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A Novel Anti-Inflammatory Formulation Comprising Celecoxib and Cannabidiol Exerts Antidepressant and Anxiolytic Effects

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Abstract

Background: Ample research shows that anti-inflammatory drugs, particularly celecoxib, exert antidepressant effects, especially in patients with microglia activation. However, substantial cardiovascular adverse effects limit celecoxib's usefulness. Given that cannabidiol (CBD) exerts anti-inflammatory, microglia-suppressive, and antidepressant effects, we hypothesized that it may potentiate the therapeutic effects of celecoxib.

Methods: The effects of celecoxib, CBD, and their combination were examined in murine models of antidepressant- and anxiolytic-like behavioral responsiveness, including the forced swim test (FST), elevated plus maze (EPM), lipopolysaccharide (LPS)-induced neuroinflammation, and chronic social defeat stress (CSDS), as well as in microglia cell cultures.

Results: Acute administration of a combination of celecoxib plus CBD, at doses that had no effects by themselves (10 and 5 mg/kg, respectively), produced significant antidepressant- and anxiolytic-like effects in the FST and EPM, in male and female mice. In the LPS model, combinations of celecoxib (10 or 20 mg/kg) plus CBD (30 mg/kg) reversed the anxiety-like behavior in the open-field test (OFT) and anhedonia in the sucrose preference test (SPT), with minimal effects of celecoxib or CBD by themselves. In the CSDS paradigm, a combination of celecoxib plus CBD (each at 30 mg/kg) reversed the deficits in the OFT, EPM, social exploration, and SPT, whereas celecoxib or CBD by themselves had partial effects. In BV2 microglia cultures stimulated with LPS or α-synuclein, CBD markedly potentiated the suppressive effects of celecoxib over TNFα (tumor necrosis factor-α) and IL (interleukin)-1β secretion.

Conclusions: Combinations of celecoxib plus CBD produce efficacious antidepressant- and anxiolytic-like effects, which may depend on their synergistic microglia-suppressive effects.

Keywords: depression; anxiety; cyclooxygenase-2; microglia; cannabinoid

Introduction

Over the past three decades, it became evident that inflammatory processes, in general, and microglia activation, in particular, play an important role in the etiology and pathophysiology of depression,[1–7] as evidenced by findings that: (1) Chronic infectious diseases and most neurological diseases are accompanied by inflammation and microglia activation along with a high incidence of depression[8–10]; (2) many depressed patients exhibit elevated levels of peripheral inflammatory cytokines and microglia activation[1,11–13]; (3) a substantial percentage of cancer or hepatitis C patients who receive inflammatory cytokine immunotherapy develop major depression[14]; (4) antidepressant drugs produce anti-inflammatory effects, particularly in drug responders[15]; (5) depression is associated with genetic variation and differential expression of microglia-relevant genes[16,17]; (6) inflammatory processes are associated with anxiety,[18] which is often comorbid with depression[19]; (7) healthy human volunteers, as well as experimental animals exposed to immune challenges, develop depressed mood and associated behavioral

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symptoms, which are highly correlated with inflammatory cytokine levels and can be reversed by antidepressant drugs or cytokine antagonists[20–23]; (8) inflammation and microglia activation are involved in the responsiveness to stress, which both in humans and in animal models has been implicated as a major trigger for depression.[24–27]

Consistently with the established role of inflammatory processes and microglia activation in the pathophysiology of depression, ample pre-clinical research and dozens of clinical trials revealed that anti-inflammatory drugs, including nonsteroidal anti-inflammatory drugs (NSAIDs), cytokine inhibitors, glucocorticoids, statins, and minocycline, can exert potent antidepressant effects.[28–30]

The most studied anti-inflammatory drug in depressed patients has been the selective cyclooxygenase (COX)-2 inhibitor celecoxib. This NSAID was first shown to be an efficacious antidepressant as add-on to the conventional antidepressant drug reboxetine in 2006,[31] and since then, it was examined in more than a dozen randomized clinical trials, inducing antidepressant and anxiolytic effects in most of these studies, as either add-on or mono-therapy.[30,32,33] Celecoxib was also found to attenuate depressive- and anxiety-like symptoms in pre-clinical models of depression, induced by acute[34,35] or chronic[36] inflammatory challenges or by chronic unpredictable mild stress (CUMS).[34] These effects were associated with the microglia-suppressive and anti-inflammatory effects of celecoxib.[34,37,38]

One factor that limits the wide use of celecoxib as an antidepressant is the substantial and sometimes severe cardiovascular adverse effects that could appear after administration of this drug, particularly for long periods.[39] Thus, it is suggested that a dose-sparing combination of celecoxib with an additional efficacious compound would be beneficial.

Previous pre-clinical research demonstrated that cannabidiol (CBD) induces antidepressant effects in almost all known models of depression, including the forced swim and tail suspension tests, chronic stress, learned helplessness, olfactory bulbectomy, genetic manipulations, and inflammatory and diabetic conditions.[40–44] CBD was also shown, in both clinical and pre-clinical research, to induce marked anxiolytic effects.[45,46] These effects of CBD were found to be mediated by modulation of the endocannabinoid system, which is involved in the etiology and pathophysiology of depression and anxiety,[47] as well as by direct effects on other depression-related processes, such as serotonergic neurotransmission.[48] Importantly, CBD was shown to suppress the synthesis of prostaglandins[49] and to exert anti-inflammatory and microglia-suppressive effects,[50,51] which were proposed to contribute to its antidepressant efficacy,[52] and thus could potentiate similar effects of celecoxib.

In the present study, we aimed to assess the possible synergistic antidepressant and anxiolytic effects of celecoxib and CBD, by themselves or in combination, in several murine models of antidepressant- and anxiolytic-like behavioral responsiveness. As a first attempt to examine the role of microglia in mediating the effects of celecoxib plus CBD, we examined the interactive effects of these compounds in microglia cell cultures stimulated with immune challenges (lipopolysaccharide [LPS] or α-synuclein). We report that formulations comprising both celecoxib and CBD induce greater antidepressant and anxiolytic effects than each of these compounds by itself, possibly due to the potentiated inhibitory effects of these combinations on microglia activation.

Materials and Methods

Subjects

In the experiments with acute models (the forced swim and elevated plus maze [EPM] tests) and the LPS model, subjects were 4- to 7-month-old male and female C57BL/6 mice (Envigo, Israel). Independent groups of mice were used for each of these behavioral tests. In the LPS-induced depressive-like behavior, subjects were 4- to 7-month-old male C57BL/6 mice (Envigo). In the chronic social defeat stress (CSDS) paradigm, 11–13 weeks old male C57BL/6 mice were used as subjects, and 4- to 6-month-old male CD-1 retired-breeder mice (Envigo) were used as aggressors. Mice were housed two to three per cage, kept in an air-conditioned room (23°C ± 2°C), and given *ad libitum* access to food and water. The mice were kept in a reversed light/dark cycle, with lights off from 7 a.m. to 7 p.m. All experiments were approved by the Hebrew University of Jerusalem Ethics Committee on Animal Care and Use.

Reagents

LPS (from *Escherichia coli* serotype O111:B4; Sigma, Rehovot, Israel) was dissolved in Dulbecco's phosphate-buffered saline (D-PBS; Sigma) to a prestock concentration of 1 mg/mL, aliquoted, and stored at −80°C. For *in vitro* studies, stock solution of LPS at 10 μg/mL was prepared using sterile D-PBS, followed by filtering using 0.22 μm membrane, aliquoting, and

storing in −20°C till further use. For *in vivo* studies LPS was injected intraperitoneally (i.p.) at a dose of 330 μg/kg.

Oligomerized α-synuclein was prepared by reconstituting lyophilized recombinant human α-synuclein (S7820; Sigma) in sterile, molecular grade water to the concentration of 50 μM (1 mg/mL), according to the manufacturer's instructions. To induce oligomerization, the α-synuclein solution was placed for 24 h on a shaker with agitation of 80 rpm and at 37°C. Oligomerized α-synuclein preparations were aliquoted and stored in −20°C. The protocol used for α-synuclein oligomerization was based on previous studies,[53,54] modified to optimize efficient oligomerization (including incubation time and agitation strength), and verified by gel electrophoresis, as well as by determination of tumor necrosis factor-α (TNFα) released by BV2 microglia cells in response to various α-synuclein preparations (as determined using enzyme-linked immunosorbent assays [ELISAs]).

Celecoxib (Sigma) powder was stored at room temperature (RT) in a desiccator compartment. For *in vitro* studies, celecoxib and CBD stock solutions at 10 mM were prepared in pure absolute ethanol 100% (BioLab Ltd, Jerusalem, Israel) and stored in −20°C. For *in vivo* studies celecoxib and CBD were first dissolved in 100% ethanol, to which Cremophor (Sigma) and saline were sequentially added, at a ratio of 1:1:18, respectively. The solution was thoroughly vortexed after each substance addition.

Plant-derived CBD was purchased from BOL Pharma (Revadim, Israel). HPLC/UV-based analysis revealed that in addition to CBD the preparation contained an overall of 0.8% impurities, with traces of CBDV (0.6%) and <0.05% of THC and any other individual impurity.

Behavioral tests and paradigms

The Porsolt forced swim test. This test measures coping strategy during exposure to inescapable stress, which is a major contributing factor for the development of depression in humans. Indeed, drugs that have efficacious antidepressant effect in humans have been shown to promote active coping strategy in the forced swim test (FST). Therefore, the measures of immobility (passive coping) and the latency to exhibit the first immobility bout in the FST are considered as measures of despair-like behavioral responses to stress.[55] In the FST, mice were individually placed for 6 min in a Plexiglass cylinder (with a 20 cm diameter and 40 cm height), containing 15 cm-depth water at 23–25°C, and their behavior was videotaped for this entire duration. The time spent in immobility, defined as the absence of all movement except motions required to maintain the animal's head above the water, and the latency to first immobility episode were recorded by an observer who was blinded with respect to the group allocation.

The EPM test. This is the most popular test for assessing anxiety-like responses in rodents, and it has been validated to assess the antianxiety effects of various pharmacological agents.[56] The apparatus, situated 40 cm above the floor, consists of a plus-like shaped maze with two white plastic closed arms and two opposite white open arms. Each arm is 30 cm long and 6 cm wide. Each mouse was placed invariably in the center of the EPM, with its face toward an open arm. Behavior in the maze was recorded for 4 min and coded using EthoVision XT video tracking system and software (Noldus, The Netherlands). The maze was thoroughly cleaned with ethanol and with tap water and dried between subjects to eliminate any odor cues. The level of anxiety was assessed by measuring the time (in sec) spent in the open arms, as well as by the number of entries into the open arms. The total distance moved and the total number of entries to all arms were assessed as indicators of locomotor and exploratory activity.

The open-field test. This is a common test for measuring the locomotor, exploratory, and anxiety-like behavior in rodents.[57] Each subject was introduced into a white 80×80 cm^2 arena, with 40 cm high walls. Mice were placed invariably in the center of the arena. The distance travelled (cm), the velocity of the movement (cm/sec), and the time spent in the center area of the arena (sec) were automatically measured over a 5-min period, using EthoVision XT video tracking system and software. The arena was thoroughly cleaned with ethanol and with tap water and dried between subjects to eliminate any odor cues. In this test, the measured locomotor activity reflects not only motor capacity but also particularly the innate motivation for spatial exploration (which increases the distance travelled) and the anxiety-like level (which reduces the distance travelled, in general, and particularly the distance and time spent in the center of the open field).

The social exploration test. This test measures social motivation, anxiety, and avoidance, which are commonly

comorbid with depressive symptomatology.[58] Each subject was placed in an observation cage and allowed to habituate to the cage for 8 min. Following the habituation, a male juvenile (3 weeks old) mouse was placed in the cage. Social exploration (SE), defined as the time of near contact between the nose of the subject and the juvenile conspecific, was then recorded for 2 min, using computerized in-house software. The cages were thoroughly cleaned with ethanol and with tap water and dried between subjects to eliminate any odor cues. SE time was encoded by an investigator who was blinded to the group allocation.

The sucrose preference test. In this test, reduction in the preference for sucrose represents the development of anhedonia, a core symptom of depression.[59] Before the initiation of each experiment, subjects underwent four daily sessions of sucrose adaptation, in which the standard water bottle was replaced, for 6 h during the dark phase of the circadian cycle, with two graduated drinking tubes, one containing tap water and the other containing 1% sucrose solution. During the sucrose preference tests (SPTs), conducted at the beginning and end of the experiment, the regular drinking bottles were replaced overnight by two graduated drinking tubes, one containing tap water and the other 1% sucrose solution. Sucrose preference was calculated as the percentage of sucrose consumption out of the total drinking volume.

Acute paradigms for assessment of antidepressant- and anxiolytic-like behaviors

The FST and the EPM paradigms were used to assess the antidepressant- and anxiolytic-like effects of Clx, CBD, and their combination. For each of the two behavioral tests, an independent group of male and female mice was randomly assigned into four treatment groups. Following body weight measurement, the mice received an i.p. injection (in a volume of 10 mg/kg) of either Vehicle, Clx 10 mg/kg (Clx10), CBD 5 mg/kg (CBD5), or a combination of celecoxib and CBD, at these doses (Clx:CBD). The specific doses were chosen based on the scientific literature on the acute effects of Clx or CBD in the FST and EPM paradigms. To investigate the possible mutual potentiation of the two drugs, we chose doses that were just below those shown to have an effect in previous reports in this area.[60–62] Control mice were injected with the vehicle only. One hour following the injections, the mice were subjected to the FST (in one experiment) or to the EPM (in another experiment), as described above.

The LPS model

The LPS model of depression was developed in rodents more than two decades ago[63] and was later validated in humans.[20] Since its invention, this model served as one of the most popular and efficient paradigms for assessing inflammatory/microglia-related behavioral/psychiatric disturbances and was utilized in hundreds of studies.[64] LPS-induced depressive-like behavior has been shown to depend on the induction of inflammation and microglia activation, as proved by the amelioration of the depressive-like symptoms by anti-inflammatory drugs and by microglia suppressive drugs.[65]

During the week before the beginning of the experiment, mice were habituated four times to sucrose drinking, as described above. Baseline sucrose preference was determined on the fifth day. Mice were assigned to seven groups, matched for baseline sucrose preference, and injected i.p. with one of the following compounds on three consecutive days: (1) vehicle (ethanol, cremophor, and saline, at a ratio of 1:1:18) (Veh), (2) vehicle (same formulation as group 1), (3) Celecoxib 10 mg/kg (Clx10), (4) Celecoxib 20 mg/kg (Clx 20), (5) CBD 30 mg/kg (CBD30), (6) Celecoxib 10 mg/kg + CBD 30 mg/kg (Clx10:CBD30), and (7) Celecoxib 20 mg/kg + CBD 30 mg/kg (Clx20:CBD 30). The Clx dose was chosen based on previous studies examining the effects of this drug at similar doses on LPS-induced behavioral symptoms.[35,66] The CBD dose was chosen based on two recent studies on the effects of this compound in the LPS model.[52,67]

One hour following the injection on the third day, one of the vehicle-treated groups was injected with saline (Sal), whereas all the other groups were injected with LPS (330 μg/kg). All injections were administered at a volume of 10 mL/kg. Four hours following the Sal/LPS injection, mice were tested in the open-field test (OFT). Sucrose preference was assessed overnight, as described above, between 8 and 24 h post-LPS injection.

The CSDS paradigm

Exposure to repeated episodes of social defeat stress in mice induces a depression-like syndrome, characterized by anhedonia, anxiety-like, and social-avoidance behaviors.[68] Before the beginning of the experiment, C57BL/6 mice were habituated four times to sucrose drinking as described above. Mice were assigned to

five groups, matched for baseline sucrose preference, including a naive untreated group (Naive) and four groups injected (i.p.) with one of the following compounds: (1) Vehicle (comprising ethanol, cremophor, and saline, at a ratio of 1:1:18), (2) Celecoxib 30 mg/Kg (Clx30), (3) CBD 30 mg/kg (CBD30), (4) Combination of celecoxib 30 mg/kg + CBD 30 mg/kg (Clx30:CBD30). A standard CSDS paradigm was utilized.[68] The dose of Clx was chosen based on a recent report, showing that at this dose (30 mg/kg) Clx produced significant, yet partial, reversal of chronic stress-induced behavioral impairments.[69] The dose of CBD was chosen based on several previous studies on the antidepressant effects of 30 mg/kg CBD in chronic stress models.[70–72]

Before the experiment, male CD-1 mice underwent a screening procedure in which their aggressive behavior was monitored during social interactions with C57BL/6 mice (which were different than those later used as subjects), for three consecutive days. Twenty-four hours before the beginning of the defeat sessions, CD-1 mice displaying high levels of aggressive behavior were housed in one side of a social defeat cage, which had a clear perforated Plexiglass divider separating the two sides of the cage. Only male subjects were used in this paradigm because the nature of the interaction, between the aggressor males and the subjects, is not suitable for examination of the development of depressive-like behavior in females.

Experimental C57BL/6 mice were subjected to 5–10 min of physical interaction sessions, once daily for 10 consecutive days, with a different CD-1 mouse in each session. Following each interaction, the C57Bl/6 mouse was removed from the CD-1's side and placed in the contiguous empty side of the cage for the remaining 24 h. The control group comprised naive untreated mice that were kept undisturbed in their home cages at a separate room. Treatment was given 1 h after the defeat session for five consecutive days starting on day 6 of defeat until day 10. Behavioral tests were conducted in the following order, OFT (day 7), SE (day 8), EPM (day 9), SPT (day 10), ~1 h after the treatment injections.

In vitro microglia activation assay

The interactive effects of Clx and CBD were examined in BV2 microglia cultures stimulated with immune challenges, as previously described.[51,73] BV2 cells were cultured at 37°C in a humidified atmosphere with 5% CO_2 in high D-glucose (4.5 g/L) Dulbecco's modified Eagle's medium (Sigma) supplemented with 5% heat-inactivated fetal bovine serum, streptomycin (100 μg/mL), and penicillin (100 units/mL) and sodium pyruvate (1 mM; all from Biological Industries Ltd., Kibbutz Beit Haemek, Israel). Twenty-four hours before experiments with LPS stimulation, the BV2 cells were split into 24-well plates, 2×10^5 cells per well, covered with 1 mL of growth medium and allowed to attach overnight. Twenty-four hours before experiments with $\alpha-$synuclein stimulation, the BV2 cells were split into 24-well plates, 5×10^4 cells per well, covered with 1 mL of growth medium and allowed to attach overnight.

Working solutions of 1 mM of the drugs used in the *in vitro* studies were prepared from the stocks in sterile cell growth medium just before the experiment. Each drug was tested at the concentrations of 1, 2.5, 5, and 10 μM, alone or in combination with each other. Each experiment was accompanied by a control group with a combination of the highest concentrations of a vehicle (ethanol) to estimate the effect of the vehicle concentration on the cytokine release. The maximal final concentration of ethanol in the cell medium was $\leq$0.1% (or 0.2% where maximal doses were combined) and did not interfere with cytokine release. Cultures of BV2 microglia cells were treated with celecoxib and CBD, at the final concentrations of 1, 2.5, 5, and 10 μM, alone or in combination with each other. Two hours after the drug addition, the BV2 cells were activated with LPS at 100 ng/mL, or with α-synuclein at 500 nM, and the plates returned to the incubator. Four hours after LPS stimulation, the culture media was collected and spun down for 5 min at 2000 rpm. Cell free media was frozen in -80°C until further analysis.

On the day of the analysis, frozen cell-free media was thawed on an ice-cold surface, vortexed, and proceeded for ELISAs. The concentrations of TNFα and interleukin (IL)-1β in the collected media were determined using mouse ELISAs, following the protocols recommended by the supplier (R&D Systems, Minneapolis, MN). The serum in the culture media did not interfere with the assays. The ELISA measurements were carried out using Tecan Sunrise absorbance plate reader, with a 450 nm filter (and 620 nm filter background correction), and the data (optical density [OD] values) processed using Magellan™ (Tecan, Switzerland) and Microsoft Excel software.

Statistical analysis

The GraphPad Prism software v9.0.0 (San Diego, CA) was used for statistical analysis. Data were expressed as the mean ± standard error of the mean. The data from the FST and EPM experiments were analyzed by two-

way analyses of variance (ANOVAs), with the treatment and the sex as between-subject factors, and the data from the experiments with the LPS and the CSDS models were analyzed by one-way ANOVAs, followed by *post hoc* tests with the Fisher PLSD test. $p < 0.05$ was considered significant.

For the *in vitro* analyses, the raw OD values obtained using absorbance microplate reader were transformed to relative percent values, with the OD value obtained for the LPS or α-synuclein exposure (without drug pretreatments) taken as 100% activation (maximal inflammatory response). The data were analyzed by one-way ANOVAs, followed by Dunnett's *post hoc* tests for comparisons of each group to the vehicle-LPS group, and by Tukey's *post hoc* tests for comparisons of each combination of drugs versus the effect of each component given individually in the corresponding concentration ($N = 3$–4 samples for each concentration).

For a more global analysis of these findings, XY plots were prepared, where the concentrations of celecoxib are plotted on the x-axis and the responses to this drug in combination with various concentrations of CBD, expressed as the % of maximal LPS or α-synuclein effect, are plotted on the y-axis. Based on these plots, the IC_{50} of celecoxib and CBD, by themselves or in combination, was computed, using GraphPad Prism software v9.0.0, using nonlinear regression method (least squares regression) where X represents a drug concentration.

Results

Effects of celecoxib, CBD, and their combination in the FST and EPM paradigms

In the FST, there was a significant overall difference in the immobility time between the treatment groups ($F_{3,66} = 3.45$, $p = 0.021$) (Fig. 1A), with no significant effects of sex or sex by treatment interaction. *Post hoc* analysis showed that treatment with the Clx:CBD combination significantly reduced the immobility time, compared with groups treated with vehicle, Clx10, or CBD5 ($p < 0.05$), with no significant effects between the latter three groups. A significant overall difference between the treatment groups was also found with respect to the latency to the first float ($F_{3,66} = 4.28$, $p = 0.008$) (Fig. 1B), with no significant effects of sex or sex by treatment interaction. *Post hoc* tests showed that treatment with the Clx:CBD combination significantly increased the latency to first float, compared with groups treated with vehicle, Clx10, or CBD5 ($p < 0.05$), with no differences between the latter three groups.

In the EPM paradigm, females spent less time in the open arms, reflected by significant effects of sex ($F_{1,61} = 5.606$, $p = 0.021$) (Fig. 1C), with no significant effects of treatment or sex by treatment interaction. *Post hoc* tests showed that overall, mice treated with the Clx:CBD combination spent more time in the open arms, compared with the vehicle-treated mice ($p = 0.031$), but there were no significant effects for each compound by itself. A significant overall difference between the treatment groups was found with respect to the total number of entries into the open arms ($F_{3,61} = 2.976$, $p = 0.038$). In this measure, females displayed a lower number of entries, but the sex effect did not reach statistical significance ($F_{1,61} = 2.541$, $p = 0.116$), with no treatment by sex interaction. *Post hoc* analysis showed that treatment with the Clx:CBD combination significantly increased the number of entries into the open arms, compared with the vehicle-treated mice ($p = 0.004$), but there were no significant effects for each compound by itself (Fig. 1D). Analysis of the total distance moved results in the EPM revealed no significant effects of treatment, sex, or treatment by sex interaction ($p > 0.1$ for all) (Fig. 1E). Analysis of the total arm entry results revealed a significant sex difference ($F_{1,61} = 5.945$, $p = 0.018$), with females showing lower number of total arm entries. *Post hoc* tests found no significant differences between the treatment groups in either males or females (Fig. 1F).

Effects of celecoxib, CBD, and their combinations on inflammation-induced behavioral alterations

In the model of LPS-induced inflammation, anxiety- and depressive-like symptoms were assessed in the OFT and SPT, respectively. In the OFT, there were significant differences between the groups in the total distance moved ($F_{6,68} = 24.63$, $p < 0.0001$) (Fig. 2A), the distance moved in the center ($F_{6,68} = 11.10$, $p < 0.0001$) (Fig. 2B), and the number of entries into the center of the open field ($F_{6,68} = 9.60$, $p < 0.0001$) (Fig. 2C). *Post hoc* tests revealed that compared with Sal-injected mice, all of the LPS-injected groups displayed significant reductions in the distance travelled, both overall (Fig. 2A) and in the center of the OF (Fig. 2B) ($p < 0.05$). All LPS-injected groups, except the group treated with Clx10:CBD30, also displayed a reduction in number of entries into the OF center ($p < 0.01$) (Fig. 2C).

Treatment with the Clx10:CBD30 combination significantly elevated the overall distance moved, distance moved in the center, and the number of entries into the center, compared with the Veh-treated group

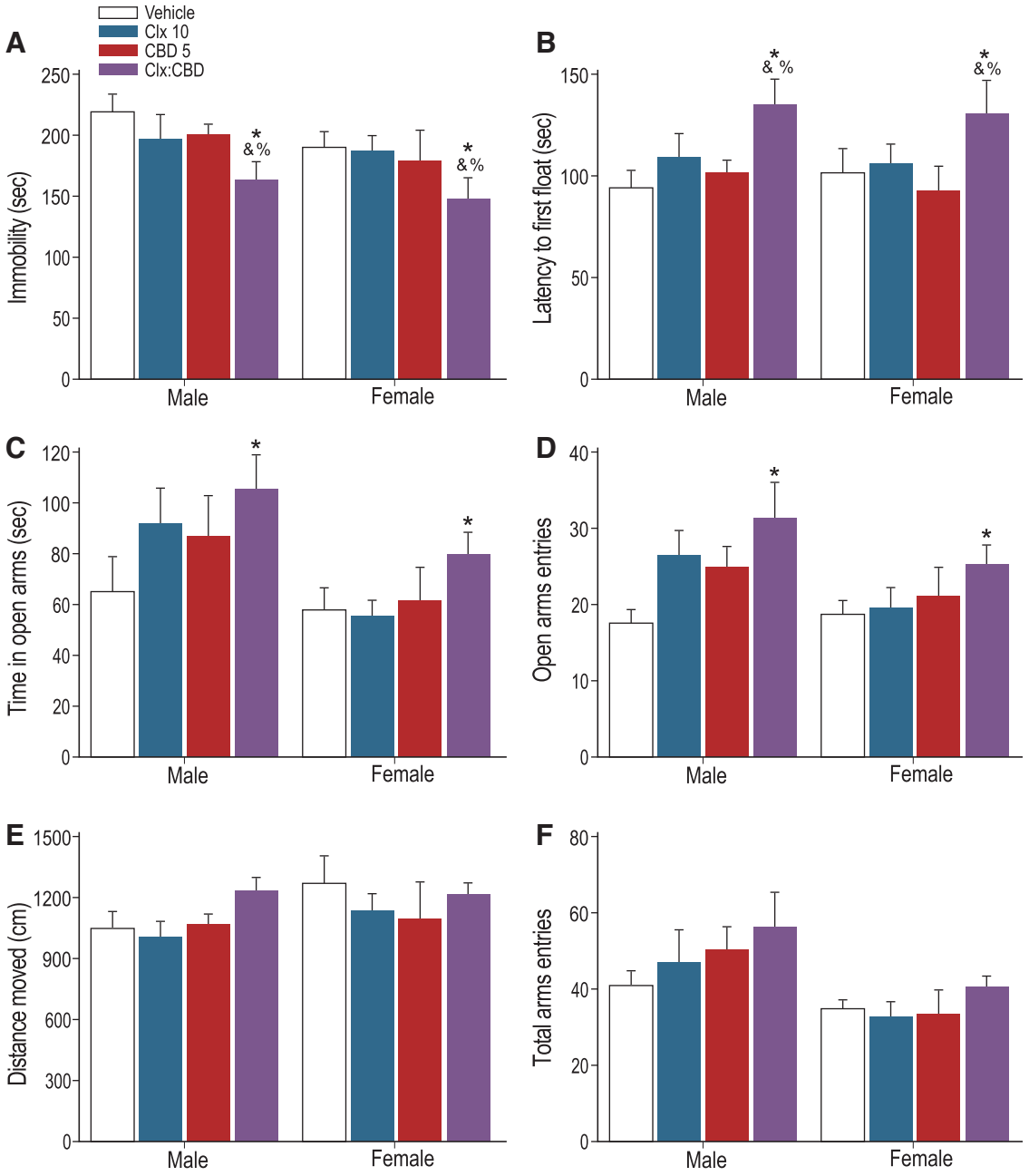


FIG. 1. Effects of celecoxib, CBD, and their combination in the FST and EPM paradigms. The effects of injections of celecoxib, 10 mg/kg (Clx10), CBD, 5 mg/kg (CBD5), the combination of the two drugs at these doses (Clx:CBD) or vehicle on behavior in the FST and EPM, measured 60 min postinjection, are depicted. Male and female mice that were injected with the Clx:CBD combination displayed a significant reduction in immobility time **(A)** and increase in the latency to first float **(B)**, compared with mice that were injected with vehicle or with each compound by itself. In the EPM, male and female mice that were injected with the Clx:CBD combination displayed an increase in the time spent in the open arms **(C)**, as well as in the number of entries into the open arms **(D)**, compared with mice that were injected with vehicle. There were no differences between the groups in the total distance moved by the mice in the EPM **(E)**, or in the total numbers of entries into the open and enclosed arms **(F)**. All data are presented as mean ± SEM ($n = 6$–13 per group). $^{*}p < 0.05$ compared with the Vehicle-treated group. $^{\&}p < 0.05$ compared with the Clx-treated group. $^{\%}p < 0.05$ compared with CBD-treated group. CBD, cannabidiol; EPM, elevated plus maze; FST, forced swim test; SEM, standard error of the mean.

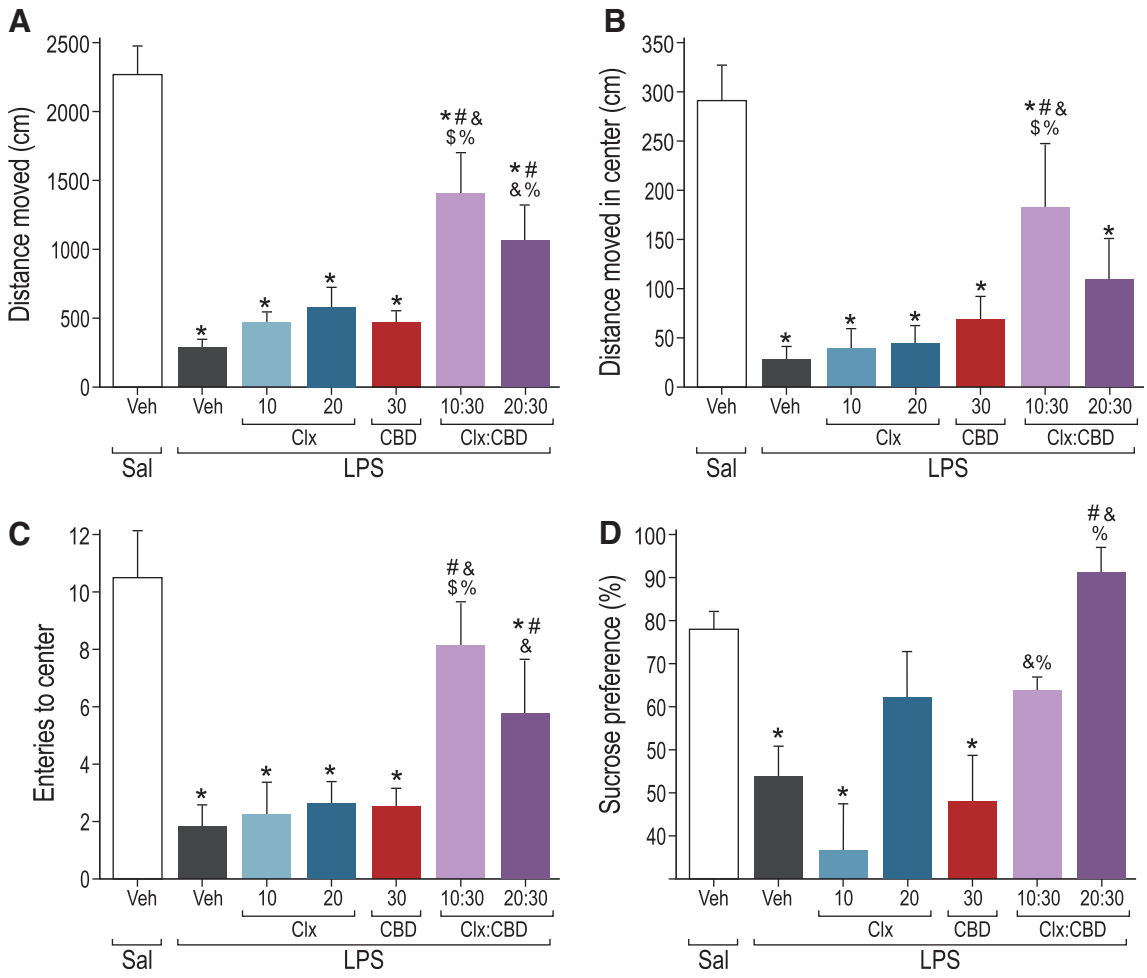


FIG. 2. Effects of celecoxib, CBD, and their combinations on LPS-induced depression- and anxiety-like symptoms. The effects of treatment of LPS-injected mice with celecoxib, 10 or 20 mg/kg (Clx10 or Clx20, respectively), CBD, 30 mg/kg (CBD30), combinations of the two drugs at these doses (Clx10:CBD30 and Clx20:CBD30) or vehicle (Veh) on behavior in the OFT and SPT, are depicted. In the OFT, LPS-injected groups treated with vehicle, Clx, or CBD by themselves displayed significant reductions in the distance moved, both overall **(A)** and in the center of the OF **(B)**, along with a decrease in the number of entries into the OF center **(C)**, compared with saline-injected mice (Sal), reflecting increased anxiety-like behavior. Treatments with the Clx10:CBD30 or Clx20:CBD30 combinations significantly elevated these measures, compared with the Veh-treated group, and with most of the groups treated by the individual components of these combinations. **(D)** In the SPT, the LPS-injected groups that were treated with Veh, Clx10, or CBD30 showed significant sucrose preference suppression, compared with the Sal-injected group. Treatment with the Clx10:CBD30 or Clx20:CBD30 combination significantly increased sucrose preference, compared with the Clx10 and CBD30 by themselves. Furthermore, the Clx20:CBD30 combination also increased sucrose preference in comparison with the Veh-treated group. All data are presented as mean + SEM ($n = 7–17$ per group). $^{*}p < 0.05$ compared with the Sal/Vehicle-treated group. $^{\#}p < 0.05$ compared with the LPS/Vehicle-treated group. $^{\&}p < 0.05$ compared with the Clx 10-treated group. $^{\$}p < 0.05$ compared with the Clx20-treated group. $^{\%}p < 0.05$ compared with CBD30-treated group. LPS, lipopolysaccharide; OFT, open-field test; SPT, sucrose preference test.

($p < 0.001$), and with the groups treated by the individual components of the combination ($p < 0.05$). Treatment with the Clx20:CBD30 combination significantly elevated the overall distance moved compared with the Veh-treated group ($p < 0.001$) and with the groups treated by the individual components of the combination ($p < 0.05$). This combination also increased the number of entries into the center, compared with the Veh- and CBD30-treated groups ($p < 0.05$), with only a trend for a similar effect with respect to the distance moved in the center of the OF ($p = 0.052$).

In the SPT, there was a significant difference between the groups ($F_{6,64} = 5.40$, $p < 0.0001$). *Post hoc* tests demonstrated that LPS-injected groups treated with Veh, Clx10, and CBD30 showed significant sucrose preference suppression, compared with the Sal-injected group ($p < 0.05$). The LPS-injected groups treated with Clx10:CBD30 or Clx20:CBD30 displayed increased sucrose preference compared with the groups treated with Clx10 and CBD30 ($p < 0.05$). Furthermore, the Clx20:CBD30 combination also increased sucrose preference compared with the Veh-treated group ($p < 0.001$).

Effects of celecoxib, CBD, and their combination on CSDS-induced behavioral alterations

In the CSDS paradigm, depressive- and anxiety-like symptoms were assessed in a battery of behavioral tests consisting of the OFT, SE, EPM, and SPT.

In the OFT, there was a significant difference between the groups in the distance moved ($F_{4,75} = 14.50$, $p < 0.0001$) (Fig. 3A). *Post hoc* tests demonstrated that all of the CSDS-exposed groups displayed a significant reduction in the distance moved, compared with the Naive group ($p < 0.001$). The group treated with the Clx30:CBD30 combination displayed significantly greater distance moved compared with the Vehicle group ($p = 0.011$). A significant difference between the groups was also found in the time spent in the center of the OF ($F_{4,75} = 6.940$, $p < 0.0001$) (Fig. 3B). *Post hoc* tests revealed that the CSDS-exposed groups treated with Vehicle or Clx30 spent significantly less time in the center compared with the Naive group ($p < 0.001$), whereas no such difference was found in the CBD30- or Clx30:CBD30-treated groups.

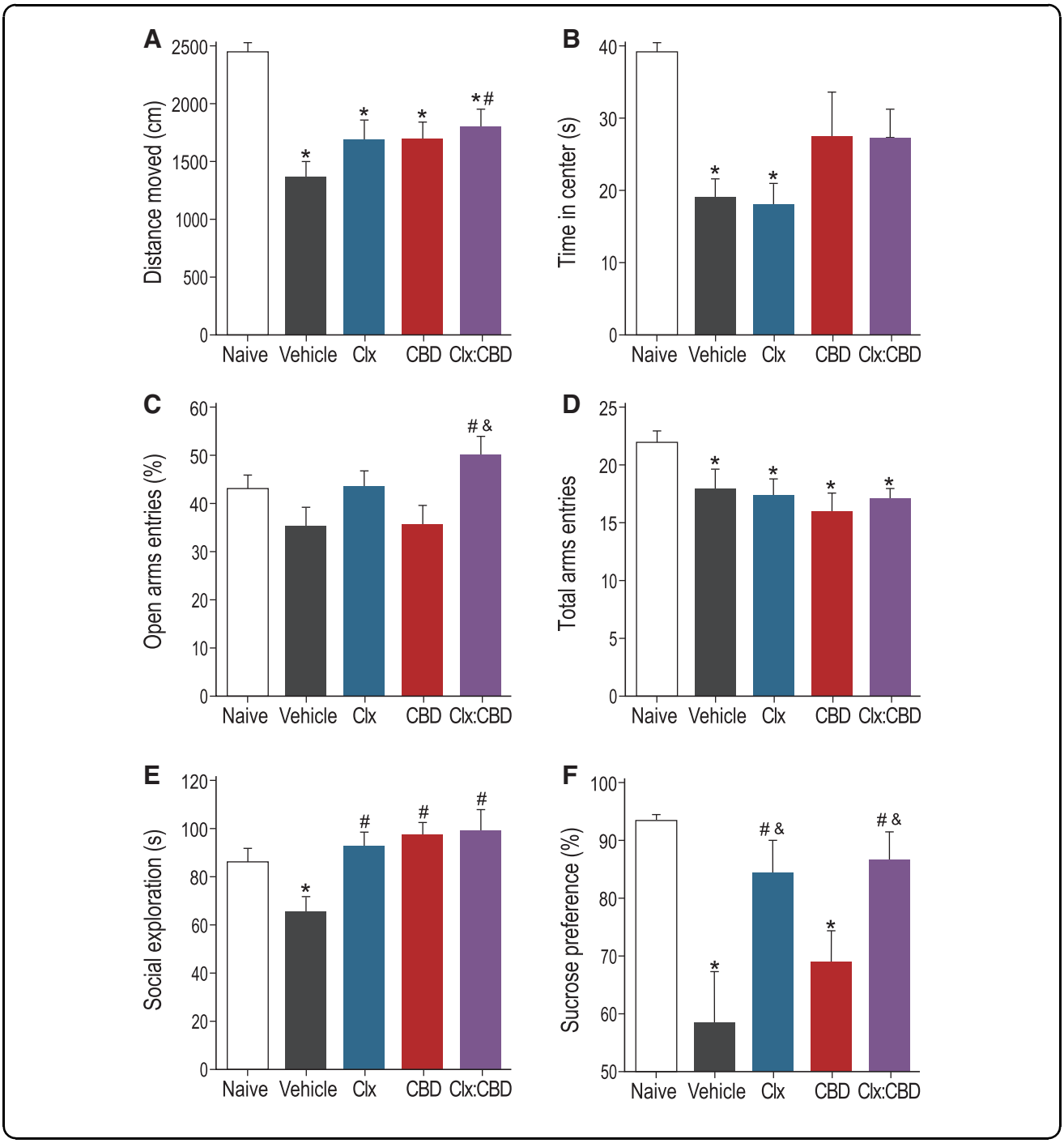
In the EPM, there was a significant difference between the groups in the ratio of entries into the open arms ($F_{4,74} = 2.837$, $p < 0.03$) (Fig. 3C). *Post hoc* tests revealed that the Clx30:CBD30-treated group displayed a significantly higher ratio of entries into the open arms compared with the Vehicle-treated group ($p < 0.005$). The total number of arms entries also differed significantly between the groups ($F_{4,74} = 4.561$, $p = 0.002$) (Fig. 3D). *Post hoc* analysis revealed that each of the CSDS-exposed groups displayed a lower number of total entries compared with the Naive group ($p < 0.05$), with no differences between these groups.

In the SE test, there was a significant difference between the groups in exploration time ($F_{4,75} = 3.973$, $p = 0.006$). *Post hoc* tests showed that exposure to CSDS significantly decreased the exploration time in

FIG. 3. Effects of celecoxib, CBD, and their combination on depression- and anxiety-like symptoms in the CSDS paradigm. The effects of treatment of CSDS-exposed mice with celecoxib, 30 mg/kg (Clx), CBD, 30 mg/kg (CBD), the combination of the two drugs at these doses (Clx:CBD), or Vehicle on behavior in the OFT, EPM, SE, and SPT are depicted. **(A)** Compared with the Naive group, all the CSDS-exposed groups moved shorter distances in the OFT; however, the Clx:CBD group showed a partial reversal of this effect, reflected by significantly higher distance moved than the Vehicle-treated group. **(B)** The CSDS-exposed groups treated with Vehicle or Clx spent significantly less time in the center of the OF, compared with the Naive group, but the groups treated with CBD or Clx:CBD displayed no such anxiogenic effect of CSDS. **(C)** In the EPM paradigm, the Clx:CBD-treated group displayed a significantly higher percentage of entries into the open arms, compared with the groups treated with vehicle or CBD. This anxiolytic-like effect was specific given that **(D)** all of the CSDS-exposed groups displayed significantly lower total number of entries (to both the open and enclosed arms of the EPM), with no differences between these groups. **(E)** In the SE test, exposure to CSDS significantly decreased the time spent in exploration in the vehicle-treated group, and this effect was completely reversed in all the other treatment groups. **(F)** In the SPT, the CSDS-exposed groups that were treated with Vehicle or CBD displayed significantly suppressed sucrose preference, compared with the Naive group, which was reversed by treatment by either Clx or the Clx:CBD combination. All data are presented as mean + SEM ($N = 10$–29 per group). $^{*}p < 0.05$ compared with the Naive group; $^{\#}p < 0.05$ compared with the vehicle-treated group; $^{\&}p < 0.05$ compared with the CBD-treated group. CSDS, chronic social defeat stress; SE, social exploration.

the Vehicle-treated group, compared with the Naive group ($p < 0.012$). This effect was completely reversed in all the other treatment groups, i.e., Clx30, CBD30, and Clx30:CBD30 ($p < 0.05$), compared with the vehicle treated group (Fig. 3E).

In the SPT, there was a significant difference between the treatment groups ($F_{4,55} = 11.97$, $p < 0.0001$). *Post hoc* tests revealed that sucrose preference was significantly suppressed in the vehicle- and CBD30-treated mice, compared with the Naive group ($p < 0.0001$). This suppression was completely reversed in the Clx30:CBD30 and Clx30 groups, which showed significantly elevated sucrose preference compared with the vehicle and CBD30 groups ($p < 0.05$) (Fig. 3F).



Effects of celecoxib, CBD, and their combination on microglia activation in BV2 cell cultures

Analysis of the effects of Clx, CBD, and their combinations on LPS-induced TNFα secretion by BV2 microglia cell cultures demonstrated an overall significant difference between the groups ($F_{19,55} = 3.389$, $p < 0.001$) (Fig. 4A). *Post hoc* tests, comparing the effects of each concentration with the Vehicle only, demonstrated that while no concentration of Clx or CBD by itself produced a significant effect, combinations of Clx (at 2.5, 5, or 10 μM) with 5 μM CBD, as well as combinations of 10 μM Clx with either 1 or 2.5 μM CBD, produced significant TNFα-inhibitory effects ($p < 0.05$). Further *post hoc* tests, comparing the

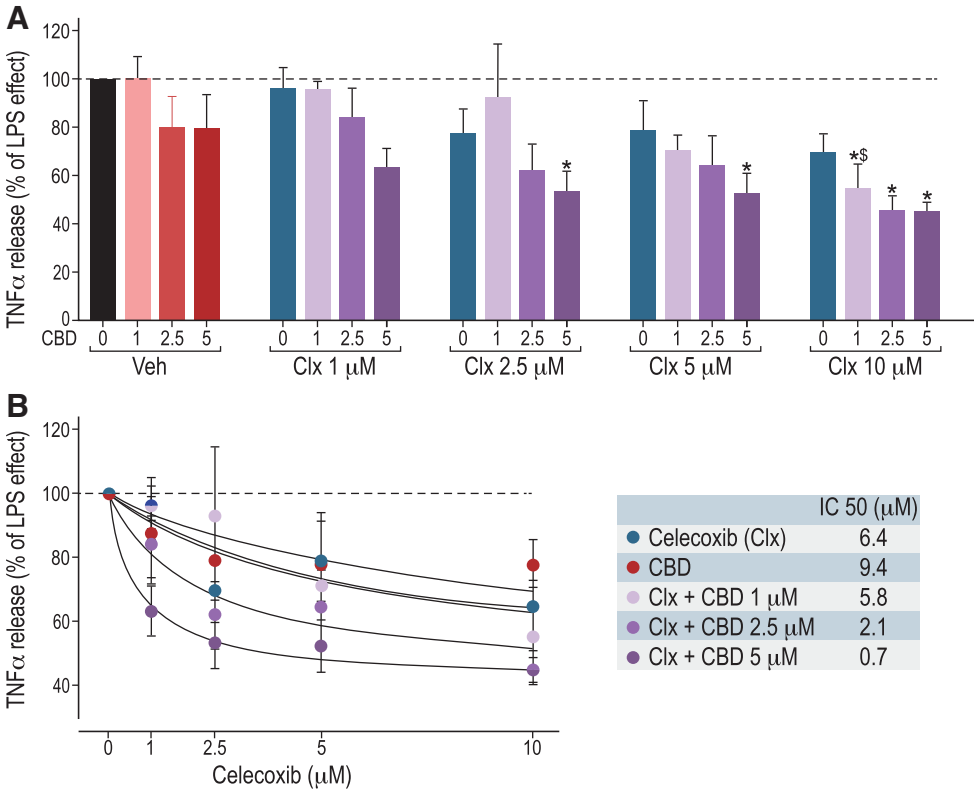


FIG. 4. Celecoxib and CBD combinations suppress LPS-induced TNFα secretion by BV2 microglia cell cultures. The inhibitory effects of various concentrations of celecoxib (Clx), CBD, and their combinations on LPS (100 ng/mL)-induced TNFα secretion by BV2 microglia cells are depicted. **(A)** Whereas no concentration of Clx or CBD by itself produced a significant effect, combinations of Clx (at either 2.5, 5, or 10 μM) with 5 μM CBD, as well as combinations of 10 μM Clx with either 1 or 2.5 μM CBD, produced significant TNFα-inhibitory effects. Furthermore, the combination of Clx (10 μM) plus CBD (1 μM) produced significantly greater inhibition than this concentration of CBD by itself. **(B)** A concentration-response (XY) plot was prepared, showing nonlinear regression curves fitted based on wide-range TNFα suppressive effects of the two compounds and their corresponding combinations. Based on these plots, the IC_{50} values were computed, defined as the concentrations of Clx and CBD, by themselves or in combinations, at which the response to LPS is reduced by half of the maximal effect. The table on the right of the XY plot demonstrates the markedly lower IC_{50} of combinations of Clx plus CBD, compared with the IC_{50} of each compound applied by itself. *$p < 0.05$ compared with LPS-exposed cultures treated with Veh. $^{\$}p < 0.05$ compared with culture treated with the corresponding concentration of CBD by itself. TNFα, tumor necrosis factor-α.

various combinations with the respective concentrations of their individual components by themselves, demonstrated that the combination of Clx (10 μM) plus CBD (1 μM) produced significantly greater inhibition than this concentration of CBD by itself ($p < 0.05$).

For a more global analysis of these findings, an XY plot was prepared, showing nonlinear regression curves fitted based on wide-range concentration-response experiments of Clx and CBD tested individually or when Clx (1 or 2.5 or 5 μM) was added to various concentrations of CBD (Fig. 4B). IC_{50}, computed based on these curves, again demonstrated that while Clx and CBD by themselves had only small effects ($IC_{50} = 6.4$ and 9.4 μM, respectively), addition of 1, 2.5, or 5 μM of CBD markedly decreased the Clx IC_{50} to 2.0, 1.7, and 1.1 μM, respectively.

Analysis of the effects of Clx, CBD, and their combinations on LPS-induced IL-1β secretion by BV2 microglia cell cultures demonstrated an overall significant difference between the groups ($F_{19,40} = 8.123$, $p < 0.001$) (Fig. 5A). *Post hoc* tests, comparing the effects of each concentration with the Vehicle only condition, demonstrated that while no concentration of Clx by itself produced a significant effect, exposure to 5 μM of CBD, either by itself or in combination with Clx, significantly suppressed IL-1β secretion ($p < 0.05$). Combinations of 10 μM of Clx plus lower concentrations of CBD (1 or 2.5 μM) also significantly suppressed IL-1β secretion ($p < 0.05$). Further *post hoc* tests, comparing the various combinations with the respective concentrations of their individual components by themselves, demonstrated that combinations of each Clx concentration with 5 μM of CBD produced suppressive effects that were significantly greater than the corresponding Clx concentration by itself ($p < 0.05$). This was also the case for the combination of 10 μM Clx plus 2.5 μM CBD ($p < 0.05$). Moreover, combinations of 10 μM Clx plus either 1 or 2.5 μM CBD produced significantly greater IL-1β suppressive effect than the corresponding CBD concentrations by themselves ($p < 0.05$).

IC_{50}s, computed based on nonlinear regression curves fitted based on wide-range concentration-response experiments of Clx and CBD tested individually or in combinations, demonstrated that Clx by itself had a small effect ($IC_{50} = 14.1$ μM) (Fig. 5B). The IC_{50}s for the Clx combination with CBD concentrations (1, 2.5, and 5 μM) were markedly lower than the IC_{50} for Clx (4.6, 3.2, and 0.6 μM, respectively), but given that the effect of CBD by itself was quite substantial ($IC_{50} = 3.8$ μM), the potentiating effect of CBD in this analysis was limited.

To increase the generalizability of these results, we examined the inhibitory effects of various concentrations of Clx, CBD, and their combinations on TNFα release by BV2 microglia cells exposed to α-synuclein aggregates. A significant difference was revealed between the concentration groups ($F_{15,32} = 4.307$, $p < 0.001$) (Fig. 6A). *Post hoc* analysis revealed that neither CBD (1, 2.5, or 5 μM) nor the lower concentrations of Clx (1 or 2.5 μM) by themselves affected the release of TNFα by microglia incubated with α-synuclein aggregates. However, combinations of Clx (2.5 μM) plus CBD (either 2.5 or 5 μM) significantly decreased the release of TNFα protein from the activated cells ($p < 0.05$ and 0.01, respectively). Clx (5 μM) alone, or in combination with all concentrations of CBD, significantly decreased the levels of TNFα released in response to α-Synuclein ($p < 0.05$). Furthermore, comparisons of the effects of each combination with its components given by themselves revealed that Clx (1 μM) plus CBD (5 μM) was significantly more efficacious in inhibiting TNFα than Clx given alone in the same dose ($p < 0.05$).

IC_{50}s, computed based on nonlinear regression curves fitted based on wide-range concentration-response experiments of Clx and CBD tested individually or in combinations, demonstrated that the Clx and CBD by themselves produced only small effects ($IC_{50} = 4.2$ and 5.1 μM, respectively); however, addition of 1, 2.5, or 5 μM of CBD markedly decreased the Clx IC_{50} to 2.0, 1.7, and 1.1 μM, respectively (Fig. 6B).

Discussion

The findings of the present study demonstrate that formulations comprising celecoxib and CBD exert potent effects in several models of depression- and anxiety-like behavior. Specifically, at doses of celecoxib and CBD that had no effects by themselves, a combination of the two compounds reduced despair-like behavior in the FST and anxiety-related behavior in the EPM paradigm. These effects were found in both male and female mice, consistently with previous reports that celecoxib had similar antidepressant effects in male and female depressed patients[31] and CBD produced similar effects in depressed-like mice.[74] In the LPS model of inflammation, combinations of celecoxib and CBD, at doses that had no effects by themselves, induced a marked anxiolytic effect in the OFT. These combinations also reversed the LPS-induced anhedonia in the SPT, whereas the same doses of each compound by itself produced only partial effect.

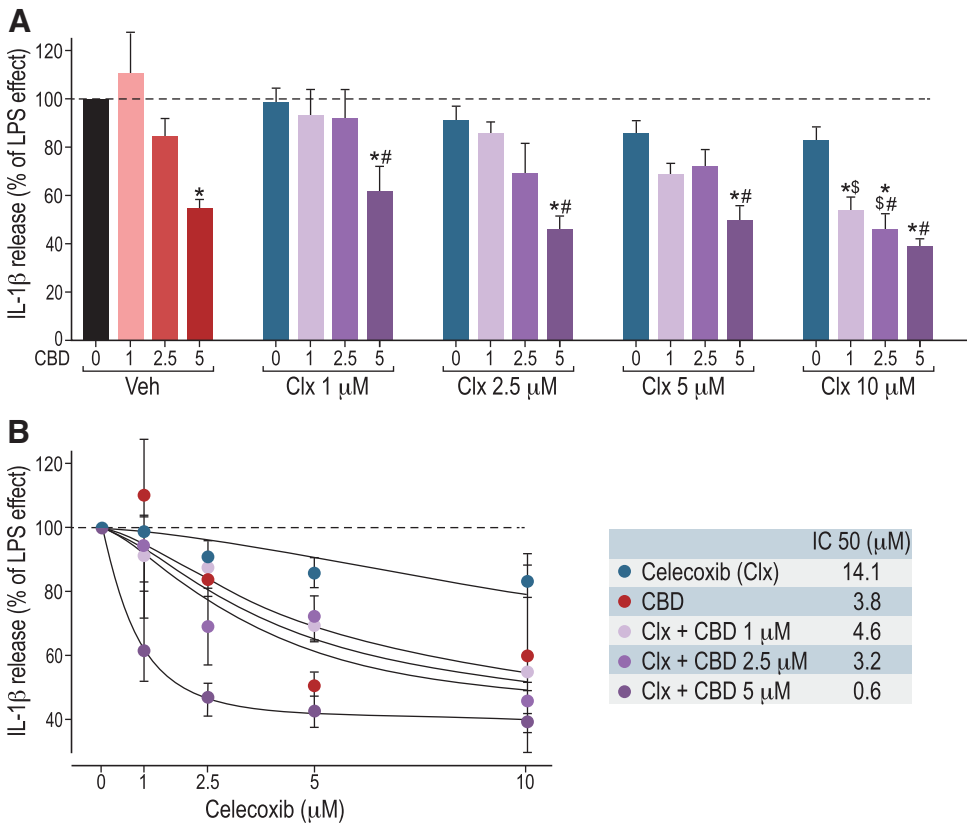


FIG. 5. Celecoxib and CBD combinations suppress LPS-induced IL-1β secretion by BV2 microglia cell cultures. The inhibitory effects of various concentrations of celecoxib (Clx), CBD, and their combinations on LPS (100 ng/mL)-induced IL-1β secretion by BV2 microglia cells are depicted. **(A)** Whereas no concentration of Clx by itself produced a significant effect, exposure to 5 μM of CBD, either by itself or in combination with Clx, significantly suppressed IL-1β secretion. Combinations of 10 μM of Clx plus lower concentrations of CBD (1 or 2.5 μM) also significantly suppressed IL-1β secretion. Combinations of each Clx concentration with 5 μM of CBD produced suppressive effects that were significantly greater than the corresponding Clx concentration by itself. This was also the case for the combination of 10 μM Clx plus 2.5 μM CBD. Moreover, combinations of 10 μM Clx plus either 1 or 2.5 μM CBD produced significantly greater IL-1β suppressive effect than the corresponding CBD concentrations by themselves **(B)** A concentration-response (XY) plot, showing nonlinear regression curves fitted based on the wide-range IL-1β suppressive effects of the two compounds and their corresponding combinations (left), and the IC_{50} values computed based on these plots are depicted. As shown, the IC_{50} for the combinations of Clx with all CBD concentrations was markedly lower than the IC_{50} of Clx by itself. However, the higher concentration of CBD (5 μM) by itself was highly efficacious, so there were only small differences between the IC_{50}s of the combinations from that of CBD by itself. $^{*}p < 0.05$ compared with LPS-exposed cultures treated with Veh. $^{\#}p < 0.05$ compared with culture treated with the corresponding concentration of Clx by itself. $^{\$}p < 0.05$ compared with culture treated with the corresponding concentration of CBD by itself. IL, interleukin.

In the CSDS model, a combination of celecoxib and CBD induced significant anxiolytic and antidepressant-like effects in all the behavioral assessments, including the OFT, EPM, SE, and SPT. In this experiment, the dose of celecoxib was higher than the one used in the other experiments and was sufficient to produce significant beneficial effects in the SE and SPT (but not in the other paradigms), whereas CBD administration by itself was sufficient to reverse the CSDS effect only in the SE test. Thus, to provide more convincing evidence for the

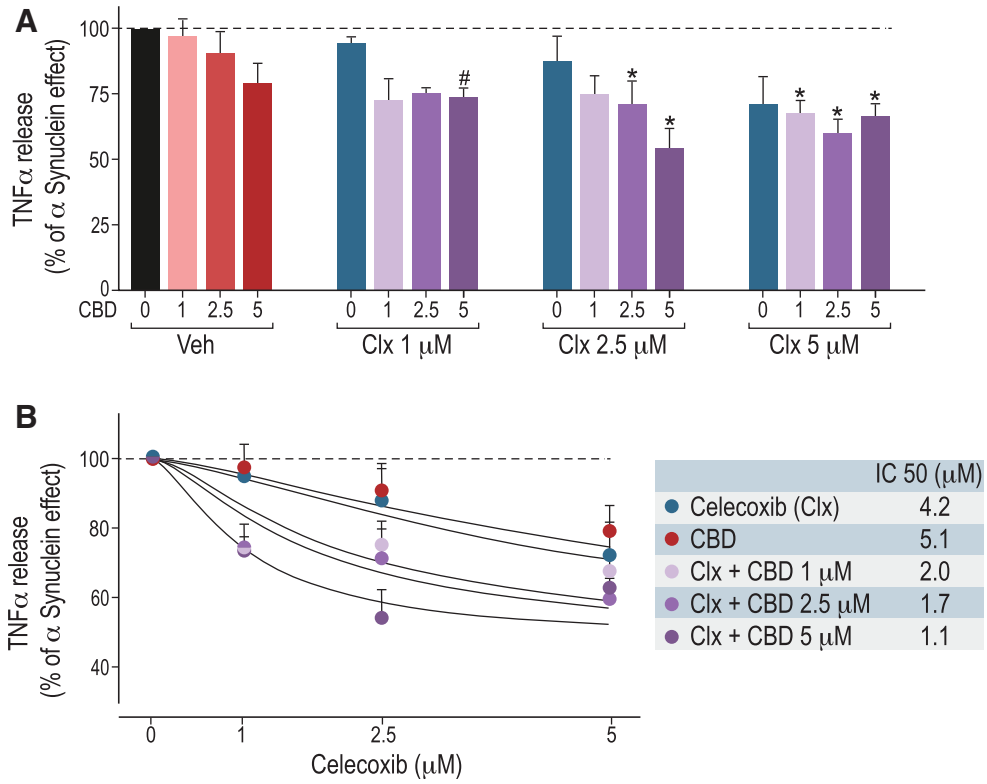


FIG. 6. Celecoxib and CBD combinations suppress α-synuclein-induced TNFα secretion by BV2 microglia cell cultures. The inhibitory effects of various concentrations of celecoxib (Clx), CBD, and their combinations on α-synuclein (500 ng/mL)-induced TNFα secretion by BV2 microglia cells are depicted. **(A)** Whereas neither CBD (1, 2.5, or 5 μM) nor the lower concentrations of Clx (1 or 2.5 μM) by themselves affected the release of TNFα by microglia incubated with α-synuclein aggregates, combinations of Clx (2.5 μM) plus CBD (either 2.5 or 5 μM) significantly decreased the release of TNFα protein from the activated cells. Clx (5 μM) alone, or in combination with all concentrations of CBD, significantly decreased the levels of TNFα released in response to α-Synuclein. Furthermore, Clx (1 μM) plus CBD (5 μM) was significantly more efficacious in inhibiting TNFα release than Clx given alone at the same dose. **(B)** An XY plot was prepared, where the Clx concentrations are plotted on the x-axis and the response, that is, release of TNFα (expressed as % of maximal effect) induced by α-synuclein aggregates/fibrils, is plotted on the y-axis. Calculation of the IC_{50} demonstrated that addition of each concentration of CBD to each of the tested concentrations of Clx markedly decreased the combination's IC_{50} compared with the IC_{50} each of these compounds by themselves. $^{*}p < 0.05$ compared with α-synuclein-exposed cultures treated with Vehicle. $^{\#}p < 0.05$ compared with culture treated with the corresponding concentration of Clx by itself.

potentiated effects of the combination of celecoxib and CBD in the CSDS model of depression, future studies should assess the effects of combining lower doses of the two compounds. Finally, combinations of celecoxib and CBD were also found to produce potent suppressive effects in a cellular model of microglia activation, suggesting that alterations in microglia activation status could underlie at least some of the behavioral effects of this formulation.

The potentiated effects of celecoxib plus CBD on depressive-like symptomatology may involve their combined COX-2 inhibitory effects and combined stimulatory effects on the endogenous cannabinoid system (Fig. 7), as evidenced by the following: (1) Pre-clinical studies demonstrated that COX-2 activity and prostaglandin E2 (PGE2) levels in the brain are elevated in several models of depression, including acute inflammation, CSDS, and CUMS.[34,75,76] The resulting

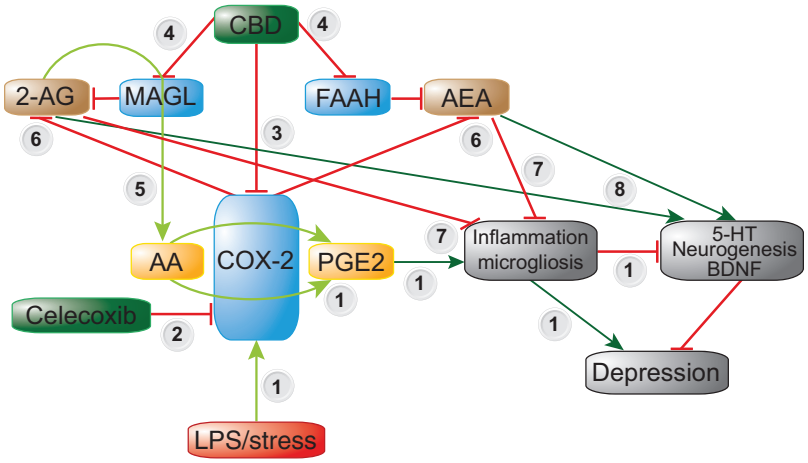


FIG. 7. Putative mechanisms underlying the interactions between celecoxib and CBD. The antidepressant effects of celecoxib plus CBD may involve their combined inhibitory effects on COX-2 activity and combined stimulatory effects on the eCB system, as evidenced by: (1) Major depression in humans, as well as exposure to stress or inflammatory challenges in experimental animals, is associated with COX-2 activation, resulting in the conversion of AA to PGE2, which contributes to the development of depression symptomatology, at least partly through microglia activation, as well as suppression of serotonergic neurotransmission, neurogenesis, and BDNF signaling. (2) Celecoxib directly inhibits COX2 activity, reducing PGE2 levels and depression severity in clinical trials, as well as in animal models of depression. (3) Similarly to celecoxib, CBD inhibits COX-2 activity, reduces PGE2 levels, and improves depressive symptomatology in animal models of depression. (4) CBD inhibits the activity of the eCB degrading enzymes FAAH and MAGL, resulting in higher levels of AEA and 2-AG, respectively. (5) Given that MAGL-dependent degradation of 2-AG is the main source for the free AA that is required for COX-2 mediated PGE2 synthesis, the CBD-induced MAGL inhibition lowers free AA levels and consequently PGE2 levels. (6) COX-2 directly contributes to the degradation of AEA and 2-AG, so its inhibition by celecoxib and CBD further increases the levels of these eCBs. Both AEA and 2-AG have direct antidepressant effects using multiple mechanisms, including anti-inflammatory and microglia suppressive effects (7), and direct stimulatory effects on serotonin (5-HT) neurotransmission, neurogenesis, and BDNF signaling (8). 2-AG, 2-arachidonoylglycerol; AA, arachidonic acid; AEA, *N*-arachidonoylethanolamine; BDNF, brain-derived neurotrophic factor; COX, cyclooxygenase; eCB, endocannabionoids; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; PGE2, prostaglandin E2.

neuroinflammation and microglia hyperactivation contribute to the depressive symptomatology using multiple mechanisms, including impairments in serotonergic neurotransmission, neurogenesis, and brain-derived neurotrophic factor (BDNF) production.[1–7] These pre-clinical findings are corroborated by clinical research showing that depressed patients exhibit elevated levels of prostaglandins in the CSF[77] and COX-2 mRNA in peripheral blood cells.[78] (2) In the CSDS and CUMS models of depression, blockade of prostaglandin signaling, by various COX-inhibitors, particularly celecoxib, reversed the elevation in PGE2 levels along with the attenuation of the depressive- and anxiety-like symptoms.[34,37,75,76,79] (3) Similarly to celecoxib, CBD can also inhibit COX-2 activity, reduce PGE2 plasma levels, and improve clinical symptoms of local inflammation.[49] (4) CBD was shown to inhibit the endocannabinoids (eCBs)-degrading enzymes fatty acid amide hydrolase and monoacylglycerol lipase (MAGL), resulting in elevated levels of the eCBs *N*-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG).[80] (5) The production of PGE2, in general[81] and particularly following CSDS exposure,[75] depends on MAGL-mediated conversion of

2-AG to free-arachidonic acid (AA), which is the substrate for COX mediated PGE2 synthesis. Importantly, MAGL blockade can abrogate the behavioral effect of CSDS.[75] Given that CBD is known to be a MAGL inhibitor,[82] it may reduce AA levels, leading to suppression of PGE2 production and its behavioral effects. (6) COX-2 takes an important part in oxygenation and inactivation of both AEA and 2-AG; thus COX-2 inhibition by celecoxib and/or CBD can directly boost the activation of the eCB and increase the levels of AEA and 2-AG.[83,84] The elevation in the levels of AEA and 2-AG is beneficial for mood regulation, and therefore, the potentiated stimulation of the eCB system by both celecoxib and CBD can produce antidepressant effects through (7) anti-inflammatory and microglia suppressive effects, as well as through (8) direct effects on other depression-related processes, such as serotonergic neurotransmission, neurogenesis, and BDNF production.[71,85–88]

The therapeutic efficacy reported here for the formulation of celecoxib and CBD in the LPS and CSDS models of depression, in which inflammatory processes and microglia activation are considered to play a causal role,[2,64] is in line with previous research that attributed the antidepressant and anxiolytic activity of the two compounds to their anti-inflammatory and microglia-suppressive actions. For example, one clinical trial demonstrated that along with the reductions in depression levels, celecoxib treatment also reduced the levels of IL-6, with a significant correlation between the decrease in IL-6 levels and the improvement of the depression scores.[89] Furthermore, celecoxib's antidepressant effect was particularly exhibited by depressed patients with elevated peripheral levels of an inflammatory marker[90] or a marker of elevated microglial activation (Translocator Protein 18 kDa [TSPO]),[91] with no effects of celecoxib in patients with low inflammatory/microglial status.

CBD was also shown to produce anti-inflammatory and microglia suppressive effects, for example, it suppressed the proliferation, secretion of pro-inflammatory cytokines, nitric oxide production, and oxidative stress in microglia cell cultures stimulated by LPS or other immune challenges,[51,92–94] and attenuated microglia activation in several animal models of diseases in which inflammatory processes play a detrimental role.[95,96] Furthermore, in the LPS model of inflammation-induced depression in mice, the reversal of depressive-like symptoms by CBD was accompanied by diminished elevations in brain NF-κB expression and cytokine production,[52,97] whereas in the CSDS model, microglia activation, along with infiltrating monocytes, was found to be an important mechanism underlying the development of the depressive- and anxiety-like symptoms.[98,99]

Taken together with these studies, the findings of the present study, showing that the combination of celecoxib plus CBD effectively reversed the behavioral effects of LPS and CSDS, as well as the stimulatory effects of LPS in BV2 microglia cultures, suggest that modulation of microglia activation status contributes to the potentiated antidepressant and anxiolytic effects of this formulation. Future studies should verify this conclusion by analysis of the potential effects of celecoxib plus CBD on microglia changes in animals exposed to LPS and CSDS.

In addition to their mediation by inflammatory processes, the potentiated effects of celecoxib plus CBD probably involve actions on neurochemical systems not directly related to inflammation. Such actions are expected to be particularly important for mediating the effects of this formulation in the FST and EPM, given the unlikelihood of changes in inflammatory processes during the short time frame of these paradigms. Alteration in serotonergic neurotransmission is a particularly probable candidate, given that both celecoxib and CBD augment the serotonergic system. For example, pre-clinical studies have shown that celecoxib increases serotonergic output in the medial prefrontal cortex, by itself, and even more so in combination with selective serotonin reuptake inhibitors.[100] Similarly, in a rat model of Alzheimer's disease celecoxib prevented the reduction in serotonin (5-HT) content in the prefrontal cortex and resultant depressive-like symptoms.[101] CBD was shown to boost serotonergic neurotransmission, and blockade of this system, by either a serotonergic 5-HT1A receptor antagonist or the serotonin synthesis inhibitor para-chlorophenylalanine, was found to abrogate the antidepressant effects of CBD in several rodent models of depression.[61,102,103] Alterations in serotonergic neurotransmission, along with additional mechanisms implicated in CBDs antidepressant effects, including elevated neurogenesis[70,71] and BDNF production,[104,105] could also contribute to the potentiated effects of celecoxib plus CBD.

A major impediment for using celecoxib as an antidepressant is that it produces marked cardiovascular adverse effects, increasing the risk of heart attack and stroke. Importantly, CBD has been found to exert potent cardiovascular protective effects. Specifically, although CBD can promote arterial relaxation, inhibit

the contraction of small resistance arteries in various animal models, and reduce arterial blood pressure in normal human volunteers, its effects on cardiovascular regulation under physiological conditions are quite small.[106,107] However, during conditions of stress and anxiety, CBD was found to prevent the increases in blood pressure and heart rate, along with beneficial effects on the stress and anxiety responses, both in rodents[108,109] and in humans.[110] Such findings suggest that CBD can not only potentiate the anti-inflammatory and antidepressant effects of celecoxib but also may reduce its adverse cardiovascular effects.

In conclusion, our findings demonstrate that relatively low doses of celecoxib, which have minimal or only partial behavioral effects by themselves, can be combined with CBD to produce potentiated dose-sparing antidepressant and anxiolytic effects. Considering that there is substantial clinical and pre-clinical evidence that celecoxib exerts potent antidepressant effects,[28,30] but is seldom used as an antidepressant because of its associated adverse effects, we propose that celecoxib should be combined with CBD, allowing celecoxib dose-sparing effects, and possibly preventing celecoxib's adverse effects. Finally, we suggest that potentiated inhibition of microglia activation may underlie the effects of the celecoxib plus CBD combination, although this mechanism of action should be further examined.

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Authors' Contributions

E.D.: Developed some aspects of the methodology, performed the experiments, analyzed data, wrote the article. H.G.: Developed aspects of the methodology, performed the experiments, analyzed data. E.R.: Performed the experiments, analyzed data. L.N.: Performed the experiments. E.K.: Developed some aspects of the methodology, performed the experiments, analyzed data, wrote the article. R.Y.: Conceptualized the research goals and aims, acquired the funding for the research leading to this publication, analyzed data and wrote the article.

Author Disclosure Statement

The authors filed an international patent application, under the PCT, which is partly based on findings presented in this publication.

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Abbreviations Used

2-AG = 2-arachidonoylglycerol
AA = arachidonic acid
AEA = *N*-arachidonoylethanolamine
ANOVA = analysis of variance
CBD = cannabidiol
COX = cyclooxygenase
CSDS = chronic social defeat stress
CUMS = chronic unpredictable mild stress
D-PBS = Dulbecco's phosphate-buffered saline
eCBs = endocannabinoids
ELISA = enzyme-linked immunosorbent assay
EPM = elevated plus maze
FAAH = fatty acid amide hydrolase
FST = forced swim test
i.p. = intraperitoneally
IC = inhibitory concentration
IL = interleukin
LPS = lipopolysaccharide
MAGL = monoacylglycerol lipase
NSAIDs = nonsteroidal anti-inflammatory drugs
OD = optical density
OFT = open-field test
PGE2 = prostaglandin E2
SE = social exploration
SEM = standard error of the mean
SPT = sucrose preference test
TNFα = tumor necrosis factor-α