Original Article

Cannabidiol improves non-motor symptoms, attenuates neuroinflammation, and favours hippocampal newborn neuronal maturation in a rat model of Parkinsonism

Bianca Andretto de Mattos[1], Jéssica Mendes Bonato[1], Maria Clara Splendor[1], Elaine Del Bel[2], Humberto Milani[1] and Rúbia Maria Weffort de Oliveira[1]

[1]Department of Pharmacology and Therapeutics, State University of Maringá, Maringá, Paraná, Brazil and [2]Department of Basic and Oral Biology, School of Medicine, USP, Ribeirão Preto, São Paulo, Brazil

Abstract

Objective: To investigate the effects of cannabidiol (CBD) on emotional and cognitive symptoms in rats with intra-nigral 6-hydroxydopamine (6-OHDA) lesions. *Methods:* Adult male Wistar rats received bilateral intranigral 6-OHDA infusions and were tested in a battery of behavioural paradigms to evaluate non-motor symptoms. The brains were obtained to evaluate the effects of CBD on hippocampal neurogenesis. *Results:* 6-OHDA-lesioned rats exhibited memory impairments and despair-like behaviour in the novelty-suppressed feeding test and forced swim test, respectively. The animals also exhibited dopaminergic neuronal loss in the substantia nigra pars compacta (SNpc), striatum, and ventral tegmental area and a reduction of hippocampal neurogenesis. CBD decreased dopaminergic neuronal loss in the SNpc, reduced the mortality rate and decreased neuroinflammation in 6-OHDA-lesioned rats. In parallel, CBD prevented memory impairments and attenuated despair-like behaviour that were induced by bilateral intranigral 6-OHDA lesions. Repeated treatment with CBD favoured the neuronal maturation of newborn neurons in the hippocampus in Parkinsonian rats. *Conclusion:* The present findings suggest a potential beneficial effect of CBD on non-motor symptoms induced by intra-nigral 6-OHDA infusion in rats.

Significant outcomes

- Cannabidiol (CBD) reduced the mortality rate, decreased dopaminergic neuronal loss in the SNpc and attenuated neuroinflammation in 6-OHDA-lesioned rats
- CBD prevented memory impairments and attenuated despair-like behaviours that were induced by bilateral intranigral 6-OHDA lesions.
- CBD favoured the neuronal maturation of newborn neurons in the hippocampus 6-OHDA-lesioned rats.

Limitations

- The 6-OHDA toxin model does not mimic the progressive dopaminergic loss and lacks Lewy-related pathology seen in Parkinson's disease (PD).
- CBD was given after intra-nigral 6-OHDA infusion. Using genetic models, for example, CBD treatment could be started before the onset of the symptoms to mimic the development of PD in humans.
- A therapeutic time window study and the use of ageing animals is necessary to address acute and long-term term effects of CBD in 6-OHDA lesioned rats.

Introduction

Parkinson's disease (PD) is a neurodegenerative condition that is associated with motor and non-motor symptoms that decrease functioning and quality of life, resulting in high rates of disability and intensive care requirements (WHO, 2022). The main non-motor symptoms of PD include sleep disorders, autonomic dysfunction, fatigue, pain, cognitive deficits, anxiety, and depression (Kalia and Lang 2016). Despite their high prevalence, non-motor symptoms of PD are scarcely recognised or reported and thus are insufficiently treated (Poewe *et al.*, 2017; Santos Garcia *et al.*, 2019). Anxiety is present in approximately 40-60% of PD patients and appears to be commonly associated with depression (Martínez-Martín and Damián, 2010; Schapira *et al.*,

2017). Depression, apathy, and anhedonia occur in approximately 35% of PD patients (Aarsland *et al.*, 2011). Cognitive impairments are seen as a sign of PD progression and are present in 25-30% of patients (Schapira *et al.*, 2017; Hayes *et al.*, 2019). Notably, dementia (Camicioli *et al.*, 2003) and depressive symptoms have been linked to a reduction of neurogenesis in patients with PD (Lim *et al.*, 2018).

The pharmacological treatment of PD involves dopamine precursors (e.g. levodopa) and dopamine degradation inhibitors (e.g. monoaminoxidase inhibitors; Alborghetti and Nicoletti, 2019). Although these drugs can attenuate many symptoms of PD, their efficacy gradually decreases over time, accompanied by potential side effects (Cilia *et al.*, 2015). Moreover, non-motor manifestations (e.g. psychosis) can be worsened by dopaminergic medications. Conventional antidepressants do not always show satisfactory clinical effectiveness in PD (Lee and Gilbert, 2016). Therefore, the identification of new therapeutic strategies that are able to counteract motor and non-motor symptoms of PD is a major challenge in the field.

Cannabidiol (CBD), one of over 100 phytocannabinoids that have been identified in *Cannabis sativa*, has received much research attention in recent years because of its multimodal pharmacological profile and remarkable safety profile (Crippa *et al.*, 2018). Several studies have reported neuroprotective actions of CBD in Alzheimer's disease (Watt and Karls, 2017), cerebral ischaemia (Mishima *et al.*, 2005; Hayakawa *et al.*, 2008; Meyer *et al.*, 2021; Khaksar *et al.*, 2022), traumatic brain injury (Singh and Neary, 2020), and spinal cord injury (Perez *et al.*, 2021). In Parkinsonian patients with no psychiatric comorbidities, CBD improved motor and general symptoms and ameliorated quality of life and well-being (Chagas *et al.*, 2014). Experimentally, CBD significantly reduced dopaminergic cell death in rats that were injected with 6-hydroxydopamine (6-OHDA) in the medial forebrain bundle (MFB; Lastres-Becker *et al.*, 2005). CBD also reduced α-synuclein accumulation and restored 6-OHDA depleted dopaminergic neurons in the transgenic *C. elegans* model of PD (Muhammad *et al.*, 2022). When administered locally in the masseter in rats, CBD attenuated the enhancement of nociception in animals with selective loss of the dopaminergic nigrostriatal pathway that was induced by 6-OHDA lesions (Vivanco-Estela *et al.*, 2021). An intrapallidal injection of CBD reduced motor asymmetry in 6-OHDA hemiparkinsonian rats and improved fine motor skills in the contralateral limb (Patricio *et al.*, 2022). However, whether CBD produces neuroprotective effects on non-motor symptoms of 6-OHDA lesions remains to be determined.

This study aimed to investigate the effect of CBD on non-motor symptoms of rats lesioned with 6-OHDA into the SNpc. Dopaminergic neuron loss was quantified by determining tyrosine hydroxylase (TH) immunoreactivity in the SNpc, ventral tegmental area (VTA), and striatum. Behavioural dysfunction was estimated using a battery of tests, including the open field test (OFT), object recognition test (ORT), novelty-suppressed feeding test (NSF), and forced swim test (FST). To investigate dopaminergic lesions that influence hippocampal neurogenesis, the expression of newborn hippocampal neurons was evaluated by the immunohistochemical detection of doublecortin (DCX)-immunoreactive (IR) neurons. Neuroinflammation was evaluated by Iba-1 (microglia marker) and GFAP (astrocyte marker) expression in the striatum and hippocampus.

Methods

Animals

Male 3-month-old Wistar rats (280-320 g) were obtained from the central *vivarium* of the State University of Maringá. The animals were acclimated to a controlled temperature (22°C ± 1°C) and a 12 h/12 h light/dark cycle (lights on at 7:00 AM) for 2 weeks before the experiments. The animals were housed in groups ($n = 5$–10/ group) in plastic cages and given standard commercial chow (Nuvilab, Quimtia, PR, Brazil) and tap water *ad libitum*. The local Ethics Committee on Animal Experimentation of the State University of Maringá approved (CEUA no. 8156290119) the experimental procedures according to the guidelines of the U.S. National Institutes of Health and Brazilian College for Animal Experimentation. All efforts were made to minimise the number of animals used and their suffering.

Drugs

CBD (THC Pharma, Frankfurt, Germany) was dissolved in 2% Tween 80 (Synth, Maringá, Brazil) in sterile isotonic saline (vehicle). Desipramine and 6-OHDA were obtained from Sigma-Aldrich (Sigma, St Louis, MO, USA). Desipramine was dissolved in sterile saline. 6-OHDA was dissolved in a vehicle (sterile saline with 0.2% ascorbic acid). 6-OHDA was prepared according to established doses that were shown to promote significant dopaminergic neuron loss in the SNpc (Bonato *et al.*, 2018).

Stereotaxic surgery

Thirty minutes before stereotaxic surgery, the animals received an intraperitoneal (i.p.) injection of desipramine (25 mg/kg) to protect noradrenergic neurons (Padovan-Neto *et al.*, 2009). The animals were then anaesthetised with Equitesin (3 ml/kg, i.p.; 1% sodium thiopental, 4.25% chloral hydrate, 2.13% magnesium sulphate, 42.8% propylene glycol, and 3.7% ethanol in water; Vieira *et al.*, 2019), and the skulls were fixed in a stereotaxic frame (Kopf 957, Kopf Instruments, Tujunga, CA, USA). Bilateral infusions of 6-OHDA were made in a single dose directly in the SNpc (4 μg/μl) using a 27-gauge stainless-steel needle that was connected to a 5 μl Hamilton syringe (Hamilton Company, Reno, NV, USA) according to the following coordinates: SNpc (anterior/posterior, −5.0 mm from bregma; medial/lateral, ± 2.1 mm from midline; dorsal/ventral, −8.0 mm from the skull) (Paxinos and Watson, 2007). The flow of the infusions was controlled by an electronic pump (Insight, Ribeirão Preto, Brazil) at a rate of 0.25 μl/min over 4 min (Bonato *et al.*, 2018). The injection needle was left in place for an additional 2 min to avoid reflux. After surgeries, the surgical wound was cleaned with chlorhexidine (Sigma-Aldrich, St Louis, MO, USA), and the animals were individually placed in clean cages on warming pads until recovery. No analgesic or anaesthetic antagonists were administered because these treatments could interfere with lesion development and thus impact functional outcomes. After recovery, the rats were returned to group housing (Padovan-Neto *et al.*, 2009). During the 7 days following surgeries, the animals had free access to laboratory rat chow that was moistened with water to make a paste. The rat chow and water were placed inside the cages to make them easily available and allow the animals to recover. Further, the animals received i.p. vehicle or CBD (10 or 30 mg/kg injections, once a day for 13 days).
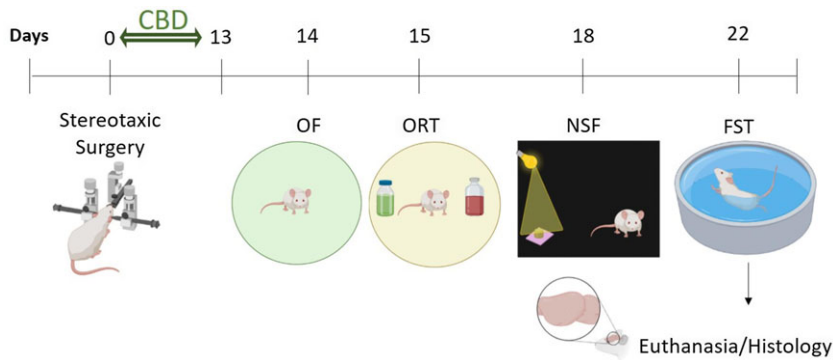
Figure 1. Experimental design. Vehicle or 6-OHDA was bilaterally injected in the substantia nigra pars compacta (SNpc) according to coordinates from the Paxinos and Watson atlas (2007). The animals received intraperitoneal cannabidiol (CBD) 10 or 30 mg/kg injections for 13 days and were sequentially evaluated in the open field (OF) test, object recognition test (ORT), novel suppressing feeding test (NSF) and forced swim test (FST). The behavioural testing was conducted from day 14 to day 22 after the surgeries. The animals were euthanised on day 22, and brains were removed for immunohistochemistry assays.

Experimental design

The animals were randomly distributed into 4 experimental groups: Saline + Vehicle (Saline + Veh or Sham grou), 6-OHDA + Veh, 6-OHDA + CBD10, and 6-OHDA + CBD30. The animals received intraperitoneal (i.p.) vehicle or CBD (10 or 30 mg/kg injections for 13 days). The CBD doses were determined by referencing prior studies that demonstrated the neuroprotective effects of CBD following brain injury (Mori *et al.*, 2017; Meyer *et al.*, 2021). Behavioural testing was conducted from 8:00 AM to 12:00 AM. The OFT, ORT, NSF, and FST were performed on days 14, 15, 19, and 22 after the 6-OHDA intra-nigral infusion, respectively (Fig. 1). The time points used for behavioural characterisation after SNpc lesion were based in previous studies (Santiago *et al.*, 2010; 2014; Bonato *et al.*, 2018). Animals' behaviours were monitored and recorded using a video camera that was positioned above the apparatuses. The images were later analysed using the ANYmaze contrast-sensitive video monitoring system (Stoelting, Wood Dale, IL, USA). After behavioural testing, the animals' brains were removed and processed for histological analyses.

Behavioural testing

Open field test. The OFT evaluates locomotion and exploratory behaviour. The OF consisted of a circular arena with an 80 cm diameter and 40 cm high wall. On day 14 after the lesion, each rat was gently moved from its home cage and immediately placed in the centre of the OF. The rat was allowed to freely explore the apparatus for 5 min. The distance travelled (in metres), number of entries, and time spent in the centre of the arena were recorded.

Object recognition test. The ORT is based on the rodent's instinctive preference for novelty, thus allowing assessments of an animal's recognition memory (Akkerman *et al.*, 2012). On the first day, the animals were placed on a round platform (80 cm diameter, 40 cm high wall) for 5 min for habituation. After two identical objects were placed symmetrically on the platform, the animal was given another 5 min for free exploration. Twenty-four hours later, the animals were returned to the platform with the objects from the previous day, and they were allowed to explore them for 4 min. After a 1 h interval, the animals were returned to the platform, but one of the objects was replaced with a new object, which started the test session. Each animal was given 4 min to explore the new and old (familiar) objects. The exploration time for each object was recorded. Exploration was considered when the animal was at least 2 cm from the object or touched it. Animals that had an exploration time of fewer than 5 s were discarded from the test because this memory assessment requires sufficient exploratory

behaviour for reliable performance (Akkerman *et al.*, 2012). The following parameters were evaluated: time spent by each animal exploring the familiar object and time spent exploring the new object. The exploration index (D2) was calculated using the following formula: (new object exploration time – familiar object exploration time) / (new object exploration time + familiar object exploration time).

Novel suppressed feeding test. The NSF has been used to assess anxiety-like and depressive-like behaviours (Blasco-Serra *et al.*, 2017). This test introduces an additional element of motivation, in which the animal is deprived of food for a long period. Twenty-four hours before the test, the animal's food was removed from the cage. The next day, the test was performed on a square platform (70 cm × 70 cm) on which each animal was placed individually. The room lights were turned off, and a spotlight was directed only towards the centre of the platform on a food pellet. The animal was allowed to remain on the platform for 10 min or until the moment when it picked up the pellet. The animal was then transferred to a home cage with previously weighed food pellets and allowed to feed for 10 min. After this period, the food was removed and weighed again to record consumption. After the end of the test, the animals were returned to their cages with food and water available *ad libitum*. The following parameters were analysed: latency (time for the animal to pick up the food) and consumption in the home cage (weight of the food before feeding – the weight of the food after feeding). The parameters were quantified manually.

Forced swim test. The FST identifies passive coping strategies in rodents (Dalla *et al.*, 2011). The test was conducted in two sessions. In the training session, the rats were placed in a tank (25 cm diameter, 65 cm height) that was filled with water at a temperature of 24°C ± 1°C and depth of 30 cm for 15 min. Twenty-four hours after the training session, the rats were subjected to the test session for 5 min, which was videotaped for subsequent quantification of the latency and immobility time (in seconds; i.e. the absence of motion of the entire body and only small movements necessary to keep the animal's head above the water). The water was changed after each animal to avoid the influence of urinary or faecal material.

Immunohistochemistry

Five to eight animals were randomly chosen from each experimental group, and their brains were processed for immunohistochemistry. Briefly, the animals were deeply anaesthetised with sodium thiopental (50 mg/kg; Thiopentax, Cristália, SP, Brazil) and then transcardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.2 M phosphate buffer. The brains were removed, postfixed in the same fixative for 2 h, and

cryoprotected by immersion in 30% sucrose for 48 h before snap freezing. Frozen tissue was serially sectioned on a cryostat (Cryocut 1800, Reichert-Jung, Heidelberg, Germany) into 30 µm coronal sections that were collected in replicates in Eppendorf tubes that contained 0.01 M PBS with 0.01% sodium azide. The brain sections were quenched in 1% H_2O_2 for 30 min and then blocked with 2% bovine serum albumin (BSA) in PBS for 60 min. The sections were incubated with mouse polyclonal anti-TH antibody (1 : 2,000, catalogue no. AB152, Merck Millipore, Darmstadt, Germany), goat polyclonal anti-DCX antibody (1 : 1,000, catalogue no. sc-8066, Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit polyclonal anti-Iba-1 antibody (1 : 500, catalogue no. 0019-19741, Wako Osaka, Japan) or rabbit anti-GFAP (1:500, catalogue no. sc-2042, Santa Cruz Biotechnology, Santa Cruz, CA, USA) in PBS that contained 0.3% Triton X-100 for 48 h at 4°C. The sections were then incubated with respective biotinylated secondary antibodies (1:500, anti-mouse [catalogue no. sc2039], anti-goat [catalogue no. sc-2042] and anti-rabbit [catalogue no. 2492] Santa Cruz Biotechnology, Santa Cruz, CA, USA), for 2 h. The signals were visualised using avidin-biotin complex (Vector Laboratories, Burlingame, CA, USA), 3,3'-diaminobenzidine (DAB) as the chromogen, and 0.05% H_2O_2. For the Iba-1 and GFAP assays, 1% Ni_2SO_4 was added to the revelation solution. The sections were then mounted on gelatin-coated slides, dehydrated in ethanol, and coverslipped with Permount mounting medium.

For semi-quantitative analysis, the slides were previously coded and evaluated by an experimenter who was blind to treatments. The slides were analysed with an optical microscope (Olympus, BX41, PA, USA) that equipped with an Olympus QColor camera. Immunoreactive cells were quantified by capturing photomicrographs from the the SNpc, VTA, hippocampus, and striatum. ImageJ software (National Institutes of Health, Bethesda, MD, USA) was utilised to count the number of immunoreactive cells or the integrated optical density (IOD), in pre-fixed areas. For IOD measurements, after acquiring the images, they were converted to a 16-bit greyscale image, the background was subtracted and the threshold for positive signals was determined. The two cerebral hemispheres were evaluated, and the average between them was obtained. The analysis was conducted with four to six sections per animal.

Doublecortin-IR cells were counted throughout the SGZ of the dentate gyrus of the hippocampus (bregma −3.14 to −4.52 mm; Paxinos and Watson, 2007). For each section, the DG area was obtained, and the number of DCX-IR cells was counted. To estimate the total number of immunoreactive cells in the SGZ, a modified unbiased stereological procedure was used where the total number of positive cells counted per DG is multiplied by the reciprocal of the sampling factor (Bonato *et al.*, 2018). Therefore, DCX-IR cells were counted on every 6th section of the hippocampus and the number of counted cells was multiplied by 6 to obtain an estimate of the total number of positive cells per SGZ. The SGZ of the DG was defined as the region between the hilus and the granular cell layer (GCL): two GCL cell widths into the hilus and the inner half of the GCL (Lau *et al.*, 2016).

Doublecortin-IR neurons were classified into six different categories according to their dendrite morphology (Pl ümpe *et al.*, 2006): A (neuron with no processes), B (neuron with very short processes), C and D (neurons with intermediate processes, with D being more developed than C), and E and F (which corresponded to more mature neurons; E neurons had apparent branches in the molecular layer of the SGZ, and F neurons had several branches along the dendritic tree). The results are presented
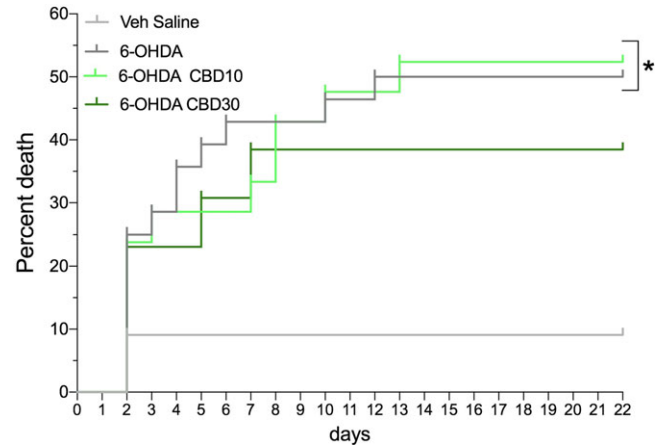

Figure 2. Mortality curve. Mortality rate expressed as a percentage over time (days) of the experimental groups. *p < 0.05 compared with saline + vehicle group (Long-rank test).

as the sum of A + B (proliferative stage), C + D (intermediate stage), and E + F (post-mitotic stage; Fig. 6A).

Statistical analysis

Statistica 8.0 software (StatSoft, Palo Alto, CA, USA) was used for the statistical analysis. A mortality curve was generated to analyse survival data and the Long-rank test ($\chi2$) was used to assess differences in animal survival. First, the behavioural and histological data were examined to verify normality (Shapiro-Wilk test) and homoscedasticity (F test to compare variances). The behavioural data were analysed using one-way analysis of variance (ANOVA), followed by the Tukey's multiple-comparison *post hoc* test. In the ORT, functional memory within groups (i.e. a D2 value that differed significantly from zero) was analysed with a two-tailed one sample *t*-test (Soares *et al.*, 2016). The data are expressed as mean ± SEM. Values of $p < 0.05$ were considered statistically significant.

Result

Mortality rate

In total, 71 animals were used: Saline + Veh ($n = 11$), 6-OHDA + Veh ($n = 25$), 6-OHDA + CBD10 ($n = 22$), and 6-OHDA + CBD30 ($n = 13$). As shown in Fig. 2, the mortality rate was: Saline + Veh 15.5 % (1 of 11 animals), 6-OHDA + Veh 52.0 % (13 of 25 animals), 6-OHDA + CBD10 50% (11 of 22 animals) and 6-OHDA + CBD30 38.46 % (5 of 13 animals). 6-OHDA + Veh ($\chi2 = 4.86$, $p = 0.027$) and 6-OHDA + CBD10 ($\chi2 = 4.66$, $p = 0.03$) groups were different from Saline + Veh group. CBD 30 mg/kg did not protect the animals from 6-OHDA-induced death since there was no significant difference between 6-OHDA + Veh and 6-OHDA + CBD30 groups ($\chi2 = 0.43$, $p = 0.51$).

CBD protects the SNpc dopaminergic neurons from bilateral 6-OHDA infusion into the SNpc

Dopaminergic neuronal loss was evaluated in the SNpc, striatum, and VTA 22 days after bilateral 6-OHDA infusion into the SNpc (Fig. 3). Animals that did not present bilateral lesions were removed from the analysis. ANOVA revealed significant
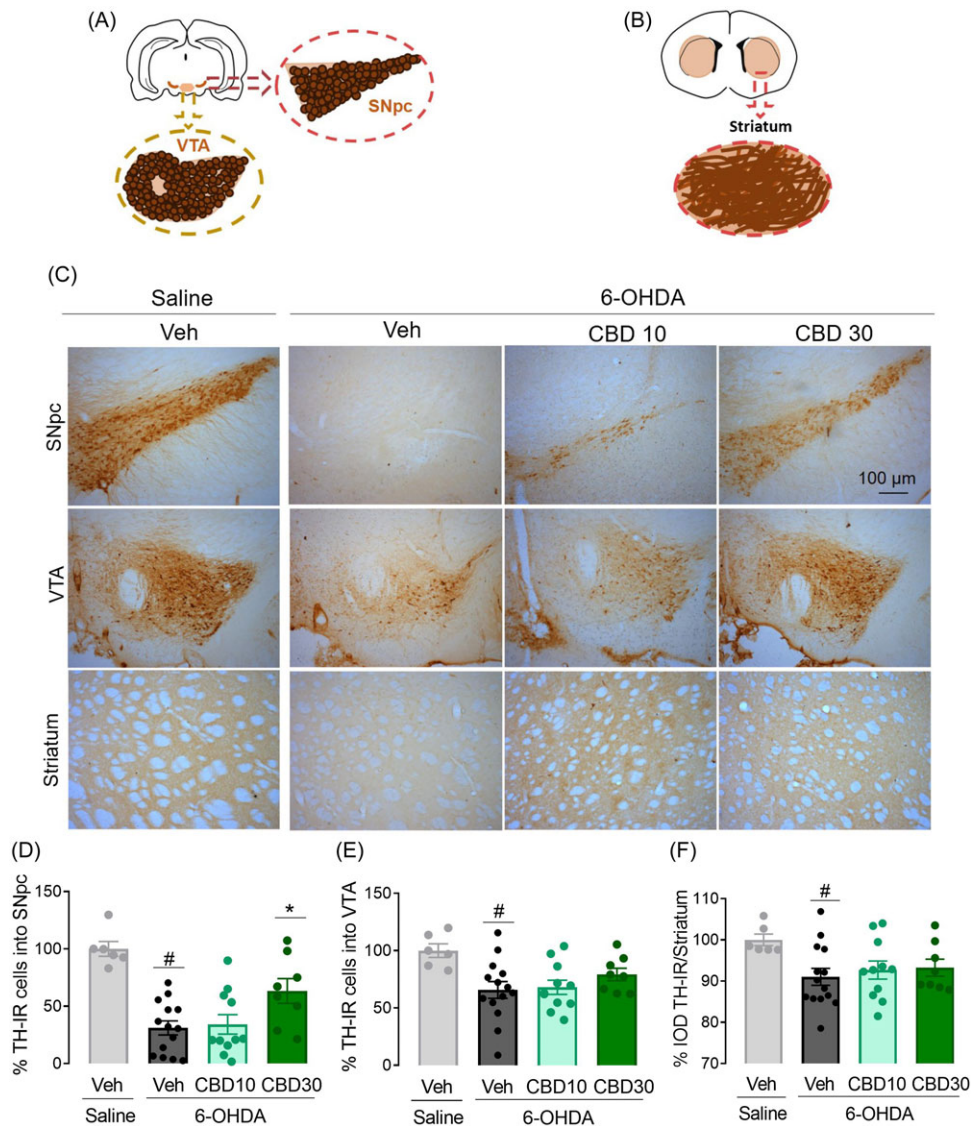
Figure 3. CBD protects the SNpc dopaminergic neurons from bilateral 6-OHDA infusion into the SNpc. Representative diagrams of tyrosine-hydroxylase-immunioreactivity (TH-IR) cells in the substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA) (A), and striatum (B) according to the Paxinos and Watson atlas (2007). Representative photomicrographs of TH-IR cells in the SNpc, VTA and striatum 22 days after 6-OHDA injection (C). The number of TH-IR cells in the SNpc (D), VTA (E) and striatum (F) are represented as % of the controls. **$p < 0.001$ when compared to saline + veh group and #$p < 0.05$ when compared to 6-OHDA + veh group (ANOVA followed by the Tukey's *post hoc* test).

differences among the experimental groups concerning TH-IR cells into SNpc ($F_{3,35} = 12.60$, $p < 0.0001$), striatum ($F_{3,35} = 2.54$, $p = 0.007$), and VTA ($F_{3,35} = 4.0$, $p = 0.02$). There was a decrease in the TH-IR in the SNpc, striatum, and, VTA in 6-OHDA + Veh group when compared to Veh + saline group ($p < 0.05$). CBD 10 mg/kg did not protect dopaminergic neurodegeneration in the SNpc, VTA or striatum ($p > 0.05$). In contrast, CBD 30 mg/Kg prevented dopaminergic neuronal loss in the SNpc ($p = 0.03$) and attenuated dopaminergic neuronal loss in the striatum and VTA, albeit this effect did not reach the 5% significance level.

Effects of CBD on non-motor symptoms following bilateral 6-OHDA infusion into the SNpc

The OF has been used to evaluate the locomotor activity of the animals as well as anxiety-related behaviours. As shown in Fig. 4A, ANOVA detected a significant difference among the experimental

groups concerning the distance travelled in the OF ($F_{3,36} = 3.75$, $p = 0.02$). The 6-OHDA + Veh group presented a decrease in the distance travelled when compared to Saline + Veh group (p, 0.05). No other significant change was observed concerning the number of entries ($F_{3,36} = 1.68$, $p = 0.19$) and time ($F_{3,36} = 1.09$, $p = 0.37$) in the OF centre between sham (Saline + Vehicle) and lesioned animals.

Fig. 4D shows the effects of 6-OHDA on memory performance in the ORT. To investigate which groups had functional recognition memory, the D2 value in the ORT was compared to 0. Only the control ($t = 7.811$, df = 18, $p < 0.0001$) and the 6-OHDA + CBD30 ($t = 5.708$, df = 16, $p < 0.0001$) groups were different from zero. During the test session, the 6-OHDA + Veh group explored less the new object compared with the Saline + Veh group ($t = 4.72$, df = 20, $p < 0.001$), indicating that lesioned rats did not retain recognition memory of the familiar object. ANOVA showed significant differences in the discrination index
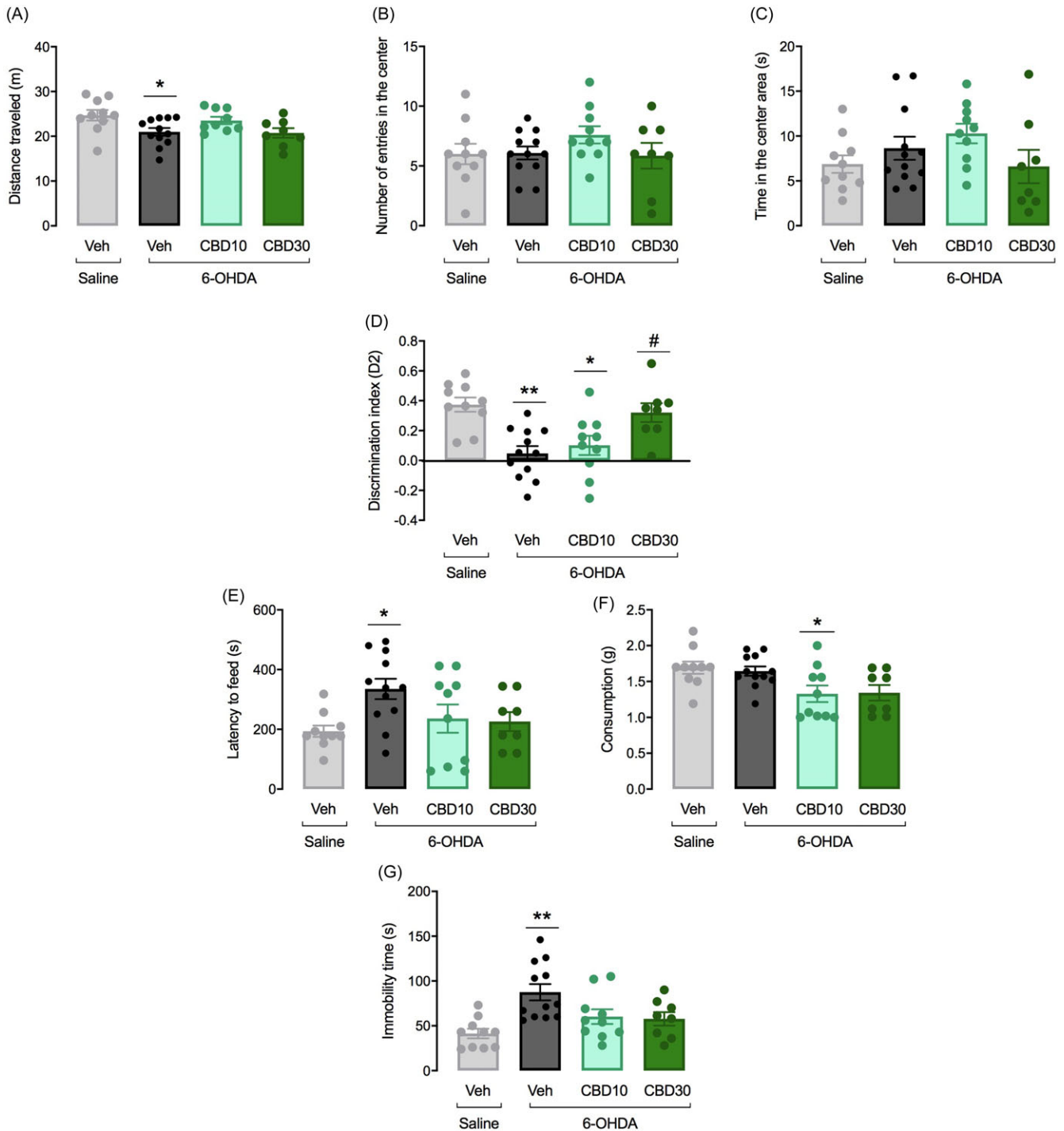
Figure 4. Effects of CBD on non-motor symptoms following bilateral 6-OHDA infusion into the SNpc. Motor activity was evaluated in the open field (OF) by measuring the distance travelled in metres (A). Anxiety-like behaviour was evaluated by measuring the number of entries (B) and the time (sec) (C) spent in the centre of the OF. Hippocampus-dependent memory was analysed in the object location test (ORT) using the D2 exploration index (new object exploration time – familiar object exploration time) / (new object exploration time + familiar object exploration time) (D). The latency to feed (E) and food consumption (F) were registered in the NSF test. Despair-like behaviours were detected in the forced swim test (FST) by measuring the immobility time (G). The bars represent the mean ± SEM in the different groups (n = 10-12/group). **$p < 0.001$ and *$p < 0.05$ different from the saline + vehicle group. #$p < 0.05$ different from 6-OHDA + veh group (one-way ANOVA followed by the Tukey's *post hoc* test).

among the groups ($F_{3,36} = 8.53$, $p < 0.001$). 6-OHDA lesioned, 6-OHDA + CBD10 and 6-OHDA + CBD30 groups presented a decrease in the discrimination index when compared to controls ($p < 0.05$). CBD 30 mg/kg prevented the memory deficits induced by SN 6-OHDA lesion ($p = 0.009$) when compared to 6-OHDA + Veh group.

In the NSF test, the 6-OHDA lesion induced changes in the latency to feed when compared to sham animals ($F_{3,36} = 3.37$, $p = 0.03$, Fig. 4E). The 6-OHDA + veh group showed an increase latency to feed when compared to the control group (p = 0.03). Also for the food consumption, there was a significant difference among the groups ($F_{3,36} = 4.33$, $p = 0.01$, Fig. 4E). While 6-OHDA
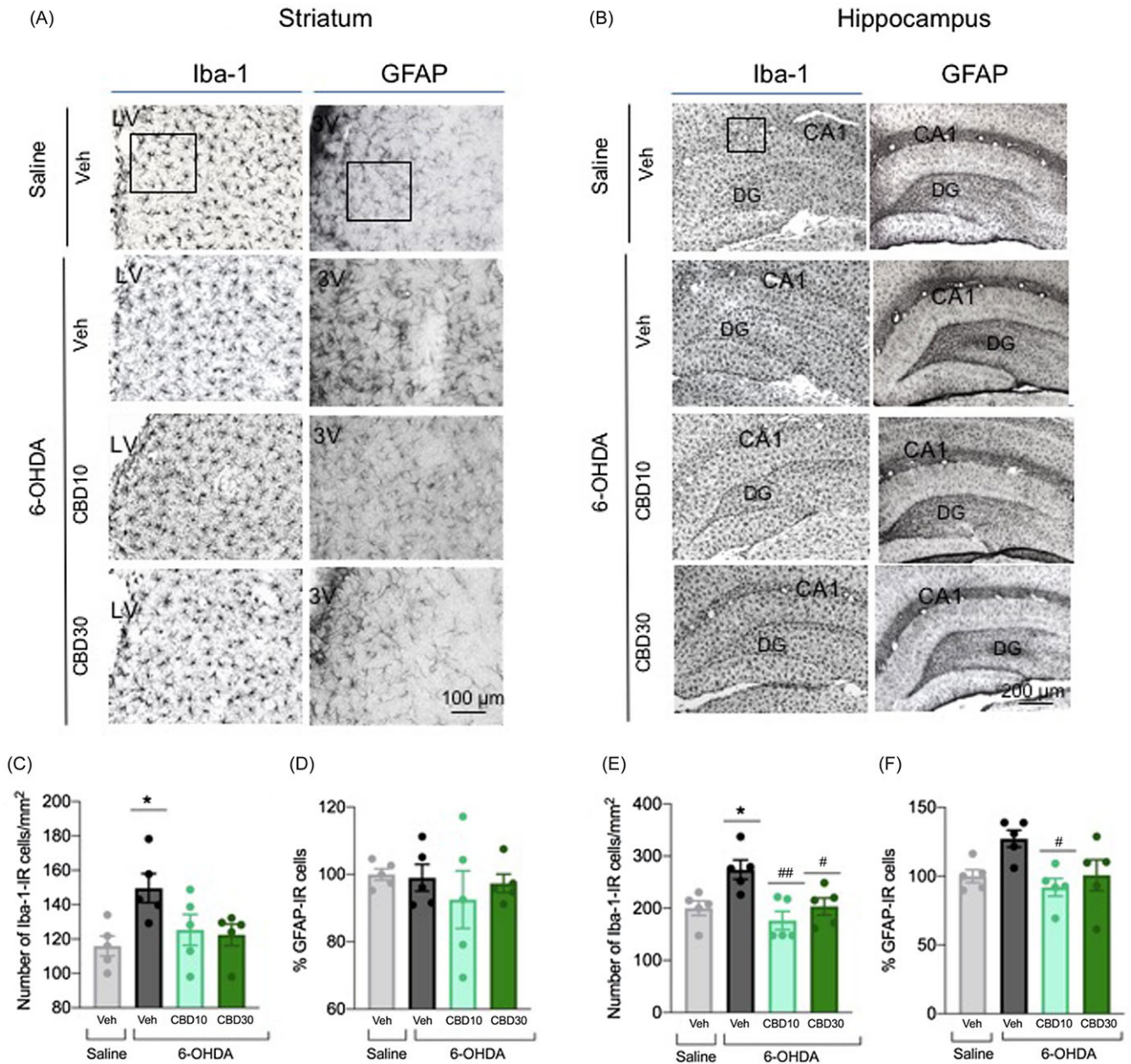
Fig. 5. CBD decreases neuroinflammation induced by bilateral 6-OHDA infusion nto the SNpc. Representative photomicrographies of Iba-1 and GFAP immunohistochemistry in coronal brain sections of the striatum (A) and CA1 hippocampal subfield (B) of rats. Number of Iba-1-IR cells in the striatum (C) and CA1 hippocampal subfield (E). % of control of IOD GFAP-IR in the striatum (D) and CA1 hippocampal subfield (F) of rats. The bars represent the mean ± SEM of the experimental groups. $*p < 0.05$ different from the saline + vehicle group. $\#p < 0.05$ different from 6-OHDA + veh group (one-way ANOVA followed by by the Tukey's *post hoc* test).

infusion did not affect the animal's food consumption ($p > 0.05$) the 6-OHDA group that received CBD10 mg/Kg presented a decrease consumptiom ($p = 0.04$) in comparison to Saline + veh group.

The immobility time in the FST reflects despair-like behaviours. ANOVA revealed significant differences among groups ($F_{3,36} = 6.48$, $p = 0.001$). As seen in Fig. 4G, bilateral 6-OHDA infusion into the SNpc results in an increased immobility time of lesioned rats when compared to controls ($p < 0.001$). Although CBD 10 and 30 mg/Kg attenuated the effects of 6-OHDA lesion, this effect did not reach statistical significance.

CBD reduces neuroinflammation induced by bilateral 6-OHDA infusion into the SNpc

Neuroinflammation was assessed by analysing the expression of Iba-1 (microglia) and GFAP (astrocytes) in the striatum and CA1 and CA3 hippocampal subfields (Fig. 5). Analysis of Iba-1-IR cells revealed significant differences in the total number of microglial cells in the striatum ($F_{3,16} = 3.82$, $p = 0.03$) and CA1 ($F_{3,16} = 6.4$, $p = 0.005$) of lesioned rats, while no significant variance was observed in the CA3 hippocampal subfield ($F_{3,16} = 0.94$, $p = 0.44$). Additionally, ANOVA demonstrated notable differences in
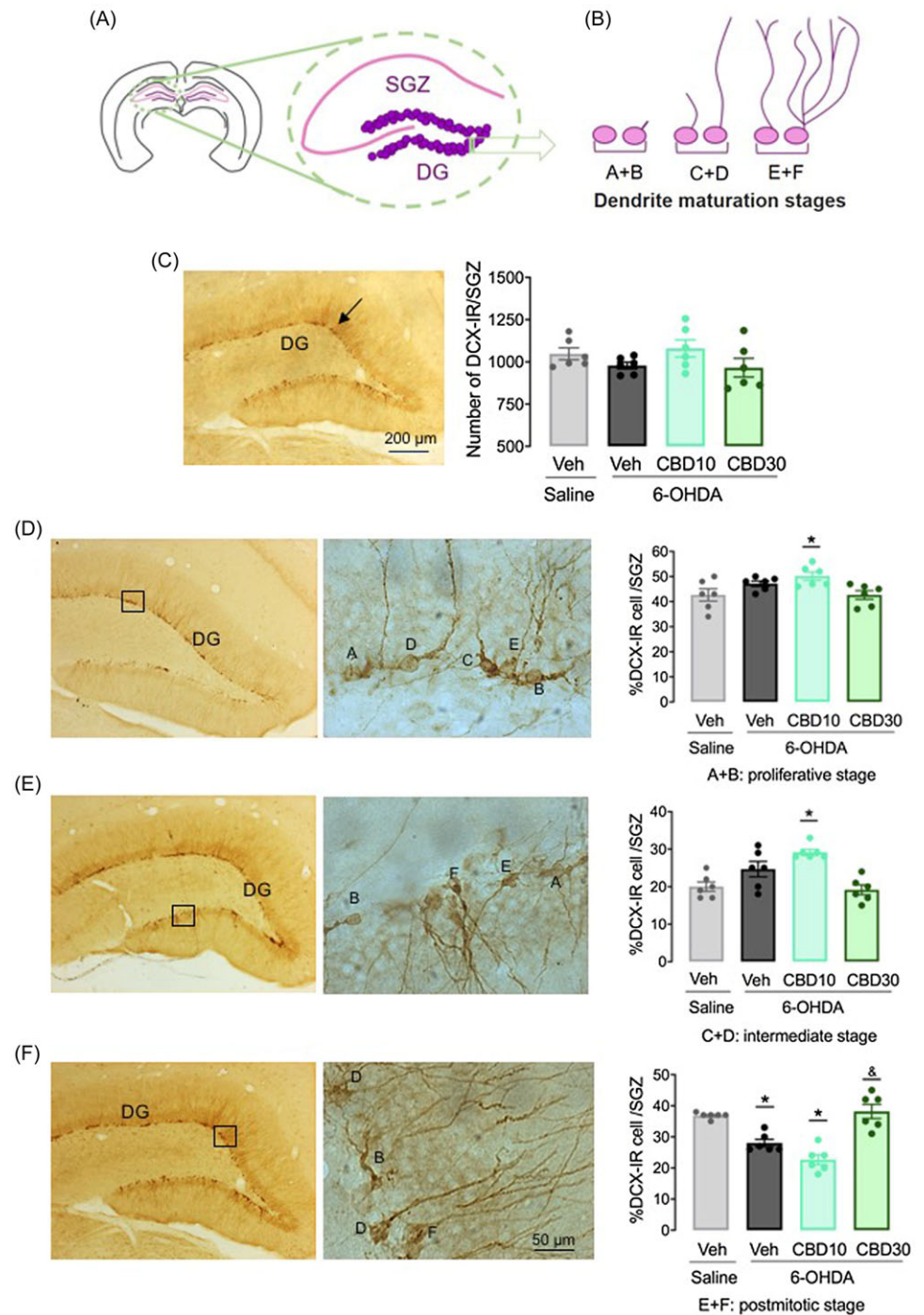
Figure 6. CBD favours newborn neurons maturation in the hippocampus of 6-OHDA lesioned rats. Representative diagrams of coronal brain sections of the hippocampus (A) and classification of DCX-IR neuron dendrite morphology (B). Number of DCX-IR cells in the subgranular zone of the hippocampus (D) and percentage of DCX-IR cells per stage, that is, proliferative stage (E), intermediate stage (F) and postmitotic stage (G). The bars represent the mean ± SEM of the experimental groups. **$p < 0.001$ and *$p < 0.05$ different from the saline + vehicle group. ##$p < 0.05$ different from 6-OHDA + veh group (one-way ANOVA followed by the Tukey's *post hoc* test).

GFAP-IR in the CA1 region of the hippocampus ($F_{3,16} = 4.1$, $p = 0.02$), with no significant changes noted in the CA3 hippocampal subfield ($F_{3,16} = 0.94$, $p = 0.44$) or the striatum ($F_{3,16} = 3.16$, $p = 0.63$) of rats infused with intra-nigral 6-OHDA.

CBD favours newborn neurons maturation in the hippocampus of 6-OHDA lesioned rats

Fig. 6 shows the DCX-IR cells in ZSG of DG, 22 days after bilateral 6-OHDA intra-nigral infusion. The number of DCX-IR neurons was not different among the experimental groups ($F_{3,20} = 1.64$,

$p = 0.21$). However, ANOVA revealed significant differences in DCX-IR cell dendrite classification among the experimental groups, in the stages A + B ($F_{3,20} = 4.19$, $p < 0.05$), C + D ($F_{3,20} = 10.58$, $p < 0.001$) and E + F ($F_{3,20} = 23.56$, $p < 0.001$). The treatment with CBD 10 mg/kg increased the number of cells in the A + B ($p < 0.05$) and C + D ($p < 0.001$) stages (Fig. 5E and 5F). In the post-mitotic stage (E + F), the groups 6-OHDA + Veh and 6-OHDA + CBD10 presented a decreased number of DCX-IR cells compared with the Saline + Veh group ($p < 0.001$; Fig. 6G). In contrast, the treatment with CBD 30 mg/kg increased the number of DCX-IR cells in the postmitotic stage when compared with 6-OHDA + Veh group ($p < 0.001$).

Discussion

The present study investigated neuroprotective effects of CBD in rats that received bilateral intranigral 6-OHDA lesions. 6-OHDA-lesioned rats exhibited memory impairments in the ORT and depressive-like behaviour in the NSF and FST paradigms. In parallel, the animals exhibited dopaminergic neuronal loss in the SNpc, striatum, and VTA. CBD decreased the mortality rate, prevented memory impairments, and attenuated despair-like behaviours in 6-OHDA-lesioned animals. Moreover, CBD decreased dopaminergic neuronal loss in the SNpc, attenuated neuroinflammation and favoured newborn neuron maturation in the hippocampus in Parkinsonian rats.

Toxin administration in specific brain regions in rodents, including the SNpc, MFB, and striatum, has been widely reported to cause the degeneration of dopaminergic neurons and mimic some clinical aspects of PD (Simola *et al.*, 2007; Chia *et al.*, 2020). Specifically, bilateral infusions of 6-OHDA in the SNpc have been shown to produce dopaminergic neurodegeneration and consistent depressive-like behaviours in rats (Santiago *et al.*, 2010; Bonato *et al.*, 2018). One drawback of this lesion model, however, is the high mortality rate after bilateral intranigral 6-OHDA infusions. In the present study, bilateral intranigral 6-OHDA infusions resulted in a 52% mortality rate. CBD (30 mg/kg) decreased the mortality rate in 6-OHDA-lesioned rats. This finding is consistent with previous studies. In an in vivo model of generalised seizures that were induced by pentylenetetrazol, CBD decreased seizures and mortality compared with vehicle-treated animals (Jones *et al.*, 2010). CBD also improved outcomes and prevented mortality in a severe seizure model of soman exposure in female mice (Kundrick *et al.*, 2021). Patra *et al.* (2020) recently reported that CBD prevented premature mortality and improved cognition and social interaction in the *Scn1a*± mouse model of Dravet syndrome. Furthermore, CBD increased the mean survival time in aluminium phosphide-poisoned rats (Hooshangi Shayesteh *et al.*, 2022) and reduced cocaine lethality in mice (Vilela *et al.*, 2015). Altogether, these findings indicate that CBD might exert a neuroprotective effect by decreasing mortality in rats under different experimental conditions.

Bilateral intranigral 6-OHDA infusions induced dopaminergic neurodegeneration in the SNpc, striatum, and VTA. Previous studies (Lindner *et al.*, 1999; Blandini *et al.*, 2007) reported that injections of 6-OHDA in the SNpc caused the retrograde degeneration of these cells. In the present study, CBD attenuated neuronal loss only in the SNpc and not in the VTA or striatum. This lack of statistical significance for the effect of CBD in the VTA and striatum may be due to the high interindividual variability observed in the 6-OHDA + Vehicle group. Results of in vivo studies of effects of CBD on dopaminergic cells in animal models of PD are conflicting. The administration of CBD (5 mg/kg) for 5 weeks did not impact dopaminergic neuronal loss in mice that were lesioned with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Celorrio *et al.*, 2017). In other study, the daily administration of CBD (3 mg/kg) for 13 days decreased dopamine levels and TH expression in the striatum in rats that received unilateral injections of 6-OHDA in the MFB (Lastres-Becker *et al.*, 2005). These conflicting results could be attributable to differences in experimental methodologies because the magnitude of dopaminergic lesions depends on the type and dose of toxins, the site of injections, whether the lesions are uni- or bilateral, and the age and species of the animals (Tieu, 2011). Yet, one should consider using different CBD dosages.

The OF was used to evaluate motor functions and anxiety-related behaviour that were induced by bilateral intranigral 6-OHDA infusions. 6-OHDA-lesioned rats exhibited a decrease in the distance travelled in the OF compared with the control group. The SNpc lesion was expected to cause hypolocomotion because the nigrostriatal pathway is related to locomotor function. However, this effect is transient, in which motor activity is recovered by compensatory mechanisms 2–3 weeks after the intranigral 6-OHDA lesion (Bové and Perier, 2012; Santiago *et al.*, 2015; Bonato *et al.*, 2018). No group differences in anxiety-related parameters were detected in the OF, which is consistent with the finding that hemiparkinsonian rats and controls exhibited similar behaviours in the elevated plus maze test of anxiety (Delaville *et al.*, 2012). However, contradictory findings of intranigral 6-OHDA lesion-induced anxiety-like behaviour in rats have been reported (Chen *et al.*, 2011; Vieira *et al.*, 2019).

In the present study, 6-OHDA-lesioned rats exhibited memory deficits in the ORT compared with controls. CBD treatment increased exploration time in Parkinsonian rats in the ORT. In agreement, the administration of CBD improved memory in rats (Meyer *et al.*, 2021) and mice (Mori *et al.*, 2017) that were subjected to cerebral ischaemia. CBD also improved memory in animal models of Alzheimer's disease including *APPxPS1* (Coles *et al.*, 2020; Chesorth *et al.*, 2022) and aged TAU58/2 transgenic female mice (Kreilaus *et al.*, 2022). Effects of CBD on memory performance have been associated with a decrease in neuroinflammation and an increase in levels of trophic factors and proteins that are involved in hippocampal neurogenesis and synaptic plasticity (Mori *et al.*, 2017; Meyer *et al.*, 2021).

Astrogliosis and microglial activation contribute to neuroinflammation-induced neuronal death in PD (Lee *et al.*, 2019). In particular, microglia activation combined with oxidative stress and apoptosis, contributes to the degeneration of dopaminergic neurons (Pabon *et al.*, 2011). In this study, CBD attenuated the glial response induced by the infusion of 6-OHDA in the SNpc of rats. Decreases in the expression of Iba-1, a microglial marker, were observed in the hippocampus and striatum of lesioned rats. Besides, CBD treatment improved neuronal maturation, potentially enhancing cognitive function in 6-OHDA lesioned rats. We speculate if these effects of CBD effects could be linked to its anti-inflammatory action. In line, in vitro and in vivo investigations have demonstrated CBD's neuroprotective impact on several experimental conditions by addressing microglia-mediated neuroinflammation. For example, Wang et al., (2022) have shown that a 14-day regimen of CBD treatment precipitates a conspicuous reduction in both apoptosis and neuroinflammation subsequent to the administration of MPTP in mice. These studies demonstrated that CBD exerts its anti-neuroinflammatory effects on microglial cells by downregulating NF-κB dependent signalling pathways and suppressing NADPH oxidase-mediated ROS expression (Dos-Santos-Pereira *et al.*, 2020; Yousaf *et al.*, 2022). Whether CBD impacts the NF-κB pathways expression in 6-OHDA lesioned rats needs further investigation.

Concerning the effects on synaptic plasticity, a pro-neurogenic effect has been attributed to CBD. The treatment with CBD (6 weeks) enhanced adult neurogenesis; however, this effect was not present in mice lacking CB1R suggesting the critical role of these receptors in the CBD-mediated actions on hippocampal neurogenesis (Wolf *et al.*, 2010). Furthermore, the anxiolytic actions of CBD in mice exposed to chronic unpredictable stress were closely associated with the pro-neurogenic effect of CBD. The authors suggested that this phenomenon depends on the

facilitation of the endocannabinoid-mediated signalling and subsequent cannabinoid receptors activation (Campos *et al.*, 2013). Likewise, repeated administration of CBD at low doses (3 mg/kg, i.p.) increased cell proliferation and neurogenesis in the DG and SVZ (Schiavon *et al.*, 2016) and improved memory in ischaemic mice.

The NSF test has been used as a measure of anxiety- and depressive-like behaviours (Blasco-Serra *et al.*, 2017). Intranigral infusions of 6-OHDA promoted an increase in the latency to feed in the NSF 18 days after the lesion. This finding was accompanied by another feature of behavioural despair, reflected by an increase in immobility time in the FST. CBD attenuated effects of 6-OHDA lesions in both the NSF test and FST, indicating an antidepressant-like effects of this treatment. Indeed, CBD has been shown to promote antidepressant-like effect in several experimental conditions. Although the pharmacological mechanism are not entirely clear, the antidepressant-like effect of CBD has been related to serotonin 5-hydroxytryptamine-1A (5-HT$_{1A}$) receptor activation (Zanelati *et al.*, 2010; Sales *et al.*, 2018).

The degeneration of midbrain dopaminergic neurons has been shown to affect several mesolimbic structures, including the hippocampus (Bonito-Oliva *et al.*, 2014; Kim *et al.*, 2022), which is critically involved in memory and emotional control. Hippocampal dysfunction has been reported in neurotoxic and genetic animals models of PD, including 6-OHDA-lesioned rats (Esmaeili-Mahani *et al.*, 2021) and α-synuclein transgenic mice (Costa *et al.*, 2012). The loss of dopaminergic neurons that was induced by intranigral 6-OHDA infusions was paralleled by the area-dependent degeneration of noradrenergic and serotonergic systems (Santiago *et al.*, 2010; Delaville *et al.*, 2012) and a decline of hippocampal synaptic plasticity (Maxreiter *et al.*, 2013; Lim *et al.*, 2018). In the present study, DCX immunoreactivity was used to study plastic changes at the level of the dendrite maturation of newborn hippocampal neurons (Brown *et al.*, 2003; Plümpe *et al.*, 2006). Intranigral 6-OHDA lesions decreased the number of DCX-IR neurons in the postmitotic stage, indicating that the lesions impacted neuronal differentiation. This effect was prevented by CBD at the dose of 30 mg/kg, but not 10 mg/kg. One possibility is that CBD facilitated neuronal maturation which could impact the establishment of synaptic connectivity and integration of newborn neurons in the hippocampal circuitry. This effect might be particularly important for hippocampus-dependent functions.

CBD exerts its neuroprotective effects through cannabinoid receptor-dependent and -independent mechanisms. In PD models, CBD has been shown to attenuate neuroinflammation and oxidative stress through cannabinoid receptor-independent actions (Lastres-Becker *et al.*, 2005; Patricio *et al.*, 2020). Moreover, CBD is known to stimulate hippocampal neurogenesis and synaptic plasticity (Campos *et al.*, 2016). However, the pharmacological profile of CBD is complex. It is known to act on different molecular targets (Patricio *et al.*, 2020). We recently reported the involvement of cannabinoid CB$_1$ and CB$_2$ receptors, serotonin 5-HT$_{1A}$ receptors, and peroxisome proliferator-activated receptor-γ (PPAR-γ) in cognitive and emotional symptoms in mice that were subjected to cerebral ischaemia (Mori *et al.*, 2021). Remaining to be determined is whether similar mechanisms are involved in neuroprotective effects of CBD observed in 6-OHDA-lesioned rats.

References

Aarsland D, Påhlhagen S, Ballard CG, Ehrt U and Svenningsson P (2011) Depression in Parkinson disease—epidemiology, mechanisms and management. *Nature Reviews Neurology* **8**(1), 35–47. DOI: 10.1038/nrneurol.2011.189.

Akkerman S, Blokland A, Reneerkens O, van Goethem NP, Bollen E, Gijselaers HJM, Lieben CKJ, Steinbusch HWM and Prickaerts J (2012) Object recognition testing: methodological considerations on exploration and discrimination measures. *Behavioural Brain Research* **232**(2), 335–347.

Akkerman S, Blokland A, Reneerkens O, van Goethem NP, Bollen E, Gijselaers HJM, Lieben CKJ, Steinbusch HWM and Prickaerts J (2012) Object recognition testing: methodological considerations on exploration and discrimination measures. *Behavioural Brain Research* **232**(2), 335–347. DOI: 10.1016/j.bbr.2012.03.022.

Alborghetti M and Nicoletti F (2019) Different generations of type-B monoamine oxidase inhibitors in Parkinson's disease: from bench to bedside. *Current Neuropharmacology* **17**(9), 861–873. DOI: 10.2174/1570159X16666180830100754.

Blandini F, Levandis G, Bazzini E, Nappi G and Armentero MT (2007) Time-course of nigrostriatal damage, basal ganglia metabolic changes and behavioural alterations following intrastriatal injection of 6-hydroxydopamine in the rat: new clues from an old model. *European Journal of Neuroscience* **25**(2), 397–405. DOI: 10.1111/j.1460-9568.2006.05285.x.

Blasco-Serra A, González-Soler EM, Cervera-Ferri A, Teruel-Martí V and Valverde-Navarro AA (2017) A standardization of the novelty-suppressed feeding test protocol in rats. *Neuroscience Letters* **658**, 73–78.

Bonato JM, Bassani TB, Milani H, Vital MABF and de Oliveira RMW (2018) Pioglitazone reduces mortality, prevents depressive-like behavior, and impacts hippocampal neurogenesis in the 6-OHDA model of Parkinson's disease in rats. *Experimental Neurology* **300**, 188–200.

Bonito-Oliva A, Masini D and Fisone G (2014) A mouse model of non-motor symptoms in Parkinson's disease: focus on pharmacological interventions targeting affective dysfunctions. *Frontiers in Behavioral Neuroscience* **8**, 290. DOI: 10.3389/fnbeh.2014.00290.

Bové J and Perier C (2012) Neurotoxin-based models of Parkinson's disease. *Neuroscience* **211**, 51–76. DOI: 10.1016/j.neuroscience.2011.10.057.

Brown JP, Couillard-Després S, Cooper-Kuhn CM, Winkler J, Aigner L and Kuhn HG (2003) Transient expression of doublecortin during adult neurogenesis. *Journal of Comparative Neurology* **467**(1), 1–10.

Camicioli R, Moore MM, Kinney A, Corbridge E, Glassberg K and Kaye JA (2003) Parkinson's disease is associated with hippocampal atrophy. *Movement Disorders* **18**(7), 784–790.

Camicioli R, Moore MM, Kinney A, Corbridge E, Glassberg K and Kaye JA (2003) Parkinson's disease is associated with hippocampal atrophy. *Movement Disorders* **18**(7), 784–790. DOI: 10.1002/mds.10444.

Campos AC, Fogaça MV, Sonego AB and Guimarães FS (2016) Cannabidiol, neuroprotection and neuropsychiatric disorders. *Pharmacological Research* **112**, 119–127. DOI: 10.1016/j.phrs.2016.01.033.

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 and Guimarães FS (2013) The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: involvement of the endocannabinoid system. *International Journal of Neuropsychopharmacology* **16**(6), 1407–1419. DOI: 10.1017/S1461145712001502.

Celorrio M, Rojo-Bustamante E, Fernández-Suárez D, Sáez E, Estella-Hermoso de Mendoza A, Müller CE, Ramírez MJ, Oyarzábal J, Franco R and Aymerich MS (2017) GPR55: a therapeutic target for Parkinson's disease? *Neuropharmacology* **125**, 319–332. DOI: 10.1016/j.neuropharm.2017.08.017.

Chagas MHN, Zuardi AW, Tumas V, Pena-Pereira MA, Sobreira ET, Bergamaschi MM, dos Santos AC, Teixeira AL, Hallak JEC and Crippa JAS (2014) Effects of cannabidiol in the treatment of patients with parkinson's disease: an exploratory double-blind trial. *Journal of Psychopharmacology* **28**(11), 1088–1098. DOI: 10.1177/0269881114550355.

Chen L, Liu J, Zhang QJ, Feng JJ, Gui ZH, Ali U, Wang Y, Fan LL, Hou C and Wang T (2011) Alterations of emotion, cognition and firing activity of the basolateral nucleus of the amygdala after partial bilateral lesions of the nigrostriatal pathway in rats. *Brain Research Bulletin* **85**(6), 329–338. DOI: 10.1016/j.brainresbull.2011.05.009.

Chesworth R, Cheng D, Staub C and Karl T (2022) Effect of long-term cannabidiol on learning and anxiety in a female alzheimer's disease mouse model. *Frontiers in Pharmacology* **13**, 931384. DOI: 10.3389/fphar.2022.931384.

Chia SJ, Tan EK and Chao YX (2020) Historical perspective: models of Parkinson's disease. *International Journal of Molecular Sciences* **21**(7), 2464.

Cilia R, Cereda E, Klersy C, Canesi M, Zecchinelli AL, Mariani CB, Tesei S, Sacilotto G, Meucci N, Zini M, Ruffmann C, Isaias IU, Goldwurm S and Pezzoli G (2015) Parkinson's disease beyond 20 years. *J Neurol Neurosurg Psychiatry* **86**(8), 849–855. DOI: 10.1136/jnnp-2014-308786.

Coles M, Watt G, Kreilaus F and Karl T (2020) Medium-dose chronic cannabidiol treatment reverses object recognition memory deficits of APPSwe/PS1ΔE9 transgenic female mice. *Frontiers in Pharmacology* **11**, 587604. DOI: 10.3389/fphar.2020.587604.

Costa C, Sgobio C, Siliquini S, Tozzi A, Tantucci M, Ghiglieri V, Di Filippo M, Pendolino V, de Iure A, Marti M, Morari M, Spillantini MG, Latagliata EC, Pascucci T, Puglisi-Allegra S, Gardoni F, Di Luca M, Picconi B and Calabresi P (2012) Mechanisms underlying the impairment of hippocampal long-term potentiation and memory in experimental Parkinson's disease. *Brain* **135**(Pt 6), 1884–1899. DOI: 10.1093/brain/aws101.

Crippa JA, Guimarães FS, Campos AC and Zuardi AW (2018) Translational investigation of the therapeutic potential of cannabidiol (CBD): toward a new age. *Frontiers in Immunology* **9**, 2009.

Dalla C, Pitychoutis PM, Kokras N and Papadopoulou-Daifoti Z (2011) Sex differences in response to stress and expression of depressive-like behaviours in the rat. *Current Topics in Behavioral Neurosciences* **8**, 97–118. DOI: 10.1007/7854_2010_94.

Delaville C, Chetrit J, Abdallah K, Morin S, Cardoit L, De Deurwaerdère P and Benazzouz A (2012) Emerging dysfunctions consequent to combined monoaminergic depletions in Parkinsonism. *Neurobiology of Disease* **45**(2), 763–773. DOI: 10.1016/j.nbd.2011.10.023.

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

Esmaeili-Mahani S, Haghparast E, Nezhadi A, Abbasnejad M and Sheibani V (2021) Apelin-13 prevents hippocampal synaptic plasticity impairment in Parkinsonism rats. *Journal of Chemical Neuroanatomy* **111**, 101884. DOI: 10.1016/j.jchemneu.2020.101884.

Hayakawa K, Mishima K, Irie K, Hazekawa M, Mishima S, Fujioka M, Orito K, Egashira N, Katsurabayashi S, Takasaki K, Iwasaki K and Fujiwara M (2008) Cannabidiol prevents a post-ischemic injury progressively induced by cerebral ischemia via a high-mobility group box1-inhibiting mechanism. *Neuropharmacology* **55**(8), 1280–1286. DOI: 10.1016/j.neuropharm.2008.06.040.

Hayes MW, Fung VS, Kimber TE and O'Sullivan JD (2019) Updates and advances in the treatment of Parkinson disease. *The Medical Journal of Australia* **211**(6), 277–283. DOI: 10.5694/mja2.50224.

Hooshangi Shayesteh MR, Haghi-Aminjan H, Baeeri M, Rahimifard M, Hassani S, Gholami M, Momtaz S, Salami SA, Armandeh M, Bameri B, Samadi M, Mousavi T, Ostad SN and Abdollahi M (2022) Modification of the hemodynamic and molecular features of phosphine, a potent mitochondrial toxicant in the heart, by cannabidiol. *Toxicology Mechanisms and Methods* **32**(4), 288–301.

Jones NA, Hill AJ, Smith I, Bevan SA, Williams CM, Whalley BJ and Stephens GJ (2010) Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. *Journal of Pharmacology and Experimental Therapeutics* **332**(2), 569–577. DOI: 10.1124/jpet.109.159145.

Kalia LV and Lang AE (2016) Parkinson disease in 2015: Evolving basic, pathological and clinical concepts in PD. *Nature Reviews Neurology* **12**(2), 65–66. DOI: 10.1038/nrneurol.2015.249.

Khaksar S, Bigdeli M, Samiee A and Shirazi-Zand Z (2022) Antioxidant and anti-apoptotic effects of cannabidiol in model of ischemic stroke in rats. *Brain Research Bulletin* **180**, 118–130. DOI: 10.1016/j.brainresbull.2022.01.00.

Khaksar S, Bigdeli M, Samiee A and Shirazi-Zand Z (2022) Antioxidant and anti-apoptotic effects of cannabidiol in model of ischemic stroke in rats. *Brain Research Bulletin* **180**, 118–130. DOI: 10.1016/j.brainresbull.2022.01.001.

Kim B, Weerasinghe-Mudiyanselage PDE, Ang MJ, Lee J, Kang S, Kim J-C, Kim S-H, Kim J-S, Jung C, Shin T and Moon C (2022) Changes in the neuronal architecture of the hippocampus in a 6-hydroxydopamine-lesioned rat model of parkinson disease. *International Neurourology Journal* **26**(Suppl 2), S94–105. DOI: 10.5213/inj.2244252.126.

Kreilaus F, Przybyla M, Ittner L and Karl T (2022) Cannabidiol (CBD) treatment improves spatial memory in 14-month-old female TAU58/2 transgenic mice. *Behavioural Brain Research* **425**, 113812. DOI: 10.1016/j.bbr.2022.113812.

Kundrick ER, Marrero-Rosado BM, de Araujo Furtado M, Stone M, Schultz CR and Lumley LA (2021) Cannabidiol reduces soman-induced lethality and seizure severity in female plasma carboxylesterase knockout mice treated with midazolam. *Neurotoxicology* **82**, 130–136. DOI: 10.1016/j.neuro.2020.12.002.

Lastres-Becker I, Molina-Holgado F, Ramos JA, Mechoulam R and Fernández-Ruiz J (2005) Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity in vivo and in vitro: relevance to Parkinson's disease. *Neurobiology of Disease* **19**(1-2), 96–107. DOI: 10.1016/j.nbd.2004.11.009.

Lastres-Becker I, Molina-Holgado F, Ramos JA, Mechoulam R and Fernández-Ruiz J (2005 Jun-Jul) Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity in vivo and in vitro: relevance to parkinson's disease. *Neurobiology of Disease* **19**(1-2), 96–107. DOI: 10.1016/j.nbd.2004.11.009.

Lau C, Hebert M, Vani MA, Walling S, Hayley S, Lagace DC and Blundell J (2016) Absence of neurogenic response following robust predator-induced stress response. *Neuroscience* **339**, 276–286. Dec https://doi.org/10.1016/j.neuroscience.2016.10.001.

Lee A and Gilbert RM (2016) Epidemiology of Parkinson disease. *Neurologic Clinics* **34**(4), 955–965. DOI: 10.1016/j.ncl.2016.06.012.

Lee Y, Lee S, Chang S-C and Lee J (2019) Significant roles of neuro-inflammation in Parkinson's disease: therapeutic targets for PD prevention. *Archives of Pharmacal Research* **42**(5), 416–425. DOI: 10.1007/s12272-019-01133-0.

Lim J, Bang Y and Choi HJ (2018) Abnormal hippocampal neurogenesis in Parkinson's disease: relevance to a new therapeutic target for depression with Parkinson's disease. *Archives of Pharmacal Research* **41**(10), 943–954.

Lindner MD, Cain CK, Plone MA, Frydel BR, Blaney TJ, Emerich DF and Hoane MR (1999) Incomplete nigrostriatal dopaminergic cell loss and partial reductions in striatal dopamine produce akinesia, rigidity, tremor and cognitive deficits in middle-aged rats. *Behavioural Brain Research* **102**(1-2), 1–16. DOI: 10.1016/s0166-4328(98)00160-0.

Martínez-Martín P and Damián J (2010) Parkinson disease: depression and anxiety in Parkinson disease. *Nature Reviews Neurology* **6**(5), 243–245. DOI: 10.1038/nrneurol.2010.49.

Marxreiter F, Regensburger M and Winkler J (2013) Adult neurogenesis in Parkinson's disease. *Cellular and Molecular Life Sciences* **70**(3), 459–473.

Meyer E, Bonato JM, Mori MA, Mattos BA, Guimarães FS, Milani H, de Campos AC and de Oliveira RMW (2021) Cannabidiol confers neuroprotection in rats in a model of transient global cerebral ischemia: impact of hippocampal synaptic neuroplasticity. *Molecular Neurobiology* **58**(10), 5338–5355.

Mishima K, Hayakawa K, Abe K, Ikeda T, Egashira N, Iwasaki K and Fujiwara M (2005) Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1A receptor-dependent mechanism. *Stroke* **36**(5), 1077–1082. DOI: 10.1161/01.STR.0000163083.59201.34.

Mori MA, Meyer E, da Silva FF, Milani H, Guimarães FS and Oliveira RMW (2021) Differential contribution of CB1, CB2, 5-HT1A, and PPAR-γ receptors to cannabidiol effects on ischemia-induced emotional and cognitive impairments. *European Journal of Neuroscience* **53**(6), 1738–1751.

Mori MA, Meyer E, Soares LM, Milani H, Guimarães FS and de Oliveira RMW (2017) Cannabidiol reduces neuroinflammation and promotes neuroplasticity and functional recovery after brain ischemia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **75**, 94–105.

Muhammad F, Liu Y, Wang N, Zhao L, Zhou Y, Yang H and Li H (2022) Neuroprotective effects of cannabidiol on dopaminergic neuro-degeneration and α-synuclein accumulation in C. elegans models of Parkinson's disease. *Neurotoxicology* **93**, 128–139. DOI: 10.1016/j.neuro.2022.09.001.

Pabon MM, Bachstetter AD, Hudson CE, Gemma C and Bickford PC (2011) CX3CL1 reduces neurotoxicity and microglial activation in a rat model of Parkinson's disease. *Journal of Neuroinflammation* **8**(1), 9. DOI: 10.1186/1742-2094-8-9.

Padovan-Neto FE, Echeverry MB, Tumas V and Del-Bel EA (2009) Nitric oxide synthase inhibition attenuates l-DOPA-induced dyskinesias in a rodent model of Parkinson's disease. *Neuroscience* **159**(3), 927–935. DOI: 10.1016/j.neuroscience.2009.01.034.

Patra PH, Serafeimidou-Pouliou E, Bazelot M, Whalley BJ, Williams CM and McNeish AJ (2020) Cannabidiol improves survival and behavioural co-morbidities of dravet syndrome in mice. *British Journal of Pharmacology* **177**(12), 2779–2792. DOI: 10.1111/bph.15003.

Patricio F, Morales Dávila E, Patricio-Martínez A, Arana Del Carmen N, Martínez I, Aguilera J, Perez-Aguilar JM and Limón ID (2022) Intrapallidal injection of cannabidiol or a selective GPR55 antagonist decreases motor asymmetry and improves fine motor skills in hemi-parkinsonian rats. *Frontiers in Pharmacology* **13**, 945836. DOI: 10.3389/fphar.2022.945836.

Patricio F, Morales-Andrade AA, Patricio-Martínez A and Limón ID (2020) Cannabidiol as a therapeutic target: evidence of its neuroprotective and neuromodulatory function in parkinson's disease. *Frontiers in Pharmacology* **11**, 595635. DOI: 10.3389/fphar.2020.595635.

Paxinos G and Watson C (2007) The rat brain in stereotaxic coordinates, 7 edn. Elsevier Science.

Perez M, Cartarozzi LP, Chiarotto GB, Guimarães FS and Oliveira ALR (2021) Short and long-term neuroprotective effects of cannabidiol after neonatal peripheral nerve axotomy. *Neuropharmacology* **197**, 108726. DOI: 10.1016/j.neuropharm.2021.108726.

Plümpe T, Ehninger D, Steiner B, Klempin F, Jessberger S, Brandt M, Römer B, Rodriguez GR, Kronenberg G and Kempermann G (2006) Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. *BMC Neuroscience* **7**(1), 77.

Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, Schrag A-E and Lang AE (2017) Parkinson disease. *Nature Reviews Disease Primers* **3**(1), 17013.

Sales AJ, Crestani CC, Guimarães FS and Joca SRL (2018) Antidepressant-like effect induced by Cannabidiol is dependent on brain serotonin levels. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **86**, 255–261. DOI: 10.1016/j.pnpbp.2018.06.002.

Santiago RM, Barbieiro J, Lima MMS, Dombrowski PA, Andreatini R and Vital MAF (2010) Depressive-like behaviors alterations induced by intranigral MPTP, 6-OHDA, LPS and rotenone models of parkinson's disease are predominantly associated with serotonin and dopamine. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **34**(6), 1104–1114. DOI: 10.1016/j.pnpbp.2010.06.004.

Santiago RM, Tonin FS, Barbiero J, Zaminelli T, Boschen SL, Andreatini R, Da Cunha C, Lima MMS and Vital MABF (2015) The nonsteroidal anti-inflammatory drug piroxicam reverses the onset of depressive-like behavior in 6-OHDA animal model os Parkinson's disease. *Neuroscience* **300**, 246–253.

Santos García D, Jesús S, Aguilar M, Planellas LL, García Caldentey J, Caballol N, Legarda I, Hernández Vara J, Cabo I, López Manzanares L, González Aramburu I, Ávila Rivera MA, Catalán MJ, López Díaz L, Puente V, García Moreno JM, Borrué C, Solano Vila B, Álvarez Sauco M, Vela L, Escalante S, Cubo E, Carrillo Padilla F, Martínez Castrillo JC, Sánchez Alonso P, Alonso Losada MG, López Ariztegui N, Gastón I, Kulisevsky J, Menéndez González M, Seijo M, Rúiz Martínez J, Valero C, Kurtis M, de Fábregues-Boixar O, González Ardura J, Prieto Jurczynska C, Martinez-Martin P, Mir P and on behalf of the COPPAIDS Study Group (2019) COPPADIS-2015 (COhort of Patients with PArkinson's DIsease in Spain, 2015): an ongoing global Parkinson's disease project about disease progression with more than 1000 subjects included. Results from the baseline evaluation. *European Journal of Neurology* **26**(11), 1399–1407. DOI: 10.1111/ene.14008.

Schapira AHV, Chaudhuri KR and Jenner P (2017) Non-motor features of Parkinson disease. *Nature Reviews Neuroscience* **18**(7), 435–450.

Schiavon AP, Bonato JM, Milani H, Guimarães FS and Weffort de Oliveira RM (2016) Influence of single and repeated cannabidiol administration on emotional behavior and markers of cell proliferation and neurogenesis in non-stressed mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **64**, 27–34. DOI: 10.1016/j.pnpbp.2015.06.017.

Simola N, Morelli M and Carta AR (2007) The 6-hydroxydopamine model of Parkinson's disease. *Neurotoxicity Research* **11**(3-4), 151–167.

Singh J and Neary JP (2020) Neuroprotection following concussion: the potential role for cannabidiol. *Canadian Journal of Neurological Sciences* **47**(3), 289–300. DOI: 10.1017/cjn.2020.23.

Soares LM, De Vry J, Steinbusch HWM, Milani H, Prickaerts J and Weffort de Oliveira RM (2016) Rolipram improves cognition, reduces anxiety- and despair-like behaviors and impacts hippocampal neuroplasticity after transient global cerebral ischemia. *Neuroscience* **326**, 69–83. DOI: 10.1016/j.neuroscience.2016.03.062.

Tieu K (2011) A guide to neurotoxic animal models of parkinson's disease, vol. **1**. Cold Spring Harb Perspect Med, a009316. DOI:10.1101/cshperspect.a009316.

Vieira JCF, Bassani TB, Santiago RM, de O. Guaita G, Zanoveli Jína M, da Cunha C and Vital MABF (2019) Anxiety-like behavior induced by 6-OHDA animal model of Parkinson's disease may be related to a dysregulation of neurotransmitter systems in brain areas related to anxiety. *Behavioural Brain Research* **371**, 111981.

Vilela LR, Gomides LF, David BA, Antunes MM, Diniz AB, Moreira Fício de A and Menezes GB (2015) Cannabidiol rescues acute hepatic toxicity and seizure induced by cocaine. *Mediators of Inflammation* **2015**, 523418. DOI: 10.1155/2015/523418.

Vivanco-Estela AN, Dos-Santos-Pereira M, Guimaraes FS, Del-Bel E and Nascimento GCD (2021) Cannabidiol has therapeutic potential for myofascial pain in female and male parkinsonian rats. *Neuropharmacology* **196**, 108700. DOI: 10.1016/j.neuropharm.2021.108700.

Watt G and Karl T (2017) In vivo evidence for therapeutic properties of cannabidiol (CBD) for Alzheimer's disease. *Frontiers in Pharmacology* **8**, 20. DOI: 10.3389/fphar.2017.00020.

WHO (2022) Launch of WHO's Parkinson disease technical brief. Available at "https://www.who.int/news/item/14-06-2022-launch-of-who-s-parkinson-disease-technical-brief" (accessed 24 July 2023).

Wolf SA, Bick-Sander A, Fabel K, Leal-Galicia P, Tauber S, Ramirez-Rodriguez G, Müller A, Melnik A, Waltinger TP, Ullrich O and Kempermann G (2010) Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. *Cell Communication and Signaling* **8**, 12. DOI: 10.1186/1478-811X-8-12.

Yousaf M, Chang D, Liu Y, Liu T and Zhou X (2022) Neuroprotection of cannabidiol, Its synthetic derivatives and combination preparations against microglia-mediated neuroinflammation in neurological disorders. *Molecules* **27**(15), 4961. DOI: 10.3390/molecules27154961.

Zanelati TV, Biojone C, Moreira FA, Guimarães FS and Joca SRL (2010) Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. *British Journal of Pharmacology* **159**(1), 122–128.