Schizophrenia Research xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol

Felipe V. Gomes [a,b,*], Ricardo Llorente [c], Elaine A. Del Bel [b,d], Maria-Paz Viveros [e], Meritxell López-Gallardo [c], Francisco S. Guimarães [a,b,*]

[a] Department of Pharmacology, Medical School of Ribeirão Preto, University of São Paulo, Brazil
[b] Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of São Paulo, Brazil
[c] Department of Physiology, Faculty of Medicine, Complutense University of Madrid, Spain
[d] Department of Physiology, Faculty of Odontology of Ribeirão Preto, University of São Paulo, Brazil
[e] Department of Physiology (Animal Physiology II), Faculty of Biology, Complutense University of Madrid, Spain

ARTICLE INFO

Article history:
Received 9 October 2014
Received in revised form 6 January 2015
Accepted 8 January 2015
Available online xxxx

Keywords:
Cannabinoid
Psychosis
Microglia
Astrocytes
NeuN
NMDA receptor
MK-801
Clozapine

ABSTRACT

NMDA receptor hypofunction could be involved, in addition to the positive, also to the negative symptoms and cognitive deficits found in schizophrenia patients. An increasing number of data has linked schizophrenia with neuroinflammatory conditions and glial cells, such as microglia and astrocytes, have been related to the pathogenesis of schizophrenia. Cannabidiol (CBD), a major non-psychotomimetic constituent of *Cannabis sativa* with anti-inflammatory and neuroprotective properties induces antipsychotic-like effects. The present study evaluated if repeated treatment with CBD (30 and 60 mg/kg) would attenuate the behavioral and glial changes observed in an animal model of schizophrenia based on the NMDA receptor hypofunction (chronic administration of MK-801, an NMDA receptor antagonist, for 28 days). The behavioral alterations were evaluated in the social interaction and novel object recognition (NOR) tests. These tests have been widely used to study changes related to negative symptoms and cognitive deficits of schizophrenia, respectively. We also evaluated changes in NeuN (a neuronal marker), Iba-1 (a microglia marker) and GFAP (an astrocyte marker) expression in the medial prefrontal cortex (mPFC), dorsal striatum, nucleus accumbens core and shell, and dorsal hippocampus by immunohistochemistry. CBD effects were compared to those induced by the atypical antipsychotic clozapine. Repeated MK-801 administration impaired performance in the social interaction and NOR tests. It also increased the number of GFAP-positive astrocytes in the mPFC and the percentage of Iba-1-positive microglia cells with a reactive phenotype in the mPFC and dorsal hippocampus without changing the number of Iba-1-positive cells. No change in the number of NeuN-positive cells was observed. Both the behavioral disruptions and the changes in expression of glial markers induced by MK-801 treatment were attenuated by repeated treatment with CBD or clozapine. These data reinforces the proposal that CBD may induce antipsychotic-like effects. Although the possible mechanism of action of these effects is still unknown, it may involve CBD anti-inflammatory and neuroprotective properties. Furthermore, our data support the view that inhibition of microglial activation may improve schizophrenia symptoms.

1. Introduction

Individuals suffering from schizophrenia manifest a range of behavioral changes, including positive (delusions and hallucinations) and negative symptoms (social withdrawal, anhedonia), as well as cognitive impairment. Cognitive deficits and negative symptoms are present even before the onset of psychosis and are frequently associated with poor long-term outcome (Elvevag and Goldberg, 2000; Lesh et al., 2011). While the existing medications have proven effective in treating positive symptoms, their efficacy on negative symptoms and cognitive deficits is limited (Elvevag and Goldberg, 2000; Hanson et al., 2010), indicating a great need for new psychopharmacologic agents.

Although the etiology of schizophrenia is still unknown, evidence suggests that an impaired function of the prefrontal cortex mediated by a glutamate NMDA receptor hypofunction could be involved in the negative and cognitive symptoms of schizophrenia (Gonzalez-Burgos and Lewis, 2012; Nakazawa et al., 2012). This proposal is based essentially on studies showing that acute and chronic administration of NMDA receptor antagonists, such as phencyclidine, ketamine, and MK-801, in animals and healthy volunteers induces schizophrenia-like

* Corresponding authors at: Department of Pharmacology, Medical School of Ribeirão Preto, University of São Paulo, 3900 Bandeirantes Ave, Ribeirão Preto, SP 14049-900, Brazil. Tel.: +55 16 36023325; fax: +55 16 36332301.
E-mail addresses: gomesfv@usp.br (F.V. Gomes), fsguimar@fmrp.usp.br (F.S. Guimarães).

http://dx.doi.org/10.1016/j.schres.2015.01.015
0920-9964/

signs (Krystal et al., 1994; Jentsch and Roth, 1999). Moreover, when administered to schizophrenia patients these drugs can worse the psychotic symptoms (Krystal et al., 2005). Thus, animal models based on administration of these drugs have been widely used. However, even if most studies have employed acute administration of NMDA receptor antagonists, the effects induced by chronic treatment with these drugs are proposed to better represent the behavioral, neurochemical and neuroanatomical changes observed in schizophrenia patients (Jentsch and Roth, 1999).

An increasing number of clinical, epidemiological, and experimental data have linked schizophrenia with inflammatory conditions. In this context, glial cells, such as microglia and astrocytes, have been related to the pathogenesis of schizophrenia (Schnieder and Dwork, 2011; Monji et al., 2013). Microglia and astrocytes are the major immune cells in the central nervous system (CNS), regulating the induction as well as the limitation of inflammatory processes (Sofroniew and Vinters, 2010; Graeber et al., 2011).

Cannabidiol (CBD), a major non-psychotomimetic compound from *Cannabis sativa*, presents potential therapeutic effects in schizophrenia with several pre-clinical studies indicating that this drug induces antipsychotic-like effects (for review see Campos et al., 2012). These effects have also been described in open-label clinical studies (Zuardi et al., 1995, 2006) and in a recent controlled, randomized, double-blind clinical trial (Leweke et al., 2012). The mechanism of these effects is still unknown (Campos et al., 2012). However, besides its antipsychotic properties, CBD also induces anti-inflammatory and neuroprotective effects, which could contribute for its beneficial effects in schizophrenia. Indeed, a considerable number of preclinical studies have indicated that CBD attenuated increased glial reactivity associated to pathological conditions (Mecha et al., 2013; Perez et al., 2013; Schiavon et al., 2014). Yet, the involvement of these mechanisms in CBD antipsychotic effects has not been evaluated in animal models of schizophrenia.

Based on these pieces of evidence, we investigated whether repeated CBD treatment would attenuate the impairment in social interaction and novel object recognition (NOR) tests induced by chronic administration of the NMDA receptor antagonist MK-801. These tests have been widely used to study the negative symptoms and cognitive deficits, respectively, in animal models of schizophrenia (Ellenbroek and Cools, 2000; Rajagopal et al., 2014). Additionally, given that neuroinflammatory processes in schizophrenia may involve abnormal astrocyte and microglia functions (Rothermundt et al., 2009; Schnieder and Dwork, 2011; Monji et al., 2013; Catts et al., 2014) and that NMDA receptor antagonists induce neuronal damage and alter the expression of astrocyte and microglial markers (Nakki et al., 1995, 1996), we also measured changes in the expression of neuronal (NeuN) and glial markers (GFAP, astrocytes; Iba-1, microglia) in brain structures related to the neurobiology of schizophrenia, such as the medial prefrontal cortex (mPFC), dorsal striatum (dSTR), nucleus accumbens (NAc) core and shell and dorsal hippocampus (dentate gyrus — DG, CA1 and CA3). CBD effects were compared to those induced by the atypical antipsychotic clozapine.

2. Material and methods

2.1. Animals

The experiments were performed using male C57BL/6J mice with 6 weeks of age at the beginning of treatment. Animals were housed in groups of four per cage (41 × 33 × 17 cm) in a temperature-controlled room (24 ± 1 °C) under standard laboratory conditions with free access to food and water and a 12 h light/dark cycle (lights on at 06:00 a.m.). Procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behavior guidelines for the care and use of laboratory animals, which are in compliance with international laws and politics. The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 165/2010).

2.2. Drugs

The following drugs were used: cannabidiol (CBD; THC Pharm, Germany), clozapine (Tocris, USA) and MK-801 (Sigma-Aldrich, USA). CBD was diluted in 2% Tween 80 in saline, while clozapine was diluted in saline supplemented with 30 μL of 0.1 M hydrochloric acid (pH was adjusted to a value close to neutrality when necessary). MK-801 was diluted in saline. Drugs were injected intraperitoneally (ip) in a 10 mL/kg volume. Body weight was measured daily.

2.3. Experimental design

We investigated whether repeated treatment with CBD (30 and 60 mg/kg) or clozapine (1 mg/kg) would attenuate social withdrawal and deficits in the novel object recognition (NOR) test induced by chronic treatment with MK-801 1 mg/kg for 28 days (n = 7–9/group). CBD or clozapine treatment began on the 6th day after the start of MK-801 administration and continued until the end of the treatment. The doses and treatment schedule were based on a previous study from our group (Gomes et al., 2015) and were those able to prevent the prepulse inhibition impairment induced by repeated MK-801 treatment. CBD, clozapine or vehicle were administered 30 min before MK-801 or saline, resulting in the following groups: vehicle + saline, CBD 60 mg/kg + saline, clozapine + saline, vehicle + MK-801, CBD 30 mg/kg + MK-801, CBD 60 mg/kg + MK-801, and clozapine + MK-801. One day after the end of the treatment, animals were submitted to the social interaction test. Four hours later they were submitted to the habituation session of the NOR test. The acquisition and test trials of the NOR test were performed 24 h later. On the day following the NOR test, all animals were euthanized and their brains were processed to assess changes in the expression of neuronal (NeuN) and glial markers (astrocytes — GFAP, and microglia — Iba-1) by immunohistochemistry. For this, only the dose of 60 mg/kg of CBD was evaluated, since it was more effective in mitigating MK-801-induced behavioral changes. A diagrammatic representation of the experimental design is presented in Fig. 1.

To evaluate a possible interference of anxiety-related behaviors and locomotor activity changes, independent groups of mice were submitted to a similar treatment schedule and evaluated in the elevated plus-maze (EPM) and open field tests 24 and 48 h, respectively, after the end of the treatments.

2.4. Procedure

2.4.1. Social interaction test

The social interaction test was carried out in a rectangular arena (28 × 17 × 13 cm). The animals (an experimental mouse and an unfamiliar conspecific mouse) were placed on opposite sides of the arena to freely explore it for 10 min. The time of active social behavior of the experimental mouse such as sniffing, following, grooming and climbing on or under the other mouse was recorded. The experimental animals had not been previously exposed to the arena and to the unfamiliar animal.

2.4.2. Novel object recognition (NOR) test

The NOR test was carried out in a Plexiglas circular arena (40 cm diameter and 40 cm height). One day before the test session each animal was habituated in the arena for 15 min. On the test day, animals were submitted to two trials separated by a 1 h-intertrial interval. During the first trial (acquisition trial, T1), mice were placed in the arena containing two identical objects for 10 min. For the second trial (test trial, T2), one of the objects presented in T1 was replaced by an unknown object (novel object). Animals were then placed back in the arena for 5 min. The behavior was recorded on video for blind scoring of object exploration, which was defined as situations where the animal is directing its face to the object in a distance of approximately 2 cm

Please cite this article as: Gomes, F.V., et al., Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol, Schizophr. Res. (2015), http://dx.doi.org/10.1016/j.schres.2015.01.015

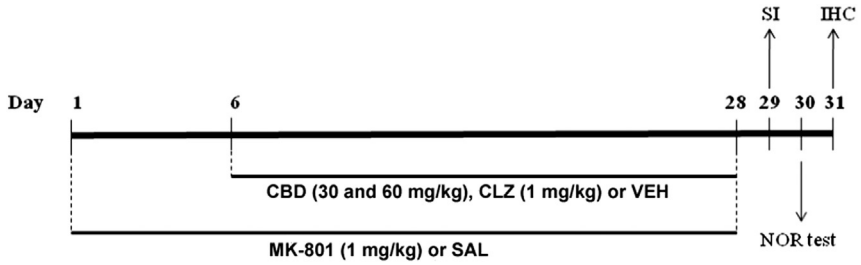

Fig. 1. Experimental design. The animals received daily ip injections of saline (SAL) or MK-801 (1 mg/kg) for 28 days. CBD (30 and 60 mg/kg) or vehicle (VEH) treatment began on the 6th day after the start of MK-801 administration and continued until the end of the treatment. One day later, animals were submitted to the social interaction (SI) test. The acquisition and test trials of the NOR test were performed 24 h later. One day later, animals were euthanized and their brains were removed and processed to assess changes in the expression of NeuN, GFAP, and Iba-1 by immunohistochemistry (IHC). CBD effects were compared to those induced by the atypical antipsychotic clozapine (CLZ; 1 mg/kg).

while watching, licking, sniffing, or touching it with the forepaws while sniffing. Exploration time (s) of each object in each trial was recorded manually by the use of two stopwatches. The arena and the objects were cleaned between each trial using alcohol 70% to avoid odor trails. The familiar and novel objects were 15 cm high, being too heavy to be displaced by the animals and having different shape, color and texture. Recognition memory was assessed using the discrimination index (discrimination index = (novel − familiar / novel + familiar)), corresponding to the difference between the time exploring the novel and the familiar object, corrected for total time exploring both objects (Bertaina-Anglade et al., 2006).

2.4.3. Elevated plus-maze (EPM)

Anxiety-like behavior was investigated using the EPM apparatus (Carobrez and Bertoglio, 2005). The EPM consisted of two opposite wooden open arms (34 × 6.5 cm), crossed at right angle by two arms of the same dimensions enclosed by 15 cm high walls with no roof. The maze was located 50 cm above the floor and a 1-cm high edge made of Plexiglas surrounded the open arms to prevent falls. The EPM was located in a sound-attenuated room. The Any-Maze software (Stoelting, USA) was employed for behavioral analysis in the EPM. It detects the position of the animal in the maze and calculates the number of entries and time spent in open and enclosed arms. Each session lasted for 5 min and after each trial the maze was cleaned using alcohol 70% v/v.

2.4.4. Open field test

The open field consisted of a Plexiglas circular arena (40 cm diameter and 40 cm height). Animals were placed in the center of the arena and the total distance traveled was determined during 5 min using the Any-Maze software.

2.4.5. Immunohistochemical detection of NeuN, GFAP and Iba-1

2.4.5.1. Tissue collection. One day after the exposure to the NOR test, animals were deeply anesthetized with a lethal dose of urethane (25%, 5 mL/kg; ip) and transcardially perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA 4%) in 0.1 M phosphate buffer (PB). The brains were removed and post-fixed in the same fixative solution (PFA 4%) for 2 h. Then, they were washed three times, 30 min each, in PB 0.1 M, pH 7.2, and cryoprotected in 11% sucrose in PBS at 4 °C during 24 h. The samples were then transferred to PB 0.1 M, pH 7.2 containing 33% sucrose and conserved at 4 °C during 24 h. Finally, the brains were embedded in Tissue Freezing Medium (Tissue-Tek® O.C.T., Sakura Finetek, USA), and kept at −30 °C until used. For immunostaining techniques, brains were frozen sectioned using a cryostat microtome at −22 °C (CM-3050, Leica, Germany) in 25 μm-thick serial coronal sections. Tissue sections were collected into five alternate series of gelatin-coated slides (6 slices per slide), air dried, and stored at −30 °C.

2.4.5.2. Immunostaining. The slides with tissue sections were allowed to dry at room temperature for 10 min then air dried for at least 15 min on a slide warmer at 36 °C, and then again 10 min at room temperature, in order to increase the adhesion of the slices to the slides. All washes and incubations were done in 0.1 M PB pH 7.4, containing 0.5% bovine serum albumin and 0.3% Triton X-100, which constituted the immunohistochemistry buffer (IB). Endogenous peroxidase was blocked for 15 min at room temperature in a solution of 2% hydrogen peroxide in IB. After three washes in IB, sections were incubated overnight at 4 °C with the primary antibody and then rinsed three times in IB and incubated for 2 h at room temperature with the secondary antibody.

In order to evaluate NeuN expression, a monoclonal antibody for NeuN was used as primary antibody, 1:50 dilution [mouse monoclonal IgG anti-NeuN (anti-*neuronal nuclei*; Millipore, USA, Ref: clone A60)] and a biotinylated rabbit anti-mouse IgG was employed as secondary antibody, 1:300 dilution (Millipore, USA). We also evaluated astroglial reaction by studying changes in GFAP, the main intermediate filament of astrocytes. It defines the astrocytic morphology, being possible to evaluate the astroglial reaction by studying changes in GFAP expression (Tagliaferro et al., 1997). We used a polyclonal antibody for GFAP as primary antibody, 1:2000 dilution [rabbit polyclonal IgG anti-GFAP (anti-glial fibrillary acidic protein; Dako, Denmark, Ref: Z0334)] and a biotinylated goat anti-rabbit IgG as secondary antibody, 1:300 dilution (Thermo Fisher Scientific, USA). For microglia, a polyclonal antibody for Iba-1 was used as primary antibody, 1:1000 dilution [rabbit polyclonal IgG anti-Iba-1 (anti-ionized calcium binding adaptor molecule 1; Wako, Japan, Ref: 019-19741)] and a biotinylated goat anti-rabbit IgG as secondary antibody, 1:300 dilution (Thermo Fisher Scientific, USA).

After incubations with the specific antibodies, sections were washed several times in IB and then incubated for 90 min at room temperature with avidin-biotin peroxidase complex (Vectastain ABC kit, Vector Laboratories, UK, 1:300 dilution). The reaction product was revealed by incubating the sections with 0.5 mg/mL of 3,3-diaminobenzidine (0.5 mg/mL; Sigma-Aldrich, Spain) and 0.2% hydrogen peroxide in PBS. Then, sections were dehydrated and coverslipped with DePeX® mounting medium (Serva, Germany). For immunohistochemical evaluation of Iba-1 and GFAP in the hippocampus, slides were counterstained with cresyl violet (0.005%) before being dehydrated in order to highlight the molecular layer of dentate gyrus (DG), and the pyramidal cell layer of CA1 and CA3 areas as anatomical reference. Sections for evaluating the medial prefrontal cortex (mPFC), dorsal striatum (dSTR), and nucleus accumbens (NAc) the slides were not counterstained. These regions were identified by using the motor cortex and corpus callosum (mPFC and dSTR), and the anterior commissure (NAc) as anatomical references.

To check the specificity of the immunoreaction, we included control preparations (omitting the primary antibody) in each immunostaining batch to rule out unspecific biding. Immunostaining batches containing all experimental groups as well as the internal control were run together.

Slides immunostaining NeuN, GFAP or Iba-1 were observed under light microscopy (Zeiss Axioplan Microscope, Germany). The microscope

Please cite this article as: Gomes, F.V., et al., Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol, Schizophr. Res. (2015), http://dx.doi.org/10.1016/j.schres.2015.01.015

had a camera attached (Zeiss Axioplan, Germany), through which the images were captured to be processed using Axiovision 40V 4.1 (Carl Zeiss vision, Germany). All lighting conditions and magnifications were kept constant during the capture process.

2.4.5.3. Quantitative evaluation. All immunostaining data related to NeuN, GFAP and Iba-1-positive cells were counted at 20× magnification by three observers unaware of the experimental groups. The following brain structures were evaluated: prelimbic and infralimbic portions of the mPFC, dSTR, NAc core and shell and dorsal hippocampus (DG, CA1 and CA3). Neuroanatomical sites were identified with the help of the Paxinos and Franklin mouse brain atlas (Paxinos and Franklin, 2008). The anterior–posterior (AP) localization from bregma of the analyzed regions was as follow: mPFC (AP: 1.98–1.70 mm); dSTR and NAc core and shell (AP: 1.54–0.98 mm); dorsal hippocampus (AP: −1.82 to −2.30 mm). For each brain structure, 3–4 tissue sections from each animal were analyzed. Since the structures evaluated are bilateral, both hemispheres were studied in each brain and the mean value between them was calculated. Immunoreactive cells were counted using ImageJ software (Research Services Branch — NIH, USA). Specific information about the quantification of NeuN, GFAP and Iba-1-positive cells is described below:

NeuN: All stained cells in a whole area were recorded. The sizes of the counting areas were 0.290 mm^2 for the mPFC (0.145 mm^2 for the prelimbic and 0.145 mm^2 for the infralimbic portion of the mPFC), 0.145 mm^2 for the dSTR and NAc core and shell. Results were expressed as the number of positive cells/0.1 mm^2. However, due to the high density and the proximity of the cells in the cellular layers of hippocampus, we performed the analysis by optical densitometry using specific ROIs for each portion (DG — upper, outer and inner blade; CA1 and CA3). Values were expressed as the percentage of optical density compared to control group.

GFAP: All immunoreactive cells in a whole area were recorded. The sizes of the counting areas were 0.290 mm^2 for the mPFC (0.145 mm^2 for the prelimbic and 0.145 mm^2 for the infralimbic portion of the mPFC), 0.145 mm^2 for the dSTR and NAc core and shell, 0.072 mm^2 for the DG (hilus); 0.055 mm^2 for the CA1 (stratum radiatum) and 0.071 mm^2 for the CA3 (stratum radiatum). Results were expressed as the number of positive cells/0.1 mm^2.

Iba-1: All immunoreactive cells in a selected area were recorded. The sizes of the counting areas were 0.092 mm^2 for the mPFC (0.046 mm^2 for the prelimbic and 0.046 mm^2 for the infralimbic portion of the mPFC), 0.046 mm^2 for the dSTR and NAc core and shell, 0.072 mm^2 for the DG (hilus); 0.055 mm^2 for the CA1 (stratum radiatum) and 0.071 mm^2 for the CA3 (stratum radiatum). Results were expressed as the number of Iba-1-positive cells/0.1 mm^2. Considering Iba-1 labels both activated and resting microglia, a quantitative assessment of microglia morphology was also conducted. Cells were classified in resting or reactive microglia according to established morphological criteria (Diz-Chaves et al., 2012; Lopez-Rodriguez et al., 2015): cells with few cellular processes (2 or less) or cells showing 3–5 short branches were considered as resting microglia; cells with numerous (>5) and longer cell processes, large soma and retracted and thicker processes, and cells with amoeboid cell body, numerous short processes, and intense Iba-1 immunostaining were considered as reactive microglia. Fig. 2 shows some examples of resting and reactive microglia.

2.5. Statistical analysis

In the NOR test, acquisition and retention trial data were analyzed by repeated measures ANOVA with treatment as the main independent factor and the object (acquisition trail: familiar object placed in the left *vs.* right side of the arena; retention trial: novel *vs.* similar object) as the repeated factor. When a significant object effect was observed specific Student t-tests were performed for each group. The discrimination index and the time of social interaction were analyzed by one-way ANOVA followed by the Student-Newman-Keuls (S-N-K) *post-hoc* test. The number of NeuN, GFAP, Iba-1-positive cells, and the percentage of Iba-1-positive cells with a reactive phenotype were analyzed by two-way ANOVA, using the first (vehicle, CBD or clozapine) and the second (MK-801 or saline) treatments as main factors, followed by the S-N-K *post-hoc* test. Both the prelimbic and infralimbic mPFC portions were quantified. However, as the results were similar, a mean value between these two regions was used to represent changes in the mPFC. Moreover, because there was no difference in both behavioral and immunohistochemical data between animals that received 2% Tween 80 in saline (used to dissolve CBD) + saline and saline supplemented with 30 μL of 0.1 M hydrochloric acid (used to dissolve clozapine) + saline, they were joined together in a control group (vehicle + saline). All data were represented as mean ± SEM. Results of statistical tests with $P < 0.05$ were considered significant.

3. Results

3.1. CBD and clozapine effects on behavioral changes induced by repeated MK-801

Repeated MK-801 treatment impaired social interaction ($F_{6,49} = 5.21$, $P < 0.0001$, one-way ANOVA followed by S-N-K *post-hoc* test; $P < 0.05$ *vs.* vehicle + saline group, Fig. 3). CBD, at the dose of 60 mg/kg, and clozapine reversed the impaired social interaction induced by MK-801 treatment (S-N-K *post-hoc* test; $P < 0.05$ *vs.* vehicle + MK-801 group, Fig. 3). This change was also attenuated by CBD 30 mg/kg + MK-801 (S-N-K *post-hoc* test; $P > 0.05$ *vs.* vehicle + saline group).

In the NOR test, no significant differences in time spent exploring the two identical objects were observed among groups in the acquisition trial ($F_{1,49} = 1.66$, $P > 0.05$, repeated measures ANOVA; Fig. 4A). On the other hand, the time spent exploring novel *vs.* familiar object was significantly different among groups in the retention trial ($F_{1,49} = 117.05$, $P < 0.0001$, repeated measures ANOVA; Fig. 4B). All groups, except vehicle + MK-801-treated animals, explored the novel object significantly longer than the familiar object ($P < 0.05$, Student t-test; Fig. 4B), indicating that the ability to discriminate novel and familiar objects was abolished by repeated MK-801 treatment. The MK-801-induced deficit was also indicated by a decreased discrimination index to the novel object ($F_{6,49} = 4.46$, $P < 0.001$, one-way ANOVA followed by S-N-K *post-hoc* test; $P < 0.05$ *vs.* vehicle + saline group, Fig. 4C). CBD at the dose of 60 mg/kg and clozapine prevented the MK-801-induced deficit (S-N-K *post-hoc* test; $P < 0.05$ *vs.* vehicle + MK-801 group, Fig. 4C). This change was also attenuated in mice treated with

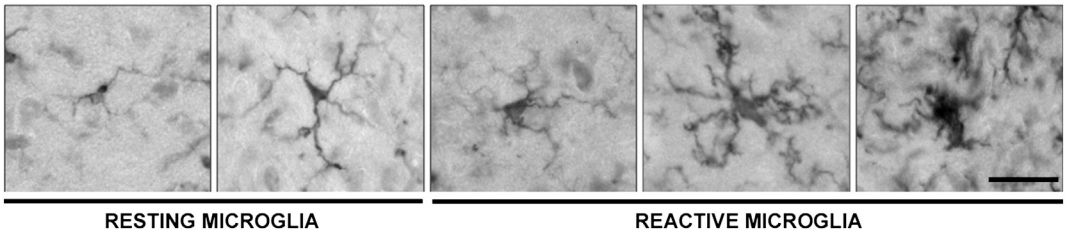

Fig. 2. Microglia cells immunostained with Iba-1 and classified according to morphological aspects in resting or reactive microglia. Images at 40× objective, bar = 25 μm.

Please cite this article as: Gomes, F.V., et al., Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol, Schizophr. Res. (2015), http://dx.doi.org/10.1016/j.schres.2015.01.015

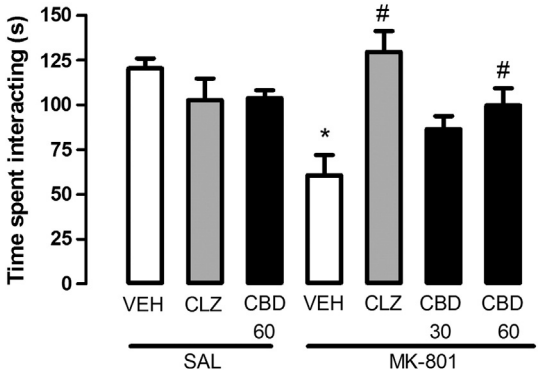

Fig. 3. CBD (30 and 60 mg/kg) prevented the decrease in social interaction time induced by repeated treatment with MK-801 (1 mg/kg) for 28 days. Similar to CBD, clozapine (CLZ; 1 mg/kg) also prevented MK-801-induced social withdraw (n = 7–9/group). Data are presented as the mean ± SEM. *P < 0.05 *vs.* VEH + SAL group, #P < 0.05 *vs.* VEH + MK-801 group; one-way ANOVA followed by S-N-K *post-hoc* test.

CBD 30 mg/kg + MK-801 (S-N-K *post-hoc* test; P > 0.05 *vs.* vehicle + saline group). Neither CBD nor clozapine induced any effect *per se.*

These treatments did not induce any change in the EPM and open field test (Supplementary Table 1), indicating that the observed effects in the social interaction and NOR tests were not due to anxiety-related behaviors or changes in locomotor activity. Additionally, no difference in body weight was found among the groups along the experiments (data not shown).

3.2. Changes in NeuN, GFAP and Iba-1 expression in specific brain regions induced by the repeated MK-801, CBD or clozapine treatment

NeuN: No change in the number of NeuN-positive cells was observed in any brain structure evaluated (Supplementary Table 2).

GFAP: Quantification of GFAP-positive cells in the mPFC revealed no significant effect of the first treatment (vehicle, clozapine or CBD; $F_{2,33} = 1.39$, P > 0.05). However, there were a significant effect of the second treatment (saline or MK-801; $F_{1,33} = 17.83$, P = 0.0001) and an interaction between the treatments ($F_{2,33} = 4.78$, P = 0.015). The number of GFAP-positive cells increased in the mPFC of vehicle + MK-801-treated mice compared to controls (S-N-K *post-hoc* test, P < 0.05 *vs.* vehicle + saline group, Fig. 5). This change was not observed in mice treated with CBD 60 mg/kg + MK-801 (S-N-K *post-hoc* test; P > 0.05 *vs.* vehicle + saline group). Moreover, clozapine was more effective in attenuating the MK-801-induced changes (S-N-K *post-hoc* test; P < 0.05 *vs.* vehicle + MK-801 group, Fig. 5). Neither CBD (60 mg/kg) nor clozapine affected GFAP expression in the mPFC *per se* (S-N-K *post-hoc* test, P > 0.05 *vs.* vehicle + saline group).

No change in GFAP expression was observed in dSTR, NAc core and shell, DG, CA1, and CA3 (Supplementary Table 3).

Iba-1: No change in the number of Iba-1-positive cells was observed in any brain structure evaluated (Supplementary Table 4). However, the evaluation of the morphology of Iba-1 immunoreactive microglia indicated a significant interaction between the first (vehicle, CBD or clozapine) *vs.* second treatment (MK-801 or saline) in the mPFC, DG and CA1 (mPFC: $F_{2,28} = 12.83$, P < 0.0001; dSTR: $F_{2,27} = 0.43$, P > 0.05; NAc core: $F_{2,26} = 0.82$, P > 0.05; NAc shell: $F_{2,27} = 1.50$, P > 0.05; DG: $F_{2,26} = 6.12$, P < 0.01; CA1: $F_{2,25} = 10.22$, P < 0.001; CA3: $F_{2,26} = 1.28$, P > 0.05). It was observed a decrease in the percentage of resting microglia and, consequently, an increase of reactive microglia in the mPFC, DG and CA1 after repeated MK-801 treatment (Fig. 6 and Supplementary Table 4). These changes were attenuated by CBD and clozapine. Neither CBD nor clozapine induced any effect *per se*.

No change in the percentage of reactive microglia was observed in the dSTR, NAc core and shell, and CA3 regions (Supplementary Table 4).

4. Discussion

The present study shows that repeated administration of the NMDA receptor antagonist MK-801 for 28 days impaired social interaction and NOR tests performed 1 and 2 days after the end of the treatment, respectively. These results are consistent with those showing a PPI disruption induced by a similar treatment schedule with MK-801 (Gomes et al., 2015). Additionally, no change was observed in the EPM and open field tests. Lacks of changes in open field activity following repeated treatment with NMDA receptor antagonists have already been reported (Rujescu et al., 2006). Together, these findings indicate that MK-801-induced behavioral disruptions observed in social interaction and NOR tests were not due to anxiety-related behaviors or changes in locomotor activity.

Besides behavioral disruption, repeated MK-801 treatment also increased the number of GFAP-positive astrocytes in the mPFC and the percentage of Iba-1-positive microglia cells with a reactive phenotype in the mPFC, DG and CA1 without changing the number of Iba-1-positive cells. No change in the number of NeuN-positive cells was observed. MK-801-induced behavioral changes were not observed in mice treated with CBD or clozapine.

The dose of 60 mg/kg of CBD was more effective in attenuating the impairment social interaction and NOR impairment induced by MK-801 treatment. Consequently, only the effects of this dose on the MK-801-induced glial changes were investigated. Similar to the behavioral changes, CBD and clozapine attenuated the changes in the expression of astrocyte and microglia markers induced by MK-801 treatment. However, clozapine was more effective in mitigating the increase in the number of GFAP-positive cells in the mPFC induced by MK-801.

Although repeated treatment with NMDA receptor antagonists can also induce behavioral responses associated with positive-like symptoms

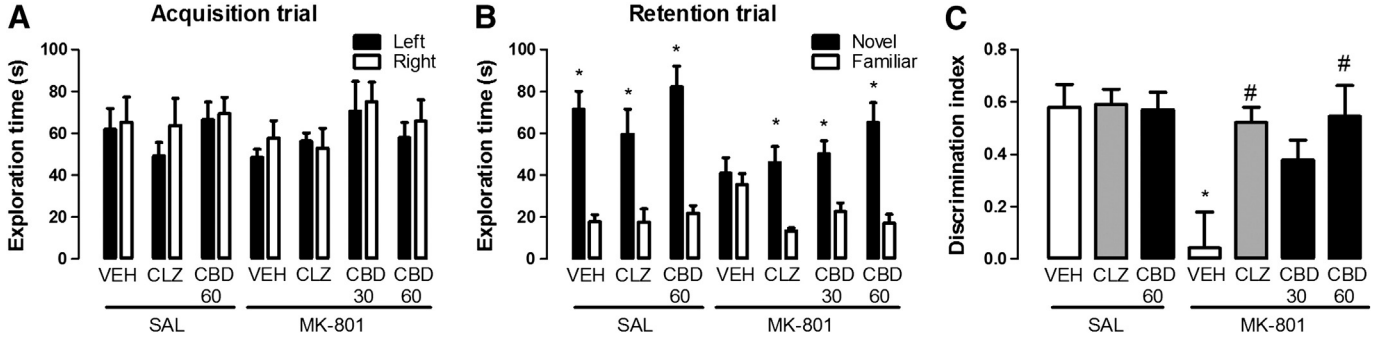

Fig. 4. Effects of chronic MK-801 (1 mg/kg), clozapine (CLZ; 1 mg/kg) and CBD (60 mg/kg) treatment in the NOR test (n = 7–9/group). Data are presented as the mean ± SEM. (A) Exploration time of two identical objects in the acquisition trial. (B) Exploration time of a novel and a familiar object in the retention trial. *P < 0.05, significant difference in time exploring the novel compared to the familiar object; repeated measures ANOVA followed by Student t-test. (C) Effects in the discrimination index. *P < 0.05 *vs.* VEH + SAL group, #P < 0.05 *vs.* VEH + MK-801 group; one-way ANOVA followed by S-N-K *post-hoc* test.

Please cite this article as: Gomes, F.V., et al., Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol, Schizophr. Res. (2015), http://dx.doi.org/10.1016/j.schres.2015.01.015

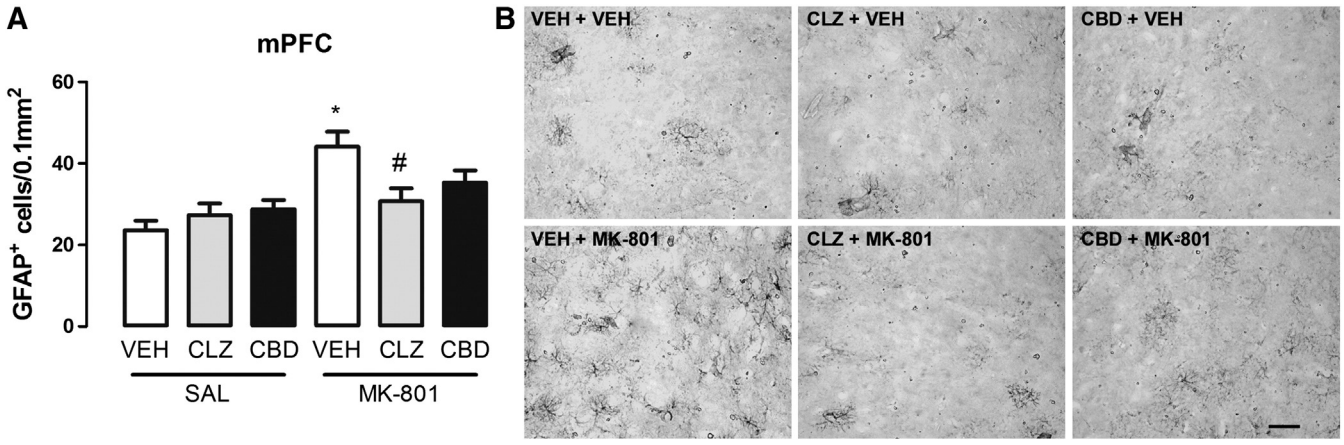

Fig. 5. Effects of chronic MK-801 (1 mg/kg), clozapine (CLZ; 1 mg/kg) and CBD (60 mg/kg) treatment on GFAP protein expression in the mice mPFC (n = 6–7/group). (A) MK-801 induced an increase in the number of GFAP-positive cells in the mPFC. CBD and clozapine attenuated GFAP increase in the mPFC. Data are presented as the mean ± SEM. $^{*}P < 0.05$ *vs.* VEH + SAL group, $^{\#}P < 0.05$ *vs.* VEH + MK-801 group; two-way ANOVA followed by S-N-K *post-hoc* test. (B) Photomicrographs of GFAP-like immunoreactivity in the mPFC (20X; bar = 50 μm).

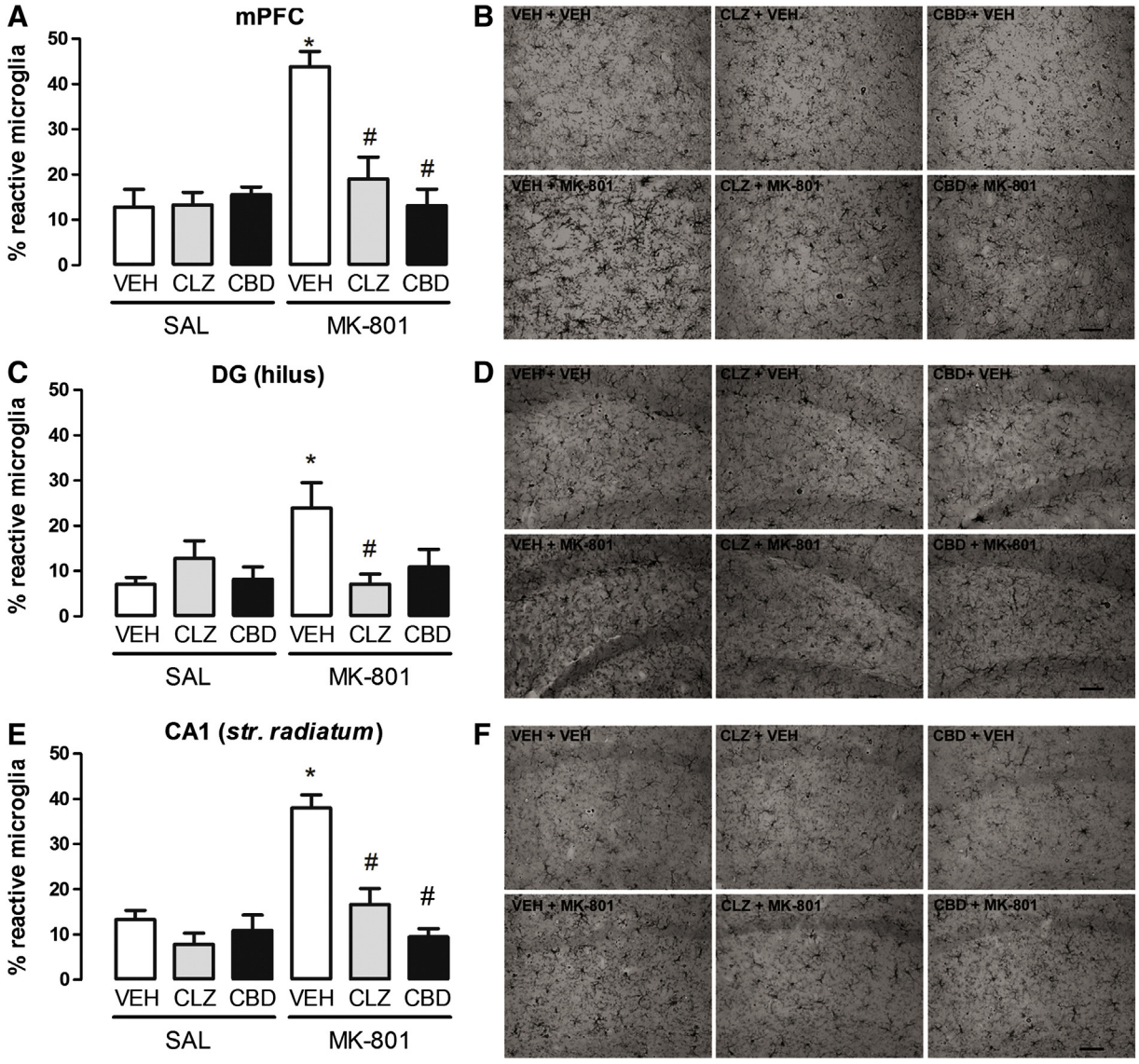

Fig. 6. Effects of chronic MK-801 (1 mg/kg), clozapine (CLZ; 1 mg/kg) and CBD (60 mg/kg) treatment on microglia reactivity in the mPFC (A–B), DG (C–D) and CA1 (n = 4–7/group) (E–F). MK-801 increased the percentage of reactive microglia in these structures. These changes were attenuated by clozapine and CBD. (A, C and E) Histograms represent the mean ± SEM. $^{*}P < 0.05$ *vs.* VEH + SAL group, $^{\#}P < 0.05$ *vs.* VEH + MK-801 group; two-way ANOVA followed by S-N-K *post-hoc* test. (B, D and F) Photomicrographs of Iba-1-immunoreactive microglia cells in the mPFC, DG and CA1, respectively (20X; bar = 50 μm).

Please cite this article as: Gomes, F.V., et al., Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol, Schizophr. Res. (2015), http://dx.doi.org/10.1016/j.schres.2015.01.015

of schizophrenia (Jentsch et al., 1998), the focus of the present study was on behavioral changes related to cognitive impairment and negative symptoms of schizophrenia. These changes, evaluated here by the NOR and social interaction tests, respectively, are also described after chronic administration of NMDA receptor antagonists in rodents (Rujescu et al., 2006; Mouri et al., 2007; Vigano et al., 2009; Guidali et al., 2011). Our results corroborated these findings and showed that these changes can be attenuated by repeated CBD and clozapine treatment.

In addition to behavioral changes, NMDA receptor antagonists induce neuronal damage and alter the activity of glial cells, mainly astrocytes and microglia, as well as the expression of their markers (Nakki et al., 1995, 1996). Although NMDA receptor antagonists can modulate neuronal birth and death, increasing neuronal proliferation and neurogenesis in the hippocampus (Genius et al., 2012), we did not observe any significant change in the expression of the neuronal marker NeuN, suggesting that MK-801 effects were not due to neuronal death or proliferation. Moreover, no change in the expression of Ki67, a nuclear protein that is associated with cellular proliferation, was observed (data not shown).

Astrocytes and microglia mediate inflammatory responses in the brain. Considering that alterations of these cells have been observed in schizophrenia patients, it has been postulated an involvement of neuroinflammatory processes in the pathogenesis of schizophrenia (Schnieder and Dwork, 2011; Monji et al., 2013). Indeed, recently Fillman et al. (2013) observed changes in inflammatory markers in the dorsolateral PFC of patients diagnosed with schizophrenia. GFAP, the major intermediate filament of astrocytes, has been the most used astrocytic marker in immunohistochemical studies to define their morphology and assess astroglial reaction (Tagliaferro et al., 1997). In the present study astrocytes were identified as cells with GFAP-positive cell bodies and processes that displayed classic astrocytic morphology. Astrocytes play an important role to keep normal neuronal function, participating in processes responsible for the uptake of neurotransmitters, the maintenance of ion homeostasis, neuronal migration during development, and synthesis, clearance and release of neuroactive amino acids (Bezzi et al., 1999; Sidoryk-Wegrzynowicz et al., 2011). In relation to microglia, we evaluated changes in the expression of Iba-1 protein, a calcium-binding protein specifically expressed in this cell type. These cells are the intrinsic immune cells of the CNS sensitive to insults (Aguzzi et al., 2013). In the healthy CNS, microglia has a distinctive ramified morphology with a small, round soma, and numerous branching processes. Upon activation, these cells may adopt different phenotypes characterized by alterations of their shape with reduction of processes length towards an amoeboid structure in response to various stimuli (Wake et al., 2011). Depending on their stage of activation, microglial cells can produce several proinflammatory cytokines and cytotoxic molecules, which may lead to neuronal apoptosis (Graeber et al., 2011). While microglia activation is a necessary and beneficial immune response to protect the brain tissue from damage, its excessive activation has been linked to the development of numerous diseases (Graeber et al., 2011; Aguzzi et al., 2013). Therefore, inhibition of these processes may result in neuroprotective effects.

Evidence suggests that neuroinflammatory changes observed in schizophrenia involve abnormal astrocyte functions (Rothermundt et al., 2009; Catts et al., 2014). However, as observed to several other alterations in the brain of schizophrenia patients, available data on the expression of astrocytic markers are conflicting, ranging from absence of alteration (Hoistad et al., 2013) to decreased (Rajkowska et al., 2002) or increased expression (Feresten et al., 2013). We observed that MK-801 treatment increased GFAP expression in the mPFC. The differences in the number of GFAP-positive cells induced by MK-801 may reflect developmental effects on the differentiation and proliferation of astrocytes or the activation of formerly quiescent astrocytes rather than from proliferation. An increased expression of GFAP and the number of GFAP-immunoreactive astrocytes are markers of astrogliosis. However, one of the possible molecular changes in reactive astrocytes is an upregulation of GFAP expression (Sofroniew and Vinters, 2010). This could make possible to detect astrocytes that would otherwise remain under the level of detection by immunohistochemistry in control mice. Therefore, part of the differences in the number of GFAP-positive cells found in the present study might reflect modifications in astrocytes reactivity, rather than their absolute number, in response to MK-801 treatment.

Another important finding of the present study was the increased percentage of Iba-1-positive microglia cells with a reactive phenotype in the mPFC, DG and CA1 regions of MK-801 treated mice. This effect was also attenuated by CBD and clozapine. These results are consistent with previous studies showing increased microglial activation after antagonism of NMDA receptors (Nakki et al., 1995, 1996; Arif et al., 2007). Similar to our results, Ribeiro et al. (2013) showed that repeated clozapine administration reversed cognitive deficits and progressive microglial activation in the hippocampus and PFC in a rodent model of schizophrenia based on neurodevelopmental disruption. Interestingly, it has recently emerged that microglia may be important to mediate or modulate inflammatory processes in the brain of schizophrenia patients. Although some studies have yielded conflicting evidence, activation and increased cellular density of microglia are shown in *postmortem* brains of schizophrenia patients (Radewicz et al., 2000). Additionally, positron emission tomography studies using [^{11}C](R)-PK11195 as an *in vivo* marker of activated microglia have indicated increased microglial activation in the brain of a subgroup of schizophrenia patients (van Berckel et al., 2008; Doorduin et al., 2009). Although it is unclear how changes in microglia activity induce abnormalities that eventually lead to schizophrenia, there seems to be an association between microglial activation and negative symptoms and/or cognitive deficits (Levkovitz et al., 2010; Ribeiro-Santos et al., 2014). Thus, the protective effects of CBD and clozapine on social interaction and NOR impairments induced by MK-801 could be related to decreased microglial activation.

In agreement with this proposition, minocycline, a potent inhibitor of microglial activation, improved negative symptoms and cognitive function as an add-on treatment in schizophrenia patients (Levkovitz et al., 2010; Chaudhry et al., 2012). Moreover, anti-inflammatory drugs, such as celecoxib and aspirin, given as an adjunct therapy to antipsychotic treatment, resulted in better outcomes regarding negative symptoms than antipsychotic treatment alone (Laan et al., 2010; Muller et al., 2010). Additionally, *in vitro* studies have indicated anti-inflammatory effects of atypical antipsychotics via the inhibition of microglial activation (Bian et al., 2008; Hu et al., 2012). In this context, CBD can also induce anti-inflammatory and neuroprotective effects. Indeed, in a mouse model of Alzheimer's disease-related neuroinflammation, CBD attenuated the expression of several glial pro-inflammatory proteins, including GFAP, inducible nitric oxide synthase (iNOS) and interleukin 1β (Esposito et al., 2007), which are major contributors of the neuroinflammatory process. Moreover, the memory restoring properties of CBD in an animal model of Alzheimer's disease were linked to a reduction in microglial activation and pro-inflammatory cytokines (Martim-Moreno et al., 2011). Additionally, similar to our results, CBD ameliorates symptomatology of multiple scleroses, brain ischemia and peripheral nerve axotomy in animal models by attenuating microglial activation and astrogliosis (Mecha et al., 2013; Perez et al., 2013; Schiavon et al., 2014).

Other possible explanation of the enhanced number of GFAP-positive cells in the mPFC induced by MK-801 is that the antagonism of NMDA receptors by this drug decreases the expression of parvalbumin (PV), a calcium binding protein expressed in a subclass of GABAergic interneurons in the mPFC (Gomes et al., 2015). This could lead to GABAergic interneuron dysfunction and, consequently, to high levels of glutamate via a disinhibition of glutamate release from pyramidal neurons targeted by those interneurons (Nakazawa et al., 2012). This change has been suggested to be directly involved in cognitive disruption (Gonzalez-Burgos and Lewis, 2012) and has been prevented by repeated treatment with

Please cite this article as: Gomes, F.V., et al., Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol, Schizophr. Res. (2015), http://dx.doi.org/10.1016/j.schres.2015.01.015

CBD or clozapine (Gomes et al., 2015). Since astrocytes have a key role in glutamate homeostasis (Bezzi et al., 1999), an increase in the number of these cells in the mPFC may be a compensatory mechanism in an attempt to normalize the levels of glutamate in this structure. Therefore, CBD and clozapine effects on mitigating the increased GFAP expression induced by MK-801 could be indirect, due to an attenuation of GABAergic interneurons dysfunction in the mPFC induced by MK-801. This possibility needs to be further investigated.

The glial changes observed in the present study could also be related to the neurotoxicity induced by MK-801 (Olney et al., 1989; Wozniak et al., 1996). If it is true, we may assume that CBD and clozapine effects are also associated to protective effects against MK-801-induced neurotoxicity. However, in the NeuN immunostaining of vehicle + MK-801-treated mice no marked neurotoxicity-like signs, such as decreased number of NeuN-positive cells, vacuolization and nuclear fragmentation, were seen.

While a microglial activation induced by repeated MK-801 treatment was observed in the mPFC, dentate gyrus, and CA1, an astroglial reaction, indicated by the increased number of GFAP-positive cells, was only seen in the mPFC. The meaning of these differences is not clear. It could be related to our previous data showing a decrease in PV expression induced by MK-801 in the mPFC, but not in the hippocampus (Gomes et al., 2015). As aforementioned, a decrease in PV expression has been associated to disturbances of the glutamatergic and GABAergic neurotransmission. Additionally, early microglial activation has been observed to precede the phenotypic changes in astrocytes (Tilleux and Hermans, 2007). However, given the heterogeneity of astrocytes in the brain, circuitry- and astrocyte type-specific manipulations are needed to get a better understanding of the role of these cells in neuropsychiatric disorders (Xia et al., in press). For example, Oberheim et al. (2012) observed that increases in Ca^{2+} were induced in cortical astrocytes by glutamate and norepinephrine, while hippocampal astrocytes show Ca^{2+} responses to ATP, GABA, acetylcholine, and endocannabinoids, suggesting that various neurotransmitters differentially affected astrocytes in the cortex and hippocampus. Additionally, differences between brain regions were also observed after the exposure to other psychotomimetic drugs such as cocaine. For example, while astrogliosis (increased number of GFAP-positive cells) was observed in the striatum and hippocampus after acute cocaine exposure, microgliosis (increased number of Iba-1-positive cells) was observed only in the hippocampus (Blanco-Calvo et al., 2014). Furthermore, there are no direct projections from the mPFC to the hippocampus in rodents (Verwer et al., 1997; Hoover and Vertes, 2007) making it unlikely that either similarities or differences in changes induced by MK-801 treatment are due to direct interactions between the regions. They may involve, instead, functional or structural differences between neurons present in these two regions.

In conclusion, the present results indicate that repeated treatment with CBD or the atypical antipsychotic clozapine attenuates or reverses the schizophrenia-like behavioral disruption and changes in the expression of astrocytic and microglial markers observed after chronic administration of the NMDA receptor antagonist MK-801. These data reinforce the proposal that CBD may induce antipsychotic-like effects. Although the possible mechanism of action of these effects is still unknown, it may involve CBD anti-inflammatory and neuroprotective properties. Furthermore, our data support the view that inhibition of microglial activation may improve schizophrenia symptoms. The mechanisms responsible for these effects need to be further investigated.

Role of funding source

This research was supported by grants from FAPESP (FSG and EDB: 2012/17626-7; FVG: 2010/17343-0; 2012/14144-1), CNPq (470311/2012-6;Brazil), and Instituto de Salud Carlos III, Redes temáticas de Investigación Cooperativa en salud, RD2012/0028/0021 GRUPO UCM 951579 (Spain). The funding sources had no involvement in the study design, in the acquisition, analysis, or interpretation of the data, in the writing of the report, and in the decision to submit the paper for publication.

Contributors

FVG designed the study, performed the experiments, and wrote the first draft of the manuscript. RL and MLG also performed the immunohistochemistry experiments and analyzed the data. EDB, MPV and FSG were involved in conception and design of the study. Statistical analyses were conducted by FVG and FSG. All authors were involved in manuscript development, critically reviewed the manuscript for important intellectual content, and approved the final version.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors thank José Carlos de Aguiar, Celia A. da Silva, Ángela A. Arrojo, and Lara Arnaldo for technical assistance. FVG has a FAPESP fellowship (2010/17343-0; 2012/14144-1). This research was supported by grants from FAPESP (FSG and EDB: 2012/17626-7), CNPq (470311/2012-6; Brazil), and Instituto de Salud Carlos III, Redes temáticas de Investigación Cooperativa en salud, RD2012/0028/0021 GRUPO UCM 951579 (Spain).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.schres.2015.01.015.

References

Aguzzi, A., Barres, B.A., Bennett, M.L., 2013. Microglia: scapegoat, saboteur, or something else? Science 339 (6116), 156–161.

Arif, M., Chikuma, T., Ahmed, M.M., Yoshida, S., Kato, T., 2007. Suppressive effect of clozapine but not haloperidol on the increases of neuropeptide-degrading enzymes and glial cells in MK-801-treated rat brain regions. Neurosci. Res. 57 (2), 248–258.

Bertaina-Anglade, V., Enjuanes, E., Morillon, D., Drieu la Rochelle, C., 2006. The object recognition task in rats and mice: a simple and rapid model in safety pharmacology to detect amnesic properties of a new chemical entity. J. Pharmacol. Toxicol. Methods 54 (2), 99–105.

Bezzi, P., Vesce, S., Panzarasa, P., Volterra, A., 1999. Astrocytes as active participants of glutamatergic function and regulators of its homeostasis. Adv. Exp. Med. Biol. 468, 69–80.

Bian, Q., Kato, T., Monji, A., Hashioka, S., Mizoguchi, Y., Horikawa, H., Kanba, S., 2008. The effect of atypical antipsychotics, perospirone, ziprasidone and quetiapine on microglial activation induced by interferon-gamma. Prog. Neuropsychopharmacol. Biol. Psychiatry 32 (1), 42–48.

Blanco-Calvo, E., Rivera, P., Arrabal, S., Vargas, A., Pavon, F.J., Serrano, A., Castilla-Ortega, E., Galeano, P., Rubio, L., Suarez, J., Fonseca, F.R., 2014. Pharmacological blockade of either cannabinoid CB_1 or CB_2 receptors prevents both cocaine-induced conditioned locomotion and cocaine-induced reduction of cell proliferation in the hippocampus of adult male rat. Front. Integr. Neurosci. 7, 106.

Campos, A.C., Moreira, F.A., Gomes, F.V., Del Bel, E.A., Guimaraes, F.S., 2012. Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. Philos. Trans. R. Soc. Lond. B Biol. Sci. 367 (1607), 3364–3378.

Carobrez, A.P., Bertoglio, L.J., 2005. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neurosci. Biobehav. Rev. 29 (8), 1193–1205.

Catts, V.S., Wong, J., Fillman, S.G., Fung, S.J., Shannon Weickert, C., 2014. Increased expression of astrocyte markers in schizophrenia: association with neuroinflammation. Aust. N. Z. J. Psychiatry 48 (8), 722–734.

Chaudhry, I.B., Hallak, J., Husain, N., Minhas, F., Stirling, J., Richardson, P., Dursun, S., Dunn, G., Deakin, J.W., 2012. Minocycline benefits negative symptoms in early schizophrenia: a randomised double-blind placebo-controlled clinical trial in patients on standard treatment. J. Psychopharmacol. 26 (9), 1185–1193.

Diz-Chaves, Y., Pernia, O., Carrero, P., Garcia-Segura, L.M., 2012. Prenatal stress causes alterations in the morphology of microglia and the inflammatory response of the hippocampus of adult female mice. J. Neuroinflammation 9, 71.

Doorduin, J., de Vries, E.F., Willemsen, A.T., de Groot, J.C., Dierckx, R.A., Klein, H.C., 2009. Neuroinflammation in schizophrenia-related psychosis: a PET study. J. Nucl. Med. 50 (11), 1801–1807.

Ellenbroek, B.A., Cools, A.R., 2000. Animal models for the negative symptoms of schizophrenia. Behav. Pharmacol. 11 (3–4), 223–233.

Elvevag, B., Goldberg, T.E., 2000. Cognitive impairment in schizophrenia is the core of the disorder. Crit. Rev. Neurobiol. 14 (1), 1–21.

Esposito, G., Scuderi, C., Savani, C., Steardo Jr., L., De Filippis, D., Cottone, P., Iuvone, T., Cuomo, V., Steardo, L., 2007. Cannabidiol in vivo blunts beta-amyloid induced neuroinflammation by suppressing IL-1beta and iNOS expression. Br. J. Pharmacol. 151, 1272–1279.

Feresten, A.H., Barakauskas, V., Ypsilanti, A., Barr, A.M., Beasley, C.L., 2013. Increased expression of glial fibrillary acidic protein in prefrontal cortex in psychotic illness. Schizophr. Res. 150 (1), 252–257.

Fillman, S.G., Cloonan, N., Catts, V.S., Miller, L.C., Wong, J., McCrossin, T., Cairns, M., Weickert, C.S., 2013. Increased inflammatory markers identified in the dorsolateral prefrontal cortex of individuals with schizophrenia. Mol. Psychiatry 18 (2), 206–214.

Genius, J., Benninghoff, J., Reuter, N., Braun, I., Giegling, I., Hartmann, A., Moller, H.J., Rujescu, D., 2012. Dysequilibrium of neuronal proliferation and apoptosis in a pharmacological animal model of psychosis. Methods 56 (4), 519–527.

Gomes, F.V., Issy, A.C., Ferreira, F.R., Viveros, M.P., Del Bel, E.A., Guimarães, F.S., 2015. Cannabidiol attenuates sensorimotor gating disruption and molecular changes induced by chronic antagonism of NMDA receptors in mice. Int. J. Neuropsychopharmacol. 18 (3), 1–10.

Gonzalez-Burgos, G., Lewis, D.A., 2012. NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr. Bull. 38 (5), 950–957.

Graeber, M.B., Li, W., Rodriguez, M.L., 2011. Role of microglia in CNS inflammation. FEBS Lett. 585 (23), 3798–3805.

Guidali, C., Vigano, D., Petrosino, S., Zamberletti, E., Realini, N., Binelli, G., Rubino, T., Di Marzo, V., Parolaro, D., 2011. Cannabinoid CB1 receptor antagonism prevents neurochemical and behavioural deficits induced by chronic phencyclidine. Int. J. Neuropsychopharmacol. 14 (1), 17–28.

Hanson, E., Healey, K., Wolf, D., Kohler, C., 2010. Assessment of pharmacotherapy for negative symptoms of schizophrenia. Curr. Psychiatry Rep. 12 (6), 563–571.

Hoistad, M., Heinsen, H., Wicinski, B., Schmitz, C., Hof, P.R., 2013. Stereological assessment of the dorsal anterior cingulate cortex in schizophrenia: absence of changes in neuronal and glial densities. Neuropathol. Appl. Neurobiol. 39 (4), 348–361.

Hoover, W.B., Vertes, R.P., 2007. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct. Funct. 212, 149–179.

Hu, X., Zhou, H., Zhang, D., Yang, S., Qian, L., Wu, H.M., Chen, P.S., Wilson, B., Gao, H.M., Lu, R.B., Hong, J.S., 2012. Clozapine protects dopaminergic neurons from inflammation-induced damage by inhibiting microglial overactivation. J. Neuroimmune Pharmacol. 7 (4), 187–201.

Jentsch, J.D., Roth, R.H., 1999. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20 (3), 201–225.

Jentsch, J.D., Taylor, J.R., Roth, R.H., 1998. Subchronic phencyclidine administration increases mesolimbic dopaminergic system responsivity and augments stress- and psychostimulant-induced hyperlocomotion. Neuropsychopharmacology 19, 105–113.

Krystal, J.H., Karper, L.P., Seibyl, J.P., Freeman, G.K., Delaney, R., Bremner, J.D., Heninger, G.R., Bowers Jr., M.B., Charney, D.S., 1994. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch. Gen. Psychiatry 51 (3), 199–214.

Krystal, J.H., Perry Jr., E.B., Gueorguieva, R., Belger, A., Madonick, S.H., Abi-Dargham, A., Cooper, T.B., Macdougall, L., Abi-Saab, W., D'Souza, D.C., 2005. Comparative and interactive human psychopharmacologic effects of ketamine and amphetamine: implications for glutamatergic and dopaminergic model psychoses and cognitive function. Arch. Gen. Psychiatry 62 (9), 985–994.

Laan, W., Grobbee, D.E., Selten, J.P., Heijnen, C.J., Kahn, R.S., Burger, H., 2010. Adjuvant aspirin therapy reduces symptoms of schizophrenia spectrum disorders: results from a randomized, double-blind, placebo-controlled trial. J. Clin. Psychiatry 71 (5), 520–527.

Lesh, T.A., Niendam, T.A., Minzenberg, M.J., Carter, C.S., 2011. Cognitive control deficits in schizophrenia: mechanisms and meaning. Neuropsychopharmacology 36 (1), 316–338.

Levkovitz, Y., Mendlovich, S., Riwkes, S., Braw, Y., Levkovitch-Verbin, H., Gal, G., Fennig, S., Treves, I., Kron, S., 2010. A double-blind, randomized study of minocycline for the treatment of negative and cognitive symptoms in early-phase schizophrenia. J. Clin. Psychiatry 71 (2), 138–149.

Leweke, F.M., Piomelli, D., Pahlisch, F., Muhl, D., Gerth, C.W., Hoyer, C., Klosterkotter, J., Hellmich, M., Koethe, D., 2012. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl. Psychiatry 2, e94.

Lopez-Rodriguez, A.B., Siopi, E., Finn, D.P., Marchand-Leroux, C., Garcia-Segura, L.M., Jafarian-Tehrani, M., Viveros, M.P., 2015. CB1 and CB2 cannabinoid receptor antagonists prevent minocycline-induced neuroprotection following traumatic brain injury in mice. Cereb. Cortex 25 (1), 35–45.

Martim-Moreno, A.M., Reigada, D., Ramirez, B.G., Mechoulam, R., Innamorato, N., Cuadrado, A., Ceballos, M.L., 2011. Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. Mol. Pharmacol. 79, 964–973.

Mecha, M., Feliu, A., Inigo, P.M., Mestre, L., Carrillo-Salinas, F.J., Guaza, C., 2013. Cannabidiol provides long-lasting protection against the deleterious effects of inflammation in a viral model of multiple sclerosis: a role for A2A receptors. Neurobiol. Dis. 59, 141–150.

Monji, A., Kato, T.A., Mizoguchi, Y., Horikawa, H., Seki, Y., Kasai, M., Yamauchi, Y., Yamada, S., Kanba, S., 2013. Neuroinflammation in schizophrenia especially focused on the role of microglia. Prog. Neuropsychopharmacol. Biol. Psychiatry 42, 115–121.

Mouri, A., Noda, Y., Enomoto, T., Nabeshima, T., 2007. Phencyclidine animal models of schizophrenia: approaches from abnormality of glutamatergic neurotransmission and neurodevelopment. Neurochem. Int. 51 (2–4), 173–184.

Muller, N., Krause, D., Dehning, S., Musil, R., Schennach-Wolff, R., Obermeier, M., Moller, H.J., Klauss, V., Schwarz, M.J., Riedel, M., 2010. Celecoxib treatment in an early stage of schizophrenia: results of a randomized, double-blind, placebo-controlled trial of celecoxib augmentation of amisulpride treatment. Schizophr. Res. 121 (1–3), 118–124.

Nakazawa, K., Zsiros, V., Jiang, Z., Nakao, K., Kolata, S., Zhang, S., Belforte, J.E., 2012. GABAergic interneuron origin of schizophrenia pathophysiology. Neuropharmacology 62 (3), 1574–1583.

Nakki, R., Koistinaho, J., Sharp, F.R., Sagar, S.M., 1995. Cerebellar toxicity of phencyclidine. J. Neurosci. 15 (3 Pt 2), 2097–2108.

Nakki, R., Nickolenko, J., Chang, J., Sagar, S.M., Sharp, F.R., 1996. Haloperidol prevents ketamine- and phencyclidine-induced HSP70 protein expression but not microglial activation. Exp. Neurol. 137 (2), 234–241.

Oberheim, N.A., Goldman, S.A., Nedergaard, M., 2012. Heterogeneity of astrocytic form and function. Methods Mol. Biol. 814, 23–45.

Olney, J.W., Labruyere, J., Price, M.T., 1989. Changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 244, 1360–1362.

Paxinos, G., Franklin, K.B.J., 2008. The Mouse Brain in Stereotaxic Coordinates. 3rd ed. Academic Press, New York.

Perez, M., Benitez, S.U., Cartarozzi, L.P., Del Bel, E., Guimaraes, F.S., Oliveira, A.L., 2013. Neuroprotection and reduction of glial reaction by cannabidiol treatment after sciatic nerve transection in neonatal rats. Eur. J. Neurosci. 38 (10), 3424–3434.

Radewicz, K., Garey, L.J., Gentleman, S.M., Reynolds, R., 2000. Increase in HLA-DR immunoreactive microglia in frontal and temporal cortex of chronic schizophrenics. J. Neuropathol. Exp. Neurol. 59 (2), 137–150.

Rajagopal, L., Massey, B.W., Huang, M., Oyamada, Y., Meltzer, H.Y., 2014. The novel object recognition test in rodents in relation to cognitive impairment in schizophrenia. Curr. Pharm. Des. 20 (31), 5104–5114.

Rajkowska, G., Miguel-Hidalgo, J.J., Makkos, Z., Meltzer, H., Overholser, J., Stockmeier, C., 2002. Layer-specific reductions in GFAP-reactive astroglia in the dorsolateral prefrontal cortex in schizophrenia. Schizophr. Res. 57 (2–3), 127–138.

Ribeiro, B.M., do Carmo, M.R., Freire, R.S., Rocha, N.F., Borella, V.C., de Menezes, A.T., Monte, A.S., Gomes, P.X., de Sousa, F.C., Vale, M.L., de Lucena, D.F., Gama, C.S., Macedo, D., 2013. Evidences for a progressive microglial activation and increase in iNOS expression in rats submitted to a neurodevelopmental model of schizophrenia: reversal by clozapine. Schizophr. Res. 151 (1–3), 12–19.

Ribeiro-Santos, R., Teixeira, A.L., Salgado, J.V., 2014. Evidence for an immune role on cognition in schizophrenia: a systematic review. Curr. Neuropharmacol. 12 (3), 273–280.

Rothermundt, M., Ahn, J.N., Jorgens, S., 2009. S100B in schizophrenia: an update. Gen. Physiol. Biophys. 28, F76–F81.

Rujescu, D., Bender, A., Keck, M., Hartmann, A.M., Ohl, F., Raeder, H., Giegling, I., Genius, J., McCarley, R.W., Moller, H.J., Grunze, H., 2006. A pharmacological model for psychosis based on N-methyl-D-aspartate receptor hypofunction: molecular, cellular, functional and behavioral abnormalities. Biol. Psychiatry 59 (8), 721–729.

Schiavon, A.P., Soares, L.M., Bonato, J.M., Milani, H., Guimaraes, F.S., Weffort de Oliveira, R.M., 2014. Protective effects of cannabidiol against hippocampal cell death and cognitive impairment induced by bilateral common carotid artery occlusion in mice. Neurotox. Res. 26 (4), 307–316.

Schnieder, T.P., Dwork, A.J., 2011. Searching for neuropathology: gliosis in schizophrenia. Biol. Psychiatry 69 (2), 134–139.

Sidoryk-Wegrzynowicz, M., Wegrzynowicz, M., Lee, E., Bowman, A.B., Aschner, M., 2011. Role of astrocytes in brain function and disease. Toxicol. Pathol. 39 (1), 115–123.

Sofroniew, M.V., Vinters, H.V., 2010. Astrocytes: biology and pathology. Acta Neuropathol. 119 (1), 7–35.

Tagliaferro, P., Ramos, A.J., Lopez, E.M., Pecci Saavedra, J., Brusco, A., 1997. Neural and astroglial effects of a chronic parachlorophenylalanine-induced serotonin synthesis inhibition. Mol. Chem. Neuropathol. 32 (1–3), 195–211.

Tilleux, S., Hermans, E., 2007. Neuroinflammation and regulation of glial glutamate uptake in neurological disorders. J. Neurosci. Res. 85, 2059–2070.

van Berckel, B.N., Bossong, M.G., Boellaard, R., Kloet, R., Schuitemaker, A., Caspers, E., Luurtsema, G., Windhorst, A.D., Cahn, W., Lammertsma, A.A., Kahn, R.S., 2008. Microglia activation in recent-onset schizophrenia: a quantitative (R)-[^{11}C]PK11195 positron emission tomography study. Biol. Psychiatry 64 (9), 820–822.

Verwer, R.W., Meijer, R.J., Van Uum, H.F., Witter, M.P., 1997. Collateral projections from the rat hippocampal formation to the lateral and medial prefrontal cortex. Hippocampus 7, 397–402.

Vigano, D., Guidali, C., Petrosino, S., Realini, N., Rubino, T., Di Marzo, V., Parolaro, D., 2009. Involvement of the endocannabinoid system in phencyclidine-induced cognitive deficits modelling schizophrenia. Int. J. Neuropsychopharmacol. 12 (5), 599–614.

Wake, H., Moorhouse, A.J., Nabekura, J., 2011. Functions of microglia in the central nervous system—beyond the immune response. Neuron Glia Biol. 7 (1), 47–53.

Wozniak, D.F., Brosnan-Watters, G., Nardi, A., McEwen, M., Corso, T.D., Olney, J.W., Fix, A.S., 1996. MK-801 neurotoxicity in male mice: histologic effects and chronic impairment in spatial learning. Brain Res. 707, 165–179.

Xia, M., Abazyan, S., Jouroukhin, Y., Pletnikov, M., 2015. Behavioral sequelae of astrocyte dysfunction: focus on animal models of schizophrenia. Schizophr. Res. http://dx.doi.org/10.1016/j.schres.2014.10.044 (in press).

Zuardi, A.W., Morais, S.L., Guimaraes, F.S., Mechoulam, R., 1995. Antipsychotic effect of cannabidiol. J. Clin. Psychiatry 56 (10), 485–486.

Zuardi, A.W., Hallak, J.E., Dursun, S.M., Morais, S.L., Sanches, R.F., Musty, R.E., Crippa, J.A., 2006. Cannabidiol monotherapy for treatment-resistant schizophrenia. J. Psychopharmacol. 20 (5), 683–686.

Please cite this article as: Gomes, F.V., et al., Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol, Schizophr. Res. (2015), http://dx.doi.org/10.1016/j.schres.2015.01.015