Research Paper

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Preclinical effects of cannabidiol in an experimental model of migraine

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Abstract

Migraine is a disabling disorder characterized by recurrent headaches, accompanied by abnormal sensory sensitivity and anxiety. Despite extensive historical use of cannabis in headache disorders, there is limited research on the nonpsychoactive cannabidiol (CBD) for migraine and there is no scientific evidence to prove that CBD is an effective treatment. The effects of CBD are examined here using a calcitonin gene-related peptide (CGRP)-induced migraine model that provides measures of cephalic allodynia, spontaneous pain, altered light sensitivity (photophobia), and anxiety-like behavior in C57BL/6J mice. A single administration of CGRP induced facial hypersensitivity in both female and male mice. Repeated CGRP treatment produced progressively decreased levels in basal thresholds of allodynia in females, but not in males. A single CBD administration protected both females and males from periorbital allodynia induced by a single CGRP injection. Repeated CBD administration prevented increased levels of basal allodynia induced by repeated CGRP treatment in female mice and did not lead to responses consistent with migraine headache as occurs with triptans. Cannabidiol, injected after CGRP, reversed CGRP-evoked allodynia. Cannabidiol also reduced spontaneous pain traits induced by CGRP administration in female mice. Finally, CBD blocked CGRP-induced anxiety in male mice, but failed in providing protection from CGRP-induced photophobia in females. These results demonstrate the efficacy of CBD in preventing episodic and chronic migraine-like states with reduced risk of causing medication overuse headache. Cannabidiol also shows potential as an abortive agent for treating migraine attacks and headache-related conditions such as spontaneous pain and anxiety.

Keywords: Cannabidiol, Migraine, CGRP, Allodynia, Photophobia, Chronic migraine, Mice, Headache, Periorbital

1. Introduction

Migraine is a complex nervous system disorder characterized by long-lasting (4-72 hours) throbbing, unilateral headache often accompanied by nausea; vomiting; sensory amplifications such as hypersensitivity to light, sound, and smell, and cutaneous allodynia, as well as comorbid anxiety and depression.[5,26] Migraine affects 12% of the people worldwide and is considered a leading cause of disability, significantly affecting work productivity, as well as well-being and quality of life of patients and their families.[22,32] Frequency of attacks differs widely, with some people experiencing episodic (1-14 days/month) and other chronic migraine attacks (>15 days with headache/month).[22] The disorder is highly sex-dependent, with roughly 70% of migraineurs being women.[6,45]

The pathophysiology of migraine is complex and only partially understood. Several lines of evidence suggest that activation of trigeminal nociceptors, which causes a release of vasodilatory neuropeptides including calcitonin gene-related peptide (CGRP) onto the dura and centrally onto the brainstem, plays an important role in the initiation of migraine pain.[30,43] Calcitonin gene-related peptide is with no doubt central to migraine pathophysiology[50]: first, CGRP levels are increased during a migraine attack,[25] returning to normal levels when the attack is resolved.[33] Second, intravenous CGRP infusions can induce migraine-like attacks in individuals who have migraine.[37] Third, CGRP induces cephalic allodynia and light-aversive behavior in animal models[19,40]; and finally, CGRP is a relevant target for migraine. Although CGRP receptor antagonists and monoclonal antibodies against CGRP or its receptor offer new options for migraine therapy with improved target specificity,[23,52] there is a great need for new therapeutics.

Early reports indicate that *Cannabis sativa* was used quite extensively as an effective prophylactic and abortive treatment for migraine and other headache disorders.[39] However, the schedule 1 classification of marijuana in 1970 has challenged the clinical evaluation of this substance and its components about headache disorders.[39] We hypothesized here that cannabidiol (CBD), the main nonintoxicating component of cannabis, has antimigraine effects because of its efficacy for pain-related conditions such as anxiety,[18] its analgesic effect associated with the transient receptor potential cation channel subfamily V member 1 (TRPV1),[16] its anti-inflammatory activity shown in an animal model of acute inflammation,[15] and its ability to modulate serotonergic transmission,[18] which is widely involved in trigeminal activation.[28]

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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We verify our hypothesis by testing CBD efficacy in relieving relevant symptoms of migraine-like states in mice. We first characterize, in our laboratory, an animal model of acute and chronic migraine that involves measures of periorbital mechanical allodynia associated with systemic administration of exogenous CGRP in female and male mice. We then examine the ability of CBD to modulate CGRP-evoked cephalic allodynia in these predictive acute and chronic migraine models. Finally, we test whether the rescued sensitivity to mechanical stimulation induced by CBD would be accompanied by other antimigraine CBD actions such as the reversal of CGRP-induced photophobia, spontaneous pain, and anxiety-like behavior.

2. Materials and methods

2.1. Animals

All animals used in the experiments were female and male C57BL/6J mice purchased from Jackson Laboratory (Bar Harbor, ME) or bred in our laboratory. All mice were group housed (3-4/cage) and used for experiments after they reached 9 weeks of age. Animals were kept on a 12-hour light–dark cycle with lights off at 7:00 PM in a quiet temperature-controlled room (24°C-25°C). The breeding mice were housed and maintained under identical environmental conditions throughout the study. All animals were naive to the behavioral procedures except where specified. Experiments were reported in compliance with the ARRIVE guidelines.[35] The total number of mice used throughout the study was N = 438. Mice were assigned into the various treatment groups using a random allocation sequence, ensuring an equal or similar group size (restricted randomization), except where specified. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, with methods preapproved by the Institutional Animal Care and Use Committee at Florida Atlantic University.

2.2. Drugs

Cannabidiol was a generous gift by Sunflora Inc. The CBD powder was obtained by extraction at 97.404% purity (total tetrahydrocannabinol [THC] = 0.000%, total cannabinoids = 97.765%). Cannabidiol was prepared in a vehicle (VEH) of 3% ethanol, 3% Tween 80, and 0.9% saline, and used at the dose of 10 and 30 mg/kg.[12] Calcitonin gene-related peptide (AnaSpec, Inc, Fremont, CA) was dissolved in PBS and used at the dose of 0.1 mg/kg.[40,47] Olcegepant (Tocris, Minneapolis, MN) was prepared in 4% DMSO, 4% Tween 80, and 0.9% saline and injected at the dose of 1 mg/kg.[19,20] Sumatriptan succinate (Fisher Scientific, Waltham, MA) was dissolved in the same VEH as CBD and used at the dose of 0.6 mg/kg.[17] All compounds were administered in a 5 mL/kg volume injection and given by intraperitoneal (i.p.) route of administration.

2.3. Assessment of periorbital mechanical allodynia

The operator who conducted the assessment was always blind to treatment conditions and performed all testing for a given experiment. In the days preceding each experiment, mice were habituated to the testing room twice for 30 minutes. During this acclimation period mice were gently handled, habituated to i.p. injection, and held as during the test, but the filaments were not applied until the test started. On the day of the experiment, 30 additional minutes of acclimation was given to the mice before starting the assessment of mechanical allodynia. To decrease the mouse withdrawal reflex, which is usually greater under novelty conditions, 2 basal points were conducted; however, only the second baseline was used for data analysis. Female and male mice were tested on separate days by the same operator. Allodynia was evaluated in the mouse periorbital region over the rostral portion of the eye and near the midline. Mice, which during the 30-minute acclimation period were left undisturbed inside Plexiglas cylinders covered on top by a meshed grid, at the time of assessment were placed on top of the grid and gently held by the tail with one hand to keep them from slipping away. With the palm of the same hand, the animal was covered without crushing it, leaving it free of all movements. With the other hand, von Frey filaments of different forces (0.02, 0.04, 0.07, 0.16, 0.4, 1.0, and 1.4 g) were applied to the periorbital area perpendicular to the skin, with sufficient force to cause slight buckling, and held for approximately 2 seconds to elicit a positive response. Mice were poked 5 times with the same filament in a uniform manner throughout the periorbital region before changing filament. The stimulation was initiated with the 0.16-g filament. A response occurred when the mouse stroked the face with its forelimb, withdrew its head from the stimulus, or shook its head.[19] Allodynia was measured according to the up–down method.[13] The absence of response led to the use of a filament with increased force, whereas a response led to the use of a lighter filament. After the first response occurred, 4 more measurements were collected for each mouse or until 4 consecutive positive or negative responses occurred. The 50% mechanical withdrawal threshold (expressed in g) was then calculated.[14] Periorbital mechanical allodynia was assessed in a total of N = 257 mice. Sample sizes were computed by a priori power analysis (G*Power 3.1[24]; F test: analysis of variance [ANOVA] repeated measure, within-between interaction) conducted using effect size f = 0.25, an $\alpha = 0.05$, and power $(1 - \beta) = 0.80$ assuming nonsphericity factor = 1 and correspondence among repeated measures = 0.5. Mice were allocated to vehicle or treatment groups by counterbalancing animal performance into the different experimental groups to ensure no threshold difference at baseline.

2.4. Grimace face assay

Female and male C57BL/6J mice were acclimated for 30 minutes inside Plexiglas cylinders covered on top by a meshed grid. Mice received a pretreatment of either CBD (30 mg/kg) or VEH, and, 30 minutes later, received a treatment of CGRP (0.1 mg/kg) or PBS. To record facial grimace, mice were videotaped for 30 seconds at 15 minutes, 30 minutes, and 1 hour postinjection. The videos were then used to take still images that included the appearance of the mouse's face, ears, and posture. The images were randomized, and a blind evaluator scored each image on a scale of 0 to 2 based on signs of a facial grimace. The criteria were based on orbital tightening, nose bulge, cheek bulge, ear position, and whisker change.[36] Scores were averaged across each treatment and analyzed to determine facial grimace. A total of N = 63 mice were used for this experiment.

2.5. Photophobia

Photosensitivity was tested in the dark–light box (DLB). Two Med Associates activity monitor chambers with dark box inserts were used. The testing chambers were placed in ventilated and sound attenuating cubicles (56 × 38 × 36 cm). Each chamber had 2 compartments equivalent in dimensions and connected to each other with an opening. One compartment was delimited by a black Plexiglas insert to prevent light entrance (dark compartment), whereas the other (light compartment) was lit using a

1000-lux lighting unit (surgical lamp, Fisher Scientific).[53] Infrared beams allowed for tracking the animal's movements and time spent in each compartment. The percent of time spent in the light compartment was calculated and served as a measure of photophobia. After the photophobia test, mice were returned to their home cages.

2.6. Anxiety-like behavior

Anxiety-like responses were assessed by the elevated plus maze (EPM) test as we previously described.[53] The EPM apparatus was a black, "plus"-shaped platform equipped with 2 open arms and 2 closed arms of the same dimensions (35.6 × 7.6 cm). The platform was at a raised height of 50 cm above the ground and placed under room lighting of 500 lux. The EPM test was conducted using the same mice (on the same drug treatments) that were previously used for the photophobia test. After 5 minutes of resting in their home cages, the mice were placed on the central platform of the EPM apparatus faced to a closed arm for 5 minutes. An operator blinded to the treatment schedule recorded the time spent onto the open arms of the maze as well as the entries onto the open and closed arms. The percent of open arm time (OAT) and entries (OAEs) served as measures of anxiety-like activity, whereas the number of closed arm entries (CAEs) was an indicator of the mouse locomotor behavior. Anxiety-like behavior and photophobia were tested in the same animals (total mice: N = 95).

2.7. Open-field test

Locomotor activity chambers from Med Associates (St Albans, Vermont) were used to track and analyze the mouse locomotor behavior. This experiment was performed in N = 23 male mice (7-8/treatment) using similar methods as we previously described.[7] On day 1, mice were habituated to the open-field chambers for 1 hour. On day 2, locomotor activity was tested for 30 minutes, 1 hour after receiving an i.p. injection of CBD (10 mg/kg, 30 mg/kg) or VEH. Total distance traveled (cm) and immobility time (sec) served as primary readouts for the mouse locomotor behavior.

2.8. Statistical analysis

Tibco Statistica (version 13.5.0.17) was used for data analysis. Nonparametric statistical tests were used to analyze thresholds of mechanical stimulation of the periorbital region and grimace face scores. Specifically, the Friedman test was used to determine overall differences in treatments across multiple measures. This test was followed up by the Wilcoxon test when significance was detected. The Kruskal–Wallis test was used to determined overall differences in multiple independent groups. The Mann–Whitney *U* test was conducted to examine comparisons between groups and to analyze differences in basal responses between female and male mice. Two-way ANOVA was used for analysis of photophobia in the DLB and anxiety-like behavior in the EPM, followed, when appropriate, by Fisher LSD post hoc comparisons. Open-field data were analyzed by means of a 1-way ANOVA using "treatment" as a between-subject factor. Linear regression analyses were conducted to determine correlations between variables. Significance was set at $P < 0.05$.

3. Results

3.1. Effects of acute calcitonin gene-related peptide treatment on periorbital allodynia in female and male mice

Female and male mice received a single i.p. injection of CGRP or PBS and periorbital mechanical allodynia was assessed (**Fig. 1A**). Friedman test conducted in PBS-treated female mice revealed similar mechanical thresholds across the various time points ($X^2(4) = 2.222$, $P = 0.694$). By contrast, significant changes in allodynic responses after CGRP administration were observed ($X^2(4) = 16.915$, $P = 0.00201$). Mechanical sensitivity was significantly increased at 0.5 hour ($P = 0.0179$) and 1 hour ($P = 0.0117$), but not

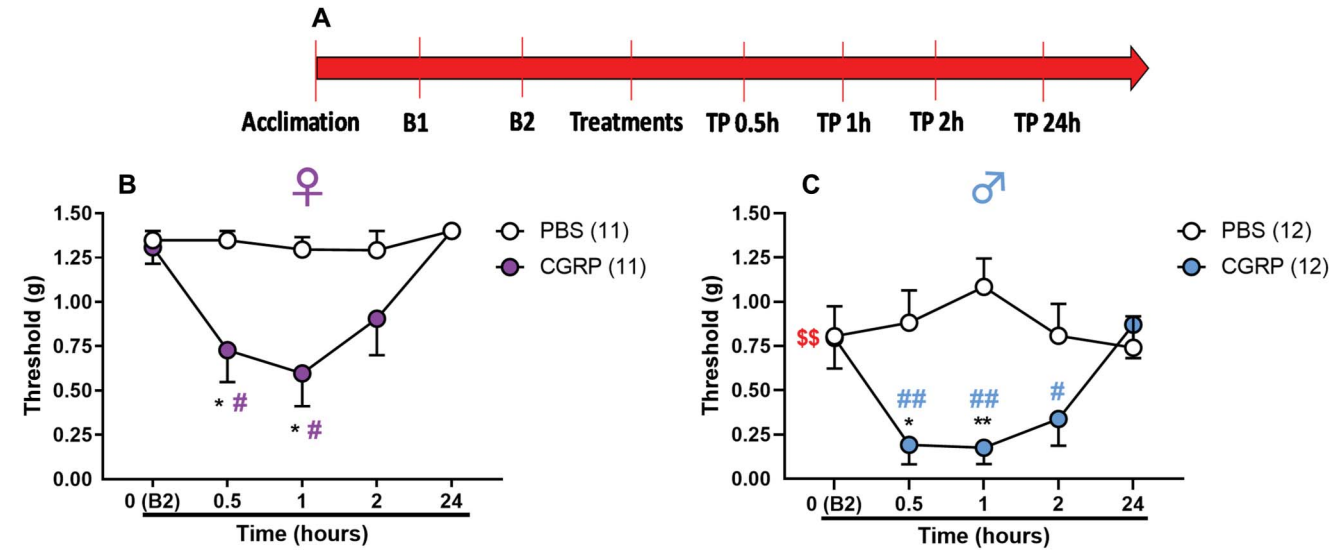

Figure 1. Single intraperitoneal (i.p.) administration of calcitonin gene-related peptide (CGRP) induces mechanical hypersensitivity in the periorbital region of C57BL/6J mice. (A) Experimental timeline: After acclimation, 2 basal points of periorbital mechanical allodynia were calculated. Female and male mice were then i.p. injected with CGRP (0.1 mg/kg) or VEH (PBS). Assessment of periorbital mechanical allodynia was evaluated again 0.5 hour, 1 hour, 2 hours, and 24 hours after treatments. von Frey hairs were applied onto the periorbital area of (B) female and (C) male mice using the up–down method. Sensitivity thresholds (force, g) were assessed at 30 minutes (0.5 hours), 1, 2, and 24 hours after acute administration of either 0.1 mg/kg CGRP or PBS (N = 11 vs 11 females and 12 vs 12 males; total mice: N = 46). *$P < 0.05$, **$P < 0.01$ difference from PBS. #$P < 0.05$, ##$P < 0.01$ difference from basal sensitivity thresholds (B2). $$$P < 0.01$ difference between sexes. TP, time point; VEH, vehicle.

at 2 hours ($P = 0.0796$) after CGRP administration. Comparisons between PBS-treated and CGRP-treated samples led to similar results (basal sensitivity threshold (B2): $P = 1.000$; 0.5 hour: $P = 0.0215$; 1 hour: $P = 0.0138$; 2 hours: $P = 0.237$; 24 hours: $P = 1.000$, **Fig. 1B**).

Friedman analysis conducted in PBS-treated male mice revealed similar mechanical thresholds across the various time points ($X^2(4) = 3.027$, $P = 0.553$), whereas significant changes in allodynic responses after CGRP administration were noticed ($X^2(4) = 21.327$, $P = 0.00027$). Changes in allodynic responses after CGRP administration were observed at 0.5 hour and 1 hour ($P = 0.00963$ for both time points), as well as at 2 hours ($P = 0.0218$). Comparisons between PBS-treated and CGRP-treated groups roughly confirmed the results (B2: $P = 0.839$; 0.5 hour: $P = 0.0153$; 1 hour: $P = 0.00149$; 2 hours: $P = 0.148$; 24 hours: $P = 0.817$, **Fig. 1C**). Subsequent analysis conducted on basal responses of female and male mice indicated that the latter were more sensitive in showing responses to von Frey hairs than their female counterparts ($U = 117.0$, $P = 0.00127$).

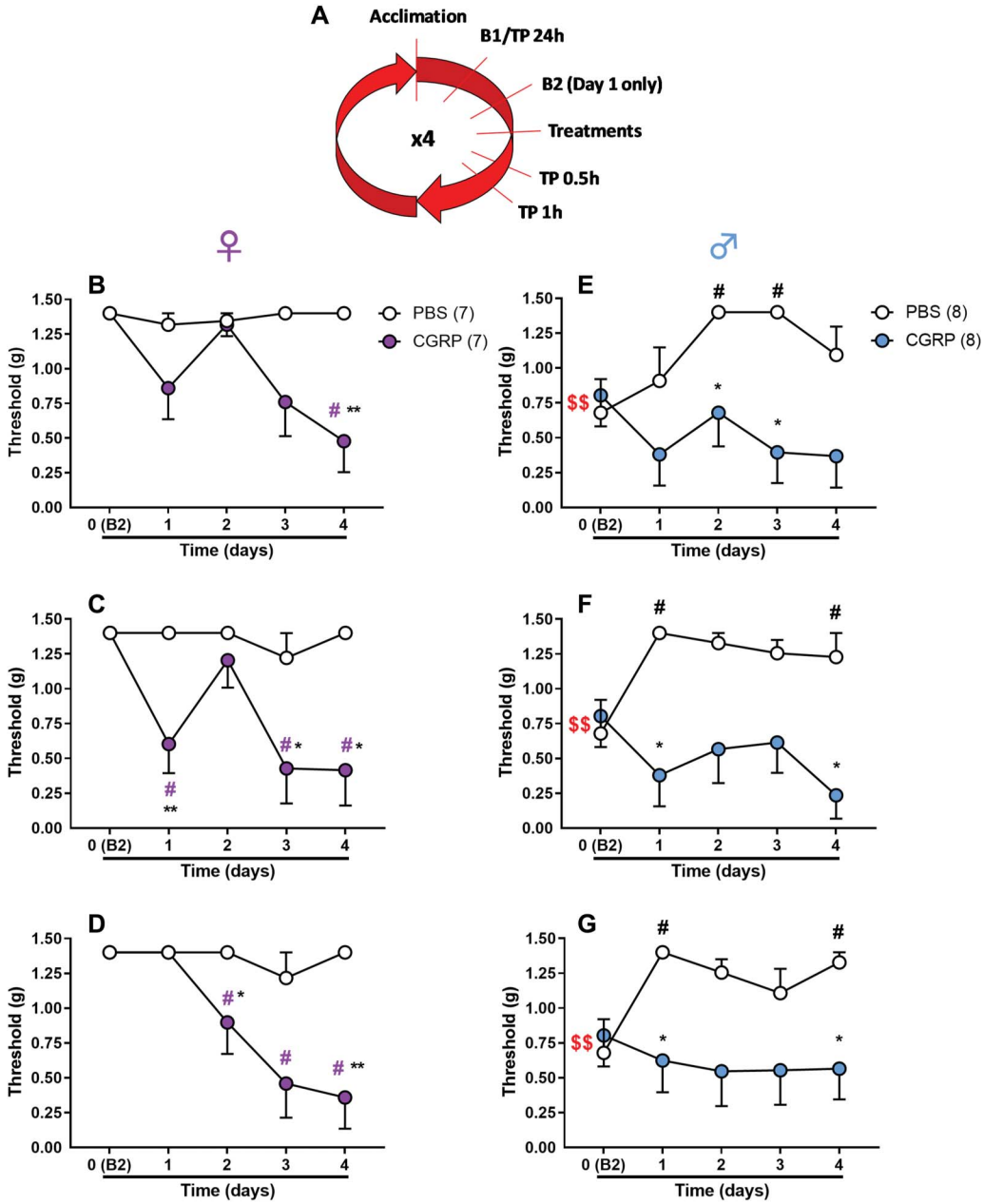

Figure 2. Repeated CGRP treatment produces progressively decreased levels of basal thresholds of allodynia in females, but not male C57BL/6J mice. (A) Experimental timeline: To model the progression from episodic to chronic migraine, CGRP (0.1 mg/kg) or PBS was administered to female and male mice every day for 4 consecutive days (x4). On day 1, after acclimation, mechanical thresholds were evaluated twice before 0.5 hour, 1 hour, and 24 hours after treatments. On days 2, 3, and 4, an identical procedure was used except that only 1 basal score was recorded before treatment. The basal score recorded from day 2 matched the 24-hour time point for each day. (B and E) Thresholds of mechanical allodynia assessed at 0.5 hour in female and male mice, respectively. (C and F) Thresholds of mechanical allodynia assessed at 1-hour posttreatment time in female and male mice, respectively. (D and G) Thresholds of mechanical allodynia assessed at 24 hours postinjections in female and male mice, respectively. Results are the mean ± SEM of force (g) applied through von Frey filaments using the up–down method to N = 14 female (7/group) and 16 male (8/group) mice (total mice: N = 30). *$P < 0.05$, **$P < 0.01$ difference from PBS. #$P < 0.05$ difference from basal threshold (B2). \$\$$P < 0.01$ difference between sexes. CGRP, calcitonin gene-related peptide; TP, time point.

3.2. Effects of long-term calcitonin gene-related peptide treatment on periorbital allodynia in female and male mice

Calcitonin gene-related peptide or PBS was administered to female and male mice every day for 4 consecutive days to model the progression from episodic to chronic migraine (**Fig. 2A**). PBS-treated female mice were insensitive to the mechanical stimulation throughout the 4 days of treatment, at all the time points examined (0.5 hour: $X^2(4)= 3.000$, $P = 0.557$; 1 hour and 24 hours: $X^2(4) = 4.000$, $P = 0.406$). Females treated with CGRP over 4 days were sensitive at 0.5 h ($X^2(4) = 15.240$, $P = 0.00423$). Mechanical sensitivity reached significance as compared to B2 ($P = 0.0277$) and PBS ($P = 0.0088$ on day 4, **Fig. 2B**). At 1 hour posttreatment, CGRP produced mechanical allodynia (X^2 (4) = 15.636, $P = 0.00355$) on days 1 ($P = 0.0277$ from B2, $P = 0.0088$ from PBS), 3 ($P = 0.0431$ and $P = 0.0409$ from B2 and PBS, respectively), and 4 ($P = 0.0431$ from B2 and $P = 0.0298$ from PBS, **Fig. 2C**). It is unclear why the mice lacked hypersensitivity in the CGRP group at 0.5 and 1 hours on day 2 in this experiment. Repetitive CGRP administration over 4 days produced progressively increased basal levels of mechanical allodynia at 24 hours posttreatment ($P = 0.0431$ on days 2 and 3, and $P = 0.0277$ on day 4 vs B2; $P = 0.0298$ on day 2, $P = 0.0552$ on day 3, $P = 0.0088$ on day 4 vs PBS-treated mice, **Fig. 2D**).

Male mice were less sensitive than female mice to the effect of CGRP across the 4 treatment days. Overall, the effect of CGRP was not significant at 0.5 hour ($X^2(4) = 8.521$, $P = 0.0742$, **Fig. 2E**), 1 hour ($X^2(4) = 9.118$, $P = 0.058$, **Fig. 2F**), and 24 hours ($X^2(4) = 4.626$, $P = 0.327$, **Fig. 2G**). Surprisingly, changes in mechanical thresholds were observed in PBS-treated males at all time points (0.5 hour: $X^2(4) = 12.833$, $P = 0.0121$; 1 hour: $X^2(4) = 11.837$, $P = 0.0186$; 24 hours: $X^2(4) = 12.888$, $P = 0.0118$) as PBS-treated male mice were more resistant to mechanical stimulation across days ($P = 0.0431$ vs B2 on days 2 and 3 at 0.5 hour, on days 1 and 4 at 1 hour and 24 hours). Further analysis displayed differences between PBS-treated and CGRP-treated males at the same time points (0.5 hour: $P = 0.0405$ and $P = 0.0135$ on days 2 and 3, respectively; 1 hour: $P = 0.0135$ on days 1 and 4; 24 hours: $P = 0.0135$ and $P = 0.0239$ on days 1 and 4, respectively). Analysis performed on basal responses between female and male mice indicated, once again, that males were spontaneously more sensitive than females (U = 42.0, $P = 0.00386$).

3.3. Effect of cannabidiol on locomotor activity

Open-field test was performed to determine whether CBD, at doses of 10 and 30 mg/kg, affected animal locomotor activity. The mean total distance traveled (cm) ± SEM was 3269.3 ± 246.7 (VEH), 3207.1 ± 366.1 (CBD 10), and 3106.3 ± 459.7 (CBD 30). The mean immobility time (sec) ± SEM was 1210.6 ± 27.3 (VEH), 1223.9 ± 47.5 (CBD 10), and 1243.7 ± 36.9 (CBD 30). Cannabidiol did not significantly alter total distance traveled [F(2,20) = 0.0506; $P = 0.950$] or time of immobility [F(2,20) = 0.183; $P = 0.834$] in the open-field assay.

3.4. Effect of cannabidiol on calcitonin gene-related peptide-induced periorbital allodynia in female and male mice

Experimental timeline is shown in **Figure 3A**. Friedman test conducted in CGRP-treated female mice revealed a significant increase in mechanical sensitivity as compared to the basal threshold ($X^2(3) = 9.842$, $P = 0.0199$) at 1 hour ($P = 0.0346$) and 2 hours ($P = 0.0464$) from the single CGRP administration. Analysis performed between all treatment groups confirmed there was a difference in the mechanical thresholds at 1 hour (H(3) = 17.790, $P = 0.0005$) and 2 hours (H(3) = 8.093, $P = 0.0441$). Single between-group comparisons indicated that administration of CGRP increased sensitivity to von Frey stimulation as compared to PBS control at 1 hour ($P = 0.0192$), but not at 2 hours ($P = 0.0773$). Both doses of CBD prevented the increased sensitivity evoked by the administration of CGRP at 1 hour (CBD 10: $P = 0.0192$; CBD 30: $P = 0.030$, **Fig. 3B**).

Overall, the analysis performed in male mice between all treatment groups revealed significant changes in allodynic responses at 0.5 hour (H(3) = 14.319, $P = 0.0025$), 1 hour (H(3) = 12.065, $P = 0.0072$), and 2 hours (H(3) = 20.092, $P = 0.0002$) from CGRP injection. Consistently, between-group comparisons indicated decreased thresholds for CGRP-treated

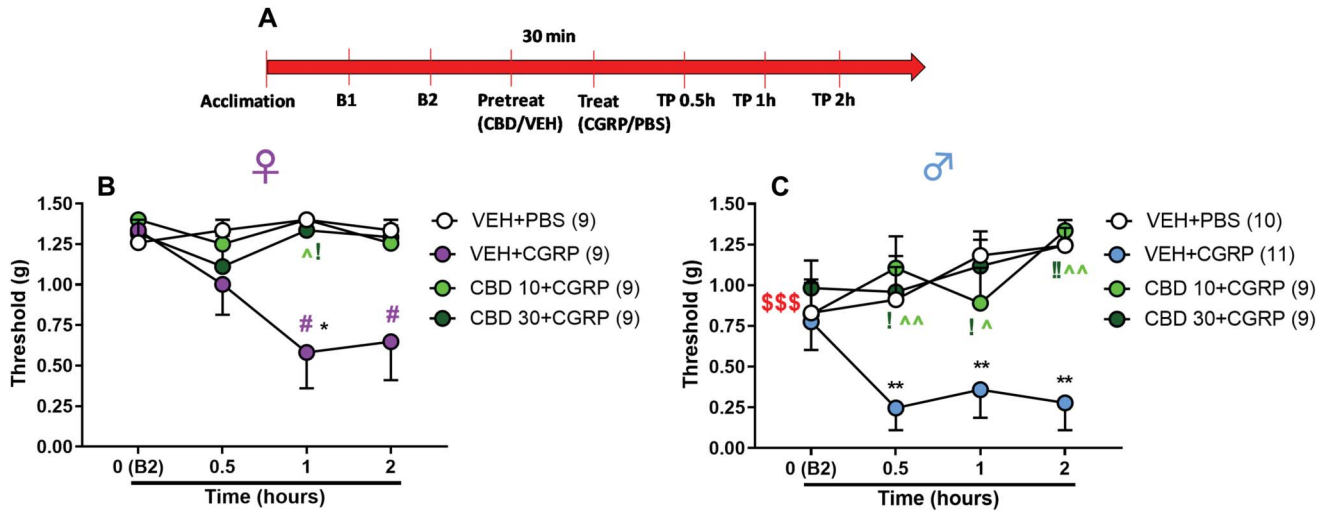

Figure 3. Cannabidiol (CBD) prevents mechanical hypersensitivity induced by a single i.p. CGRP injection in C57BL/6J mice. (A) Experimental timeline: After determination of 2 basal threshold measures, female and male mice were i.p. administered with CBD (10 mg/kg or 30 mg/kg) or VEH of CBD, 30 minutes before i.p. administration of CGRP (0.1 mg/kg) or PBS. Periorbital mechanical allodynia was evaluated 0.5 hour, 1 hour, and 2 hours after the last drug administration. Results are the mean ± SEM of force (g) applied using the up–down method to (B) N = 36 female (9/group) and (C) 39 male (9-11/group) mice (total mice: N = 75). *$P < 0.05$, **$P < 0.01$ difference from PBS. #$P < 0.05$ difference from basal thresholds (B2). ^,!$P < 0.05$, ^^,!!$P < 0.01$ difference from CGRP. $$$$P < 0.001$ difference between sexes. CGRP, calcitonin gene-related peptide; TP, time point; VEH, vehicle.

mice as compared to PBS-treated mice at all time points examined (0.5 hour: $P = 0.00406$, 1 hour: $P = 0.00541$, and 2 hours: $P = 0.00276$). This effect was prevented by both CBD doses at all time points (CBD 10: $P = 0.00493$ (0.5 hour), $P = 0.030$ (1 hour), $P = 0.00304$ (2 hours); CBD 30: $P = 0.0109$ (0.5 hour), $P = 0.0275$ (1 hour), $P = 0.00493$ (2 hours), **Fig. 3C**). Further analysis of basal responses conducted between sexes confirmed a significant difference in the spontaneous nociceptive response to mechanical stimulation of the mouse periorbital region ($U = 308.0$, $P = 0.00003$).

3.5. Effect of repeated cannabidiol administration on increased levels of basal periorbital allodynia induced by repeated calcitonin gene-related peptide treatment in female mice

Experimental timeline is shown in **Figure 4A**. At 1 hour postinjection, the CGRP-treated group showed increased sensitivity to mechanical stimulation ($X^2(4) = 10.851$, $P = 0.0282$) across all treatment days (day 1: $P = 0.0277$; days 2, 3, and 4: $P = 0.0425$). Multiple independent group analysis was significant for day 1 ($H(3) = 7.931$, $P = 0.0475$), day 2 ($H(3) = 11.041$, $P = 0.0115$), day 3 ($H(3) = 10.754$, $P = 0.0131$), and day 4 ($H(3) = 13.047$, $P = 0.0045$). Between-group comparisons indicated a significant difference between the groups receiving PBS and CGRP on treatment day 3 ($P = 0.0239$), which was blocked by both CBD doses (CBD 10: $P = 0.0239$; CBD 30: $P = 0.0313$, **Fig. 4B**).

At 24 hours postinjection, when the acute effects of the treatments wear off, repetitive CGRP administration over 4 days produced a significant decrease in basal thresholds of mechanical allodynia ($X^2(4) = 14.305$, $P = 0.00638$) with progressively increased levels of allodynia that became significant on day 4 ($P = 0.0250$). Multiple independent group analysis was significant for days 3 ($H(3) = 7.954$, $P = 0.0470$) and 4 ($H(3) = 19.883$, $P = 0.00002$) with group comparisons indicating a remarkable effect of repeatedly injected CGRP on day 4 ($P = 0.00457$), which was prevented by pretreatment of both CBD 10 ($P = 0.00387$) and 30 mg/kg ($P = 0.00538$), when administered 24 hours before testing (**Fig. 4C**).

3.6. Effect of sustained cannabidiol administration in female mice not receiving calcitonin gene-related peptide

Female mice not treated with CGRP received repeated injections of CBD, sumatriptan, or VEH (**Fig. 5A**) and periorbital mechanical allodynia was examined. At 1 hour postinjection, repeated treatment with CBD did not alter periorbital withdrawal thresholds ($X^2(6) = 6.000$, $P = 0.423$) across days, whereas repeated sumatriptan injections produced significant reductions of the mechanical thresholds ($X^2(6) = 22.787$, $P = 0.00087$) on days 3 ($P = 0.0277$), 4 and 8 ($P = 0.0431$), and 5 ($P = 0.0179$), as compared to B2. On days 3 ($P = 0.0238$) and 5 ($P = 0.00813$), mechanical thresholds were significantly decreased as compared to VEH (**Fig. 5B**).

Evaluation at 24 hours after injections led to similar results with repeated CBD treatment that did not alter sensory thresholds ($X^2(6) = 8.366$, $P = 0.212$) while sumatriptan decreased mechanical thresholds ($X^2(6) = 23.793$, $P = 0.00057$) on days 4 ($P = 0.0179$), 7, and 8 ($P = 0.0277$ both days) as compared to B2. On day 4, mechanical thresholds were significantly decreased as compared to VEH ($P = 0.00813$, **Fig. 5C**). Thresholds remained lower for several days after the termination of sumatriptan (days 10 and 16: $P = 0.0179$ vs B2 and $P = 0.00813$ vs VEH; day 22: $P = 0.108$ vs B2 and $P = 0.272$ vs VEH).

3.7. Effect of cannabidiol as an abortive treatment for calcitonin gene-related peptide-evoked periorbital allodynia in female mice

In this experiment, mice received CBD after CGRP (**Fig. 6A**). Calcitonin gene-related peptide-treated female mice showed a significant increase in mechanical sensitivity as compared to the basal threshold ($X^2(2) = 10.800$, $P = 0.00452$), as assessed 0.5 hour and 1 hour ($P = 0.0277$ both times) after VEH injection. Multiple group analysis indicated differences in allodynic responses at 0.5 hour ($H(3) = 9.924$, $P = 0.0192$) and 1 hour ($H(3) = 14.423$, $P = 0.0024$). As shown in **Figure 6B**, the CGRP + VEH group displayed increased mechanical sensitivity as compared to the PBS + VEH group at both 0.5 hour and 1 hour time points ($P = 0.0208$). Cannabidiol, administered 30 minutes after CGRP successfully blocked the CGRP-evoked allodynia at both doses of 10 (1 hour: $P = 0.0356$) and 30 mg/kg (0.5 hour: $P = 0.0356$; 1 hour: $P = 0.0135$).

In a similar experiment conducted with the CGRP receptor antagonist olcegepant (experimental timeline in **Fig. 6C**), female

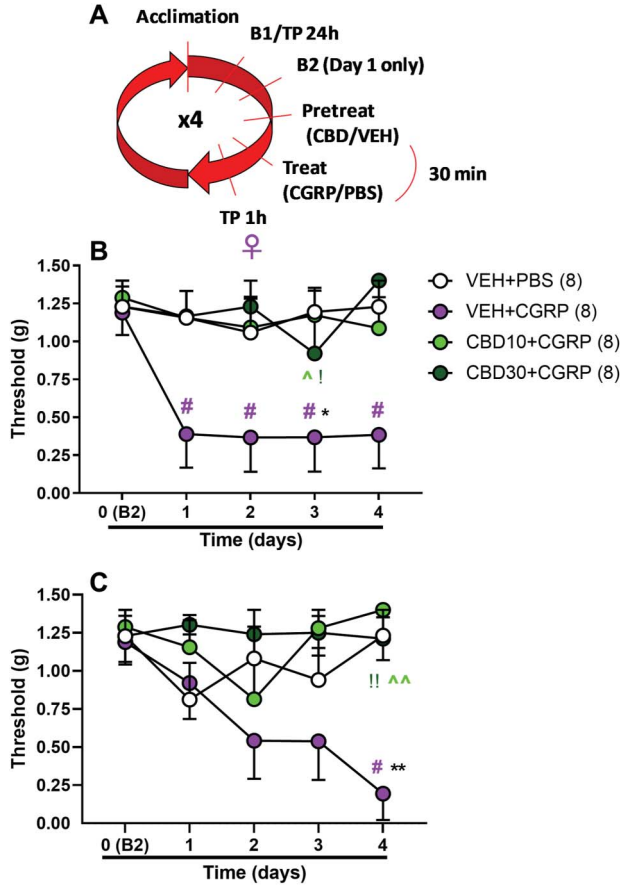

Figure 4. Repeated CBD administration prevents increased levels of basal allodynia induced by repeated CGRP treatment in female C57BL/6J mice. (A) Experimental timeline: Female mice were i.p. administered daily with CBD (10 mg/kg, 30 mg/kg) or VEH and with CGRP (0.1 mg/kg) or PBS 30 minutes later for 4 consecutive days (x4). On day 1, 2 basal measures of facial allodynia were determined immediately before the treatments as well as at 1 hour and 24 hours posttreatments. On days 2, 3, and 4, the same procedure was repeated except that only 1 basal measure of allodynia was taken, matching the 24-hour posttreatment time point. Thresholds of facial allodynia were assessed (B) 1 hour and (C) 24 hours after the injections. Results are the mean ± SEM of force (g) applied through von Frey filaments using the up–down method to N = 32 (8/group) female mice. *$P < 0.05$, **$P < 0.01$ difference from PBS. #$P < 0.05$ difference from basal thresholds (B2). ^,!$P < 0.05$, ^^,!!$P < 0.01$ difference from CGRP. CBD, cannabidiol; CGRP, calcitonin gene-related peptide; VEH, vehicle; TP, time point.

mice treated with CGRP displayed a significant increase in mechanical sensitivity as compared to the basal threshold (overall, $X^2(3) = 18.6$, $P = 0.00033$; 0.5 hour: $P = 0.0277$; 1 hour: $P = 0.0179$; 2 hours: $P = 0.0117$). Multiple group analysis confirmed differences in allodynic responses at 0.5 hour ($H(2) = 6.253$, $P = 0.0439$), 1 hour ($H(2) = 11.946$, $P = 0.0025$), and 2 hours ($H(2) = 15.837$, $P = 0.0004$). Calcitonin gene-related peptide was effective across all time points (0.5 hour: $P = 0.045$; 1 hour: $P = 0.000982$; 2 hours: $P = 0.00241$). Olcegepant successfully blocked allodynia at 1 hour ($P = 0.00865$) and 2 hours ($P = 0.00136$) postinjection (**Fig. 6D**).

3.8. Effect of cannabidiol on calcitonin gene-related peptide-induced facial grimace in female and male mice

Experimental timeline is shown in **Figure 7A**. Overall changes in grimace scores were observed in female mice 15 and 60 minutes after treatments ($H(3) = 21.833$, $P = 0.0001$ and $H(3) = 15.581$, $P = 0.0014$, respectively). Specifically, clear signs of facial discomfort were induced by peripherally administered CGRP at 15 minutes ($P = 0.00457$) and 60 minutes ($P = 0.00644$). These signs were attenuated at 60 minutes by systemic administration of CBD ($P = 0.0405$, **Fig. 7B**), suggesting that CBD contributes to reduce spontaneous pain, a response consistent with migraine, while failing to completely prevent this response. In male mice, differences in grimace scores were evident only at the 15-minute time point ($H(3) = 10.044$, $P = 0.0182$) due to CGRP treatment ($P = 0.0206$). Signs of facial pain were not rescued by CBD ($P = 0.772$, **Fig. 7C**). **Figure 7D** shows representative images used to calculate the grimace scores.

3.9. Effect of cannabidiol on calcitonin gene-related peptide-induced photophobia in male and female mice using the dark–light box

Experimental timeline is shown in **Figure 8A**. Analysis of variance revealed a main "treatment" (CGRP vs PBS) effect [$F(1,44) = 6.654$, $P = 0.0133$] accompanied by lack of a "pretreatment" (CBD vs VEH) effect [$F(1,44) = 0.653$, $P = 0.423$] or interaction

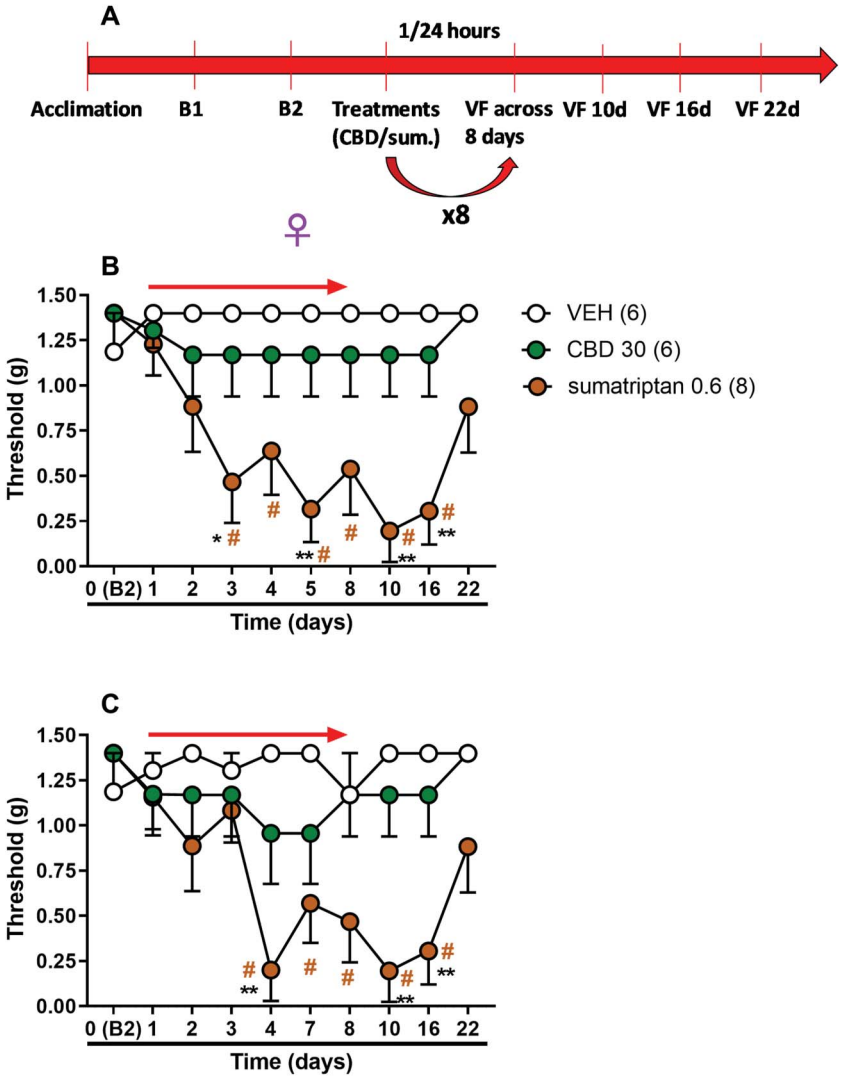

Figure 5. As opposed to sumatriptan, 8-day exposure to CBD does not alter sensory thresholds to mechanical stimulation of the periorbital region. (A) Experimental timeline: Female mice were i.p. administered daily for 8 days (x8) with CBD (30 mg/kg), sumatriptan (0.6 mg/kg), or VEH, and withdrawal thresholds were measured 1 hour and 24 hours after the injections. Periorbital withdrawal thresholds were evaluated again after the treatment period on days 10, 16, and 22. (B) Assessment of periorbital mechanical allodynia at 1 hour postinjection. (C) Assessment of allodynia 24 hours postinjection. Results are the mean ± SEM of force (g) applied through von Frey filaments using the up–down method to N = 20 mice (6-8/group). *$P < 0.05$, **$P < 0.01$ difference from VEH; #$P < 0.05$ difference from basal thresholds (B2). Red arrow, 8-day treatment period; CBD, cannabidiol; VEH, vehicle; VF, von Frey.

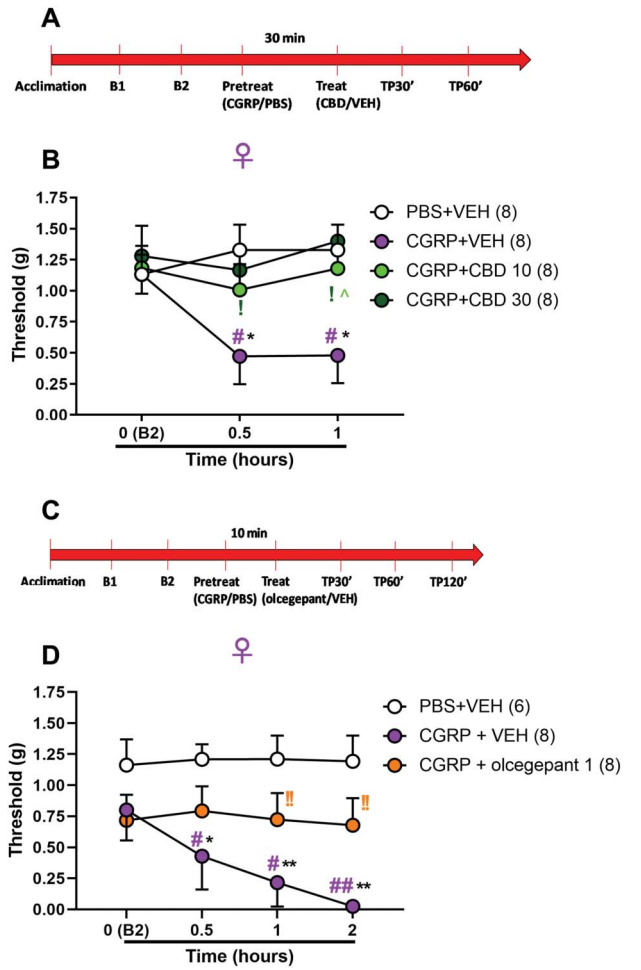

Figure 6. CBD can serve as an abortive treatment for CGRP-evoked hypersensitivity in female C57BL/6J mice. (A and C) Experimental timeline: Basal threshold of responses to mechanical stimulation was recorded in all mice. A single injection of CGRP (0.1 mg/kg) or PBS was then conducted 30 minutes before administration of CBD (10 mg/kg and 30 mg/kg) or VEH. Mechanical allodynia was assessed again at 0.5 hour and 1 hour from the last injection, that is, 1 hour and 1.5 hours from the CGRP or PBS injection. In a separate experiment, olcegepant (1 mg/kg) was injected 10 minutes after PBS or CGRP (0.1 mg/kg), with cephalic allodynia assessed 0.5 hour, 1 hour, and 2 hours later. (B and D) Both CBD and olcegepant, successfully blocked the CGRP-evoked allodynia. Results are the mean ± SEM of force (g) applied through von Frey filaments using the up–down method to female mice (total: N = 54, 32 for CBD and 22 for olcegepant testing; 6-8/group). *$P < 0.05$, **$P < 0.01$ difference from PBS. #$P < 0.05$, ##$P < 0.01$ difference from basal thresholds (B2). ^,!$P < 0.05$, !!$P < 0.01$ difference form CGRP. CBD, cannabidiol; CGRP, calcitonin gene-related peptide; TP, time point; VEH, vehicle.

[F(1,44) = 0.177, $P = 0.675$] in female mice, suggesting that CGRP led the animals to spend significantly less time in the light compartment of the DLB and that CBD failed to reverse the aversion to light induced by CGRP (**Fig. 8B**). The same analysis conducted in male mice led to a lack of a significant "treatment" effect [F(1,43) = 3.421, $P = 0.0712$], accompanied by no significant "pretreatment" effect [F(1,43) = 0.224, $P = 0.638$] or interaction [F(1,43) = 1.090, $P = 0.302$] (**Fig. 8C**).

3.10. Effect of cannabidiol on calcitonin gene-related peptide-induced anxiety in male and female mice using the elevated plus maze

Analysis of variance conducted on the EPM-related variables revealed no changes in % OAT in female mice ("pretreatment" [F(1,44) = 0.749, $P = 0.391$], "treatment" [F(1,44) = 0.687, $P = 0.411$], interaction [F(1,44) = 0.005, $P = 0.941$], **Fig. 8D**). By contrast, the same analysis conducted in male mice revealed changes in % OAT ("pretreatment x treatment" interaction [F(1,43) = 4.430, $P = 0.0411$], **Fig. 8E**). Similarly, analysis of % OAE led to no changes in females ("pretreatment" [F(1,44) = 0.117, $P = 0.733$], "treatment" [F(1,44) = 0.240, $P = 0.626$], interaction [F(1,44) = 0.701, $P = 0.406$], **Fig. 8F**) and rescued CGRP-induced reduction in % OAEs in male mice ("pre-treatment x treatment" interaction [F(1,43) = 5.225, $P = 0.0272$], **Fig. 8G**). On post hoc analysis, CGRP led to anxiogenic-like activity ($P < 0.05$ for both % OAT and OAEs), which was blocked by CBD pretreatment ($P < 0.05$ for both variables). Analysis of CAEs, an indicator of the mouse locomotor behavior, led to similar results in females and males, with CGRP eliciting a reduction in the CAEs as compared to PBS-treated mice (females: [F(1,44) = 8.646, $P = 0.00520$], males: [F(1,43) = 7.018, $P = 0.011$], **Figs. 8H and I**), which was not reversed by CBD.

3.11. Anxiety–photophobia correlations

Linear regression analyses were conducted in VEH/PBS-treated groups to determine correlations between photophobia-related and anxiety-related variables. In neither female nor male mice there was significant correlation between the % of time spent in the light compartment of the DLB and the % OAT on the EPM (**Fig. 9A**, females: $R^2 = 0.00580$, $P = 0.814$; **Fig. 9B**, males: $R^2 = 0.0471$, $P = 0.497$) or % OAEs (**Fig. 9C**, females: $R^2 = 0.0147$, $P = 0.706$; **Fig. 9D**, males: $R^2 = 0.0502$, $P = 0.483$) and CAEs (**Fig. 9E**, females: $R^2 = 0.0981$, $P = 0.321$; **Fig. 9F**, males: $R^2 = 0.0802$, $P = 0.372$), suggesting that photophobia and anxiety were likely separated, not interdependent measures. As expected, a significant correlation was observed between the EPM end points % OAT and % OAEs both in female ($R^2 = 0.466$, $P = 0.0144$, **Fig. 9G**) and male ($R^2 = 0.829$, $P = 0.0000$, **Fig. 9H**) mice.

4. Discussion

Several preclinical models have been developed to stimulate/sensitize trigeminal sensory afferents surrounding cranial blood vessels and mimic migraine in humans. A widely used model is meningeal stimulation by direct application of inflammatory compounds.[8] Other methods use systemic injection of the nitric oxide donor, nitroglycerin,[3,46] or repeated injections of the migraine relief medication sumatriptan, which paradoxically can increase sensitivity to head pain.[17] These etiologically diverse models have been used to understand the pathophysiology of headaches and to develop new therapeutics.

Considerable evidence implicates CGRP in pathophysiology of migraine.[49–51] Consistently, exogenously administered CGRP, whether delivered directly into the dura through a guide cannula, injected subcutaneously into the periorbital area, or delivered by systemic i.p. injections, can induce periorbital mechanical allodynia in laboratory animals.[2,19,20] Our findings of CGRP-induced cephalic allodynia are largely in agreement with previous studies,[19,20] as we replicate that a single CGRP exposure induces periorbital allodynia in C57BL/6J mice. However, some differences exist between our findings and others. For example, we observed that i.p. CGRP injection induced allodynia in both female and male mice, whereas dural CGRP administration elicited facial hypersensitivity only in females.[2] Another study showed no sex differences after i.p. CGRP administration of the same CGRP dose that we administered (0.1 mg/kg).[20] By

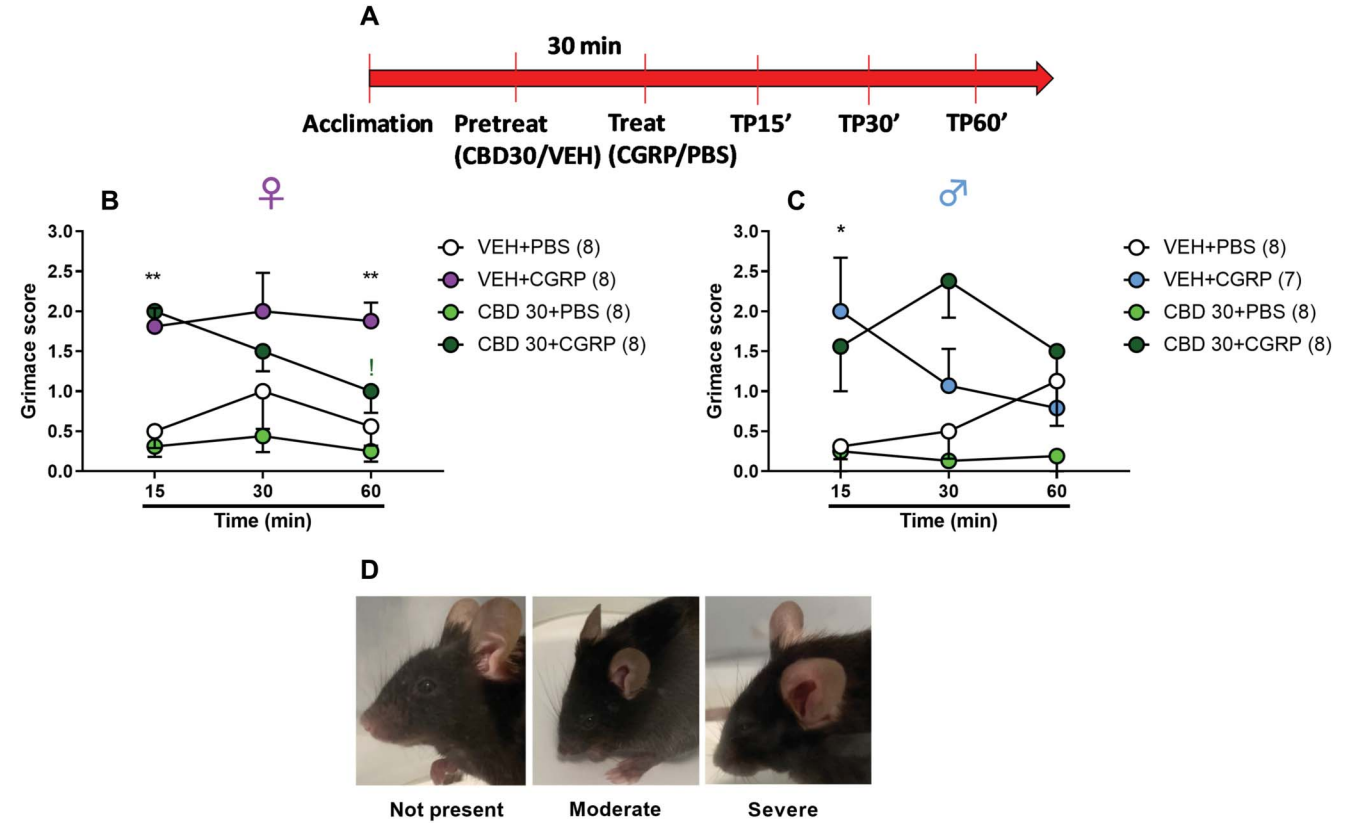

Figure 7. CBD reduces spontaneous pain traits induced by CGRP administration in female C57BL/6J mice. (A) Experimental timeline: Mice received an i.p. injection of CBD (30 mg/kg) or VEH before i.p. administration of CGRP (0.1 mg/kg) or PBS. Five facial features such as orbital tightening, nose bulge, cheek bulge, ear position, and whisker change were scored 0 to 2 (0: not present; 1: moderately visible; and 2: severe) by 2 blinded individuals 15, 30, and 60 minutes after the last injection. Values are the mean ± SEM of the grimace scores (previous score averages of individual readers were averaged for an overall score at each time point) observed in (B) female (N = 32, 8/group) and (C) male (N = 31, 7-8 group) mice (total: N = 63). (D) Three-point scale (not present, 0; moderate, 1; and severe, 2) used to calculate the grimace scores. *$P < 0.05$, **$P < 0.01$ difference from VEH + PBS. !$P < 0.05$ difference from CGRP. CBD, cannabidiol; CGRP, calcitonin gene-related peptide; TP, time point; VEH, vehicle.

contrast, we observed that levels of basal allodynic responses in female and male mice were different, with females surprisingly showing less sensitivity to tactile stimulation than males. Differences in routes of CGRP administration, methods used to assess cephalic allodynia, and acclimation procedures are critical factors that can account for such discrepancies.

One important topic in migraine research is understanding the progression of migraine from an episodic to a chronic disorder. Animal models of chronic migraine-associated pain are critical tools for studying the transition from episodic to chronic migraine, as well as for identifying and screening novel acute and preventive migraine therapies.[46,55] We approached the problem of developing a model of chronic migraine by performing daily injections of the known migraine trigger CGRP. We found different responses in female and male mice, with females not always showing acute mechanical allodynia after each CGRP exposure but displaying a progressively increased basal sensitivity, which reached its maximum after 4 continuous CGRP exposures. The treatment was therefore discontinued after 4 days. This effect was not due to possible associative learning resulting from repeated testing,[41,46] as demonstrated by stable responses observed in vehicle-treated female mice. By contrast, male mice, which once again showed increased levels of basal mechanical sensitivity as compared to females, maintained similar levels of basal allodynia across the 4 days of CGRP treatment; however, CGRP-treated males showed somehow different allodynic responses from those not receiving CGRP because the latter decreased mechanical responses after repeated mechanical stimulation across days. Our findings are consistent with a previous study showing that female mice developed increased levels of basal mechanical allodynia more quickly than males after long-term intermittent administration of nitroglycerin.[46] The sexually dimorphic responses we observed after repeated CGRP administration, with females that seem more vulnerable than males in displaying progressively increased headache-like responses, support the translational relevance of our chronic migraine model.

Except for a few clinical studies where combinations of CBD and Δ^9-THC were tested,[42,48] there is no available information on the use of CBD as migraine treatment.[39] We show here that CBD, administered to mice before a single exogenous CGRP exposure, successfully reversed periorbital mechanical allodynia. Because facial cutaneous allodynia is a symptom that can be found in almost 80% of patients experiencing a migraine attack,[10] our data strongly suggest a beneficial effect of CBD in protecting from occurrence of episodic migraine attacks. Notably, the antiallodynic effect of CBD was observed in mice of both sexes and occurred at doses that did not alter parameters of locomotor activity, thus excluding the possibility that the CBD antiallodynic effects were secondary to possible nonspecific effects on the mouse locomotor behavior. However, no complete dose–response curve for CBD has been performed, so the present data do not allow the estimation of CBD potency in this response. Remarkable CBD effects were also observed in female mice

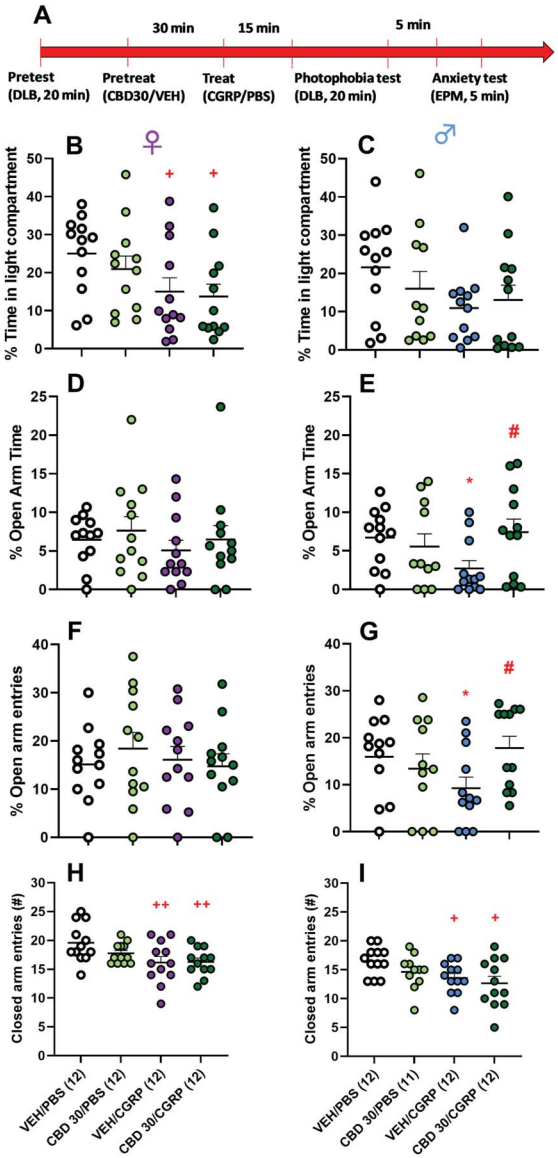

Figure 8. CBD fails to block CGRP-induced photophobia in female, whereas successfully reverses CGRP-induced anxiety-like behavior in male C57BL/6J mice. (A) Experimental timeline: Mice (total: N = 95) of both sexes (females: N = 12/group; males: N = 11-12/group) were allowed to explore the dark–light box (DLB) in a 20-minute pretest session. The day after, the mice received i.p. pretreatment of CBD (30 mg/kg) or VEH and, 30 minutes later, i.p. treatment of CGRP (0.1 mg/kg) or PBS. Fifteen minutes after the last injection, mice were allowed to re-experience the DLB for 20 minutes. After the photophobia test, mice were returned to their home cages. Five minutes later, a 5-minute elevated plus maze (EPM) was performed in the same animals. (B) CGRP (0.1 mg/kg) induced significant photophobia that was not prevented by CBD pretreatment in female mice. (C) Same CGRP dose did not produce a significant light-aversive behavior in male mice. (D) CGRP failed to produce an anxiogenic-like effect in female mice as shown by the percent (%) of time spent exploring the open arms of the EPM (open arm time). However, (E) CGRP led to reduced % open arm time, rescued by CBD pretreatment, in male mice. (F) CGRP failed to produce changes in the % open arm entries in females, whereas (G) induced reduced % open arm entries in males, an effect reversed by CBD pretreatment. (H) CGRP-treated females showed decreased exploration of the EPM maze (decreased closed arm entries). (I) Elevated anxiety of CGRP-treated male mice was accompanied by a decreased number of closed arm entries. Values are presented as mean percent (%) of time spent in the light compartment (DLB) and % ± SEM of open arm time and entries and mean ± SEM number of closed arm entries (EPM). $+P < 0.05$, $++P < 0.01$ difference from PBS-treated groups; $*P < 0.05$ vs VEH/PBS; $\#P < 0.05$ difference VEH/CGRP-CBD 30/CGRP. CBD, cannabidiol; CGRP, calcitonin gene-related peptide; VEH, vehicle.

tested on the chronic CGRP model, with both doses of CBD examined being able to reliably prevent acute mechanical allodynia after each CGRP exposure, as well as progressive and sustained basal allodynia. These results suggest that CBD may be effective both in re-establishing the lowered thresholds that result from CGRP-induced changes to meningeal perivascular nociceptors and in preventing the increase in the responsiveness (sensitization) of central neurons responsible for pain processing,[9,10] which is one of the mechanisms underlying the progression from episodic to chronic migraine.[1] After determining that CBD can be effective in preventing chronic headache, we assessed whether CBD could also be effective as a treatment for ongoing migraine-like attacks. We found in female mice that CBD abolished cephalic allodynia even when administered after CGRP, producing an effect similar to that of olcegepant, a CGRP receptor antagonist designed as acute treatment of migraine, whose development was discontinued due to side effects.[21] Collectively, these data suggest that CBD can serve as both a preventive tool and an abortive treatment for migraine. Importantly, exposure of uninjured female mice to CBD over several days demonstrated that CBD can be safely and repeatedly administered with reduced risk of causing medication overuse headache, an effect that can result from repeated exposure to triptans and opioids.[4,17]

Cannabidiol effects were then examined in migraine-like symptoms other than allodynia. Facial grimace scale[36] was used as a surrogate readout of spontaneous pain. Increase in facial signs of discomfort after peripherally administered CGRP was reported in a study that did not distinguish between female and male mice.[47] In agreement with that study, we found a significantly greater CGRP-induced pain response in mice of both sexes. However, only females showed sustained facial grimace (up to 60 minutes), which was significantly attenuated by CBD. Systemically administered CGRP also increased light sensitivity in female mice to a larger extent than in males under our experimental conditions, which supports previous evidence of CGRP-induced photophobia.[34,40] As the DLB is a test commonly used to assess anxiety-like behaviors, and also used to examine light sensitivity, making interpretations of the photophobia test particularly challenging,[56] we have taken steps to keep the photophobic and anxious components of the CGRP-induced migraine model experimentally separated and detected. First, we conducted the photophobia assay in conditions of habituation to the DLB testing chambers to avoid the neophobia or anxiety due to novelty. Second, an EPM test was conducted right after the photophobia test using the same mice under the same treatment conditions for both assays to determine whether treatment with CGRP, expected to reduce the time spent in the light compartment of the DLB, also led to anxiogenic-like responses in the EPM. We found differences in the responses to the 2 tests (eg, CGRP elicited light-aversive behavior, but not anxiogenic-like behavior in female mice), suggesting that photophobia data cannot be explained for CGRP-induced changes in anxiety-like activity. Furthermore, linear regression analyses did not show correlations between photophobia-related and anxiety-related variables, which is another indication of proper assessment of both symptoms.

The finding that CBD fails to protect from photophobia is surprising because CBD has proved very effective in managing another sensory amplification of migraine such as cutaneous allodynia. However, the neural circuits underlying migraine-relevant pain circuits and photophobia are different[5] and it is possible that CBD produces effects through the former network,

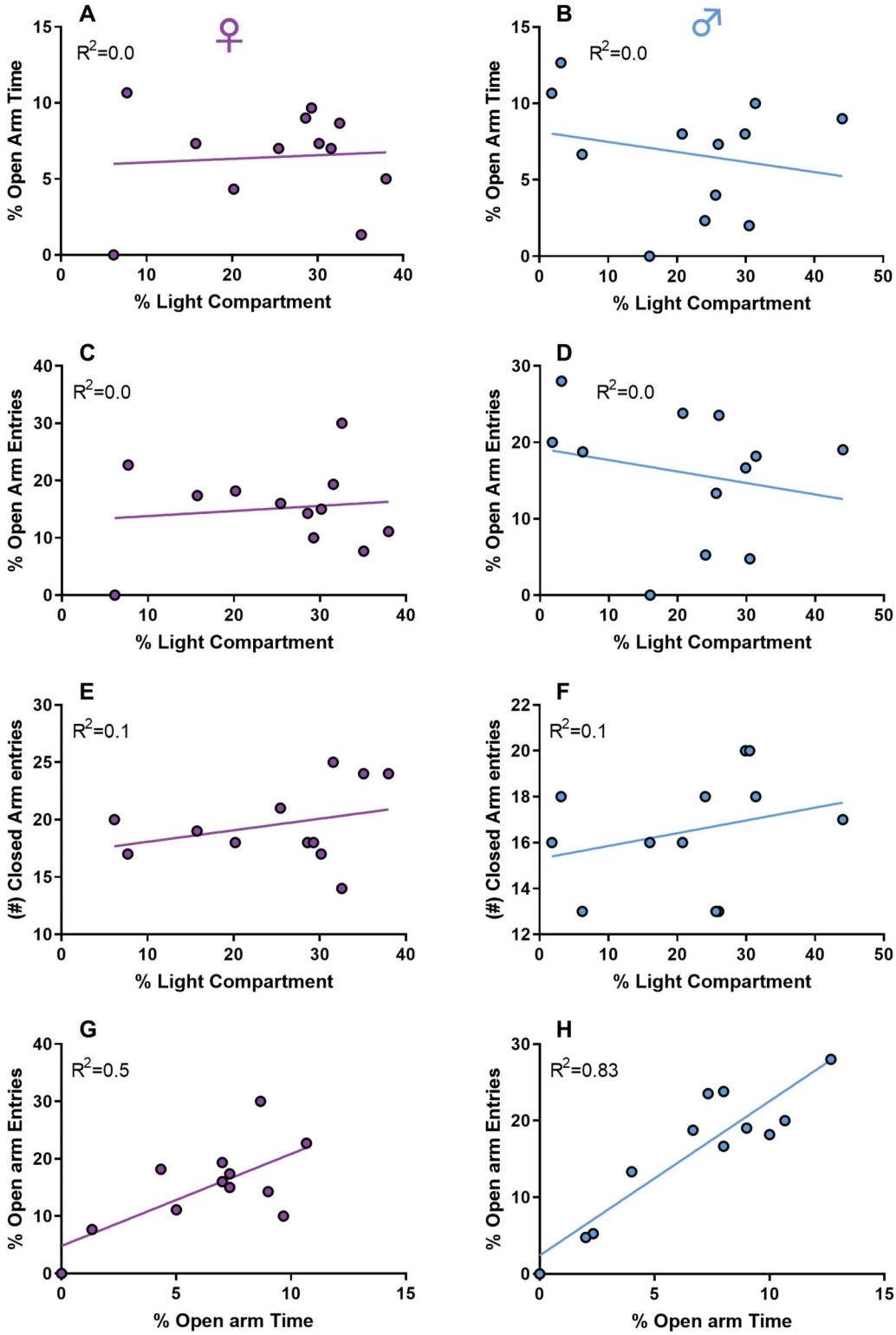

Figure 9. Anxiety parameters do not correlate with photophobia. Linear regression analyses were conducted to determine correlations between photophobia-related and anxiety-related variables (N = 24, 12 females and 12 males). There was no significant correlation between (A and B) % of time spent on the light compartment of the dark–light box (DLB) and the % of time spent on the open arm of the elevated plus maze (EPM) in female or male mice, respectively. Neither there was a significant correlation between (C and D) % of time spent on the light compartment of the DLB and the % of entries onto the open arms of the EPM in females and males, as well as between (E and F) % of time spent in the light compartment and exploration of the EPM closed arms in female or male mice, respectively. As expected, panels (G and H) show positive correlations between the % of open arm time and entries both in female and male mice.

but not through the latter. Because more than 65 molecular targets of CBD have been identified, although many of these seem to be activated at very high concentrations,[29] it is hard to establish the exact mechanism through which CBD exerts its therapeutic effects. Within the cannabinoid system, CBD is a CB_1 receptor antagonist and a CB_2 inverse agonist.[54] Cannabidiol can

also interact with various TRPV channels[29] and is an inhibitor of the fatty acid amide hydrolase, which has been demonstrated to be a potential target for antimigraine therapy.[27] Furthermore, CBD may also act through the serotonin 1A (5-HT_{1A}) receptors,[11] the orphan receptor GPR 55,[38] and PPAR activation.[44] Thus, one of these mechanisms or a combination of them may underlie the CBD's beneficial effects we observed on migraine-like states. In blocking somatic allodynia, it is probable that CBD interferes with the mechanism of CGRP-induced peripheral sensitization of nociceptive signaling from the dura mater, which is considered a necessary event in the headache phase of attacks.[31]

In conclusion, by modeling acute and chronic migraine-like states in mice, we report that CBD blocks head sensitivity when injected before or after the migraine-triggering substance CGRP, suggesting that CBD can be effective both as a preventive tool and as a treatment for headaches. Cannabidiol can also be effective in preventing chronic migraine and seems to be devoid of the risk to induce medication overuse headache on repeated administrations.

Conflict of interest statement

L. Toll is stockholder of Phoenix PharmaLabs. The other authors declare no conflicts of interest.

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References

[1] Andreou AP, Edvinsson L. Mechanisms of migraine as a chronic evolutive condition. J Headache Pain 2019;20:117.
[2] Avona A, Burgos-Vega C, Burton MD, Akopian AN, Price TJ, Dussor G. Dural calcitonin gene-related peptide produces female-specific responses in rodent migraine models. J Neurosci 2019;39:4323–31.
[3] Bates EA, Nikai T, Brennan KC, Fu YH, Charles AC, Basbaum AI, Ptacek LJ, Ahn AH. Sumatriptan alleviates nitroglycerin-induced mechanical and thermal allodynia in mice. Cephalalgia 2010;30:170–8.
[4] Bigal ME, Serrano D, Buse D, Scher A, Stewart WF, Lipton RB. Acute migraine medications and evolution from episodic to chronic migraine: a longitudinal population-based study. Headache 2008;48:1157–68.
[5] Brennan KC, Pietrobon D. A systems neuroscience approach to migraine. Neuron 2018;97:1004–21.
[6] Broner S, Bobker S, Klebanoff L. Migraine in women. Semin Neurol 2017; 37:601–10.
[7] Brunori G, Weger M, Schoch J, Targowska-Duda K, Barnes M, Borruto AM, Rorick-Kehn LM, Zaveri NT, Pintar JE, Ciccocioppo R, Toll L, Cippitelli A. NOP receptor antagonists decrease alcohol drinking in the dark in C57BL/6J mice. Alcohol Clin Exp Res 2019;43:2167–78.
[8] Burstein R, Jakubowski M. Analgesic triptan action in an animal model of intracranial pain: a race against the development of central sensitization. Ann Neurol 2004;55:27–36.
[9] Burstein R, Jakubowski M, Garcia-Nicas E, Kainz V, Bajwa Z, Hargreaves R, Becerra L, Borsook D. Thalamic sensitization transforms localized pain into widespread allodynia. Ann Neurol 2010;68:81–91.
[10] Burstein R, Yarnitsky D, Goor-Aryeh I, Ransil BJ, Bajwa ZH. An association between migraine and cutaneous allodynia. Ann Neurol 2000;47:614–24.
[11] Campos AC, Guimaraes FS. Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. Psychopharmacology (Berl) 2008;199: 223–30.
[12] Campos AC, Moreira FA, Gomes FV, Del Bel EA, Guimaraes FS. Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. Philos Trans R Soc B Biol Sci 2012; 367:3364–78.
[13] Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994; 53:55–63.
[14] Christensen SL, Hansen RB, Storm MA, Olesen J, Hansen TF, Ossipov M, Izarzugaza JMG, Porreca F, Kristensen DM. Von Frey testing revisited: provision of an online algorithm for improved accuracy of 50% thresholds. Eur J Pain 2020;24:783–90.
[15] Costa B, Colleoni M, Conti S, Parolaro D, Franke C, Trovato AE, Giagnoni G. Oral anti-inflammatory activity of cannabidiol, a non-psychoactive constituent of cannabis, in acute carrageenan-induced inflammation in the rat paw. Naunyn Schmiedebergs Arch Pharmacol 2004;369:294–9.
[16] Costa B, Giagnoni G, Franke C, Trovato AE, Colleoni M. Vanilloid TRPV1 receptor mediates the antihyperalgesic effect of the nonpsychoactive cannabinoid, cannabidiol, in a rat model of acute inflammation. Br J Pharmacol 2004;143:247–50.
[17] De Felice M, Ossipov MH, Wang R, Lai J, Chichorro J, Meng I, Dodick DW, Vanderah TW, Dussor G, Porreca F. Triptan-induced latent sensitization: a possible basis for medication overuse headache. Ann Neurol 2010;67:325–37.
[18] De Gregorio D, McLaughlin RJ, Posa L, Ochoa-Sanchez R, Enns J, Lopez-Canul M, Aboud M, Maione S, Comai S, Gobbi G. Cannabidiol modulates serotonergic transmission and reverses both allodynia and anxiety-like behavior in a model of neuropathic pain. PAIN 2019;160:136–50.
[19] De Logu F, Landini L, Janal MN, Li Puma S, De Cesaris F, Geppetti P, Nassini R. Migraine-provoking substances evoke periorbital allodynia in mice. J Headache Pain 2019;20:18.
[20] De Logu F, Nassini R, Hegron A, Landini L, Jensen DD, Latorre R, Ding J, Marini M, Souza Monteiro de Araujo D, Ramirez-Garcia P, Whittaker M, Retamal J, Titiz M, Innocenti A, Davis TP, Veldhuis N, Schmidt BL, Bunnett NW, Geppetti P. Schwann cell endosome CGRP signals elicit periorbital mechanical allodynia in mice. Nat Commun 2022;13:646.
[21] de Vries T, Villalon CM, MaassenVanDenBrink A. Pharmacological treatment of migraine: CGRP and 5-HT beyond the triptans. Pharmacol Ther 2020;211:107528.
[22] Diener HC, Solbach K, Holle D, Gaul C. Integrated care for chronic migraine patients: epidemiology, burden, diagnosis and treatment options. Clin Med (Lond) 2015;15:344–50.
[23] Edvinsson L, Haanes KA, Warfvinge K, Krause DN. CGRP as the target of new migraine therapies—successful translation from bench to clinic. Nat Rev Neurol 2018;14:338–50.
[24] Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods 2009;41:1149–60.
[25] Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. Ann Neurol 1990;28:183–7.
[26] Goadsby PJ, Holland PR, Martins-Oliveira M, Hoffmann J, Schankin C, Akerman S. Pathophysiology of migraine: a disorder of sensory processing. Physiol Rev 2017;97:553–622.
[27] Greco R, Demartini C, Zanaboni AM, Piomelli D, Tassorelli C. Endocannabinoid system and migraine pain: an update. Front Neurosci 2018;12:172.
[28] Hoskin KL, Kaube H, Goadsby PJ. Sumatriptan can inhibit trigeminal afferents by an exclusively neural mechanism. Brain 1996;119:1419–28.

[29] Ibeas Bih C, Chen T, Nunn AV, Bazelot M, Dallas M, Whalley BJ. Molecular targets of cannabidiol in neurological disorders. Neurotherapeutics 2015;12:699–730.
[30] Iyengar S, Johnson KW, Ossipov MH, Aurora SK. CGRP and the trigeminal system in migraine. Headache 2019;59:659–81.
[31] Iyengar S, Ossipov MH, Johnson KW. The role of calcitonin gene-related peptide in peripheral and central pain mechanisms including migraine. PAIN 2017;158:543–59.
[32] Jensen R, Stovner LJ. Epidemiology and comorbidity of headache. Lancet Neurol 2008;7:354–61.
[33] Juhasz G, Zsombok T, Jakab B, Nemeth J, Szolcsanyi J, Bagdy G. Sumatriptan causes parallel decrease in plasma calcitonin gene-related peptide (CGRP) concentration and migraine headache during nitroglycerin induced migraine attack. Cephalalgia 2005;25:179–83.
[34] Kaiser EA, Kuburas A, Recober A, Russo AF. Modulation of CGRP-induced light aversion in wild-type mice by a 5-HT(1B/D) agonist. J Neurosci 2012;32:15439–49.
[35] Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. J Pharmacol Pharmacotherapeutics 2010;1:94–9.
[36] Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AM, Ferrari MD, Craig KD, Mogil JS. Coding of facial expressions of pain in the laboratory mouse. Nat Methods 2010;7:447–9.
[37] Lassen LH, Haderslev PA, Jacobsen VB, Iversen HK, Sperling B, Olesen J. CGRP may play a causative role in migraine. Cephalalgia 2002;22:54–61.
[38] Leimuranta P, Khiroug L, Giniatullin R. Emerging role of (Endo) Cannabinoids in migraine. Front Pharmacol 2018;9:420.
[39] Lochte BC, Beletsky A, Samuel NK, Grant I. The use of cannabis for headache disorders. Cannabis Cannabinoid Res 2017;2:61–71.
[40] Mason BN, Kaiser EA, Kuburas A, Loomis MCM, Latham JA, Garcia-Martinez LF, Russo AF. Induction of migraine-like photophobic behavior in mice by both peripheral and central CGRP mechanisms. J Neurosci 2017;37:204–16.
[41] Moye LS, Pradhan AAA. Animal model of chronic migraine-associated pain. Curr Protoc Neurosci 2017;80:9.60.1–9.
[42] Nicolodi MSV, Terrine A. Therapeutic use of cannabinoids—dose finding, effects, and pilot data of effects in chronic migraine and cluster headache.Abstract presentation at 3rd congress of the European Academy of Neurology (EAN); 24—27 June, 2017, Amsterdam.
[43] Noseda R, Burstein R. Migraine pathophysiology: anatomy of the trigeminovascular pathway and associated neurological symptoms, cortical spreading depression, sensitization, and modulation of pain. PAIN 2013;154(suppl 1):S44–53.
[44] O'Sullivan SE. An update on PPAR activation by cannabinoids. Br J Pharmacol 2016;173:1899–910.
[45] Peterlin BL, Gupta S, Ward TN, Macgregor A. Sex matters: evaluating sex and gender in migraine and headache research. Headache 2011;51:839–42.
[46] Pradhan AA, Smith ML, McGuire B, Tarash I, Evans CJ, Charles A. Characterization of a novel model of chronic migraine. PAIN 2014;155:269–74.
[47] Rea BJ, Wattiez AS, Waite JS, Castonguay WC, Schmidt CM, Fairbanks AM, Robertson BR, Brown CJ, Mason BN, Moldovan-Loomis MC, Garcia-Martinez LF, Poolman P, Ledolter J, Kardon RH, Sowers LP, Russo AF. Peripherally administered calcitonin gene-related peptide induces spontaneous pain in mice: implications for migraine. PAIN 2018;159:2306–17.
[48] Rhyne DN, Anderson SL, Gedde M, Borgelt LM. Effects of medical marijuana on migraine headache frequency in an adult population. Pharmacother J Hum Pharmacol Drug Ther 2016;36:505–10.
[49] Russell FA, King R, Smillie SJ, Kodji X, Brain SD. Calcitonin gene-related peptide: physiology and pathophysiology. Physiol Rev 2014;94:1099–142.
[50] Russo AF. Calcitonin gene-related peptide (CGRP): a new target for migraine. Annu Rev Pharmacol Toxicol 2015;55:533–52.
[51] Russo AF. CGRP as a neuropeptide in migraine: lessons from mice. Br J Clin Pharmacol 2015;80:403–14.
[52] Silberstein S, Lenz R, Xu C. Therapeutic monoclonal antibodies: what headache specialists need to know. Headache 2015;55:1171–82.
[53] Targowska-Duda KM, Ozawa A, Bertels Z, Cippitelli A, Marcus JL, Mielke-Maday HK, Zribi G, Rainey AN, Kieffer BL, Pradhan AA, Toll L. NOP receptor agonist attenuates nitroglycerin-induced migraine-like symptoms in mice. Neuropharmacology 2020;170:108029.
[54] Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG. Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. Br J Pharmacol 2007;150:613–23.
[55] Tipton AF, Tarash I, McGuire B, Charles A, Pradhan AA. The effects of acute and preventive migraine therapies in a mouse model of chronic migraine. Cephalalgia 2016;36:1048–56.
[56] Vuralli D, Wattiez AS, Russo AF, Bolay H. Behavioral and cognitive animal models in headache research. J Headache Pain 2019;20:11.