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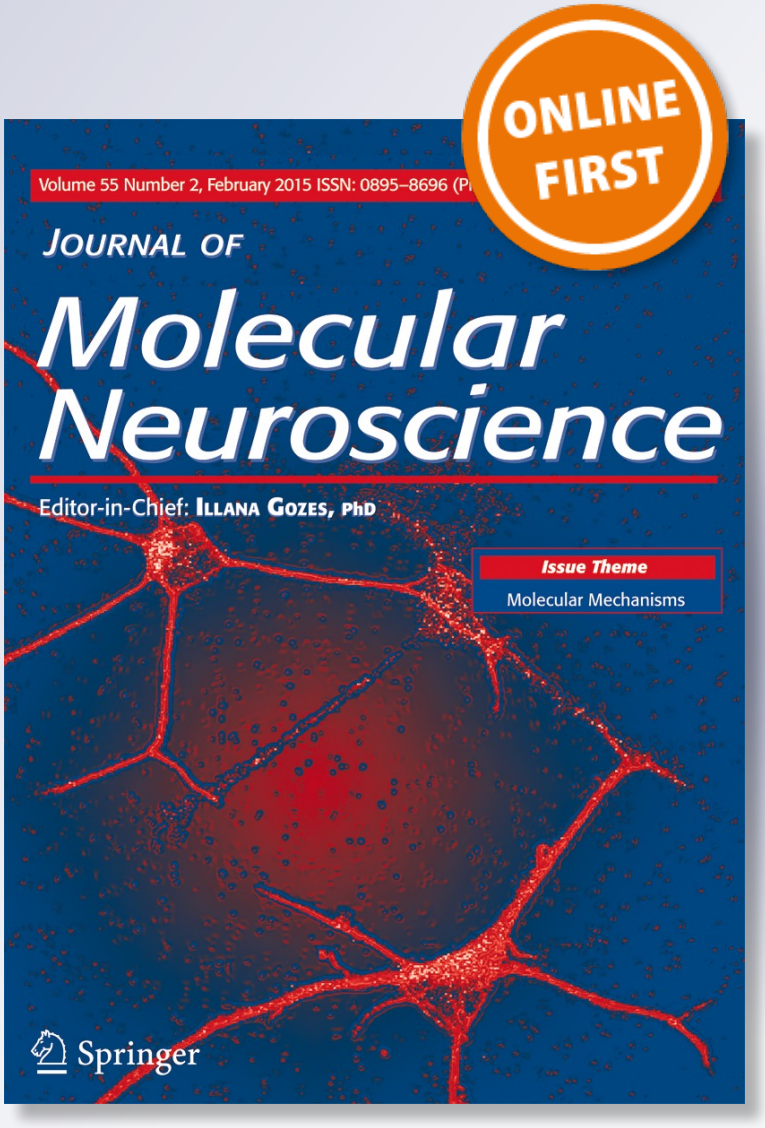
Journal of Molecular Neuroscience

ISSN 0895-8696

J Mol Neurosci
DOI 10.1007/s12031-015-0499-4



Springer

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Further Evidence for the Neuroplastic Role of Cannabinoids: A Study in Organotypic Hippocampal Slice Cultures

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Received: 28 October 2014 / Accepted: 15 January 2015

Abstract Endocannabinoid receptors CB1R and CB2R are present in the CNS and modulate synaptic activity. By using an in vitro model, two concentrations of CB1R agonist ACEA at 0.5 and 5 μM doses and CB1R antagonist AM251 at 1 and 10 μM doses were administered in organotypic slice cultures of mouse hippocampus, and their effects on neurons and glial cells were analyzed at different time points. Exposure to low concentrations of ACEA (0.5 μM) did not seem to affect tissue organization, neuronal morphology, or glial response. In contrast, at a higher concentration of ACEA, many neurons in the dentate gyrus exhibited strong caspase-3 immunoreactivity. After treatment with AM251, we observed an increase in caspase-3 immunoreactivity and a downregulation of CB1R expression. Results show that long-term hippocampal slice cultures respond to both CB1R activation and inactivation by changing neuronal protein expression patterns. In the present study, we demonstrate that CB1R agonist ACEA promotes alterations in the neuronal cytoskeleton as well as changes in CB1R expression in organotypic hippocampal slice cultures, and that CB1R antagonist AM251 promotes neuronal death and astroglial reaction.

Keywords Cannabinoids · Neuron · Neurotoxicity

Introduction

The endocannabinoid system consists of lipid mediators including endogenous ligands (anandamide and 2-arachidonoylglycerol), their target receptors (cannabinoid receptor type 1, CB1R, and cannabinoid receptor type 2, CB2R), associated synthetic and metabolic enzymes, and transport proteins (Ashton et al. 2008). The neural actions of endocannabinoids (eCBs) are mainly mediated by the activation of CB1R, which is highly expressed in brain regions including cerebral cortex, hippocampus, amygdala, basal ganglia outflow tracts, and cerebellum, thus playing relevant neuromodulatory roles in brain physiology. CB2R is mainly expressed by cells of the immune system, although its expression in neurons, astrocytes, and endothelial cells has also been reported (Onaivi et al. 2006; Brusco et al. 2008). Both the CB1R and CB2R are G-protein coupled receptors, which inhibit the accumulation of cyclic adenosine monophosphate (cAMP) within cells via their associated G-proteins Gi/o.

The CB1R activation has been demonstrated to decrease the release of a wide variety of neurotransmitters, including acetylcholine, GABA, glutamate, and others, at synapses into the central nervous system (CNS) and periphery (Mackie 2006). In turn, neuronal activity has been identified as a potent stimulus for the release of eCBs, which were found to act as retrograde messengers, regulating synaptic transmission through presynaptic CB1R. eCBs have also been characterized as triggers for short- and long-term plasticity at synapses throughout the brain, in agreement with the ubiquitous expression of CB1R (Heifets and Castillo 2009).

Glial cells express CB1R and respond to CB1R agonists by releasing glutamate and influencing synaptic transmission. eCBs may have opposite neuromodulatory effects, depressing synaptic transmission by directly acting on presynaptic CB1R or potentiating synaptic transmission by acting on astrocytic CB1R, which stimulates glutamate release from astrocytes (Navarrete and Araque 2010).

The characteristic distribution of CB1R in the CNS is linked to the most prominent behavioral effects of cannabis consumption. As it expresses high levels of cannabinoid

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Published online: 03 February 2015

receptors, the hippocampus has received much attention as a site of action for both endogenous and exogenous cannabinoids, especially considering their cognitive effects. The first autoradiographic studies found very high levels of CB1R in all subfields of the hippocampus as well as in the dentate gyrus (DG) (Herkenham et al. 1990 and Mackie 2005), whose molecular layer exhibits the highest expression levels and contains many CB1R immunoreactive fibers but very few labeled cell bodies (Katona et al. 1999). In addition, CB1R is known to be accumulated in the terminal portion of mossy cell axons (Uchigashima et al. 2011).

The eCB system modulates a wide range of physiological processes in mammals, including pain and inflammation, appetite and energy regulation, and learning and memory (Pacher et al. 2006). It has been shown that both endogenous and exogenous agonists produce neuroprotection in CNS injury models in vivo and in vitro through different mechanisms, e. g., preventing excitotoxicity by inhibiting glutamatergic neurotransmission, reducing calcium influx (Kim and Thayer 2000 and Gilbert et al. 2007), and inducing neurotrophin synthesis and transcription factors (van der Stelt and Di Marzo 2005). It has also been reported that chronic cannabinoid administration to rats causes distinct morphological changes in the hippocampus, indicating a neurotoxic action (Scallet 1991). In addition, great interest has been generated in the effects of cannabinoids on memory and cognition. Chronic exposure to Δ9-tetrahydrocannabinol (THC) or marijuana extracts have been proven to persistently alter the structure and function of the rat hippocampus, a paleocortical brain region involved in learning and memory processes in both rats and humans (Scallet 1991). Several of the well-known actions of cannabinoids have been considered involved in the inhibition of long-term potentiation and memory impairment.

This study tests the hypothesis that chronic cannabinoid agonist use results in morphological changes in the hippocampus. Although CB1R agonist has been proven to produce morphological alterations in the hippocampal dendrites (Tagliaferro et al. 2006), reports on morphological evidence of neuronal plasticity and synaptic modifications by synthetic cannabinoids remain scarce. In the present work, we have chosen synthetic cannabinoid arachidonyl-2-chloroethylamide (ACEA) because of its high potency and solubility, as compared with THC itself. ACEA, an analog of endogenous N-arachidonylethanolamine, binds to CB1R with very high affinity and possesses selectivity ratios for CB1R over CB2R of 325 and 2200, respectively (Hillard et al. 1999). The goal of the present work is thus to analyze the effects of CB1R agonist ACEA and CB1 antagonist 1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide (AM251) on neuronal survival, changes in the neuronal cytoskeleton measured by MAP2+ fibers, alterations in CB1R expression in organotypic hippocampal slice cultures and the associated glial response in each case.

Materials and Methods

Preparation of Organotypic Slice Cultures

Hippocampal slice cultures (HSCs) were prepared from 3- to 5-day-old C57 BL/6 mice (for details see Brinks et al. 2004; Humar et al. 2009 and Lacour et al. 2007). Briefly, brains were aseptically removed from the skulls and transferred into culture dishes containing cold buffer solution. Under binocular observation, hippocampi were dissected and cut into 400-μm slices perpendicular to the longitudinal axis by means of a tissue chopper. The slices were placed on translucent porous membranes (Millipore CM cell culture inserts) and were transferred into six-well plates filled with 1.2 ml culture medium supplemented with 2 mM glutamine at pH 7.3. HSC was maintained at 37 °C in an incubator in an atmosphere of humidified air and 5 % CO_2. CB1R agonist ACEA (0.5 or 5 μM) (Rubio Araiz et al. 2008) or CB1R antagonist AM251 (1 and 10 μM) (Goncalves et al. 2008) was later applied 1, 4, 6, or 8 days after the culture medium was changed. The drugs were dissolved in DMSO (final concentration, 5 μM) and stored at −20 °C.

Staining Procedures

HSC selected for immunofluorescence analysis was fixed at 6 or 12 days in vitro (DIV) with 4 % paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, for 2 h. After several rinses with PB, the Millipore membrane with the HSC was cut-off, mounted on an agar block and re-sliced into 50-μm sections with a vibratome. Free-floating sections were then incubated in PB containing 5 % normal goat serum and permeabilized with 0.1 % Triton X-100 in PB for 30 min. Primary antibodies mouse anti-NeuN (Chemicon; 1:1000), rabbit anti-caspase-3a (cell signaling; 1:500), chicken anti-MAP2 (Abcam; 1:10, 000), mouse anti-GFAP (Abcam, 1:500), rabbit anti-ionized calcium-binding adapter molecule 1 (Iba, 1 Wako; 1:1000), or rabbit anti-CB1R (Cayman; 1:5000) in phosphate-buffered saline (PBS) containing 1 % normal goat serum were applied and incubated at 4 °C overnight. After being washed with PBS, sections were incubated with a secondary antibody (Cy3-conjugated goat anti-rabbit immunoglobulin G [IgG] 1:800 and Alexa 488-conjugated goat anti-mouse IgG 1:200, respectively) for 2 h at room temperature in the dark. Sections were then washed with PBS followed by Hoechst nuclear staining. After being thoroughly rinsed in PB, slices were mounted onto gelatin-coated slides, embedded with immunomount and coverslipped.

Morphometric Analysis by Digital Image Processing

Tissue images were acquired on a Zeiss ApoTome or Confocal Microscope Olympus FV1000. In each tissue section, each

microscopic field was selected within the limits of anatomical area of interest to be morphometrically analyzed. The morphometric analysis was done in the DG.

IBA1+ and NeuN+ cells were quantified. For GFAP+ astrocytes, the cell area was evaluated by interactively measuring and determining each cell limit. In order to evaluate the CB1R+ and MAP2+ fibers, the total area of the immunolabeled fibers was related to the total area of the corresponding microscopic field (×20 primary magnification), thus rendering a relative area parameter.

Western Blot Analysis

On average, four individual slice culture sections were pooled and resuspended in Laemmli buffer, followed by ultrasonication. Protein extracts were size-fractionated by 12 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto polyvinylidene difluoride (Millipore) for Western blot analysis. The membrane was blocked in blocking solution and then incubated with either rabbit antibody directed against CB1R (Cayman; 1:5000), CB2R (Cayman; 1:1000), or a rabbit anti-actin (Sigma; 1:5000). After being washed, the blot was incubated with a 1:2000 dilution of a peroxidase-coupled donkey anti-rabbit polyclonal antiserum for 1 h at room temperature. Finally, bound enzymatic activity was detected by using the enhanced chemiluminescence system (ECL+) from Amersham.

Statistical Analysis

Individual experiments were composed of six to ten tissue sections for each treatment group. Reported values represent the mean±SD of experiments performed for each marker and each hippocampal area. Differences among the means of the different groups were statistically analyzed by one-way analysis of variance (ANOVA). A Dunnett comparison test was conducted following the significance/non-significance of the overall ANOVA. Statistical significance was set at $P<0.05$ and GraphPad Prism v5.00 software (GraphPad Software Inc.) was used. In order to simplify graphic presentation, values of the different groups are expressed as percentages of DMSO-treated values (100 %).

Results

CB1R Expression

Neuronal CB1R is localized in the presynaptic boutons, where it is activated by retrograde neurotransmission of eCBs (Pertwee 2005). CB1R-mediated signal transduction is associated with increased pro-survival gene expression and synaptic plasticity in presynaptic neurons (Howlett et al. 2002), and several studies have reported cannabinoid-mediated increases in CB1R messenger RNA (mRNA) and protein expression (Borner et al. 2007a; Mukhopadhyay et al. 2010; Proto et al. 2011).

To evaluate the expression of CB1R, we analyzed the area covered by CB1R+ fibers and studied the levels of expression by Western blot. At DIV6, results show an increase in CB1R expression at a low dose of ACEA (0.5 μM) and no changes at a high dose of ACEA (5 μM) or AM251 (1 and 10 μM) (Fig. 1). DIV12 assays showed different effects, as CB1R expression was higher at the high doses of ACEA, while the low dose of ACEA induced a decrease in CB1R expression. As complementary analysis, CB2R expression was analyzed and found to be increased only by exposure to low doses of AM251 at DIV6 (Fig. 1).

Neuronal Cells

Although many studies have demonstrated either the beneficial or deleterious effects of cannabinoids on the survival of different cell types in culture (Galve-Roperh et al. 2008; van der Stelt and Di Marzo 2005), the basis for these opposite effects on cell survival is not yet understood.

To evaluate the effects of CB1R agonists on neuronal survival in HSC, we measured the number of NeuN+ cells in the DG and the relationship between the number of neurons and CB1R expression. At DIV6, the changes in CB1R expression could be related to the number of neurons and their dendritic arborization. A low dose of ACEA did not produce alterations in the number of neurons, although the HSC exposed to 5 μM ACEA and 1 and 10 μM AM251 showed a decrease in this parameter (Fig. 2b), indicating that higher doses of the CB1R agonist produce toxic effects similar to those produced by the antagonist. In all experimental groups, the ratio CB1+fibers/NeuN+ cells was higher than in the control group (Fig. 2c).

Cell Death

CB1R agonists may act through two mechanisms to 'protect' neurons. First, through a G-protein coupled mechanism, they may reduce NMDA-controlled calcium influx; second, protein kinase A (PKA) can stimulate the ryanodine receptor. Then, CB1R agonists may effectively reduce calcium efflux from the endoplasmic reticulum by reducing PKA (Grant and Cahn 2005).

Studies using caspase-3 activity (Casp3a) as a marker for apoptotic cell death rendered a small number of Casp3a+cells in the DG at DIV6. ACEA induced cell death (Fig. 3) only at high concentrations and within the DG, while AM251 produced an increase in cell death at both low and high concentrations, which suggests that CB1R stimulation has a dose-dependent effect (Fig. 3).

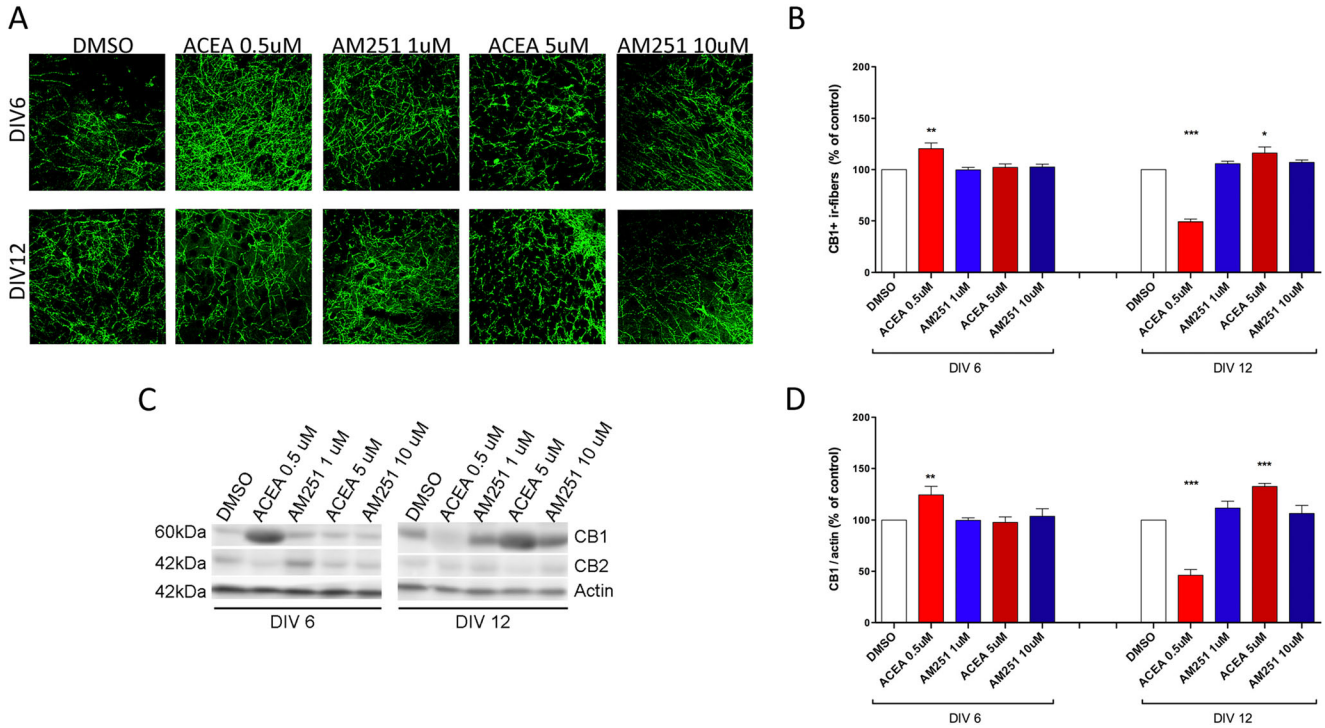

Fig. 1 CB1R expression in HSC after CB1R stimulation. **a** Confocal imaging of CB1R IF of HSC, primary magnification ×60. **b** CB1R expression levels, measured as relative area of CB1R+ fibers. **c** WB of CB1R and CB2R. **d** CB1R expression levels, measured as relative optical density of CB1R/actin. Significance between treatments after one-way ANOVA and Dunnett post-test: *$p<0.05$; **$p<0.01$; ***$p<0.001$

Dendritic Arborization

Arborization was assessed by means of MAP2+ fibers, a microtubule-associated protein found in dendrites and commonly used as a specific marker to visualize these neuronal processes and analyze the dendritic tree and its extension (Tagliaferro et al. 2006).

After DIV6, ACEA at 0.5 μM and AM251 at 1 and 10 μM increased the dendritic arborization of the DG of HSC labeled with MAP2 (Fig. 4b), while the relationship between the expression of CB1R and the area of MAP2+ fibers (Fig. 4c) did not change when compared to the control group (DMSO). The decrease in MAP2+ fibers could be dependent on ACEA concentration and unrelated to the coexpression of CB1 (Fig. 4a, b, c).

Astrocytes

Astroglial reaction was analyzed by studying changes in the area of GFAP expression, the main intermediate filament defining astrocytic morphology. Exposure to low doses of CB1R agonist ACEA did not produce glial response at DIV6, but a low dose of CB1R antagonist AM251 produced astrocytic reaction. After DIV6, low concentrations of ACEA did not modify the number of astrocytes (data not shown) or the astrocytic area covered. At DIV6, AM251 produced astrocytic hyperthopia (Fig. 5), as astrocytes showed an enlarged cell body with more tortuous and ramified cytoplasmic processes and a more intense immunostaining, as compared with ACEA-treated HSC.

Microglial Cells

The eCB system is thought to regulate many aspects of the inflammatory response in the CNS, such as the release of pro-inflammatory cytokines and the modulation of microglial activation (Klein 2005; Marchalant et al. 2007).

IBA1 immunoreactivity was then used to asses the extent of microglial activation. At DIV6, no changes were found in the number or morphology of IBA1+ microglial cells after CB1R agonist or antagonist exposure (Fig. 6). These cells showed a typically ramified cell body with multiple branches.

Discussion

In the context of current controversial data about the effects of CB1R stimulation regarding the toxic effects of cannabinoids (Landucci et al. 2011; Parmentier-Batteur et al. 2002; van der Stelt and Di Marzo 2005), this study analyzes the effects of exogenous CB1R agonist on mouse HSC, a simple technique convenient for the explant of nervous tissue and particularly well suited for developmental studies of synaptic connectivity and plasticity in hippocampus (Stoppini et al. 1991). In this

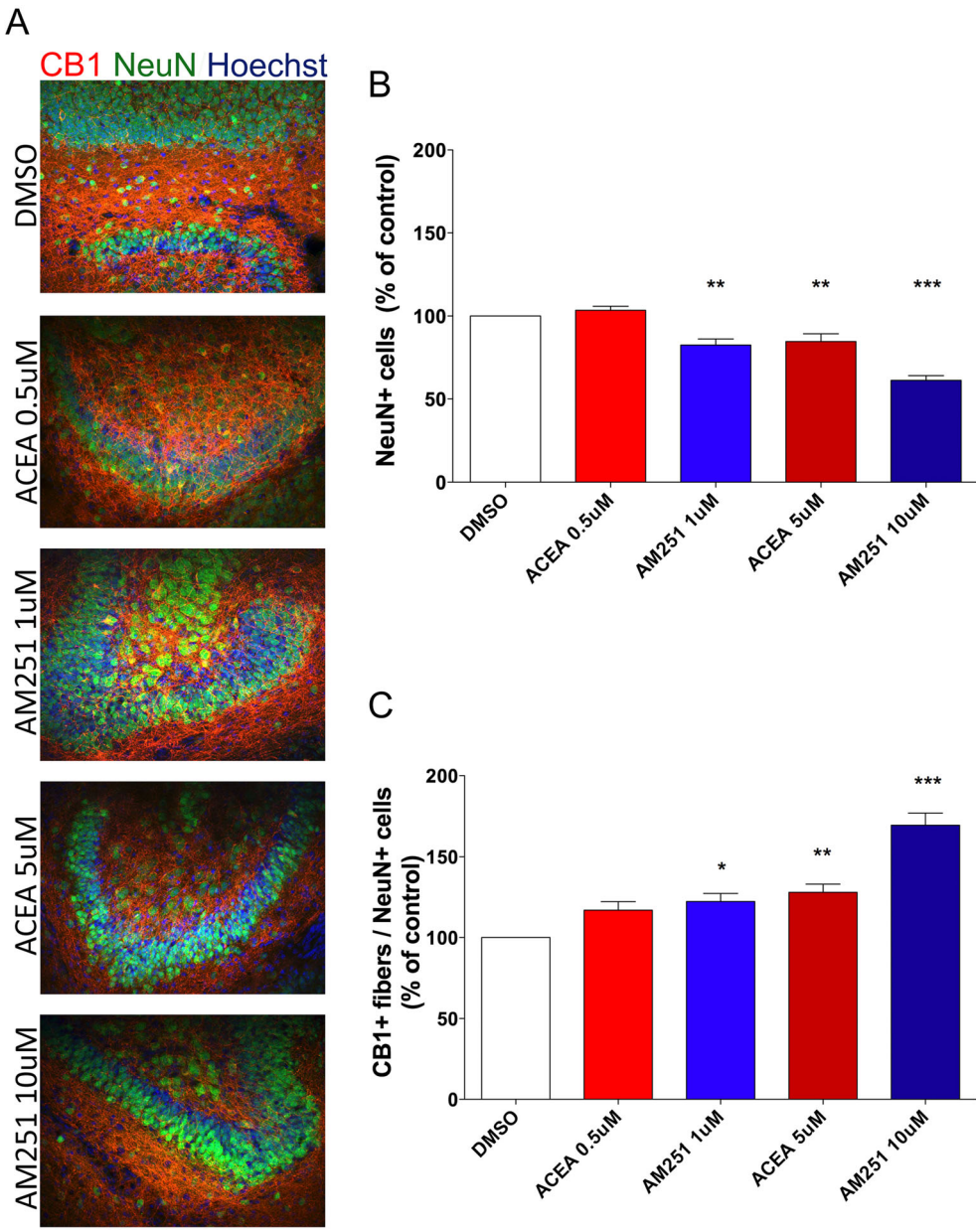

Fig. 2 **a** CB1R and NeuN double IF in the DG of HSC, primary magnification ×20. **b** Numbers of NeuN+ cells. **c** Relationship between CB1R expression and NeuN+ cells in the DG of HSC. Significance between treatments after one-way ANOVA and Dunnett post-test: *$p<0.05$; **$p<0.01$; ***$p<0.001$

way, we demonstrate changes in the neuronal cytoskeleton following chronic treatment with synthetic CB1R agonist ACEA.

eCBs are actively secreted and inactivated by neurons, microglial cells, and astrocytes, and the cannabinoid system is involved in the communication between neurons. The effects of CBs are mediated by CB1R, expressed in neurons and astrocytes, or by CB2R, highly expressed in immunocompetent cells and neurons (Onaivi et al. 2006). In turn, the widespread distribution of cannabinoid receptors in the brain is well correlated with cannabinoid effects on memory, perception, and control of movement (Ameri 1999). In particular,

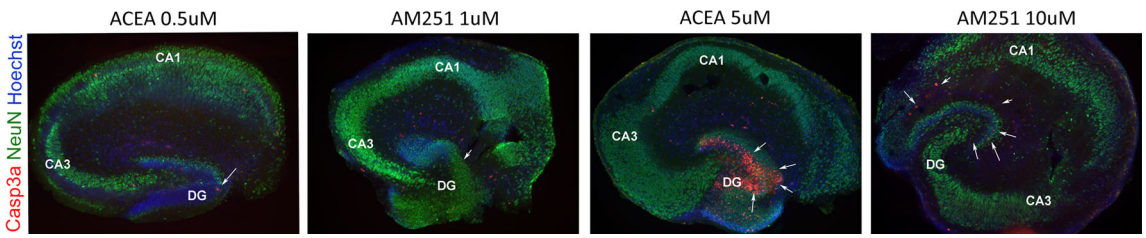

Fig. 3 Caspase-3a expression after CB1R agonist and antagonist treatments in HSC. Primary magnification ×5. *Arrows* show casp3a+cells

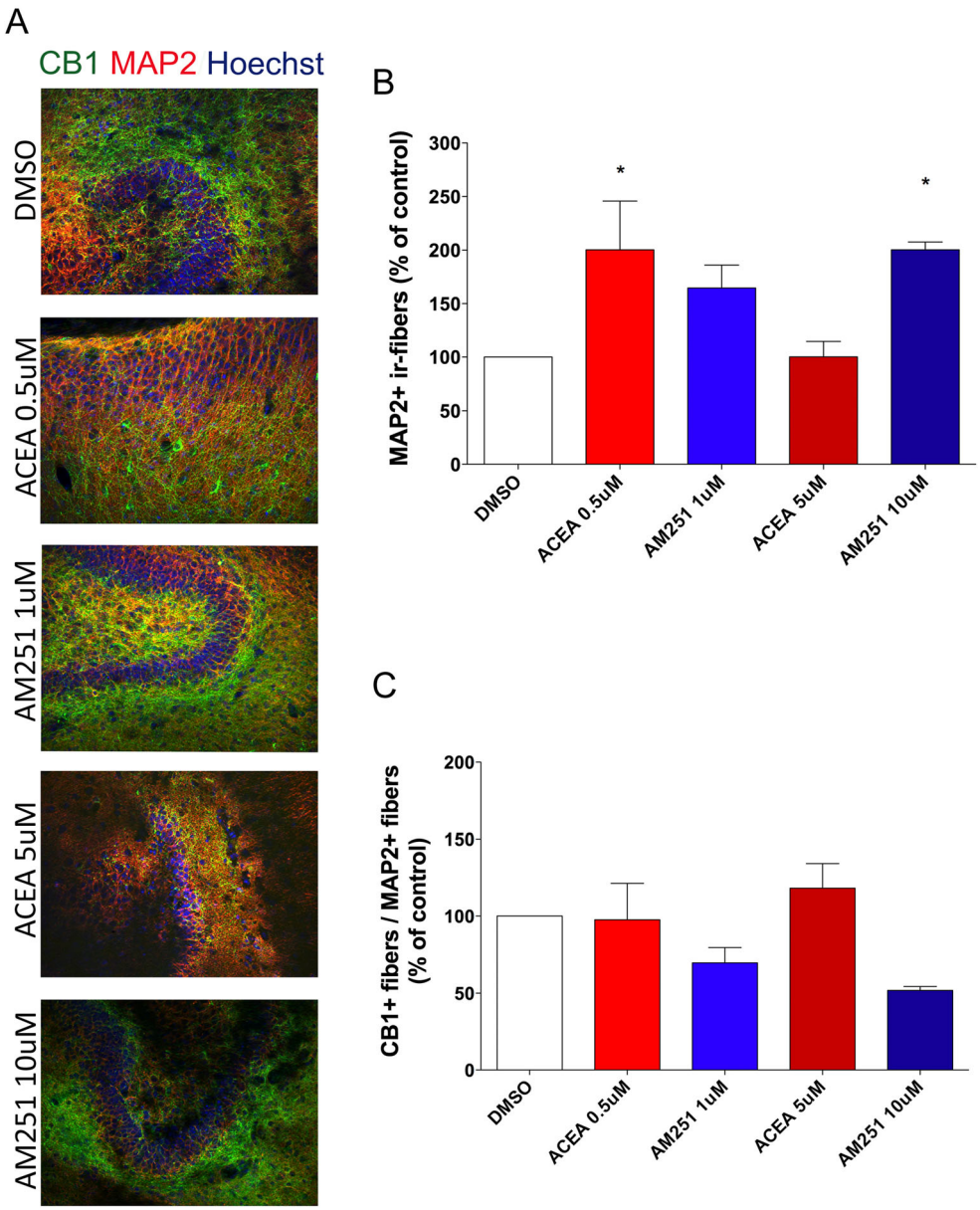

Fig. 4 **a** CB1R and MAP2 double IF in the DG of HSC, primary magnification ×20. **b** Levels of MAP2+ fibers. **c** Relationship between CB1R expression and MAP2 in the DG of HSC. Significance between treatments after one-way ANOVA and Dunnett: *$p<0.05$

CB1R has been implicated in different forms of synaptic plasticity involving the coupling of postsynaptic activation with a decrease in presynaptic neurotransmitter release (Iversen 2003; Tagliaferro et al. 2006), which may play an important role in the control of neuronal circuits.

Several authors have reported cannabinoid-mediated increases in CB1R mRNA and protein expression in different cell types including T cells and hepatocytes (Borner et al. 2007; Mukhopadhyay et al. 2010). Here, we show that CB1R expression also increases after stimulation in neurons in a dose-dependent manner.

As cannabimimetic drugs are known to inhibit adenylyl cyclase and impair memory, cannabinoid effects on synaptic plasticity (Kim and Thayer 2001) have been previously proposed. During CNS development, the administration of THC induces impairment of synapse formation through CB1R activation and disrupts the proper positioning, postsynaptic target selectivity, and functional differentiation of developing axons (Berghuis et al. 2007). Also, in cultured mouse Neuro2A cells, CB1R stimulation has been shown to induce neurite outgrowth, triggering activation of multiple transcription factors (Jordan et al. 2005). It has also been proven that CB1R agonists protect neurons from synaptically mediated excitotoxicity, and that agonist efficacy and receptor desensitization affect this protection. These effects on neuronal survival may prove particularly important when evaluating pharmacological treatments that modulate the endocannabinoid system (Gilbert et al. 2007).

Our data show that ACEA treatment at low concentrations did not produce changes in the number of neurons or caspase-

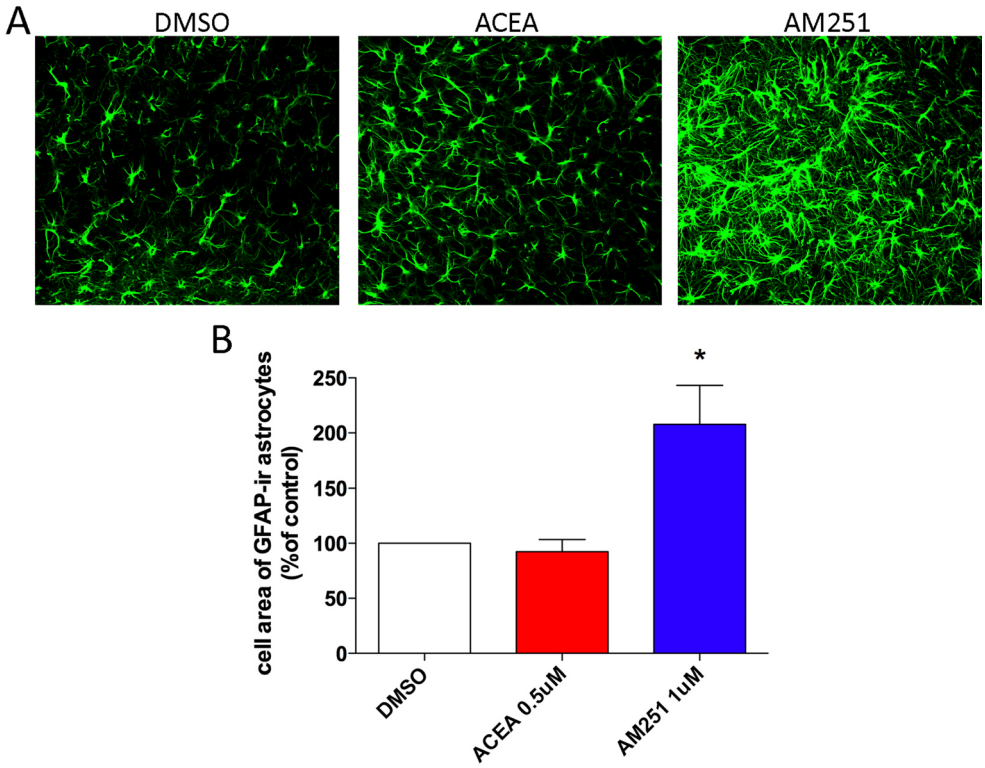

Fig. 5 **a** GFAP IF in the DG of HSC, primary magnification ×60. **b.** Cell area of GFAP+ astrocytes at DIV6. Significance between treatments after one-way ANOVA and Dunnett post-test: *$p<0.05$

3+ dead cells, but generated an increase in the number of dendrites, which could be related to the increase in CB1R expression. These sprouting phenomena, reflected as increased dendrite thickness and/or increased branching processes, are probably due to the upregulation of CB1R. On the other hand, AM251 exposure produced a strong decrease in the number of neurons but an increase in CB1R expression. This is consistent with the fact that CB1R is synthesized de novo in the neuronal somata or with an increase in CB1R expression in glial cells.

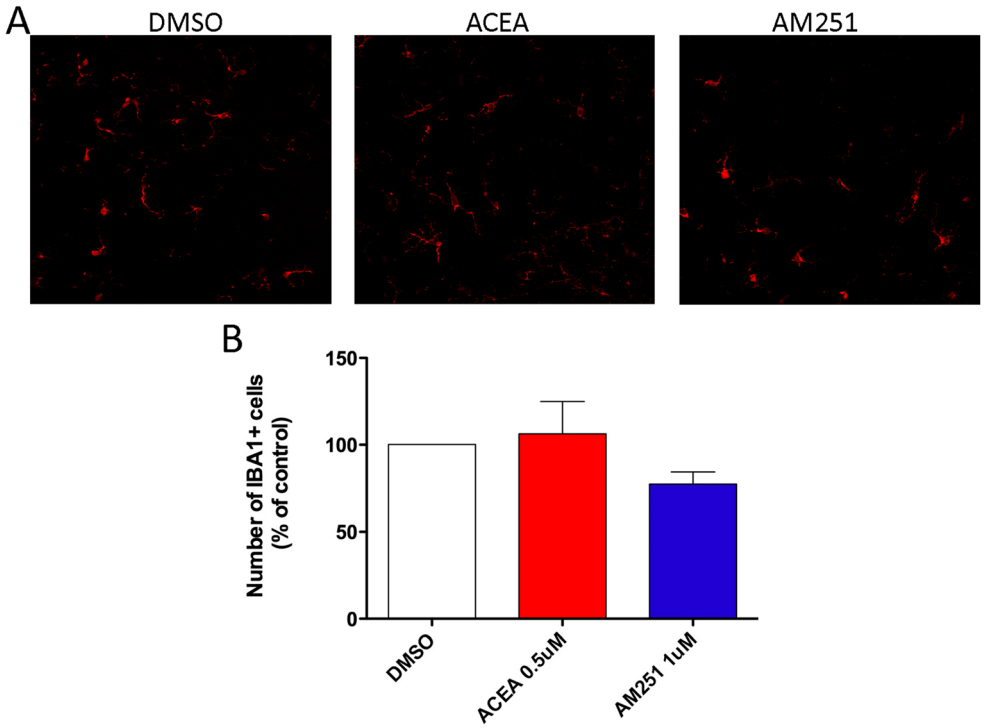

Fig. 6 **a** IBA1 IF in the DG of HSC, primary magnification ×60. **b.** Number of IBA1+ cells at DIV6. Significance between treatments after one-way ANOVA and Dunnett post-test

Astroglial reaction is characterized by an increase in cell size, the presence of thicker, and longer cellular processes and alterations in the expression pattern of some specific proteins, especially GFAP. This protein is a very sensitive parameter to evaluate neuronal damage but also relatively minor alterations in the brain environment (Evrard et al. 2006). In our study, no alterations were observed in the expression pattern of GFAP, which means our treatment schedule did not produce astroglial response in HSC. Then, with no astrocytic or microglial alterations observed at DIV6, neurons can be regarded as the cell type most sharply affected by ACEA exposure.

Considering that eCBs are released after a triggering signal—when it is necessary to maintain homeostasis—these findings open the way for research into the physiological and pathophysiological roles of the endocannabinoid system, with a view to searching for new compounds that could modulate, when administered exogenously, its regulatory abilities and serve as pharmacotherapeutical agents. Finally, as this study shows a plastic phenomenon involving neuronal survival and sprouting but no glial alterations after dose-dependent ACEA exposure, the CB1R agonists could be considered good therapeutic agents for certain brain injuries, focusing their neuroprotective effects only on altered neurons.

Acknowledgments The authors would like to thank Simone Zenker for her kind technical support.

Financial Support UBACYT 20020130300033BA (L.C.) and UBACYT 20020100100093BA (A.B...), UBACYT 20020130100258BA (A.B...), PIP CONICET 00269 (A.B...)

References

Ameri A (1999) The effects of cannabinoids on the brain. Prog Neurobiol 58(4):315–348

Ashton J, Wright J, McPartland J, Tyndall J (2008) Cannabinoid CB1 and CB2 receptor ligand specificity and the development of CB2-selective agonists. Curr Med Chem 15(14):1428–1443

Berghuis P, Rajnicek AM, Morozov YM, Ross RA, Mulder J, Urbán GM, Monory K, Marsicano G, Matteoli M, Canty A, Irving AJ, Katona I, Yanagawa Y, Rakic P, Lutz B, Mackie K, Harkany T (2007) Hardwiring the brain: Endocannabinoids shape neuronal connectivity. Science 316:1212–16

Borner C, Holt V, Kraus J (2007) Activation of human T cells induces upregulation of cannabinoid type 1 transcription. Neuroimmunomodulation 14:281–286

Brinks H, Conrad S, Vogt J, Oldekamp J, Sierra A, Deitinghoff L, Bechmann I, Alvarez-Bolado G, Heimrich B, Monnier PP, Mueller BK, Skutella T (2004) The repulsive guidance molecule RGMa is involved in the formation of afferent connections in the dentate gyrus. J Neurosci 24(15):3862–3869

Brusco A, Tagliaferro PA, Saez T, Onaivi ES (2008) Ultrastructural localization of neuronal brain CB2 cannabinoid receptors. Ann N Y Acad Sci 1139:450–7

Evrard S, Duhalde-Vega M, Tagliaferro P, Mirochnic S, Caltana L, Brusco A (2006) A low chronic ethanol exposure induces morphological changes in the adolescent rat brain that are not fully recovered even after a long abstinence: an immunohistochemical study. Exp Neurol 200(2):438–459

Galve-Roperh I, Aguado T, Palazuelos J, Guzman M (2008) Mechanisms of control of neuron survival by the endocannabinoid system. Curr Pharm Des 14:2279–2288

Gilbert GL, Kim HJ, Waataja JJ, Thayer SA (2007) Delta9-tetrahydrocannabinol protects hippocampal neurons from excitotoxicity. Brain Res 1128(1):61–9

Goncalves M, Suetterlin P, Yip P, Molina Holgado F, Walker D, Oudin M, Zentar M, Pollard S, Yanez-Muñoz R, Williams G, Walsh F, Pangalos M, Doherty P (2008) A diacylglycerol lipase-CB2 cannabinoid pathway regulates adult subventricular zone neurogenesis in an age-dependent manner. Mol Cell Neurosci 38(4):526–536

Grant I, Rael Cahn B (2005) Cannabis and endocannabinoid modulators: Therapeutic promises and challenges. Clin Neurosci Res 5(2-4): 185–199

Heifets B, Castillo P (2009) Endocannabinoid signaling and long-term synaptic plasticity. Annu Rev Physiol 71:283–306

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC (1990) Cannabinoid receptor localization in brain. Proc Natl Acad Sci U S A 87(5):1932–6

Hillard CJ, Manna S, Greenberg MJ, DiCamelli R, Ross RA, Stevenson LA, Murphy V, Pertwee RG, Campbell WB (1999) Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). J Pharmacol Exp Ther 289(3):1427–33

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54:161–202

Humar M, Graetz C, Roesslein M, Goebel U, Geiger K, Heimrich B, Pannen B (2009) Heterocyclic thioureylenes protect from calcium-dependent neuronal cell death. Mol Pharmacol 75:667–676

Iversen L (2003) Cannabis and the brain. Brain 126(Pt 6):1252–1270

Jordan JD, He JC, Eungdamrong NJ, Gomes I, Ali W, Nguyen T, Bivona TG, Philips MR, Devi LA, Iyengar R (2005) Cannabinoid receptor induced neurite outgrowth is mediated by Rap1 activation through Galphao/I-triggered proteasomal degradation of Rap1GAPII. J Biol Chem 280(12):11413–21

Katona I, Sperlágh B, Sík A, Käfalvi A, Vizi ES, Mackie K, Freund TF (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci 19(11):4544–58

Kim D, Thayer S (2000) Activation of CB1 cannabinoid receptors inhibits neurotransmitter release from identified synaptic sites in rat hippocampal cultures. Brain Res 852:398–405

Kim D, Thayer SA (2001) Cannabinoids inhibit the formation of new synapses between hippocampal neurons in culture. J Neurosci 21(10):RC146

Klein TW (2005) Cannabinoid-based drugs as anti-inflammatory therapeutics. Nat Rev Immunol 5:400–411

Lacour P, Heimrich B, Pröls F (2007) Induction of cellular stress and chaperone activation in organotypic slice cultures of hippocampus. J Neurosci Methods 166:24–31

Landucci E, Scartabelli T, Gerace E, Moroni F, Pellegrini-Giampietro D (2011) CB1 receptors and post-ischemic brain damage: Studies on the toxic and neuroprotective effects of cannabinoids in rat organotypic hippocampal slices. Neuropharmacology 60:674–682

Mackie K (2005) Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb Exp Pharmacol 168:299–325

Mackie K (2006) Mechanisms of CB1 receptor signaling: Endocannabinoid modulation of synaptic strength. Int J Obes (Lond) 30(Suppl 1):S19–23

Marchalant Y, Rosi S, Wenk GL (2007) Anti-inflammatory property of the cannabinoid agonist WIN-55212-2 in a rodent model of chronic brain inflammation. Neuroscience 144:1516–1522

Mukhopadhyay B, Liu J, Osei-Hyiaman D, Godlewski G, Mukhopadhyay P, Wang L, Jeong WI, Gao B, Duester G, Mackie K, Kojima S, Kunos G (2010) Transcriptional regulation of cannabinoid receptor-1 expression in the liver by retinoic acid acting via retinoic acid receptor-gamma. J Biol Chem 285:19002–19011

Navarrete M, Araque A (2010) Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. Neuron 68:113–126

Onaivi ES, Ishiguro H, Gong JP, Patel S, Perchuk A, Meozzi PA, Myers L, Mora Z, Tagliaferro P, Gardner E, Brusco A, Akinshola BE, Liu QR, Hope B, Iwasaki S, Arinami T, Teasenfitz L, Uhl GR (2006) Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. Ann N Y Acad Sci 1074:514–536

Pacher P, Bátkai S, Kunos G (2006) The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 58:389–462

Parmentier-Batteur S, Jin K, Mao XO, Xie L, Greenberg DA (2002) Increased severity of stroke in CB1 cannabinoid receptor knock-out mice. J Neurosci 22:9771–9775

Pertwee RG (2005) Pharmacological actions of cannabinoids. Handb Exp Pharmacol (168):1-51

Proto MC, Gazzerro P, Di Croce L, Santoro A, Malfitano AM, Pisanti S, Laezza C, Bifulco M (2011) Interaction of endocannabinoid system and steroid hormones in the control of colon cancer cell growth. J Cell Physiol 227:250–258

Rubio-Araiz A, Arevalo-Martín A, Gomez-Torres O, Navarro-Galve B, Garcia-Ovejero D, Suetterlin P, Sanchez-Heras E, Molina-Holgado E, Molina-Holgado F (2008) The endocannabinoid system modulates a transient TNF pathway that induces neural stem cell proliferation. Mol Cell Neurosci 38:374–380

Scallet AC (1991) Neurotoxicology of cannabis and THC: a review of chronic exposure studies in animals. Pharmacol Biochem Behav 40(3):671–6

Stoppini L, Buchs P, Muller D (1991) A simple method for organotypic cultures of nervous tissue. J Neurosci Methods 37:173–182

Tagliaferro P, Ramos AJ, Onaivi ES, Evrard SG, Lujilde J, Brusco A (2006) Neuronal cytoskeleton and synaptic densities are altered after a chronic treatment with the cannabinoid receptor agonist WIN 55, 212-2. Brain Res 1085(1):163–76

Uchigashima M, Yamazaki M, Yamasaki M, Tanimura A, Sakimura K, Kano M, Watanabe M (2011) Molecular and morphological configuration for 2-arachidonoylglycerol-mediated retrograde signaling at mossy cell-granule cell synapses in the dentate gyrus. J Neurosci 31(21):7700–14

Van der Stelt M, Di Marzo V (2005) Cannabinoid receptors and their role in neuroprotection. Neuromol Med 7(1–2):37–50