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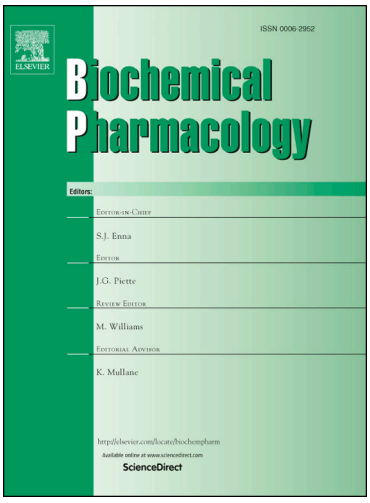

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Cannabidiol antidepressant-like effect in the lipopolysaccharide model in mice: modulation of inflammatory pathways

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ABSTRACT

Major Depression is a severe psychiatric condition with a still poorly understood etiology. In the last years, evidence supporting the neuroinflammatory hypothesis of depression has increased. In the current clinical scenario, in which the available treatments for depression is far from optimal, there is an urgent need to develop fast-acting drugs with fewer side effects. In this regard, recent pieces of evidence suggest that cannabidiol (CBD), the major non-psychotropic component of *Cannabis sativa* with anti-inflammatory properties, appears as a drug with antidepressant properties.

In this work, CBD 30 mg/kg was administered systemically to mice 30 min before lipopolysaccharide (LPS; 0.83 mg/kg) administration as a neuroinflammatory model, and behavioral tests for depressive-, anhedonic- and anxious-like behavior were performed. NF-κB, IκBα and PPARγ levels were analyzed by western blot in nuclear and cytosolic fractions of cortical samples. IL-6 and TNFα levels were determined in plasma and prefrontal cortex using ELISA and qPCR techniques, respectively. The precursor tryptophan (TRP), and its metabolites kynurenine (KYN) and serotonin (5-HT) were measured in hippocampus and cortex by HPLC. The ratios KYN/TRP and KYN/5-HT were used to estimate indoleamine 2,3-dioxygenase (IDO) activity and the balance of both metabolic pathways, respectively.

CBD reduced the immobility time in the tail suspension test and increased sucrose preference in the LPS model, without affecting locomotion and central activity in the open-field test. CBD diminished cortical NF-κB activation, IL-6 levels in plasma and brain, and the increased KYN/TRP and KYN/5-HT ratios in hippocampus and cortex in the LPS model.

Our results demonstrate that CBD produced antidepressant-like effects in the LPS neuroinflammatory model, associated to a reduction in the kynurenine pathway activation, IL-6 levels and NF-κB activation. As CBD stands out as a promising antidepressant drug, more research is needed to completely understand its mechanisms of action in depression linked to inflammation.

KEYWORDS: cannabidiol, antidepressant, kynurenine, IL-6, NF-κB

1. INTRODUCTION

Major Depressive Disorder (MDD) is a common and severe medical illness characterized by symptoms like sadness, anhedonia, psychomotor agitation or retardation, feeling worthless or guilty, difficulty concentrating, and thoughts of death or suicide, among others (American Psychiatric Association, 2013). The etiology of this disease is poorly known, and different hypotheses have been proposed. The classical *monoaminergic hypothesis* of depression postulates that there is a deregulation in monoamine neurotransmission (Schildkraut, 1965), which is complemented by the fact that most of the antidepressants currently used in clinic target these neurotransmitters systems (*rev. in* Harmer et al., 2017). However, the *monoaminergic hypothesis* does not explain the totality of alterations present in depression and novel hypotheses have been proposed.

One of the hypotheses that has gained evidence in recent years is the *neuroinflammatory hypothesis*, as some inflammatory diseases present comorbid depression (Dregan et al. 2019), and pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα) and interleukin 6 (IL-6) have been found increased in the blood of MDD patients (Dowlati et al., 2010). This peripheral inflammation, via the cross-talk with the central immune system, can induce a pro-inflammatory response in the brain (Xiao and Link, 1998; Maier, 2003; Connor et al., 2008). In this line, it has been reported that there is an activation of microglia and an increase of pro-inflammatory cytokines in cerebrospinal fluid and postmortem brain tissue of MDD patients (Enache et al., 2019).

One of the peripheral stimuli that can activate the immune response is lipopolysaccharide (LPS), a component of the bacterial wall. The administration of LPS induces not only sickness behavior, but also a depressive-like state, anhedonia, and

anxiety-like behavior in animals (Leonard and Maes, 2012; Maes et al., 2012), and depressive symptoms in humans (Engler et al., 2016; 2017). LPS administration activates toll-like receptor 4 (TLR4), initiating the canonical nuclear factor kappa B (NF-κB) pathway (Kawai et al., 2001). NF-κB is a transcription factor that mediates the inflammatory response. Under basal conditions, NF-κB is located in the cytosol by its association with the inhibitor of nuclear factor kappa B (IκBα). When LPS activates TLR4, IκBα is phosphorylated and undergoes proteasome degradation. This allows NF-κB translocation to the nucleus and the transcription of its target genes, which initiate the inflammatory response (Covert et al., 2005). Some of the proteins induced by NF-κB activation are pro-inflammatory cytokines like IL-6 and interferon-gamma (IFNγ) (Kawai et al., 2001). Moreover, LPS reduces the levels of the peroxisome proliferator-activated receptor gamma (PPARγ) in cells (Juknat et al., 2013; Choi et al., 2017), and rat cortex (Perez-Nievas et al., 2010; MacDowell et al., 2013). Conversely, the activation of PPARγ can antagonize the transcription activity of NF-κB, consequently producing anti-inflammatory effects (Ricote et al., 1998).

The increase in IL-6 and IFNγ induced by LPS activates the indoleamine 2,3-dioxygenase (IDO) enzyme (Godbout et al., 2008; Lestage et al., 2002). In mice, the increase in brain IDO activity has been associated to the appearance of depressive-like behavior (André et al., 2008; Dantzer et al., 2008). In addition, the increased activity of the IDO enzyme, which transforms the precursor tryptophan into kynurenine, leads to a skewed pathway toward the formation of kynurenine instead of serotonin (Moroni et al., 1991, Cervenka et al., 2017). Importantly, in recent years kynurenine and its metabolites have been broadly associated with the pathophysiology of depression, leading to the proposal of a new hypothesis of depression that integrates

stress, inflammation, the kynurenine pathway and serotonergic and glutamatergic neurotransmission (Maes et al., 2011; Oxenkrug, 2013; Barone, 2019).

In the last years, cannabidiol (CBD), the main non-psychotomimetic component of *Cannabis sativa* (Mechoulam et al., 2002), has shown antidepressant-like properties in predictive behavioral tests (El-Alfy et al., 2010; Zanelati et al., 2010), and in animal models of depression such as the olfactory bulbectomy (Linge et al., 2016), and the chronic unpredictable stress (Campos et al., 2013), following acute or chronic administration. CBD is known to produce changes in some neuroplasticity markers such as BDNF (Magen et al., 2010), and in hippocampal proliferation in chronically stressed animals (Campos et al., 2013), which has been associated to its antidepressant-like effect. This drug also presents anti-inflammatory and immunomodulatory effects, as reported in animal models of other pathologies such as Alzheimer's disease, which was mediated by PPARγ activation (Esposito et al., 2011). However, the link between the modulation of inflammatory pathways by cannabidiol and its antidepressant-like effects in an inflammatory model is yet to be explored (Silote et al., 2019).

Here we have studied the effect of acute cannabidiol administration in the depressive and anxious-like behavior in an animal model of inflammation induced by the systemic injection of lipopolysaccharide. We have also evaluated the effect of cannabidiol on different inflammatory markers as the NF-κB pathway, pro-inflammatory cytokines and the kynurenine pathway in order to figure out the mechanism of action of cannabidiol in this neuroinflammatory model.

2. MATERIAL AND METHODS

2.1 Animals

Male NMRI mice (2–3 months old, 30–35 g) (Envigo, Indiana, USA) were housed in groups of 3-4 with a 12-h light-dark cycle, and food and water were provided *ad libitum*. All procedures were carried out with the previous approval of the Animal Care Committee of the University of Cantabria according to the Spanish legislation and the European Communities Council Directive on “Protection of Animals Used in Experimental and Other Scientific Purposes” (86/609/EEC).

2.2 Drugs

Cannabidiol (CBD, Tocris, Bristol, United Kingdom) was dissolved in 2% Tween 80®: 5% Propilenglycol®: saline, and used at a dose of 30 mg/kg (Zanelati et al., 2010). Lipopolysaccharide (LPS from Escherichia coli O127:B8, Sigma-Aldrich, Darmstadt, Germany) was dissolved in saline and used at a dose of 0.83 mg/kg (O’Connor et al., 2009). Both drugs were administered i.p. (10 µl/g body weight) just starting the dark period. Animals that did not receive CBD or LPS were injected with the corresponding vehicle.

2.3 Experimental design

A graphical representation of the experimental procedure is shown in Figure 1. One week before the experiment, the animals were individually housed and habituated to a free choice of 2% sucrose solution and water. CBD was administered 30 min before the LPS injection. The open-field test (OFT) and the tail suspension test (TST) were performed 12 h after LPS administration. Sucrose preference was assessed during

that 12 h period. Thirty min after the end of the behavioral assessment, a set of animals used for western blot and HPLC was sacrificed by cervical dislocation and brain samples were rapidly collected and stored at -80ºC until used. Another set of animals, used for cytokine determination in plasma (ELISA) and brain (qPCR), was sacrifized by pentobarbital administration (Figure 1).

2.4 Behavioral assays

Tests were performed during the light phase. Animals were placed in the experimental room 1 h before the beginning of the procedures for habituation. Behavioral tests were performed during the light phase and were arranged from the least to most stressful ones (sucrose preference, open-field, and tail suspension test) (Figure 1).

2.4.1 Sucrose Preference Test (SPT)

This test was used to evaluate anhedonia. Animals were individualized and given a free choice of water and a 2% sucrose solution during one week before the experiment in order to habituate to the availability of both bottles, as previously described (Vidal et al., 2019). The positions of the two bottles were switched every day to avoid any potential position bias. The sucrose preference was calculated 12h after LPS injection as the percentage of the amount of sucrose solution consumed compared to the total amount of liquid intake during that period.

2.4.2 Open-Field Test (OFT)

The open-field apparatus was a brightly lit (350 lx) grey wooden box (40 × 40 × 30 cm). The center was defined as a 20 × 20 cm area. Mice were placed in one corner of the apparatus, and the behavior was video tracked by a computerized system (Any-maze Video-Tracking software, Stoelting Co., USA) for 5 minutes (Pilar-Cuéllar et al., 2019).

The parameters ‘total distance traveled’ and ‘time spent in the center’ were obtained to study locomotion and anxiety-like behavior, respectively.

2.4.3 Tail Suspension Test (TST)

Five minutes after the OFT, mice were suspended by the tail and video recorded for 6 min (Ferrés-Coy et al. 2016). The time spent immobile was determined using an automated software (Biobserve GmbH, Bonn, Germany). The settings were previously adjusted by an experienced observer to count as immobility only when mice were hanging passively and completely motionless, which is indicative of behavioral despair.

2.5 Western Blot (WB)

Cortical samples were processed for subcellular fractionation following the protocol described in García-Bueno (2005) with minor modifications. A buffer containing 10 mM HEPES (USB, Ohio, USA), pH 7.9, 1 mM EDTA, 1 mM EGTA, 10 mM KCl, 1 mM dithiothreitol (DTT) (Sigma Aldrich, Missouri, USA), 0.5 M sucrose (Scharlab S.L., Barcelona, Spain), 10 mM sodium molybdate and supplemented with a protease inhibitors cocktail (Sigma-Aldrich, Missouri, USA) was used for the homogenization of the samples. After 15 min on ice, 0.5% Igepal was added and samples were gently vortexed for 15 s. The cytosolic fraction was obtained by collecting the supernatant after a 5 min 8000 x g centrifugation at 4ºC. The remaining pellet was resuspended with 100 µl of the previous buffer supplemented with 20% glycerol (Scharlab S.L., Barcelona, Spain) and 0.4 M KCl and gently shaken for 30 min at 4ºC. The nuclear fraction was obtained by collecting the supernatant after a 5 min 13000 x g centrifugation at 4ºC.

Duplicates of 60-90 µg of cytosolic and nuclear protein, respectively, were size-separated with SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad, California, USA). For IκBα detection, membranes were blocked with 5% powder skimmed milk in TBS-T (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween-20) (Sigma Aldrich, Missouri, USA), and for NF-κB and PPARγ in 5% BSA (Sigma Aldrich, Missouri, USA) in TBS-T 0.1 (0.1% Tween-20). The following primary antibodies were incubated with the corresponding blocking solution at 4ºC over-night: IκBα (1:2000, AB3016, Chemicon, Massachusetts, USA), NF- κB (1:1000, #6956, Cell signaling, Massachusetts, USA), PPARγ (1:1000, MA5-14889, Invitrogen, California, USA), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:2000, sc-32233, Santa Cruz Biotechnology, Texas, USA), and proliferating cell nuclear antigen (PCNA) (1:1000, sc-56, Santa Cruz Biotechnology, Texas, USA). LI-COR® fluorescent secondary antibodies (700 and 800CW) were used at 1:15000, and the signal was detected with an Odyssey® CLx Imaging System (LI-COR Biosciences, Nebraska, USA). Protein quantification was performed using Image Studio™ Software (LI-COR Biosciences, Nebraska, USA). GAPDH and PCNA were used as cytosolic and nuclear housekeeping proteins, respectively, and the average of the duplicates of each sample was calculated. Purity of subcellular fractions was assessed analyzing GAPDH and PCNA protein expression. All blots were performed at least 3 times in separate assays. Results are represented in percentage *versus* the vehicle group.

2.6 Enzyme-linked Immunosorbent Assay (ELISA)

Mice were deeply anesthetized with a pentobarbital injection (40 mg/kg, i.p.) and the blood collected directly from the heart with an EDTA-treated syringe, centrifuged 10 min at 2000 x g, 4ºC, and the supernatant collected and stored at -80ºC until used.

TNFα and IL-6 ELISA kits were purchased from Invitrogen (Life Technologies Corporation, California, USA), and the procedure specified by the manufacturer was followed. The optical density was determined at 450 nm with a Mithras LB 940 (Berthold technologies, Baden-Württemberg, Germany). The data are represented as pg/ml.

2.7 RNA extraction and reverse transcription

RNA extraction was performed as previously described (Pilar-Cuéllar et al., 2017). RNA was extracted from prefrontal cortex (PFC) samples using TRI reagent (Merck KGaA, Darmstadt, Germany) following manufacturer's instructions. RNA purity assessment (ratios 260/280 and 260/230) and quantification were performed with a NanoDrop 1000 (Thermo Fischer Scientific S.L., Madrid, Spain). 600 ng of total RNA were used in the reverse transcriptase reaction performed with a high capacity cDNA reverse transcriptase kit (Applied Biosystems, California, USA) following manufacturer's instructions.

2.8 Quantitative PCR (qPCR)

Quantitative PCR was performed in a StepOne™ Real-Time PCR System (Applied Biosystems, California, USA) using TaqMan gene expression assays for TNFα (category no. Mm00443258_m1; O'Connor et al., 2009), IL-6 (category no. Mm00446190_m1; Martin et al., 2013), beta-actin (actb, category no. Mm00607939_s1), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, category no. Mm99999915_g1) that were purchased from Applied Biosystems (California, USA). Reactions were performed in triplicate using 125 ng cDNA template

per reaction. GAPDH and Actb were used as endogenous housekeeping control genes. Non-template controls were included in the experiment. The average of the triplicates of each sample was calculated and the relative quantitative measurement of target gene levels was performed using the ΔΔCt method (Livak and Schmittgen, 2001).

2.9 High Performance Liquid Chromatography (HPLC)

Samples from PFC and hippocampus (Hp) were diluted 1:7 (weight/volume) in milli-Q water and homogenized with a pellet pestle motor. For each 100 µl of sample, 25 µl of 6% perchloric acid was added. Samples were centrifuged at 16,000 xg for 15 min at 4ºC, and the supernatants were collected and filtered with a 0.45 µm filter. Serotonin (5-HT), tryptophan (TRP), and kynurenine (KYN) levels were quantified by HPLC. The HPLC system used was an ALEXYS® Neurotransmitter Analyzer (Antec Scientific, Leiden, The Netherlands) with an Acquity UPLC® BEH C18, 1.7 µm, 1 x 100 mm column (Waters, Massachusetts, USA). The oxidation potential was set at 0.7V, and the mobile phase consisted of 100 mM citric acid, 100 mM phosphoric acid, pH 6.0, 0.1 mM EDTA, 950 mg/L octanesulfonic acid, and 5% acetonitrile. The ratios KYN/TRP and KYN/5-HT are represented.

2.10 Statistical analysis

Results are expressed as mean ± standard error of the mean (S.E.M.). As CBD treatment was administered to both *naïve* and LPS mice, the statistical analysis was performed using two-way ANOVA followed by Newman-Keuls *posthoc* test. For the correlation studies, the Pearson's correlation coefficient (r) was used. Graphs and

statistical analyses were made using the GraphPad Prism software, version 6.1 (GraphPad Software Inc., California, USA). The level of significance was set at $p<0.05$. The number of animals used in each experimental group is indicated in the figure legends.

3. RESULTS

3.1 Antidepressant-like effect of CBD in the LPS model.

In the tail suspension test (TST), the LPS group showed an increased immobility time (126.8±20.3 s) compared to the vehicle group (53.8±10.7 s, $p<0.01$). The administration of CBD prevented the increase in the immobility time induced by LPS (72.6±10.3 s, $p<0.01$) (Figure 2A). A two-way ANOVA showed a significant effect of the model [$F(1,22)=10.26$, $p<0.01$], and the interaction [$F(1,22)=5.54$, $p<0.05$].

In the sucrose preference test, the LPS group presented a decreased sucrose preference (49.9±3.9%), compared to the vehicle group (81.4±2.2%, $p<0.001$). The administration of CBD to LPS mice (63.3±3.4%) increased the sucrose preference compared to the LPS group (49.9±3.9%, $p<0.01$) (Figure 2B). A two-way ANOVA showed a significant effect of the model [$F(1,28)=51.89$, $p<0.001$] and the interaction [$F(1,28) = 7.00$, $p<0.05$].

In the open-field test (OFT), the LPS group showed a decrease in the time spent in the center (2.4±1.4 s) compared to the vehicle group (18.9±4.7 s, $p<0.001$). The administration of CBD to LPS mice did not modify the central time compared to the LPS group (1.5±1.3 s) (Figure 2C). A two-way ANOVA showed a significant effect of the model [$F(1,22)= 44.86$, $p<0.001$]. LPS mice also traveled less distance in this test (7.41±1.37 s) compared to the vehicle group (16.32±2.46 s, $p<0.001$), and CBD

administration to LPS mice did not alter the distance travelled (3.88±2.1 s) (Figure 2D). A two-way ANOVA showed a significant effect of the model [$F(1,22)=42.31$, $p<0.001$].

CBD administration to *naïve* mice did not modify any of the behavioral parameters studied.

Correlations were made to check if the reduced locomotion induced by LPS could interfere in the behavioral tests' interpretation. There was no correlation between the total distance traveled in the OFT and the time immobile in the TST (Figure 2E). Conversely, a significant correlation was found between the total distance traveled and the time spent in the center in the OFT (Figure 2F).

3.2 CBD-induced changes in the LPS model: NF-κB pathway activation

The activation of the NF-κB pathway and PPARγ was assessed by determining NF-κB, IκBα and PPARγ protein levels in the nuclear and cytosolic fractions from cortical samples. Twelve hours post-LPS injection, there was an increase of NF-κB nuclear levels (197.9±32.6%) in comparison to the vehicle group (100±9.3%, $p<0.001$), and CBD pre-treatment significantly attenuated this increase in LPS mice (153±37.7%, $p<0.01$) (Figure 3A). A two-way ANOVA showed a significant effect of the LPS model [$F(1,22)= 32.33$, $p<0.001$] and the interaction [$F(1,22)=11.35$, $p<0.01$].

No changes were observed in the cytosolic levels of IκBα in any of the experimental groups in the post-test analysis (Figure 3B). A two-way ANOVA showed an effect of the LPS model [$F(1,22)=6.56$, $p<0.05$].

CBD administration in *naïve* animals produced an increase of PPARγ in the nucleus (161±50.9%) compared to the vehicle group (100±19.8%, $p<0.05$) (Figure 3C) and a decrease in the cytosolic fraction (75.5±14.4% in CBD *vs* 100±6.1% in the vehicle

group, $p<0.01$) (Figure 3D). Neither the LPS nor the CBD+LPS groups presented differences in PPARγ protein levels compared to the vehicle group (Figure 3C and D). A two-way ANOVA revealed a significant effect of CBD on PPARγ nuclear levels [$F(1,22)=5.01$, $p<0.05$] and a significant effect of the interaction on PPARγ cytosolic levels [$F(1,22)=13.54$, $p<0.01$].

3.3 CBD-induced changes in the LPS model: pro-inflammatory cytokines IL-6 and TNFα in plasma and brain

LPS administration induced a mild increase in plasma TNFα levels (9.3±1.3 pg/ml) compared to the vehicle group (6.3±0.2 pg/ml, $p<0.05$). No changes were observed in the LPS mice treated with CBD (Figure 4A). A two-way ANOVA showed a significant effect of the model [$F(1,25)=9.06$, $p<0.01$]. LPS injection produced a huge increase in plasma IL-6 levels in LPS (1143±191 pg/ml) *versus* the vehicle group (27.6±4.3 pg/ml, $p<0.001$). The CBD+LPS group showed a significant reduction of IL-6 levels (573±44 pg/ml) compared to the LPS group ($p<0.001$) (Figure 4B). A two-way ANOVA showed a significant effect of the model [$F(1,25)=76.64$, $p<0.001$], treatment [$F(1,25)=9.00$, $p<0.01$], and the interaction [$F(1,25)= 9.14$, $p<0.01$].

In PFC, the LPS administration increased TNFα mRNA levels (9.8±1.8) compared to the vehicle group (1.0±0.1, $p<0.001$), and the previous CBD administration did not modify the TNFα expression (Figure 4C). A two-way ANOVA showed a significant effect of the model [$F(1,25)=45.57$, $p<0.001$]. IL-6 mRNA levels in PFC were higher in the LPS group (3.6±0.6) compared to the vehicle group (1.0±0.1, $p<0.001$), and CBD administration reduced LPS-induced IL-6 increase (2.2±0.4, $p<0.05$) (Figure 4D). A two-way ANOVA showed a significant effect of the model [$F(1,25)=22.83$, $p<0.001$],

and the interaction [F(1,25)=4.40, p<0.05]. CBD administration to *naïve* mice did not induce changes in the pro-inflammatory cytokines studied.

3.4 CBD-induced changes in the LPS model: kynurenine pathway

The kynurenine/tryptophan (KYN/TRP) ratio is used as a marker of the IDO activity (Fuchs et al., 1990; Widner et al., 2002; O'Connor et al., 2009). In the hippocampus of the LPS group a higher KYN/TRP ratio was found (0.557±0.041) compared to the vehicle group (0.360±0.062, p<0.05). The administration of CBD prevented the increase in the KYN/TRP ratio in the LPS group (0.373±0.048, p<0.05) (Figure 5A). A two-way ANOVA showed a significant effect of the interaction [F(1,19)=8.389, p<0.01]. Similar results were obtained in cortex, as the LPS group presented a higher KYN/TRP ratio (3.449±0.217) *versus* the vehicle group (2.084±0.066, p<0.001). CBD attenuated this increase in the LPS group (2.931±0.189, p<0.05) (Figure 5C). A two-way ANOVA showed a significant effect of the model [F(1,28)=35.47, p<0.001], and a strong tendency of the interaction [F(1,28)=4.060, p=0.054].

To examine the effects of LPS and CBD administration in the balance between the two major tryptophan metabolic pathways, we determined the kynurenine/serotonin (KYN/5-HT) ratio (Mohamed et al., 2013; Li et al., 2020). In the hippocampus of LPS mice, the KYN/5-HT ratio was higher (1.069±0.097) than in the vehicle group (0.650±0.156, p<0.01), while the administration of CBD prevented this increase (0.534±0.066, p<0.01) (Figure 5B). A two-way ANOVA showed a significant effect of the model [F(1,19)=8.160, p<0.05], and of CBD [F(1,19)=17.04, p<0.001]. In cortex, the KYN/5-HT ratio in the LPS group was higher (4.204±0.315) than the vehicle group (2.127±0.115, p<0.001), and CBD attenuated that increase (2.786±0.184, p<0.001)

(Figure 5D). A two-way ANOVA showed a significant effect of the model [$F(1,28)=47.30$, $p<0.001$], CBD [$F(1,28)=14.39$, $p<0.001$], and the interaction [$F(1,28)=8.062$, $p<0.01$]. CBD administration to *naïve* mice did not alter the kynurenine pathway.

4. DISCUSSION

The present study shows the antidepressant-like effect elicited by an acute cannabidiol administration in a neuroinflammatory model induced by LPS administration, in parallel with the reduction of different inflammatory markers in the brain and periphery.

The systemic administration of LPS induces a depressive-like state, as evidenced by the increase in behavioral despair and anhedonia. Since the immobility time in the tail suspension test and the locomotion observed in the open field test did not show a statistically significant correlation in *naïve* and LPS animals, we can infer that the sickness behavior is not interfering with the depressive-like behavior at this time-point. The administration of a single dose of cannabidiol prior to LPS prevented the LPS-induced behavioral despair. This agrees with the effect elicited by the acute administration of SSRIs as fluoxetine and paroxetine (Oghi et al., 2013), drugs with fast antidepressant effect as ketamine (Walker et al., 2013), and the chronic administration of tricyclic antidepressants such as imipramine (Renault and Aubert, 2006) in this inflammatory model. The reduction in immobility elicited by cannabidiol in the tail suspension test contrasts with the lack of effect on locomotion observed in the open field test, supporting the impact of cannabidiol on the depressive-like behavior. Moreover, the anhedonic state induced by LPS is partially prevented by cannabidiol, supporting its role in the remission of the different depressive manifestations observed

in this model, in line with the results obtained using CBD in other mouse (Linge et al., 2016) and rat (Shoval et al., 2016) models of depression.

However, cannabidiol treatment did not show anxiolytic-like effects in the LPS model, as it has been described in Wistar Kyoto rats (Shoval et al., 2016). In contrast, there is an acute anxiolytic-like effect in naïve animals (reviewed in Blessing et al., 2015), and in the olfactory bulbectomy model (Linge et al., 2016). Nevertheless, we obtained a significant correlation between locomotion and the anxious-like behavior in our inflammatory model, which reveals the impossibility to separate the anxious from the sickness behavior. Therefore, we cannot draw conclusions about the anxious-like behavior in this study.

The LPS model of neuroinflammation is characterized by an initial sickness behavior that resolves in less than 24 h, followed by a depressive-like behavior that overlaps with sickness in the initial phases, and lasts longer (Dantzer et al., 2008). At the time-point studied in this work, there were still some manifestations characteristic of the sickness behavior (decreased locomotor activity, reduced body weight, etc.) (Dantzer, 2001; O'Connor et al., 2009). However, this behavior can be differentiated from the depressive-like behavior (behavioral despair and anhedonia), as some molecules as kynurenine induce depressive-like but not sickness behavior, and the blockade of IDO prevents the manifestation of the depressive-like behavior, without altering the sickness behavior induced by LPS (O'Connor et al., 2009).

To investigate the mechanism of action of CBD responsible for the behavioral findings, we studied the NF-κB pathway, as it takes part in the pro-inflammatory mechanism of LPS (Kawai et al., 2001). The LPS model presented increased NF-κB nuclear levels as previously described (Perez-Nievas et al., 2010; MacDowell et al., 2013), evidencing the activation of the NF-κB pathway. CBD administration prior to LPS

significantly attenuated the increase in nuclear NF-κB levels, in line with the inhibitor effect of CBD on this pathway reported in cell culture studies (Esposito et al., 2006; Juknat et al., 2019; dos-Santos-Pereira et al., 2020) and in an animal model of Alzheimer's disease (Esposito et al., 2011). Interestingly, the widely used antidepressant fluoxetine also reversed the NF-κB-induced increase in a chronic LPS model in mice (Rodrigues et al., 2018). The direct inhibition of NF-kB also presents antidepressant-like effects in stress models (Koo et al., 2010). The results obtained in NF-κB and IκBα levels fit with the time course of NF-κB pathway activation/deactivation described by Hobbs et al. (2018). They reported that LPS activation of TLR4 rapidly produces a decrease in IκBα cytoplasmic levels and NF-κB nuclear translocation. However, NF-κB deactivation is much slower, as this protein is still increased in the nucleus 10 h after LPS exposure, when IκBα cytoplasmic levels have returned to its basal expression (Hobbs et al., 2018). All this suggests that the reduction of NF-κB pathway activation could participate in the antidepressant-like effect of cannabidiol in this neuroinflammatory model induced by LPS administration.

Regarding PPARγ, it has been reported that LPS produces a reduction in its expression in BV2 cells (Juknat et al., 2013; Choi et al., 2017), and in rat cortex (Perez-Nievas et al., 2010; MacDowell et al., 2013) in a few hours. Thus, the lack of changes observed in our experiments 12 hours after LPS exposure may be due to the recovery of PPARγ basal levels. In contrast, the administration of CBD induced an increase in PPARγ nuclear levels and a decrease in its cytosolic levels. As CBD is a PPARγ ligand (O'Sullivan et al., 2009; reviewed in O'Sullivan, 2016), the CBD-mediated activation of PPARγ in naïve animals would promote its translocation to the nucleus, as previously described (Khan and Abu-Amer, 2003; Kelly et al., 2004; Umemoto and Fujiki, 2012). In this regard, other authors have reported that chronic CBD treatment induced an

increase in PPARγ levels in the spinal cord of an animal model of multiple sclerosis (Giacoppo et al., 2017) and an increase in PPARγ coactivator 1-alpha (PGC-1α) mRNA levels in the striatum of an animal model of dyskinesia (Sonego et al., 2018). Thus, this is the first time, to our knowledge, that an increase in PPARγ nuclear levels is reported with a single CBD administration *in vivo*. Although PPARγ nuclear levels were increased in the CBD naïve group, this was not associated to changes in nuclear NF-κB levels. This could be explained by the lack of activation of the NF-κB pathway in this experimental group. The lack of changes in nuclear PPARγ levels in the CBD+LPS group could be due to the role of PPARγ in the downregulation of the pathway. PPARγ can bind to NF-κB in the nucleus and induce the nuclear export of PPARγ-NF-κB complex (Chung et al., 2000; Hou et al., 2012), promoting its translocation to the cytosol (Kelly et al., 2004). Nevertheless, we cannot exclude the possibility that CBD could produce its effects on the NF-κB pathway independently of PPARγ activation, as CBD has also shown anti-inflammatory effects that are not dependent on PPARγ (Alhamoruni et al., 2012; dos-Santos-Pereira et al., 2020).

In the evaluation of pro-inflammatory cytokines, LPS administration induced an elevation of TNFα plasma levels to a lesser extent than IL-6 levels, as TNFα peaks earlier than IL-6 in response to LPS (Andreasen et al., 2008). In contrast, the increase in TNFα mRNA expression was higher than the one observed in IL-6 mRNA levels in the brain, as previously described (André et al., 2008). The coexistence of depressive-like behavior and increased plasma IL-6 levels in this neuroinflammatory model, agrees with the elevated IL-6 levels observed in animal models of depression (Sukoff Rizzo et al., 2012) and in meta-analyses of studies in MDD patients (Dowlati et al., 2010; Haapakoski et al., 2015). In addition, the dysregulation of both IL-6 production and serotonergic neurotransmission, as observed in our model by the increase in the

ratio kynurenine/serotonin, is associated with a greater vulnerability to suffering depression (Bull et al., 2009). In line with the effect of cannabidiol on the depressive-like behavior and IL-6 levels presented here, it has been reported that IL-6 knockout animals exhibit reduced behavioral despair and resistance to stress-induced depression (Chourbaji et al., 2006). In addition, the increased IL-6 levels observed in acute depressed patients are reverted to control levels after antidepressant treatment (Frommberger et al., 1997). The reduction of IL-6 could be of great importance, as high IL-6 levels are associated not only with depressive-like behavior but also with treatment resistance (Sukoff Rizzo et al., 2012). Regarding TNFα, previous studies demonstrated that its administration induces sickness behavior and neuroinflammation, but not depressive symptoms as anhedonia (Biesmans et al., 2015). In addition, a cumulative meta-analysis of studies in MDD patients revealed that the association of TNFα with the disease is inconsistent, while other inflammatory markers as IL-6 are clearly associated to MDD (Haapakoski et al., 2015). This is in accordance with the fact that, in this work, CBD exerted antidepressant-like effects in the LPS model without producing changes in TNFα levels. These pieces of evidence suggest that the antidepressant-like effect of cannabidiol in the LPS model may be partially due to its effect over IL-6 levels.

To further investigate the mechanism of action of CBD responsible for the findings presented here, we studied the kynurenine pathway. Our results show an increase in the kynurenine/tryptophan ratio in the LPS model, which is indicative of an increased IDO activity (Fuchs et al., 1990; Widner et al., 2002; O'Connor et al., 2009). This IDO activation is further confirmed by the increased kynurenine/serotonin ratio in these animals, showing a tilted balance toward kynurenine synthesis. Moreover, the coexistence of behavioral despair and anhedonia with elevated kynurenine levels in

our animal model is in line with the behavioral despair (O'Connor et al., 2009) and anhedonia (Salazar et al., 2012) induced by kynurenine administration. Conversely, CBD administration produced antidepressant-like effects and a decrease in kynurenine/tryptophan and kynurenine/serotonin ratios, which is in line with the antidepressant-like effect described following IDO enzyme inhibition (O'Connor et al., 2009; Dobos et al., 2012). Moreover, the inhibition of this enzyme is also associated to a lower anhedonic state, as observed in IDO knockout animals (Lawson et al., 2013). This body of evidence, together with the reversion of the behavioral and molecular changes within the kynurenine pathway promoted by cannabidiol in the LPS model, suggest that cannabidiol could be producing its antidepressant- and hedonic-like effects in this model by the reduction of brain IDO activity. This could be due to the direct inhibition of the enzyme (Jenny et al., 2009), or to the decrease in pro-inflammatory cytokines (Lestage et al., 2002). Within the kynurenine pathway, recent basic and clinical research highlight the involvement of the kynurenine metabolites in depression (reviewed in Maes et al., 2011; Barone, 2019). In animal models such as LPS-induced neuroinflammation and in MDD patients, the neurotoxic branch of the KYN pathway [3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN)] is increased, while the neuroprotective branch [kynurenic acid (KYNA)] is decreased (Verdonk et al., 2019; reviewed in Barone, 2019). This imbalance between the two KYN branches has been related to a depressive-like behavior (Parrott et al., 2016; Laumet et al., 2017). As it has been recently found that CBD can increase KYNA levels and decrease 3-HK and QUIN levels after a neurotoxic stimulus (di Giacomo et al., 2020), all these pieces of evidence suggest that the restoration of this neurotoxic/neuroprotective balance could be also participating in the antidepressant-like effects elicited by CBD in the LPS model presented in this work.

In summary, our results show for the first time that cannabidiol exerts antidepressant-like effects in the LPS model of depression, together with a reduction of brain NF-κB pathway activation, plasma and brain IL-6 levels and IDO activity. However, we cannot exclude the possibility that other mechanisms of action proposed for cannabidiol could have also participated in the observed effects. In view of the anti-neuroinflammatory actions of CBD associated to its antidepressant-like effect, more research is needed to better characterize it as a potential drug in the treatment of depression.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Alhamoruni A, Wright KL, Larvin M, O'Sullivan SE. Cannabinoids mediate opposing effects on inflammation-induced intestinal permeability. Br J Pharmacol. 2012;165(8):2598-2610. doi:10.1111/j.1476-5381.2011.01589.x.

American Psychiatric Association, DSM-5 Task Force. Diagnostic and statistical manual of mental disorders: DSM-5™ (5th ed.). American Psychiatric Publishing, Inc. 2013. doi: 10.1176/appi.books.9780890425596.

André C, O'Connor JC, Kelley KW, Lestage J, Dantzer R, Castanon N. Spatio-temporal differences in the profile of murine brain expression of proinflammatory cytokines and indoleamine 2,3-dioxygenase in response to peripheral lipopolysaccharide administration. J Neuroimmunol. 2008;200(1-2):90-9. doi: 10.1016/j.jneuroim.2008.06.011.

Andreasen AS, Krabbe KS, Krogh-Madsen R, Taudorf S, Pedersen BK, Møller K. Human endotoxemia as a model of systemic inflammation. Curr Med Chem. 2008;15(17):1697-705. doi: 10.2174/092986708784872393.

Barone P. The 'Yin' and the 'Yang' of the kynurenine pathway: excitotoxicity and neuroprotection imbalance in stress-induced disorders. Behav Pharmacol. 2019;30(2 and 3-Spec Issue):163-186. doi: 10.1097/FBP.0000000000000477.

Biesmans S, Bouwknecht JA, Ver Donck L, Langlois X, Acton PD, De Haes P, Davoodi N, Meert TF, Hellings N, Nuydens R. Peripheral Administration of Tumor Necrosis Factor-Alpha Induces Neuroinflammation and Sickness but Not Depressive-Like Behavior in Mice. Biomed Res Int. 2015;2015:716920. doi: 10.1155/2015/716920.

Blessing EM, Steenkamp MM, Manzanares J, Marmar CR. Cannabidiol as a Potential Treatment for Anxiety Disorders. Neurotherapeutics. 2015;12(4):825-36. doi: 10.1007/s13311-015-0387-1.

Bull SJ, Huezo-Diaz P, Binder EB, Cubells JF, Ranjith G, Maddock C, Miyazaki C, Alexander N, Hotopf M, Cleare AJ, Norris S, Cassidy E, Aitchison KJ, Miller AH, Pariante CM Functional polymorphisms in the interleukin-6 and serotonin transporter genes, and depression and fatigue induced by interferon-alpha and ribavirin treatment. Mol Psychiatry. 2009;14(12):1095-104. doi: 10.1038/mp.2008.48.

Campos AC, Ortega Z, Palazuelos J, Fogaça MV, Aguiar DC, Díaz-Alonso J, Ortega-Gutiérrez S, Vázquez-Villa H, Moreira FA, Guzmán M, Galve-Roperh I, Guimarães FS. The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: involvement of the endocannabinoid system. Int J Neuropsychopharmacol. 2013;16(6):1407-19. doi: 10.1017/S1461145712001502.

Cervenka I, Agudelo LZ, Ruas JL. Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. Science. 2017;357(6349). pii: eaaf9794. doi: 10.1126/science.aaf9794.

Choi MJ, Lee EJ, Park JS, Kim SN, Park EM, Kim HS. Anti-inflammatory mechanism of galangin in lipopolysaccharide-stimulated microglia: Critical role of PPAR-γ signaling pathway. Biochem Pharmacol. 2017;144:120-131. doi:10.1016/j.bcp.2017.07.021.

Chourbaji S, Urani A, Inta I, Sanchis-Segura C, Brandwein C, Zink M, Schwaninger M, Gass P. IL-6 knockout mice exhibit resistance to stress-induced development of depression-like behaviors. Neurobiol Dis. 2006;23(3):587-94. doi: 10.1016/j.nbd.2006.05.001.

Chung SW, Kang BY, Kim SH, Pak YK, Cho D, Trinchieri G, Kim TS. Oxidized low density lipoprotein inhibits interleukin-12 production in lipopolysaccharide-activated mouse macrophages via direct interactions between peroxisome proliferator-activated receptor-gamma and nuclear factor-kappa B. J Biol Chem. 2000;275(42):32681-32687. doi:10.1074/jbc.M002577200.

Connor TJ, Starr N, O'Sullivan JB, Harkin A. Induction of indolamine 2,3-dioxygenase and kynurenine 3-monooxygenase in rat brain following a systemic inflammatory challenge: a role for IFN-gamma?. Neurosci Lett. 2008;441(1):29-34. doi: 10.1016/j.neulet.2008.06.007.

Covert MW, Leung TH, Gaston JE, Baltimore D. Achieving stability of lipopolysaccharide-induced NF-kappaB activation. Science. 2005;309(5742):1854-7. doi: 10.1126/science.1112304.

Dantzer R. Cytokine-induced sickness behavior: mechanisms and implications. Ann N Y Acad Sci. 2001;933:222-34. doi: 10.1111/j.1749-6632.2001.tb05827.x.

Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008;9(1):46-56. doi: 10.1038/nrn2297.

di Giacomo V, Chiavaroli A, Recinella L, Orlando G, Cataldi A, Rapino M, Di Valerio V, Ronci M, Leone S, Brunetti L, Menghini L, Zengin G, Ak G, Abdallah HH, Ferrante C. Antioxidant and Neuroprotective Effects Induced by Cannabidiol and Cannabigerol in Rat CTX-TNA2 Astrocytes and Isolated Cortexes. Int J Mol Sci. 2020;21(10):3575. doi: 10.3390/ijms21103575.

Dobos N, de Vries EF, Kema IP, Patas K, Prins M, Nijholt IM, Dierckx RA, Korf J, den Boer JA, Luiten PG, Eisel UL. The role of indoleamine 2,3-dioxygenase in a mouse model of neuroinflammation-induced depression. J Alzheimers Dis. 2012;28(4):905-15. doi: 10.3233/JAD-2011-111097.

dos-Santos-Pereira M, Guimarães FS, Del-Bel E, Raisman-Vozari R, Michel PP. Cannabidiol prevents LPS-induced microglial inflammation by inhibiting ROS/NF-κB-dependent signaling and glucose consumption. Glia. 2020;68(3):561-573. doi:10.1002/glia.23738

Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctôt KL. A meta-analysis of cytokines in major depression. Biol Psychiatry. 2010;67(5):446-57. doi: 10.1016/j.biopsych.2009.09.033.

Dregan A, Matcham F, Harber-Aschan L, Rayner L, Brailean A, Davis K, Hatch S, Pariante C, Armstrong D, Stewart , Hotopf M. Common mental disorders within chronic inflammatory disorders: a primary care database prospective investigation. Ann Rheum Dis. 2019;78(5):688-695. doi: 10.1136/annrheumdis-2018-214676.

Duman RS, Li N. A neurotrophic hypothesis of depression: role of synaptogenesis in the actions of NMDA receptor antagonists. Philos Trans R Soc Lond B Biol Sci. 2012;367(1601):2475-84. doi: 10.1098/rstb.2011.0357.

El-Alfy AT, Ivey K, Robinson K, Ahmed S, Radwan M, Slade D, Khan I, ElSohly M, Ross S. Antidepressant-like effect of delta9-tetrahydrocannabinol and other cannabinoids isolated from Cannabis sativa L. Pharmacol Biochem Behav. 2010;95(4):434-42. doi: 10.1016/j.pbb.2010.03.004.

Enache D, Pariante CM, Mondelli V. Markers of central inflammation in major depressive disorder: A systematic review and meta-analysis of studies examining cerebrospinal fluid, positron emission tomography and post-mortem brain tissue. Brain Behav Immun. 2019;81:24-40. doi: 10.1016/j.bbi.2019.06.015.

Engler H, Benson S, Wegner A, Spreitzer I, Schedlowski M, Elsenbruch S. Men and women differ in inflammatory and neuroendocrine responses to endotoxin but not in the severity of sickness symptoms. Brain Behav Immun. 2016;52:18-26. doi: 10.1016/j.bbi.2015.08.013.

Engler H, Brendt P, Wischermann J, Wegner A, Röhling R, Schoemberg T, Meyer U, Gold R, Peters J, Benson S, Schedlowski M. Selective increase of cerebrospinal fluid IL-6 during experimental systemic inflammation in humans: association with depressive symptoms. Mol Psychiatry. 2017;22(10):1448-1454. doi: 10.1038/mp.2016.264.

Esposito G, De Filippis D, Maiuri MC, De Stefano D, Carnuccio R, Iuvone T. Cannabidiol inhibits inducible nitric oxide synthase protein expression and nitric oxide production in beta-amyloid stimulated PC12 neurons through p38 MAP kinase and NF-kappaB involvement. Neurosci Lett. 2006;399(1-2):91-95. doi:10.1016/j.neulet.2006.01.047.

Esposito G, Scuderi C, Valenza M, Togna GI, Latina V, De Filippis D, Cipriano M, Carratù MR, Iuvone T, Steardo L. Cannabidiol reduces Aβ-induced neuroinflammation

and promotes hippocampal neurogenesis through PPARγ involvement. PLoS One. 2011;6(12):e28668. doi: 10.1371/journal.pone.0028668.

Ferrés-Coy A, Galofré M, Pilar-Cuéllar F, Vidal R, Paz V, Ruiz-Bronchal E, Campa L, Pazos Á, Caso JR, Leza JC, Alvarado G, Montefeltro A, Valdizán EM, Artigas F, Bortolozzi A. Therapeutic antidepressant potential of a conjugated siRNA silencing the serotonin transporter after intranasal administration. Mol Psychiatry. 2016;21(3):328-38. doi: 10.1038/mp.2015.80.

Frommberger UH, Bauer J, Haselbauer P, Fräulin A, Riemann D, Berger M. Interleukin-6-(IL-6) plasma levels in depression and schizophrenia: comparison between the acute state and after remission. Eur Arch Psychiatry Clin Neurosci. 1997;247(4):228-33. doi: 10.1007/BF02900219.

Fuchs D, Möller AA, Reibnegger G, Stöckle E, Werner ER, Wachter H. Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symptoms. J Acquir Immune Defic Syndr (1988). 1990;3(9):873-6.

García-Bueno B, Madrigal JL, Lizasoain I, Moro MA, Lorenzo P, Leza JC. Peroxisome proliferator-activated receptor gamma activation decreases neuroinflammation in brain after stress in rats. Biol Psychiatry. 2005;57(8):885-94. doi: 10.1016/j.biopsych.2005.01.007.

Giacoppo S, Pollastro F, Grassi G, Bramanti P, Mazzon E. Target regulation of PI3K/Akt/mTOR pathway by cannabidiol in treatment of experimental multiple sclerosis. Fitoterapia. 2017;116:77-84. doi:10.1016/j.fitote.2016.11.010.

Godbout JP, Moreau M, Lestage J, Chen J, Sparkman NL, O'Connor J, Castanon N, Kelley KW, Dantzer R, Johnson RW. Aging exacerbates depressive-like behavior in

mice in response to activation of the peripheral innate immune system. Neuropsychopharmacology. 2008;33(10):2341-51. doi: 10.1038/sj.npp.1301649.

Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimäki M. Cumulative meta-analysis of interleukins 6 and 1β, tumour necrosis factor α and C-reactive protein in patients with major depressive disorder. Brain Behav Immun. 2015;49:206-15. doi: 10.1016/j.bbi.2015.06.001.

Harmer CJ, Duman RS, Cowen PJ. How do antidepressants work? New perspectives for refining future treatment approaches. Lancet Psychiatry. 2017;4(5):409-418. doi: 10.1016/S2215-0366(17)30015-9.

Hobbs S, Reynoso M, Geddis AV, Mitrophanov AY, Matheny RW Jr. LPS-stimulated NF-κB p65 dynamic response marks the initiation of TNF expression and transition to IL-10 expression in RAW 264.7 macrophages. Physiol Rep. 2018;6(21):e13914. doi:10.14814/phy2.13914.

Hou Y, Moreau F, Chadee K. PPARγ is an E3 ligase that induces the degradation of NFκB/p65. Nat Commun. 2012;3:1300. doi:10.1038/ncomms2270.

Jenny M, Santer E, Pirich E, Schennach H, Fuchs D. D9-tetrahydrocannabinol and cannabidiol modulate mitogen-induced tryptophan degradation and neopterin formation in peripheral blood mononuclear cells in vitro. J Neuroimmunol. 2009;207(1-2):75-82. doi: 10.1016/j.jneuroim.2008.12.004.

Juknat A, Pietr M, Kozela E, Rimmerman N, Levy R, Gao F, Coppola G, Geschwind D, Vogel Z. Microarray and pathway analysis reveal distinct mechanisms underlying cannabinoid-mediated modulation of LPS-induced activation of BV-2 microglial cells. PLoS One. 2013;8(4):e61462. doi:10.1371/journal.pone.0061462.

Juknat A, Gao F, Coppola G, Vogel Z, Kozela E. miRNA expression profiles and molecular networks in resting and LPS-activated BV-2 microglia-Effect of cannabinoids. PLoS One. 2019;14(2):e0212039. doi:10.1371/journal.pone.0212039.

Kawai T, Takeuchi O, Fujita T, Inoue J, Mühlradt PF, Sato S, Hoshino K, Akira S. Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. J Immunol. 2001;167(10):5887-94. doi: 10.4049/jimmunol.167.10.5887.

Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AG, Pettersson S, Conway S. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol. 2004;5(1):104-112. doi:10.1038/ni1018.

Khan E, Abu-Amer Y. Activation of peroxisome proliferator-activated receptor-gamma inhibits differentiation of preosteoblasts. J Lab Clin Med. 2003;142(1):29-34. doi:10.1016/S0022-2143(03)00058-1.

Koo JW, Russo SJ, Ferguson D, Nestler EJ, Duman RS. Nuclear factor-kappaB is a critical mediator of stress-impaired neurogenesis and depressive behavior. Proc Natl Acad Sci U S A. 2010;107(6):2669-74. doi: 10.1073/pnas.0910658107.

Laumet G, Zhou W, Dantzer R, Edralin JD, Huo X, Budac DP, O'Connor JC, Lee AW, Heijnen CJ, Kavelaars A. Upregulation of neuronal kynurenine 3-monooxygenase mediates depression-like behavior in a mouse model of neuropathic pain. Brain Behav Immun. 2017;66:94-102. doi: 10.1016/j.bbi.2017.07.008.

Lawson MA, Parrott JM, McCusker RH, Dantzer R, Kelley KW, O'Connor JC. Intracerebroventricular administration of lipopolysaccharide induces indoleamine-2,3-

dioxygenase-dependent depression-like behaviors. J Neuroinflammation. 2013;10:87. doi: 10.1186/1742-2094-10-87.

Leonard B, Maes M. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. Neurosci Biobehav Rev. 2012;36(2):764-85. doi: 10.1016/j.neubiorev.2011.12.005.

Lestage J, Verrier D, Palin K, Dantzer R. The enzyme indoleamine 2,3-dioxygenase is induced in the mouse brain in response to peripheral administration of lipopolysaccharide and superantigen. Brain Behav Immun. 2002;16(5):596-601. doi: 10.1016/s0889-1591(02)00014-4.

Li CC, Jiang N, Gan L, Zhao MJ, Chang Q, Liu XM, Pan RL. Peripheral and cerebral abnormalities of the tryptophan metabolism in the depression-like rats induced by chronic unpredicted mild stress. Neurochem Int. 2020;138:104771. doi: 10.1016/j.neuint.2020.104771.

Linge R, Jiménez-Sánchez L, Campa L, Pilar-Cuéllar F, Vidal R, Pazos A, Adell A, Díaz Á. Cannabidiol induces rapid-acting antidepressant-like effects and enhances cortical 5-HT/glutamate neurotransmission: role of 5-HT1A receptors. Neuropharmacology. 2016;103:16-26. doi: 10.1016/j.neuropharm.2015.12.017.

Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-408. doi: 10.1006/meth.2001.1262.

MacDowell KS, García-Bueno B, Madrigal JL, Parellada M, Arango C, Micó JA, Leza JC. Risperidone normalizes increased inflammatory parameters and restores anti-

inflammatory pathways in a model of neuroinflammation. Int J Neuropsychopharmacol. 2013;16(1):121-135. doi:10.1017/S1461145711001775.

Maes M, Leonard BE, Myint AM, Kubera M, Verkerk R. The new '5-HT' hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. Prog Neuropsychopharmacol Biol Psychiatry. 2011;35(3):702-21. doi: 10.1016/j.pnpbp.2010.12.017.

Maes M, Berk M, Goehler L, Song C, Anderson G, Gałecki P, Leonard B. Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. BMC Med. 2012;10:66. doi: 10.1186/1741-7015-10-66.

Magen I, Avraham Y, Ackerman Z, Vorobiev L, Mechoulam R, Berry EM. Cannabidiol ameliorates cognitive and motor impairments in bile-duct ligated mice via 5-HT1A receptor activation. Br J Pharmacol. 2010;159(4):950-7. doi: 10.1111/j.1476-5381.2009.00589.x.

Maier SF. Bi-directional immune-brain communication: Implications for understanding stress, pain, and cognition. Brain Behav Immun. 2003;17(2):69-85. doi: 10.1016/s0889-1591(03)00032-1.

Martin SA, Pence BD, Greene RM, Johnson SJ, Dantzer R, Kelley KW, Woods JA. Effects of voluntary wheel running on LPS-induced sickness behavior in aged mice. Brain Behav Immun. 2013;29:113-23. doi: 10.1016/j.bbi.2012.12.014.

Mechoulam R, Parker LA, Gallily R. Cannabidiol: an overview of some pharmacological aspects. J Clin Pharmacol. 2002;42(S1):11S-19S. doi: 10.1002/j.1552-4604.2002.tb05998.x.

Mohamed BM, Aboul-Fotouh S, Ibrahim EA, Shehata H, Mansour AA, Yassin NA, El-Eraky W, Abdel-Tawab AM. Effects of pentoxifylline, 7-nitroindazole, and imipramine on tumor necrosis factor-α and indoleamine 2,3-dioxygenase enzyme activity in the hippocampus and frontal cortex of chronic mild-stress-exposed rats. Neuropsychiatr Dis Treat. 2013;9:697-708. doi: 10.2147/NDT.S41020.

Moroni F, Russi P, Gallo-Mezo MA, Moneti G, Pellicciari R. Modulation of quinolinic and kynurenic acid content in the rat brain: effects of endotoxins and nicotinylalanine. J Neurochem. 1991;57(5):1630-5. doi: 10.1111/j.1471-4159.1991.tb06361.x.

O'Connor JC, Lawson MA, André C, Moreau M, Lestage J, Castanon N, Kelley KW, Dantzer R. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. Mol Psychiatry. 2009;14(5):511-22. doi: 10.1038/sj.mp.4002148.

Ohgi Y, Futamura T, Kikuchi T, Hashimoto K. Effects of antidepressants on alternations in serum cytokines and depressive-like behavior in mice after lipopolysaccharide administration. Pharmacol Biochem Behav. 2013;103(4):853-9. doi: 10.1016/j.pbb.2012.12.003.

O'Sullivan SE, Sun Y, Bennett AJ, Randall MD, Kendall DA. Time-dependent vascular actions of cannabidiol in the rat aorta. Eur J Pharmacol. 2009;612(1-3):61-68. doi:10.1016/j.ejphar.2009.03.010.

O'Sullivan SE. An update on PPAR activation by cannabinoids. Br J Pharmacol. 2016;173(12):1899-1910. doi:10.1111/bph.13497.

Oxenkrug G. Serotonin-kynurenine hypothesis of depression: historical overview and recent developments. Curr Drug Targets. 2013;14(5):514-21. doi: 10.2174/1389450111314050002.

Parrott JM, Redus L, Santana-Coelho D, Morales J, Gao X, O'Connor JC. Neurotoxic kynurenine metabolism is increased in the dorsal hippocampus and drives distinct depressive behaviors during inflammation. Transl Psychiatry. 2016;6(10):e918. doi: 10.1038/tp.2016.200.

Pérez-Nievas BG, Madrigal JL, García-Bueno B, Zoppi S, Leza JC. Corticosterone basal levels and vulnerability to LPS-induced neuroinflammation in the rat brain. Brain Res. 2010;1315:159-168. doi:10.1016/j.brainres.2009.12.014.

Pilar-Cuéllar F, Vidal R, Díaz Á, Garro-Martínez E, Linge R, Castro E, Haberzettl R, Fink H, Bert B, Brosda J, Romero B, Crespo-Facorro B, Pazos Á. Enhanced Stress Response in 5-HT1AR Overexpressing Mice: Altered HPA Function and Hippocampal Long-Term Potentiation. ACS Chem Neurosci. 2017;8(11):2393-2401. doi: 10.1021/acschemneuro.7b00156.

Pilar-Cuellar F, Castro E, Bretin S, Mocaer E, Pazos Á, Díaz Á. S 47445 counteracts the behavioral manifestations and hippocampal neuroplasticity changes in bulbectomized mice. Prog Neuropsychopharmacol Biol Psychiatry. 2019;93:205-213. doi: 10.1016/j.pnpbp.2019.04.005.

Renault J, Aubert A. Immunity and emotions: lipopolysaccharide increases defensive behaviours and potentiates despair in mice. Brain Behav Immun. 2006;20(6):517-26. doi: 10.1016/j.bbi.2006.03.003.

Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature. 1998;391(6662):79-82. doi: 10.1038/34178.

Rodrigues FTS, de Souza MRM, Lima CNC, da Silva FER, Costa DVDS, Dos Santos CC, Miyajima F, de Sousa FCF, Vasconcelos SMM, Barichello T, Quevedo J, Maes

M, de Lucena DF, Macedo D. Major depression model induced by repeated and intermittent lipopolysaccharide administration: Long-lasting behavioral, neuroimmune and neuroprogressive alterations. J Psychiatr Res. 2018;107:57-67. doi:10.1016/j.jpsychires.2018.10.003.

Salazar A, Gonzalez-Rivera BL, Redus L, Parrott JM, O'Connor JC. Indoleamine 2,3-dioxygenase mediates anhedonia and anxiety-like behaviors caused by peripheral lipopolysaccharide immune challenge. Horm Behav. 2012;62(3):202-9. doi: 10.1016/j.yhbeh.2012.03.010.

Schildkraut JJ. The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry. 1965;122(5):509-22. doi: 10.1176/ajp.122.5.509.

Shoval G, Shbiro L, Hershkovitz L, Hazut N, Zalsman G, Mechoulam R, Weller A. Prohedonic Effect of Cannabidiol in a Rat Model of Depression. Neuropsychobiology. 2016;73(2):123-9. doi: 10.1159/000443890.

Silote GP, Sartim A, Sales A, Eskelund A, Guimarães FS, Wegener G, Joca S. Emerging evidence for the antidepressant effect of cannabidiol and the underlying molecular mechanisms. J Chem Neuroanat. 2019;98:104-116. doi: 10.1016/j.jchemneu.2019.04.006.

Sonego AB, Prado DS, Vale GT, Sepulveda-Diaz JE, Cunha TM, Tirapelli CR, Del Bel EA, Raisman-Vozari R, Guimarães FS. Cannabidiol prevents haloperidol-induced vacuos chewing movements and inflammatory changes in mice via PPARγ receptors. Brain Behav Immun. 2018;74:241-251. doi:10.1016/j.bbi.2018.09.014.

Sukoff Rizzo SJ, Neal SJ, Hughes ZA, Beyna M, Rosenzweig-Lipson S, Moss SJ, Brandon NJ. Evidence for sustained elevation of IL-6 in the CNS as a key contributor

of depressive-like phenotypes. Transl Psychiatry. 2012;2:e199. doi: 10.1038/tp.2012.120.

Umemoto T, Fujiki Y. Ligand-dependent nucleo-cytoplasmic shuttling of peroxisome proliferator-activated receptors, PPARα and PPARγ. Genes Cells. 2012;17(7):576-596. doi:10.1111/j.1365-2443.2012.01607.x.

Verdonk F, Petit AC, Abdel-Ahad P, Vinckier F, Jouvion G, de Maricourt P, De Medeiros GF, Danckaert A, Van Steenwinckel J, Blatzer M, Maignan A, Langeron O, Sharshar T, Callebert J, Launay JM, Chrétien F, Gaillard R. Microglial production of quinolinic acid as a target and a biomarker of the antidepressant effect of ketamine. Brain Behav Immun. 2019;81:361-373. doi: 10.1016/j.bbi.2019.06.033.

Vidal R, Garro-Martínez E, Díaz Á, Castro E, Florensa-Zanuy E, Taketo MM, Pazos Á, Pilar-Cuéllar F. Targeting β-Catenin in GLAST-Expressing Cells: Impact on Anxiety and Depression-Related Behavior and Hippocampal Proliferation. Mol Neurobiol. 2019;56(1):553-566. doi: 10.1007/s12035-018-1100-2.

Walker AK, Budac DP, Bisulco S, Lee AW, Smith RA, Beenders B, Kelley KW, Dantzer R. NMDA receptor blockade by ketamine abrogates lipopolysaccharide-induced depressive-like behavior in C57BL/6J mice. Neuropsychopharmacology. 2013;38(9):1609-16. doi: 10.1038/npp.2013.71.

Widner B, Laich A, Sperner-Unterweger B, Ledochowski M, Fuchs D. Neopterin production, tryptophan degradation, and mental depression--what is the link? Brain Behav Immun. 2002;16(5):590-5. doi: 10.1016/s0889-1591(02)00006-5.

Xiao BG, Link H. IFN-gamma production of adult rat astrocytes triggered by TNF-alpha. Neuroreport. 1998;9(7):1487-90. doi: 10.1097/00001756-199805110-00044.

Zanelati TV, Biojone C, Moreira FA, Guimarães FS, Joca SR. Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. Br J Pharmacol. 2010;159(1):122-8. doi: 10.1111/j.1476-5381.2009.00521.x.

FIGURE LEGENDS

Figure 1. Experimental design of drug administration and behavioral tests performed in this study, and sample collection for the different molecular techniques. CBD: cannabidiol, Cx: cortex, Hp: hippocampus, HPLC: high performance liquid chromatography, LPS: lipopolysaccharide, OFT: open-field test, PFC: prefrontal cortex, qPCR: quantitative PCR, TST: tail suspension test, veh: vehicle, WB: western blot.

Figure 2. Behavioral effect of cannabidiol (CBD) administration in the lipopolysaccharide (LPS) model. Immobility time in the tail suspension test (TST) (A), sucrose preference test (B), time in the center in the open-field test (OFT) (C), and total distance traveled in the OFT (D). Results are expressed as mean ± S.E.M. Two-way ANOVA followed by Newman-Keuls *posthoc* test. $^{**}p<0.01$, and $^{***}p<0.001$. Correlation between the total distance traveled in the OFT and the immobility time in the TST (E). Correlation between the total distance traveled and the time spent in the center in the OFT (F). r: Pearson's correlation coefficient. ns: not significant. veh: vehicle group. n=6-9 mice *per* group.

Figure 3. NF-κB (A), IκBα (B) and PPARγ (C and D) levels in nuclear (A and C) and cytoplasmic (B and D) fractions of cortical samples from lipopolysaccharide (LPS) mice with cannabidiol (CBD) treatment. PCNA and GAPDH were used as nuclear and cytoplasmic housekeeping proteins, respectively. Representative bands from the western blot of each marker and the corresponding housekeeping proteins are shown. Results are expressed in percentage *vs* the vehicle group (veh) and as mean ± S.E.M.

Two-way ANOVA followed by Newman-Keuls *posthoc* test, **p*<0.05, ***p*<0.01, ****p*<0.001. n=6-7 animals *per* group.

Figure 4. TNFα (A) and IL-6 (B) levels in plasma, and TNFα (C) and IL-6 (D) mRNA expression levels in the prefrontal cortex (PFC) of lipopolysaccharide (LPS)-treated mice with cannabidiol (CBD) administration. Results are expressed as mean ± S.E.M. Two-way ANOVA followed by Newman-Keuls *posthoc* test, **p*<0.05, ****p*<0.001. veh: vehicle group. n=6-9 animals *per* group.

Figure 5. Ratio kynurenine/tryptophan (KYN/TRP) (A and C) and kynurenine/serotonin (KYN/5-HT) (B and D) in hippocampus (Hp) (A and B) and cortex (Cx) (C and D) after cannabidiol (CBD) treatment in the lipopolysaccharide (LPS) neuroinflammatory model in mice. Results are expressed as mean ± S.E.M. Two-way ANOVA followed by Newman-Keuls *posthoc* test. **p*<0.05, ***p*<0.01, ****p*<0.001. veh: vehicle group. n=5-9 mice *per* group.