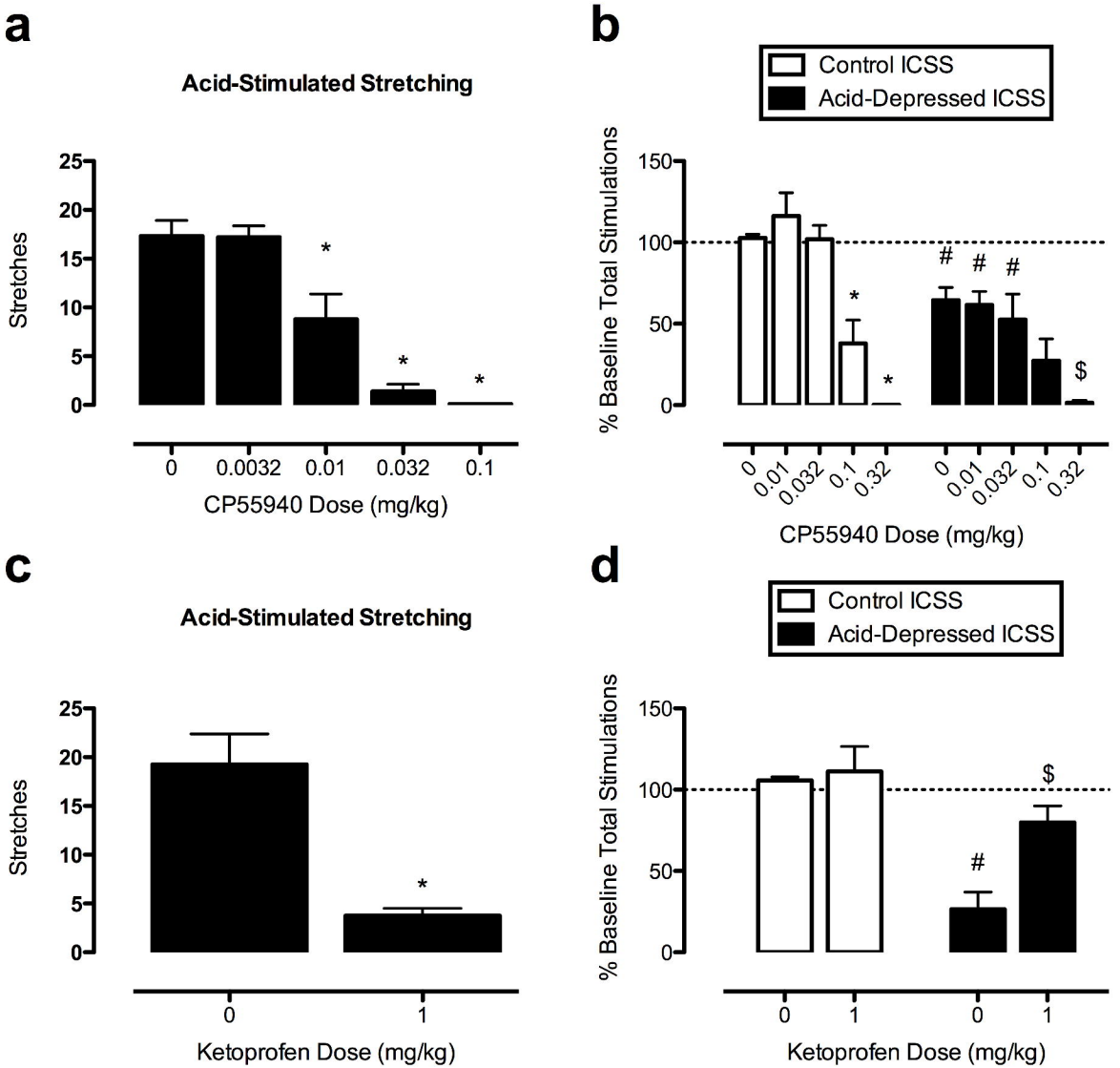

a
Acid-Stimulated Stretching
Stretches
25
20
15
10
5
0
*
*
*
0
0.0032
0.01
0.032
0.1
CP55940 Dose (mg/kg)
b
Control ICSS
Acid-Depressed ICSS
% Baseline Total Stimulations
150
100
50
0
*
*
#
#
#
$
0
0.01
0.032
0.1
0.32
0
0.01
0.032
0.1
0.32
CP55940 Dose (mg/kg)
c
Acid-Stimulated Stretching
Stretches
25
20
15
10
5
0
*
0
1
Ketoprofen Dose (mg/kg)
d
Control ICSS
Acid-Depressed ICSS
% Baseline Total Stimulations
150
100
50
0
#
$
0
1
0
1
Ketoprofen Dose (mg/kg)

Figure 6

a

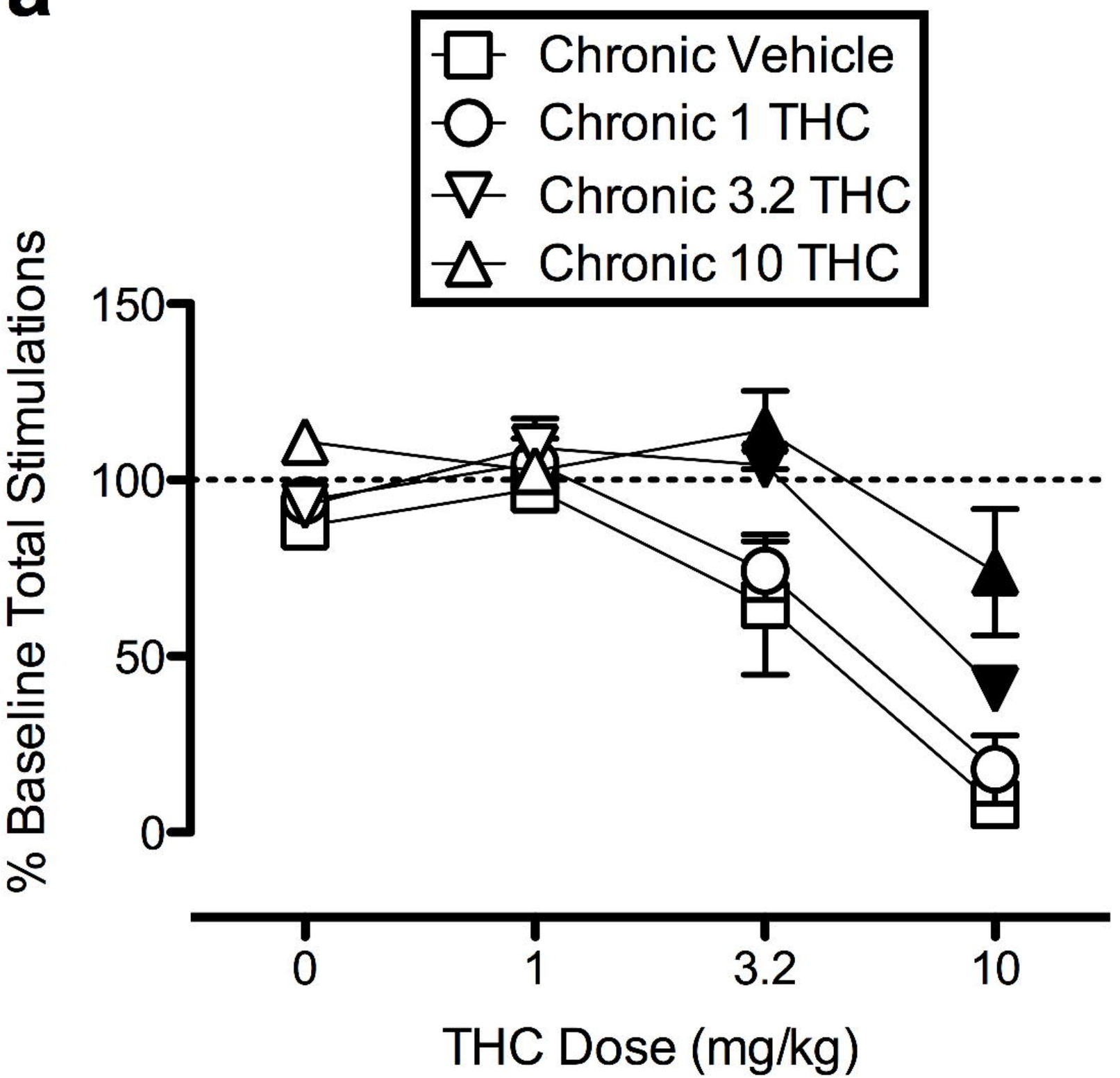

Chronic Vehicle
Chronic 1 THC
Chronic 3.2 THC
Chronic 10 THC
% Baseline Total Stimulations
150
100
50
0
0
1
3.2
10
THC Dose (mg/kg)

Figure 7

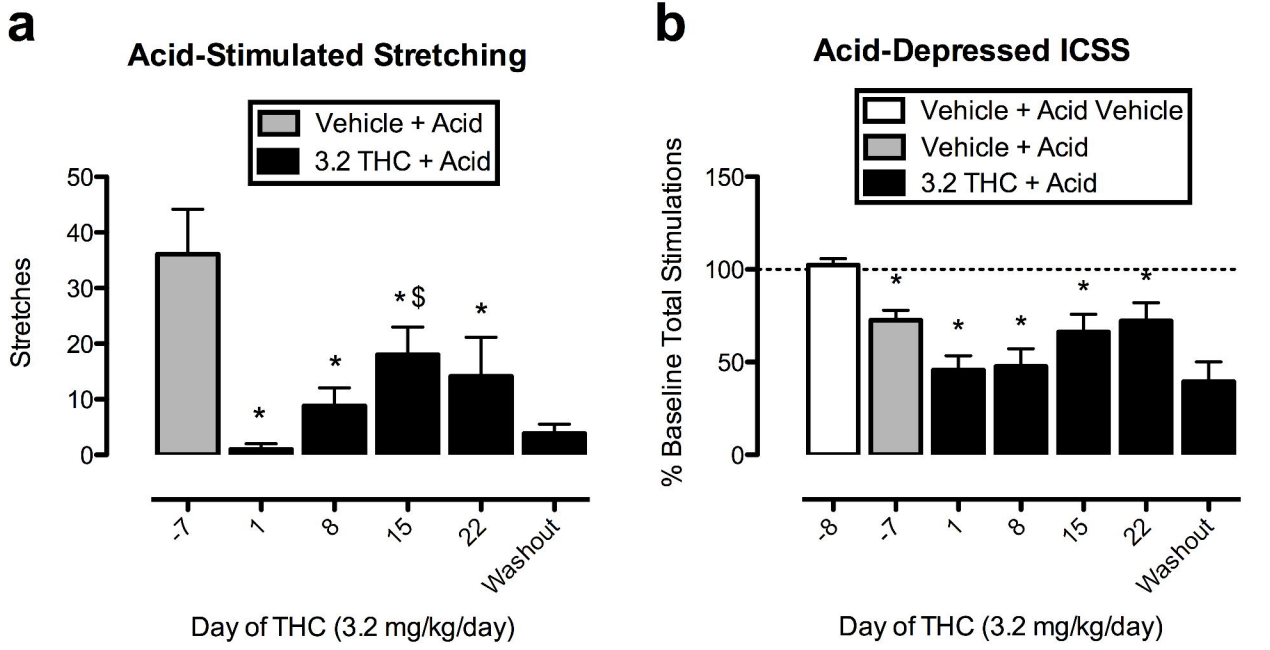
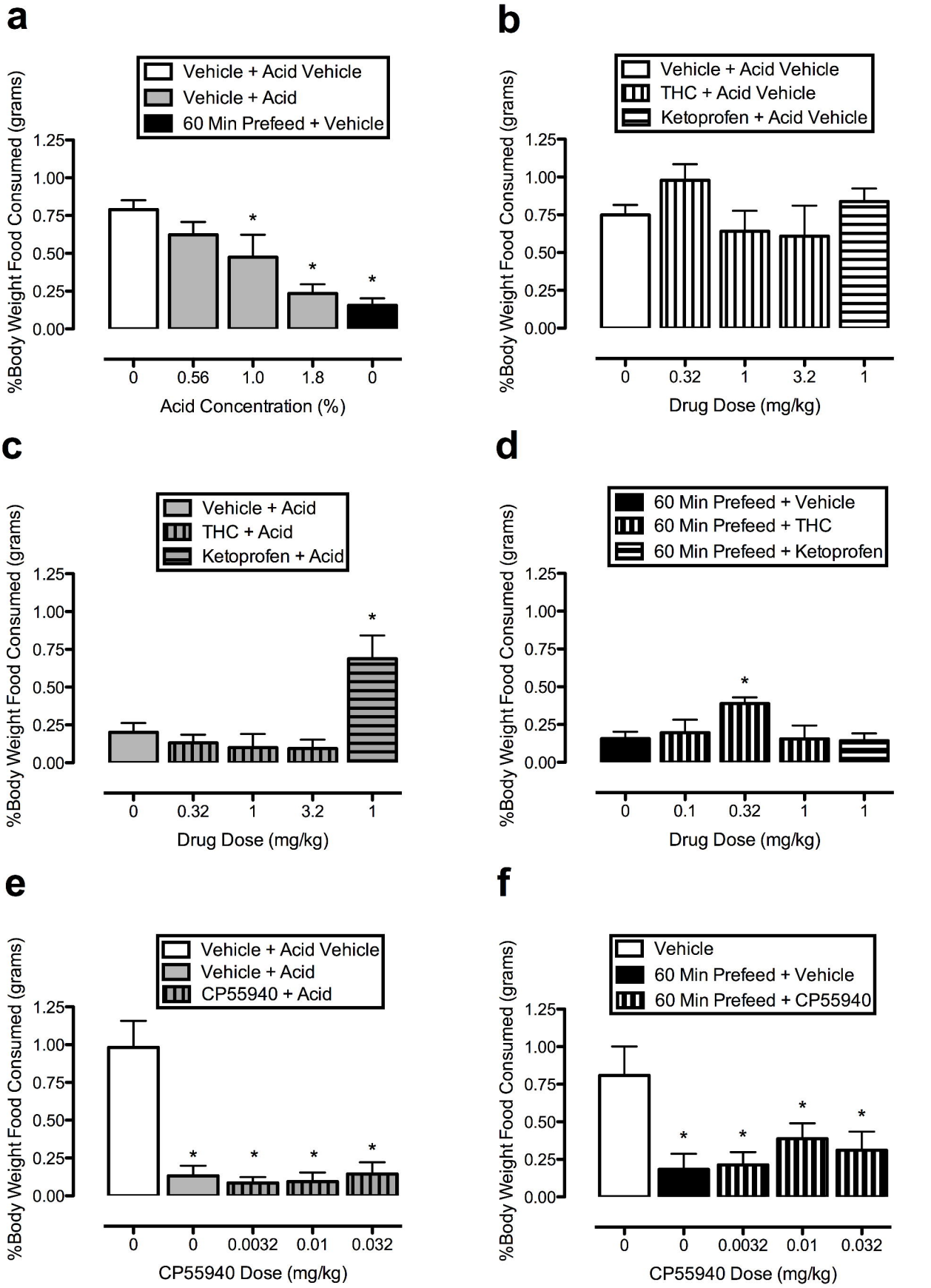

a
Acid-Stimulated Stretching
Vehicle + Acid
3.2 THC + Acid
Stretches
50
40
30
20
10
0
*
*
*$
*
-7
1
8
15
22
Washout
Day of THC (3.2 mg/kg/day)
b
Acid-Depressed ICSS
Vehicle + Acid Vehicle
Vehicle + Acid
3.2 THC + Acid
% Baseline Total Stimulations
150
100
50
0
*
*
*
*
*
-8
-7
1
8
15
22
Washout
Day of THC (3.2 mg/kg/day)

Figure 8


a
Vehicle + Acid Vehicle
Vehicle + Acid
60 Min Prefeed + Vehicle
%Body Weight Food Consumed (grams)
1.25
1.00
0.75
0.50
0.25
0.00
*
*
*
0
0.56
1.0
1.8
0
Acid Concentration (%)
b
Vehicle + Acid Vehicle
THC + Acid Vehicle
Ketoprofen + Acid Vehicle
%Body Weight Food Consumed (grams)
0
0.32
1
3.2
1
Drug Dose (mg/kg)
c
Vehicle + Acid
THC + Acid
Ketoprofen + Acid
%Body Weight Food Consumed (grams)
*
0
0.32
1
3.2
1
Drug Dose (mg/kg)
d
60 Min Prefeed + Vehicle
60 Min Prefeed + THC
60 Min Prefeed + Ketoprofen
%Body Weight Food Consumed (grams)
*
0
0.1
0.32
1
1
Drug Dose (mg/kg)
e
Vehicle + Acid Vehicle
Vehicle + Acid
CP55940 + Acid
%Body Weight Food Consumed (grams)
*
*
*
*
0
0
0.0032
0.01
0.032
CP55940 Dose (mg/kg)
f
Vehicle
60 Min Prefeed + Vehicle
60 Min Prefeed + CP55940
%Body Weight Food Consumed (grams)
*
*
*
*
0
0
0.0032
0.01
0.032
CP55940 Dose (mg/kg)