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Anti-migraine effect of Δ⁹-tetrahydrocannabinol in the female rat

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Abstract

Current anti-migraine treatments have limited efficacy and many side effects. Although anecdotal evidence suggests that marijuana is useful for migraine, this hypothesis has not been tested in a controlled experiment. Thus, the present study tested whether administration of Δ^9-tetrahydrocannabinol (THC) produces anti-migraine effects in the female rat. Microinjection of the TRPA1 agonist allyl isothiocyanate (AITC) onto the dura mater produced migraine-like pain for 3 h as measured by depression of home cage wheel running. Concurrent systemic administration of 0.32 but not 0.1 mg/kg of THC prevented AITC-induced depression of wheel running. However, 0.32 mg/kg was ineffective when administered 90 min after AITC. Administration of a higher dose of THC (1.0 mg/kg) depressed wheel running whether rats were injected with AITC or not. Administration of a CB_1, but not a CB_2, receptor antagonist attenuated the anti-migraine effect of THC. These data suggest that: 1) THC reduces migraine-like pain when administered at the right dose (0.32 mg/kg) and time (immediately after AITC); 2) THC's anti-migraine effect is mediated by CB_1 receptors; and 3) Wheel running is an effective method to assess migraine treatments because only treatments producing antinociception without disruptive side effects will restore normal activity. These findings support anecdotal evidence for the use of cannabinoids as a treatment for migraine in humans and implicate the CB_1 receptor as a therapeutic target for migraine.

Keywords

headache; antinociception; marijuana; wheel running; pain-depressed behaviour

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Declaration of conflicting interests

The authors declare no conflicts of interest.

1. Introduction

Migraine is characterized by severe headache and heightened sensitivity to sensory stimuli that results in depression of normal daily activities. Despite the prevalence and severity of primary headache disorders, treatments for migraine are surprisingly limited. Currently available prophylactic and abortive therapies manage less than 50% of migraine cases due to lack of efficacy or adverse side effects (e.g., nausea, dizziness, drowsiness) (Diener et al., 2015; Stovner et al., 2009). Furthermore, efficacious drugs that are used repeatedly (e.g., triptans, ergots, NSAIDs) can lead to medication-overuse headache, a condition in which headaches transform from an episodic to a chronic and more intense condition (Dodick and Freitag, 2006). Thus, there is a critical need to identify and employ novel anti-migraine agents.

Given the reported therapeutic benefits of cannabinoids such as Δ^9-tetrahydrocannabinol (THC) for a wide range of pain conditions (Chiou et al., 2013; Craft et al., 2013; Karst et al., 2010; Kraft, 2012; Maione et al., 2013; Milstein et al., 1975; Noyes and Baram, 1974; Noyes et al., 1975), it is not surprising that some people use marijuana as a treatment for migraine (el-Mallakh, 1989). A survey investigating reasons for self-medication with cannabis in Germany, Austria, and Switzerland revealed that 10.2% of respondents used it for migraine and headache (Schnelle et al., 1999). Medical marijuana has also been reported to reduce the frequency of migraines (Rhyne et al., 2016). Preclinical research suggests that cannabinoids may modulate migraine pain by inhibiting the activity of A- and C-fiber inputs from the dura mater via activation of cannabinoid type 1 (CB_1) receptors (Akerman et al., 2007). Although these data are promising, we are not aware of any study that systematically examined the antinociceptive efficacy of THC in an animal model of migraine.

Migraine is difficult to study in laboratory animals because pain occurs in the absence of tissue injury (Strassman and Burstein, 2013). Mechanical allodynia has served as the primary dependent measure for headache in laboratory animals, but allodynia is a marker of migraine progression (Burstein et al., 2004; Harris et al., 2017; Louter et al., 2013) rather than headache per se, and allodynia is rarely assessed clinically (Mathew et al., 2004). Moreover, mechanical allodynia may outlast the headache and occur during interictal periods (Aguggia, 2012). In contrast to allodynia, the reduction in routine physical activity caused by migraine is a diagnostic criterion that may be more clinically relevant, and it is easy to assess in laboratory animals. Several studies have used depression of activity to assess pain resulting from headache in humans (Mannix et al., 2016) and rodents (Melo-Carrillo and Lopez-Avila, 2013). However, the limited observation periods in these studies, often 60 min or less, make it difficult to quantify the duration and magnitude of migraine. Home cage wheel running is particularly advantageous because it is a voluntary behavior that shows diurnal rhythms that can be continuously and objectively quantified in the rat in a stress-free environment. We have previously shown that activation of dural afferents using the TRPA1 agonist allyl isothiocyanate (AITC) depresses home cage wheel running and this depression is prevented by the anti-migraine treatment sumatriptan (Kandasamy et al., 2017b). Thus, home cage wheel running provides an objective, sensitive, and clinically relevant measure of migraine pain in rats. The present study will test the hypothesis that THC will prevent migraine-depressed wheel running in a CB_1-dependent manner.

2. Materials and Methods

2.1 Subjects

Data were collected from 48 adult female Sprague-Dawley rats bred at Washington State University Vancouver (Vancouver, WA, USA). Female rats were selected because migraine is much more common in women than men (Vetvik and MacGregor, 2016). All rats were 50–70 days old at the start of the study and randomly assigned to treatment groups. Within-subjects designs were used to reduce the number of animals needed, as outlined in the Guide for the Care and Use of Laboratory Animals(National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). All procedures were approved by the Washington State University Institutional Animal Care and Use Committee and conducted in accordance with the International Association for the Study of Pain's Policies on the Use of Animals in Research.

2.2 Surgery

Prior to surgery, rats were housed in pairs in a 22–24 °C colony room on a 12/12-h light/dark cycle (lights off at 1700 h). Animals were anesthetized with pentobarbital (50 mg/kg, i.p.) and implanted with a guide cannula (18 gauge; 4 mm long) aimed above the dura mater (AP: +1.0 mm; ML: +1.0 mm; DV: 0.8 mm, from lambda). Loctite® super glue was used to form a tight seal around the guide cannula, and dental cement anchored the guide cannula to two screws in the skull. Rats were maintained under a heat lamp until awake. Following surgery, each rat was housed individually in an extra tall cage (36 × 24 × 40 cm) with a running wheel. The cage was located in a sound-attenuating booth (2.1 × 2.2 m; Industrial Acoustics Company, Inc., Bronx, NY, USA) for the remainder of the experiment to limit the influence of outside stimuli. Food and water were available *ad libitum*.

2.3 Running wheel

A Kaytee Run-Around Giant Exercise Wheel (Kaytee Products, Inc., Chilton, WI, USA) with a diameter of 27.9 cm was suspended from the top of the rat's home cage. The floor of the cage was covered with cellulose bedding (BioFresh™, Ferndale, WA, USA). A thin aluminum plate (0.8 mm × 5.08 cm × 3.81 cm; K&S Precision Metals, Chicago, IL, USA) was attached to one spoke of the running wheel to interrupt a photobeam projecting across the cage with each rotation. The beam was set 18 cm above the floor of the cage so that only the rotation of the wheel, not the normal activity of the rat, would interrupt the beam. The number of wheel revolutions was summed over 5 min bins for 23 h each day using Multi-Varimex software (Columbus Instruments, Columbus, OH, USA). Recording began at 1700 h, the onset of the dark phase of the light cycle when rats are most active. A full description of the running wheel with video is available in our previous publication (Kandasamy et al., 2016).

Rats were allowed unrestricted access to the wheel for 23 h/day for 8 days following surgery. The number of wheel revolutions that occurred during the 23 h prior to the first dural injection of AITC was used as the baseline activity. Rats that ran less than 400 revolutions on the baseline day (Kandasamy et al., 2016) were not included in further testing (n = 5 of 53).

2.4 Drugs

Allyl isothiocyanate (AITC; Sigma-Aldrich, Inc., St. Louis, MO, USA) was mixed in mineral oil at a concentration of 10% and injected into the periosteal space in a volume of 10 μl. Microinjection of 10% AITC onto the dura has previously been shown to mimic migraine-like pain in rodents (Edelmayer et al., 2012; Kandasamy et al., 2017b). Δ^9-tetrahydrocannabinol (Sigma-Aldrich, Inc., St. Louis, MO, USA) was dissolved in vehicle (1:1:18; ethanol:cremophor:saline) and injected intraperitoneally at doses of 0.1, 0.32, and 1.0 mg/kg in a volume of 1 ml/kg. The CB_1 receptor antagonist SR141716A (1.0 mg/kg) and the CB_2 receptor antagonist SR144528 (3.2 mg/kg) (Tocris Bioscience, Minneapolis, MN, USA) were dissolved in the same vehicle as THC and injected intraperitoneally in a volume of 1 ml/kg. These drugs are highly selective for their target receptor (Rinaldi-Carmona et al., 1995; 1998) and the doses used are known to block the antinociceptive effects of systemically administered THC in female rats(Craft et al., 2012).

2.5 Determination of estrous cycle

Vaginal lavage samples were collected from all females prior to dural injections. Proestrus was characterized by a predominance (>75%) of nucleated epithelial cells in the sample; estrus by dense sheets of cornified epithelial cells; and diestrus by scattered, nucleated and cornified epithelial cells, and leukocytes.

2.6 Experiment 1: THC administration during migraine onset

The objective of this experiment was to determine whether THC administration prevents AITC-induced depression of wheel running. Following baseline testing on Day 8, the rat was injected with 10 μl of 10% AITC or mineral oil onto the dura mater using an injection cannula inserted into the guide cannula. The rat was injected i.p. with either vehicle or THC (0.1, 0.32, 1.0 mg/kg) immediately following AITC administration. All injections were completed by 1650 h so the rats could be returned to their home cages prior to the start of the 23 h of recording beginning at 1700 h. This procedure was repeated every other day with the THC doses and vehicle administered in a counterbalanced order. No rat was injected with AITC more than three times and no rat received more than two THC doses. We have previously demonstrated that repeated injections of AITC using this dose and procedure did not change the magnitude nor duration of depressed wheel running (Kandasamy et al., 2017b). Rats were euthanized 48 h after the last injection.

2.7 Experiment 2: THC administration 90 min after migraine onset

The objective of this experiment was to determine whether THC administration 90 min after AITC injection reverses depression of wheel running. Surgical implantation of the cannula and baseline testing were identical to Experiment 1. In this experiment, the rat was removed from its cage at approximately 1500 h and injected with 10 μl of 10% AITC or mineral oil onto the dura mater. Ninety min later, the rat was injected with either vehicle or THC (0.1, 0.32 mg/kg, i.p.). All injections were completed by 1650 h. The rat was returned to its home cage and wheel running was recorded for the next 23 h beginning at 1700 h. This procedure was repeated every other day with the THC doses and vehicle administered in a counterbalanced order.

2.8 Experiment 3: Cannabinoid receptor mediation of the anti-migraine effects of THC

The goal of this experiment was to determine whether CB_1 or CB_2 receptors mediate the anti-migraine effects of THC. Surgical implantation of the cannula, baseline testing, and drug injections were identical to Experiment 1. Rats were injected with either vehicle, the CB_1 receptor antagonist SR141716A (1.0 mg/kg, i.p.), or the CB_2 receptor antagonist SR144528 (3.2 mg/kg, i.p.) 30 min prior to administration of AITC or mineral oil and then THC (0.32 mg/kg, i.p.) or vehicle. All injections were completed by 1650 h. The rats were returned to their home cages and wheel running was recorded for the next 23 h beginning at 1700 h. This procedure was repeated every other day with the different cannabinoid receptor antagonists or vehicle administered in a counterbalanced order.

2.9 Data analysis

The experiments were conducted in a completely objective manner by not entering the animal housing room while the wheel running data were collected. An average hourly nighttime running rate was used as the baseline for h-by-h analyses. Given individual differences in wheel running, all wheel running data are presented as a percent change from each rat's baseline value. All data are expressed as mean ± S.E.M. Nearly all running occurs during the dark phase of the light cycle, so only data collected during the dark phase when drugs were administered are presented. Data were analyzed with two-way ANOVA (dose × hour) followed by Bonferroni post-hoc analysis over the 3-h period following injection of AITC or THC. Because each rat was only tested in three of the four conditions within each experiment, data were treated conservatively as independent samples. Statistical significance was defined as a probability of <0.05.

3. Results

The average baseline running for the 48 rats over 23 h was 3004 revolutions. The median number of revolutions was 1818 with a range of 443 to 10354. Given that a within-subjects design was used, 38 of 48 rats were tested three times with a recovery day between each test. The mean number of wheel revolutions on these recovery days did not differ from the mean baseline activity prior to testing [Fig. 1; ($F(2,111) = 0.483$, $P = 0.618$)].

3.1 Experiment 1: THC administration during migraine onset

Microinjection of AITC onto the dura caused a reduction in wheel running that lasted for 3 h (Fig. 2, top panel). Concurrent administration of 0.32 mg/kg THC prevented AITC-induced depression of wheel running compared to lower (0.1 mg/kg) or higher (1.0 mg/kg) doses, or administration of vehicle (Fig. 2). Analysis of the magnitude of wheel running during this 3-h period revealed a significant difference between THC doses ($F(3,40) = 7.594$, $P < 0.001$). Post-hoc analysis revealed that wheel running was significantly higher following administration of 0.32 mg/kg THC compared to all other doses (Bonferroni test: Vehicle vs. 0.32 mg/kg, $P < 0.001$; 0.1 mg/kg vs. 0.32 mg/kg, $P = 0.008$; 1.0 mg/kg vs. 0.32 mg/kg, $P = 0.008$).

Microinjection of mineral oil onto the dura as a control had no effect on wheel running (Fig. 3). Likewise, wheel running was relatively stable following administration of 0.1 and 0.32

mg/kg of THC. In contrast, wheel running was consistently lower in rats injected with 1.0 mg/kg of THC. Analysis of variance over the 3-h period following administration of mineral oil revealed a significant difference between groups ($F(3,28) = 3.181$, $P = 0.039$). Post-hoc analysis revealed that wheel running was significantly lower following administration of 1.0 mg/kg THC compared to vehicle (Bonferroni test: $P = 0.045$).

3.2 Experiment 2: THC administration 90 min after migraine onset

Rats were injected with vehicle or THC (0.1 and 0.32 mg/kg) 90 min after AITC microinjection to determine whether THC reverses AITC-induced depression of wheel running. Neither dose of THC reversed AITC-induced depression of wheel running (Fig. 4). Analysis of the magnitude of wheel running during the 3 h following THC administration revealed no significant differences in wheel running between groups ($F(2,18) = 0.220$, $P = 0.805$).

3.3 Experiment 3: Cannabinoid receptor mediation of the anti-migraine effects of THC

To determine which cannabinoid receptor contributes to the anti-migraine effect of 0.32 mg/kg THC, rats were treated with vehicle, a CB_1, or a CB_2 receptor antagonist 30 min prior to the AITC and THC injections. The anti-migraine effect of THC was attenuated in animals treated with the CB_1 receptor antagonist compared to animals treated with vehicle or the CB_2 receptor antagonist (Fig. 5). Analysis of the magnitude of wheel running during this 3-h period revealed a significant difference in wheel running between groups [Fig. 5, ($F(2,17) = 5.384$, $P = 0.015$)]. Post-hoc analysis revealed that this difference was driven by significantly less wheel running in rats treated with the CB_1 receptor antagonist compared to vehicle-treated rats given THC (Bonferroni test, $P = 0.013$). Animals treated with the CB_2 receptor antagonist did not differ from rats treated with vehicle (Bonferroni test, $P = 0.402$). Administration of the cannabinoid receptor antagonists alone had no effect on wheel running in animals treated with mineral oil onto the dura mater [Fig. 6; ($F(2,15) = 0.602$, $P = 0.561$)].

4. Discussion

The present data show that administration of THC prevents depression of home cage wheel running caused by migraine-like pain in a time- and dose-dependent manner. AITC-induced activation of dural afferents produced a reduction in wheel running that persisted for approximately three h, as we have shown before (Kandasamy et al., 2017b). Administration of 0.32 mg/kg THC immediately after the onset of headache prevented AITC-induced depression of wheel running. This anti-migraine effect was absent if THC was administered 90 min after AITC microinjection, or if lower or higher doses of THC were administered. Administration of the CB_1, but not the CB_2, receptor antagonist blocked the anti-migraine effect of THC.

Preclinical studies show that THC is effective in reducing multiple types of pain, including pain caused by acute noxious stimuli (Tseng and Craft, 2001), chronic inflammation (Craft et al., 2013), lactic acid (Kwilasz and Negus, 2012), and neuropathy (Harris et al., 2016). THC also suppresses the propagation velocity, amplitude, and duration of cortical spreading depression, a key component of migraine pathophysiology (Kazemi et al., 2012). Despite

these diverse effects previously reported, this is the first preclinical study to show that THC reduces migraine-like pain in an awake animal. Our data indicate that THC reduces migraine pain if administered at the right dose (0.32 mg/kg) and time (immediately after AITC).

Anecdotal evidence indicates that medical marijuana may also be effective in aborting migraine attacks after they have started (Rhyne et al., 2016). Our data did not demonstrate an abortive effect of THC on migraine, at least when THC is administered 90 min after administration of AITC. Similarly, administration of the anti-migraine medication sumatriptan had no effect on AITC-induced depression of wheel running when administered 90 min after AITC (Kandasamy et al., 2017b). These findings are consistent with the well-known limitations of sumatriptan to abort migraine in humans if administered after migraine onset (Diener et al., 2008). One difference between our study and anecdotal reports from migraine patients is that we focused on THC specifically, whereas marijuana contains over 100 different cannabinoids as well as non-cannabinoid constituents (Atakan, 2012). Thus, it is possible that constituents other than THC can reverse migraine pain that has progressed to a stage that is unaffected by THC alone. It is also possible that abortive effects of THC in human migraineurs are mediated by mechanisms that precede the direct activation of dural afferents (e.g., cortical spreading depression) used in the present study.

Our finding that the CB_1 receptor mediates the anti-migraine effects of THC confirms previous studies indicating a role for the CB_1 receptor in migraine. Activation of CB_1 receptors in the ventrolateral periaqueductal gray attenuates activation of trigeminovascular afferents evoked by noxious stimulation of the dura mater (Akerman et al., 2013; Knight and Goadsby, 2001). Human data indicate that genetic mutations that limit the expression of the CB_1 receptor increase the risk of migraine (Juhasz et al., 2009). These findings suggest that the CB_1 receptor may be a useful therapeutic target for the treatment of migraine.

CB_1 receptors may inhibit migraine via a central mechanism or by direct inhibition of dural afferents. CB_1 receptors are present on fibers in the trigeminal tract and trigeminal nucleus caudalis (Tsou et al., 1998). Activation of these receptors via THC likely inhibits the release of neuropeptides associated with migraine such as calcitonin gene-related peptide (CGRP). CB_1 receptor agonists also inhibit dural blood vessel dilation induced by electrical stimulation or administration of CGRP, capsaicin, or nitric oxide (Akerman et al., 2004). Cannabinoids may also interact with serotonin, a neurotransmitter implicated in migraine, to modulate migraine pain (Akerman et al., 2013; Bartsch et al., 2004; Haj-Dahmane and Shen, 2009; Voth and Schwartz, 1997). Given the complex mechanisms of action underlying the effects of cannabinoids (Greco and Tassorelli, 2015) and the complex mechanisms underlying migraine (Goadsby et al., 2017), THC may modulate migraine pain through multiple mechanisms.

A major limitation of the use of cannabinoid analgesics is the centrally mediated side effects such as sedation. This limitation was evident in the present study, in that administration of 1 mg/kg of THC did not prevent AITC-induced decreases in activity. Our data show that this problem can be avoided by using a low dose (0.32 mg/kg) of THC. Another strategy may be to develop selective CB_1 receptor agonists that do not cross the blood-brain barrier. The widespread effects of cannabinoids suggest that peripherally acting compounds may provide

relief of migraine pain without the side effects mediated by central CB_1 receptor activation. An important goal of future research is to identify the sites of action for the analgesic effects of cannabinoids.

Migraine is three times more common in women than men (Vetvik and MacGregor, 2016); however, the majority of preclinical studies of migraine use male subjects. Thus, finding effective anti-migraine therapies for women and using female subjects in preclinical studies remains a priority. Previous studies have demonstrated that female rats are more sensitive to the antinociceptive effects of THC than male rats against acute (Tseng and Craft, 2001) and chronic inflammatory pain (Craft et al., 2013). Given the high prevalence of migraine in females, cannabinoids may be an especially effective therapy for women.

It has been suggested that the higher incidence of migraine in women may be due to changes in hormone levels across the menstrual cycle. The trigeminal system is sensitized when rats are in late proestrus (Martin et al., 2007). In the present study, tracking of estrous stage revealed very few females in proestrus at the time of testing (2 of 21 rats in Experiment 2). It is possible that AITC-induced depression of wheel running may have been greater if more females had been in proestrus. Additionally, there may be estrous cycle-related fluctuations in females' sensitivity to the antinociceptive effects of THC (Craft and Leitl, 2008). A large-scale study examining estrous stage modulation of both migraine and the anti-migraine effect of THC is needed.

The present study supports our previous finding that depression of home cage wheel running is an objective method to assess the duration and magnitude of migraine-like pain (Kandasamy et al., 2017b). Assessment of home cage wheel running is especially useful in evaluating drug treatments because the goal is restoration of function, which requires that an effective drug reduces pain without inducing disruptive side effects. For example, high doses of morphine block mechanical allodynia induced by inflammatory pain, but do not restore depressed wheel running because of disruptive side effects (Kandasamy et al., 2017a; 2017c). Likewise, the present data show that the highest dose of THC (1.0 mg/kg) does not restore migraine-depressed wheel running, and in fact, depresses wheel running in pain-free rats. Other tests of pain-depressed behavior, such as intracranial self-stimulation, show a similar depression of behavior following administration of high doses of THC (i.e., 1.0 mg/kg) (Leitl and Negus, 2015).

In conclusion, we demonstrate that THC, when given at the right dose and time, prevents migraine-like pain as measured by home cage wheel running. An important finding is that although higher doses of THC probably reduce migraine-like pain, disruptive side effects prevent the restoration of normal activity. Further, we demonstrate that the anti-migraine effects of THC are mediated by the CB_1 receptor. The present study builds a firm foundation for the behavioral analysis of cannabinoids such as THC as a treatment for migraine in humans. Additional controlled studies in both humans and animals are needed to more fully characterize the anti-migraine effects of THC and other cannabinoids.

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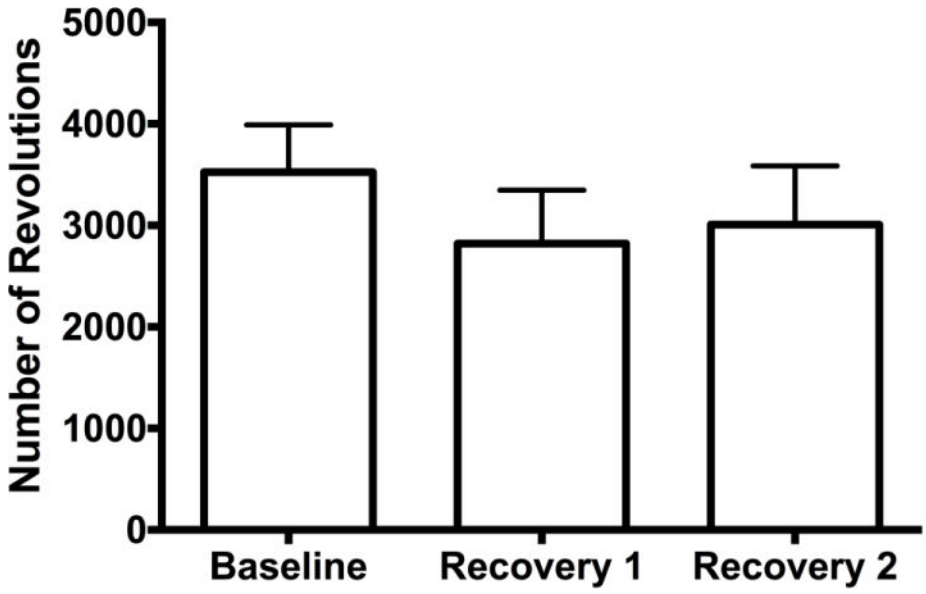

Fig. 1. Baseline levels of running are consistent across trials

Mean levels of running on the recovery days between tests did not differ from baseline running levels. Only rats tested on all three days are included in this analysis (n = 38/group).

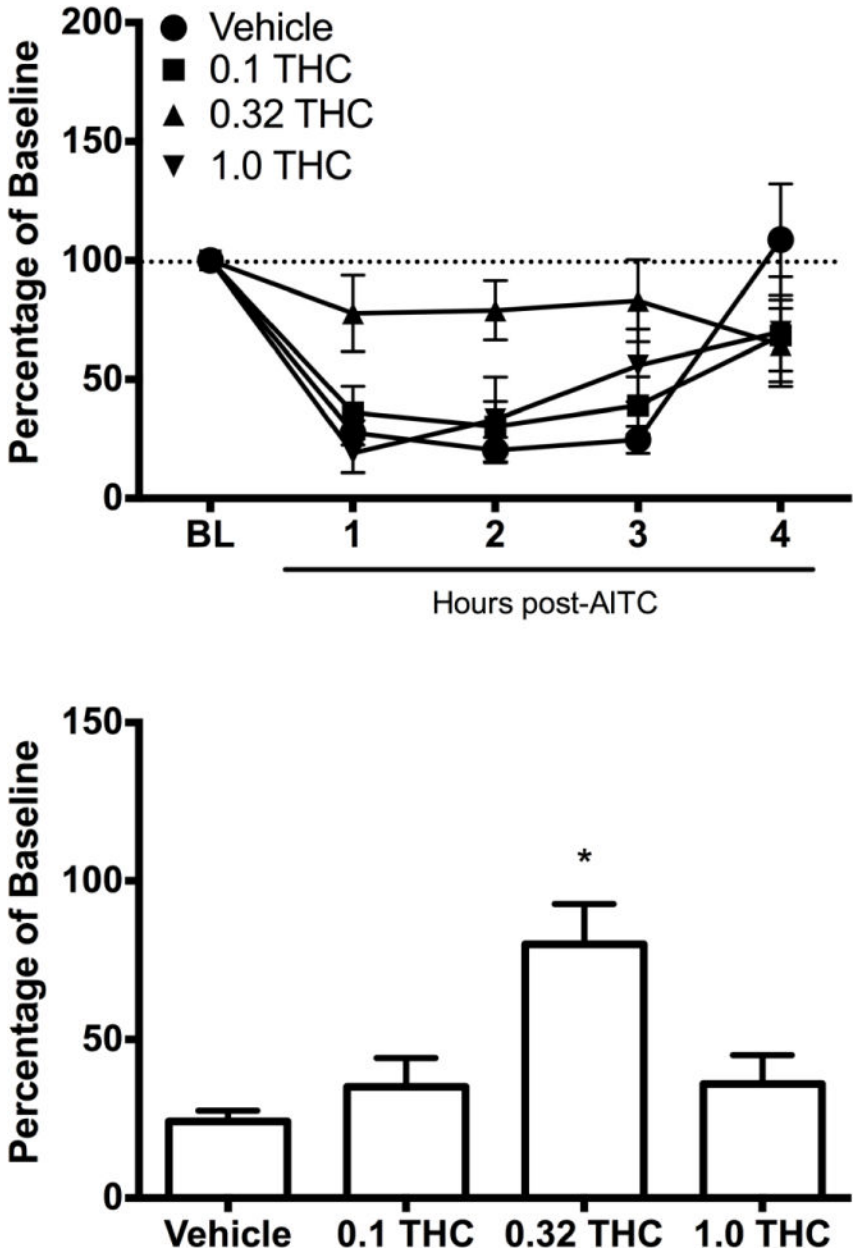

Fig. 2. THC dose dependently prevents AITC-induced depression of wheel running

Top: Time course showing that microinjection of AITC onto the dura mater produced migraine-like pain indicated by depression of wheel running that lasted 3 h. Administration of 0.32 mg/kg THC immediately after AITC administration prevented depressed wheel running. Administration of lower and higher doses of THC (0.1 and 1.0 mg/kg) did not prevent AITC-induced depression of wheel running. Bottom: Analysis of mean wheel running activity for the 3-h duration of the migraine shows reversal of migraine-like pain by 0.32 mg/kg THC (n = 10–12/group). * indicates significant difference from vehicle-treated animals (Bonferroni test, $P < 0.05$).

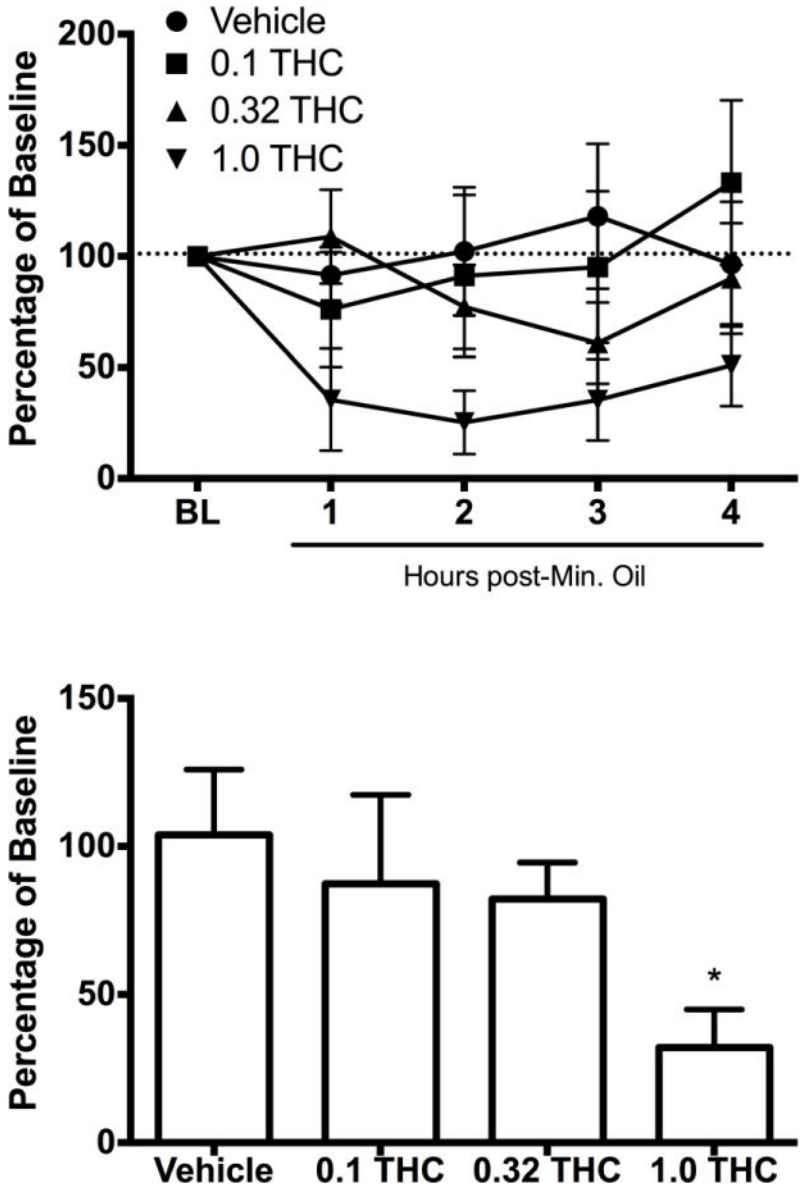

Fig. 3. The highest dose of THC depresses running in pain-free animals

Top: Time course showing that microinjection of mineral oil onto the dura as a control for AITC had no effect on wheel running. Likewise, low (0.1 mg/kg) and medium (0.32 mg/kg) doses of THC had no significant effect on wheel running in rats treated with mineral oil, whereas 1.0 mg/kg THC depressed running to about 50% of baseline levels for approximately 4 h. Bottom: Analysis of mean wheel running during the 3 h following microinjection of mineral oil onto the dura shows that 1 mg/kg THC significantly decreased wheel running compared to vehicle (n = 6–10/group).* indicates significant difference from vehicle-treated animals (Bonferroni test, $P < 0.05$).

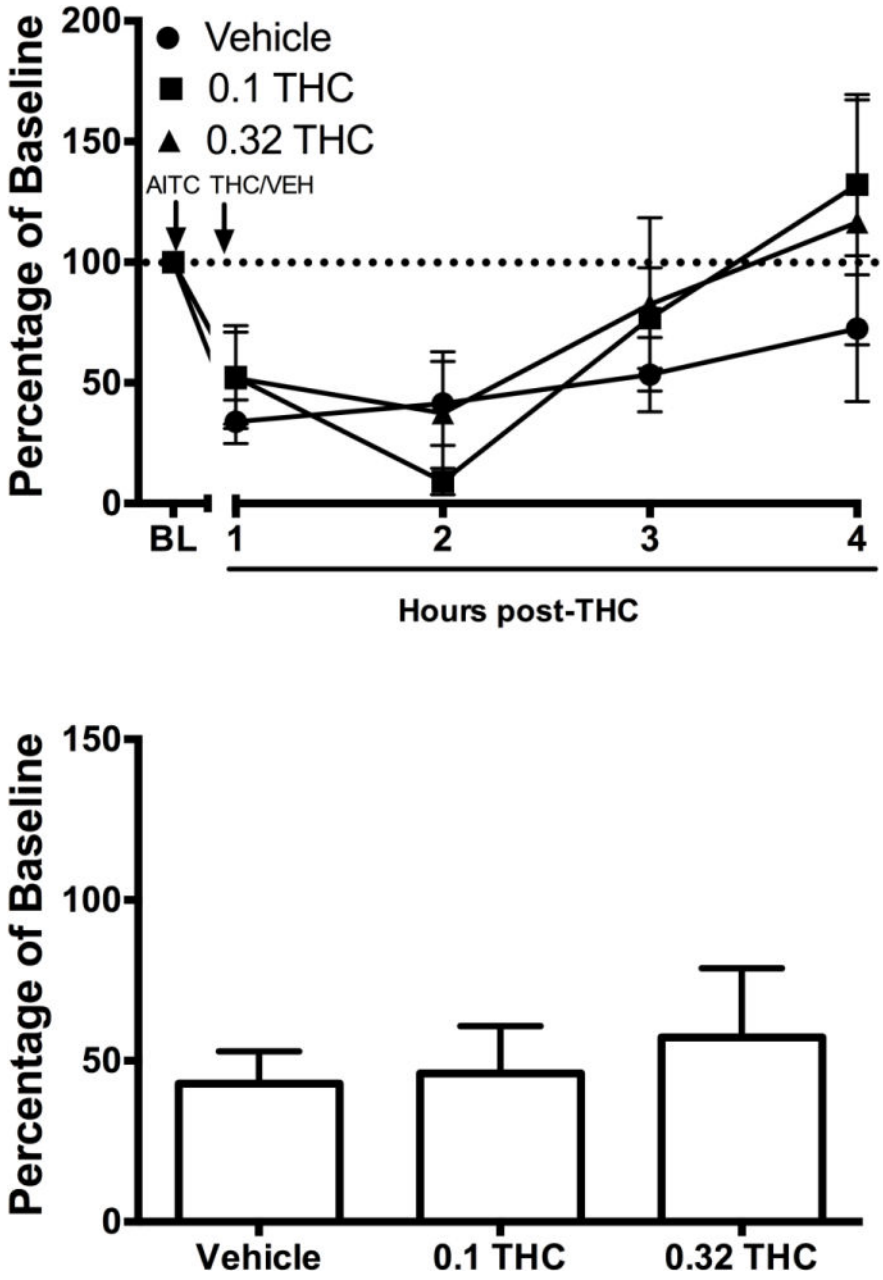

Fig. 4. Ninety minute THC post-treatment does not restore migraine-depressed running

Top: Administration of THC (0.1 and 0.32 mg/kg) 90 min after microinjection of AITC onto the dura mater did not reverse AITC-induced depression of wheel running. Bottom: Data averaged over the 3-h period following AITC administration revealed no significant differences between groups (n = 7/group).

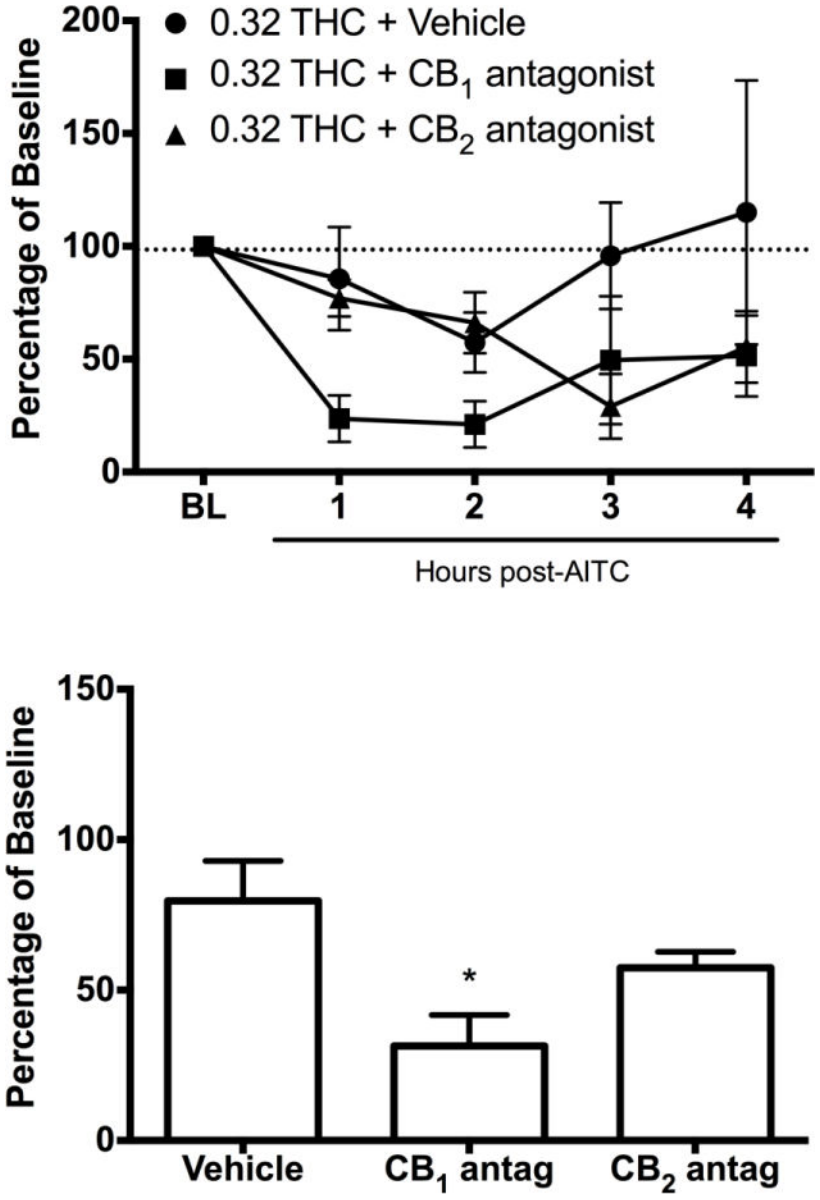

Fig. 5. Administration of a CB_1 receptor antagonist blocks the anti-migraine effects of THC

Top: Time course showing that administration of a CB_1 (SR141716A) but not CB_2 (SR144528) receptor antagonist 30 min before AITC and THC (0.32 mg/kg) injections blocked the anti-migraine effect of THC. Bottom: A significant decrease in wheel running is evident in rats injected with the CB_1 receptor antagonist compared to vehicle-treated rats when analyzed over the 3-h time course for AITC-induced migraine. All rats were injected with AITC and THC (n = 6–7/group). * indicates significant difference from vehicle-treated animals (Bonferroni test, $P < 0.05$).

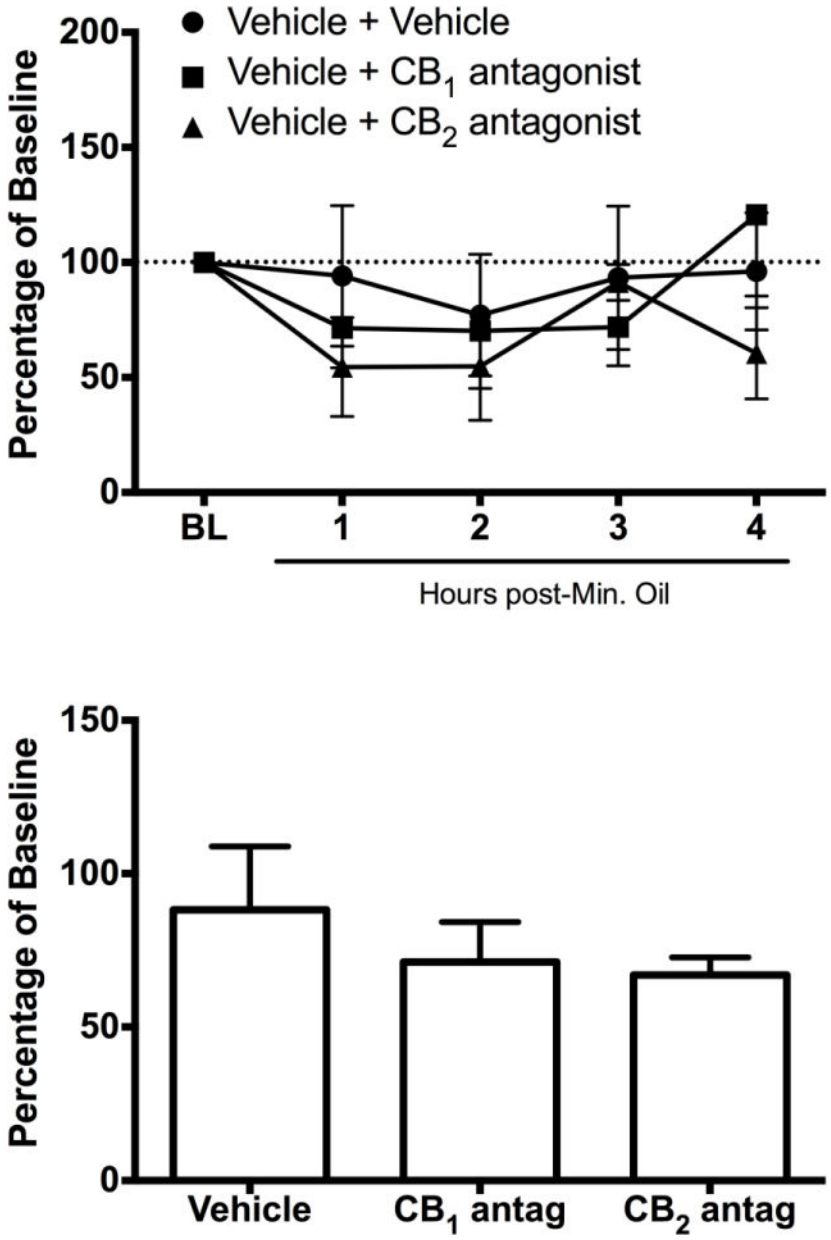

Fig. 6. Administration of cannabinoid receptor antagonists have no effect on wheel running in the absence of AITC and THC administration

Top*:* Time course for wheel running following administration of vehicle and the CB_1, or CB_2 receptor antagonist 30 min before a control injection of mineral oil onto the dura mater. Bottom: Administration of CB receptor antagonists had no significant effect on wheel running in rats without an AITC-induced migraine. Mean wheel running was analyzed over 3 h following administration of mineral oil onto the dura (n = 6/group).