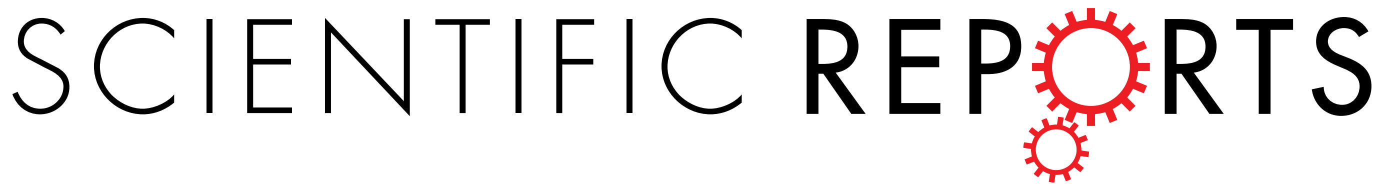

OPEN

Received: 23 June 2015
Accepted: 04 November 2015
Published: 04 December 2015

# $CB_1$ cannabinoid receptor enrichment in the ependymal region of the adult human spinal cord

Beatriz Paniagua-Torija[1], Angel Arevalo-Martin[1], Isidro Ferrer[2], Eduardo Molina-Holgado[1] & Daniel Garcia-Ovejero[1]

**Cannabinoids are involved in the regulation of neural stem cell biology and their receptors are expressed in the neurogenic niches of adult rodents. In the spinal cord of rats and mice, neural stem cells can be found in the ependymal region, surrounding the central canal, but there is evidence that this region is largely different in adult humans: lacks a patent canal and presents perivascular pseudorosettes, typically found in low grade ependymomas. Using Laser Capture Microdissection, Taqman gene expression assays and immunohistochemistry, we have studied the expression of endocannabinoid system components (receptors and enzymes) at the human spinal cord ependymal region. We observe that ependymal region is enriched in $CB_1$ cannabinoid receptor, due to high $CB_1$ expression in GFAP+ astrocytic domains. However, in human spinal cord levels that retain central canal patency we found ependymal cells with high $CB_1$ expression, equivalent to the $CB_1^{HIGH}$ cell subpopulation described in rodents. Our results support the existence of ependymal $CB_1^{HIGH}$ cells across species, and may encourage further studies on this subpopulation, although only in cases when central canal is patent. In the adult human ependyma, which usually shows central canal absence, $CB_1$ may play a different role by modulating astrocyte functions.**

The Endocannabinoid System (ECBS) is formed by lipid ligands (endocannabinoids), the enzymatic machinery for their synthesis and degradation and their specific G-protein coupled $CB_1$ and $CB_2$ receptors. The most important endocannabinoids are 2-arachydonoylglycerol (2-AG) and anandamide (AEA)[1]. These compounds are involved in the control of neural stem cell biology[2], and many of their effects are mediated by the cannabinoid receptor $CB_1$. $CB_1$ receptor is expressed in all neurogenic niches in rodents, including the ependymal region of the spinal cord (reviewed in[3]). In this region, that holds neural stem cell potential[4,5], a subpopulation of cells expresses high levels of $CB_1$ receptor ($CB_1^{HIGH}$ cells), and proliferate after lesion or during postnatal development in rats[6]. However, the ependymal region of the adult human spinal cord is strikingly different from that of rodents and other primates: although it contains ependymal cells, lacks a patent central canal and shows perivascular pseudorosettes[7–9]. This means that observations made in rodents should be validated in human tissue to understand the composition and the regenerative potential of this niche. Here we have explored the presence of the ECBS and searched for an equivalent of rat and mice $CB_1^{HIGH}$ cells in adult human spinal cord.

[1]Laboratory of Neuroinflammation, Hospital Nacional de Paraplejicos (SESCAM), Toledo, Spain. [2]Institut de Neuropatologia, Servei d'Anatomia Patològica, IDIBELL-Hospital Universitari de Bellvitge, Universitat de Barcelona, L'Hospitalet de Llobregat, Spain. Correspondence and requests for materials should be addressed to E.M.-H. (email: eduardom@sescam.jccm.es) or D.G.-O. (email: dgarciao@sescam.jccm.es)

| *GENE* | *Assay* | *ΔCt Ependymal Region vs18S (average)* | *ΔCt Ventral Horn vs18S (average)* | *RQ vs Ventral Horn* | *p* |
|---|---|---|---|---|---|
| **CNR1** | *Hs01038522_s1* | *8,379754298* | *13,57232086* | **57,27** | **0,012**** |
| CNR2 | *Hs00361490_m1* | – | – | *ND#* | – |
| GPR55 | *Hs00271662_s1* | *17,13233* | *18,721182* | *NCD#* | – |
| DAGLA | *Hs00391374_ml* | *11,7639845* | *13,04953063* | 2,34 | 0,8 |
| DAGLB | *Hs00373700_ml* | *11,1895161* | *8,199532* | 14,6 | 0,51 |
| MGLL | *Hs00200752_ml* | *10,5709992* | *9,519471* | 0,25 | 0,44 |
| ABHD6 | *Hs00977889_m1* | *12,6129394* | *14,365685* | 1,48 | 0,75 |
| ABHD12 | *Hs01018047_m1* | *12,6756672* | *12,6676875* | 4,7 | 0,095 |
| NAPEPLD | *Hs00419593_m1* | *13,0519701* | *6,908164* | *NCD#* | – |
| FAAH | *Hs01038660_m1* | *20,2593* | *19,962125* | *NCD#* | – |

**Table 1. Relative expression of endocannabinoid system related genes in the adult human ependymal region compared with ventral horn.** *Significantly enriched in Ependymal region vs Ventral Horn (Student T-test). #ND: Non detected in the Ependymal region of any individual; NCD: Non consistently detected (detected in less than 3 of the 4 individuals).

## Results and Discussion

We found that human ependymal region consistently expresses $CB_1$ cannabinoid receptor (CNR1 gene; Table 1). $CB_1$ receptor could be the target of locally produced 2-AG, since we also found expression (although non enrichment) of enzymes related with 2-AG synthesis and degradation: diacylglycerol lipase $\alpha$ (DAGLA), diacylglycerol lipase $\beta$ (DAGLB), monoacylglycerol lipase (MGLL) and abhydrolase domain-containing proteins −6 (ABHD6) and −12 (ABHD12). On the contrary, we could not find consistent expression of enzymes related with direct anandamide synthesis or degradation (NAPE-phospholipase D and fatty acid amide hydrolase, respectively). However, it should be noted that alternative enzymatic routes have been described for AEA, involving glycerophosphodiester phosphodiesterase and N-acylethalnolamine-hydrolyzing acid amidase that have been not tested here[2]. We also did not find expression of $CB_2$ cannabinoid receptor or the related GPR55 receptor. In previous works, we observed expression (but not enrichment) of PPAR-$\alpha$, another cannabinoid-related receptor[1], in human ependymal region[9].

When compared with ventral horn, only CNR1 ($CB_1$ receptor) was significantly enriched at the ependymal region (Table 1). Accordingly, we found a strong $CB_1$ immunoreactivity in central gray matter by immunohistochemistry (Fig. 1B–J). But $CB_1$ enrichment in adult humans ependyma is not equivalent to that found in rodents: In humans, $CB_1$ is expressed by astrocytes, forming part of the gliosis that accompanies central canal closure (Fig. 1C–E) and in the $GFAP^+$ hypocellular ribbon of perivascular pseudorosettes (Fig. 1F–K)[9,10]. $CB_1$ receptor is also expressed in astrocytes from other spinal cord areas (Fig. 2), and its intensity is apparently related to high GFAP expression. Accordingly, a strong $CB_1$ expression has been reported in reactive astrocytes of human pathologies like spinocerebellar ataxia[11] or temporal lobe epilepsia[12]. The role of astrocytic $CB_1$ could be multiple: protection[13], metabolism increase[14], control of inflammation[15–17], inhibition of glutamate transporters[18] or release of neurotransmitters such as glutamate[19], ATP and D-serine[20].

Interestingly, we obtained some sections from adult individuals in which parts of the central canal were patent. In those sections, we found ependymal cells with high expression of $CB_1$ receptors lining the canal (Fig. 1L–N), resembling those $CB_1^{HIGH}$ cells described for rats and mice[6]. These cells were mostly GFAP-, except for a very dim expression at the apical pole (Fig. 1N), in contrast with strongly $GFAP^+$ cells embeded in the ependymal layer (Fig. 1M).

Our results support the existence of ependymal $CB_1^{HIGH}$ cells across species, and may encourage further studies on this subpopulation, although only in cases when there is central canal patency, i.e. childhood and upper cervical levels[8,9]. But in the majority of adult ependyma, $CB_1$ is enriched in astrocyte domains, and cannabinoids may play a different role, that still might be relevant, in terms of homeostasis maintenance and response to injury.

## Methods

Human tissue was obtained from the HUFA BioBank (Alcorcon, Spain) and the HUB-ICO-IDIBELL BioBank (Hospitalet de Llobregat, Spain). Samples were obtained from donor individuals deceased without clinical or histopathological involvement of the spinal cord (Table 2). Donation always included a written informed consent from donors while alive or from their families after death. Data from donors and handling of samples were carried out after approval by the Clinical Research Ethical Committee (CEIC) in Toledo (Spain), in accordance with the Spanish law and International Guidelines (LOPD 15/1999; RD 1720/2007; Helsinki declaration, 2008).

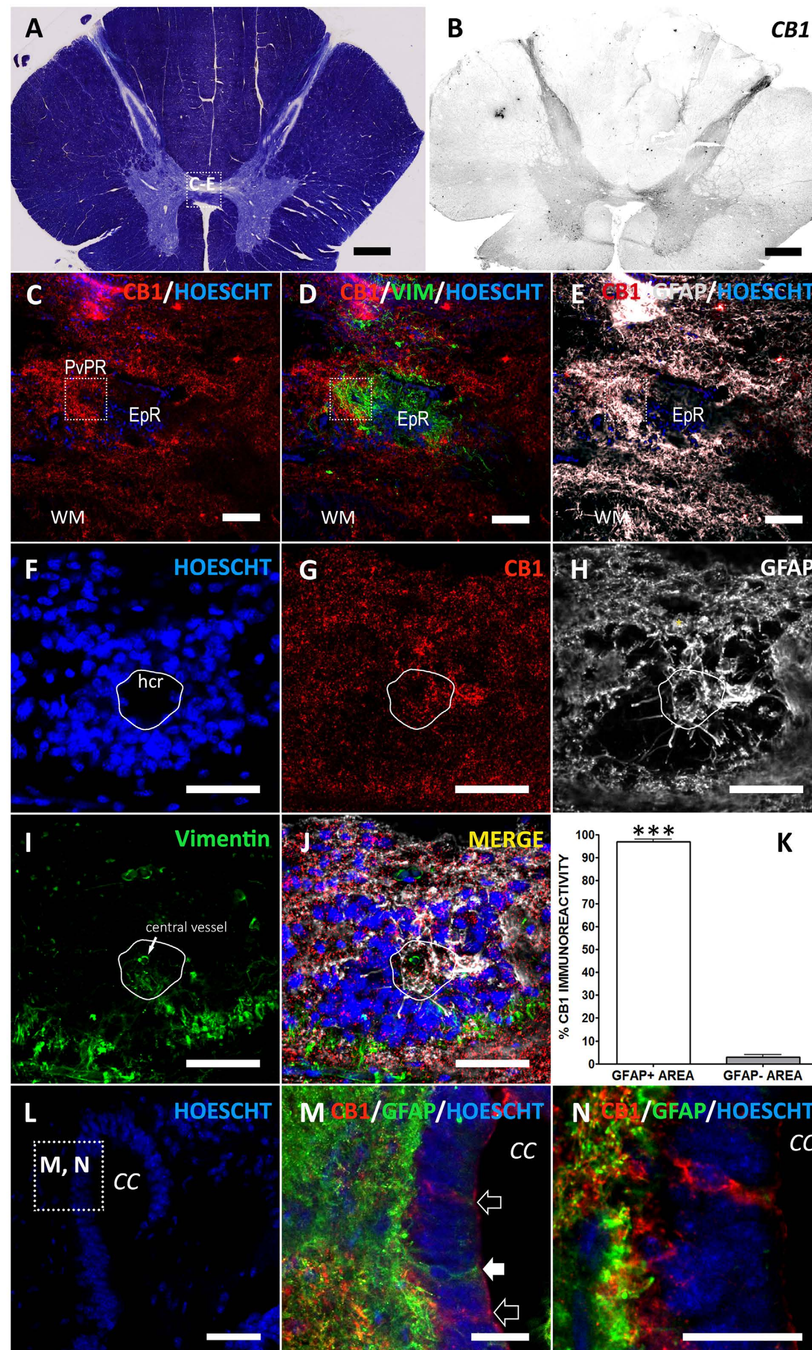


**Figure 1. $CB_1$ cannabinoid receptor in adult human spinal cord.** (**A**) Myelin staining of a representative thoracic spinal cord section. Square depicts the area shown in (**C–E**). (**B**) In low magnification a strong $CB_1$ immunoreactivity can be found in dorsal horn, lamina X and ventral gray matter. (**C–E**) Higher magnification of central gray shows $CB_1$ expression in GFAP+ areas surrounding the Vimentin$^+$ cells at the ependymary region (EpR). Square highlights the location of a perivascular pseudorosette (PvPR). (**F–J**) Strong $CB_1$ immunoreactivity is found at the GFAP$^+$ domains around and inside perivascular pseudorosettes, including the GFAP ribbon at the hypocellular region of the pseudorosette (hcr, outlined in white). In PvPRs cells are arranged around a central vessel ((**I**) arrow). (**K**) Quantification supports qualitative observations: CB1 immunoreactivity is significantly accumulated in GFAP+ areas. (**L**) Detail of the dorsal aspect of an ependymal region with a patent central canal. Square depicts location of images M and N. (**M**) $CB_1^{HIGH}$ ependymal cells (empty arrows) can be found intermingled with ependymocytes lining the central canal. GFAP$^+$ cells contacting central canal lumen (arrowhead) are $CB_1^-$. (**N**) Detail of M, showing a $CB_1^{HIGH}$ cell with a dim staining of GFAP in the apical region . ****T student, $p < 0.001$; CC, Central Canal; EpR, ependymal region; hcr, hypocellular region; PvPR, perivascular pseudorosette; WM, white matter. Scale bars: A,B = 1 mm; C-E: 100 µm; F-L = 50 µm; M,N = 25 µm.*

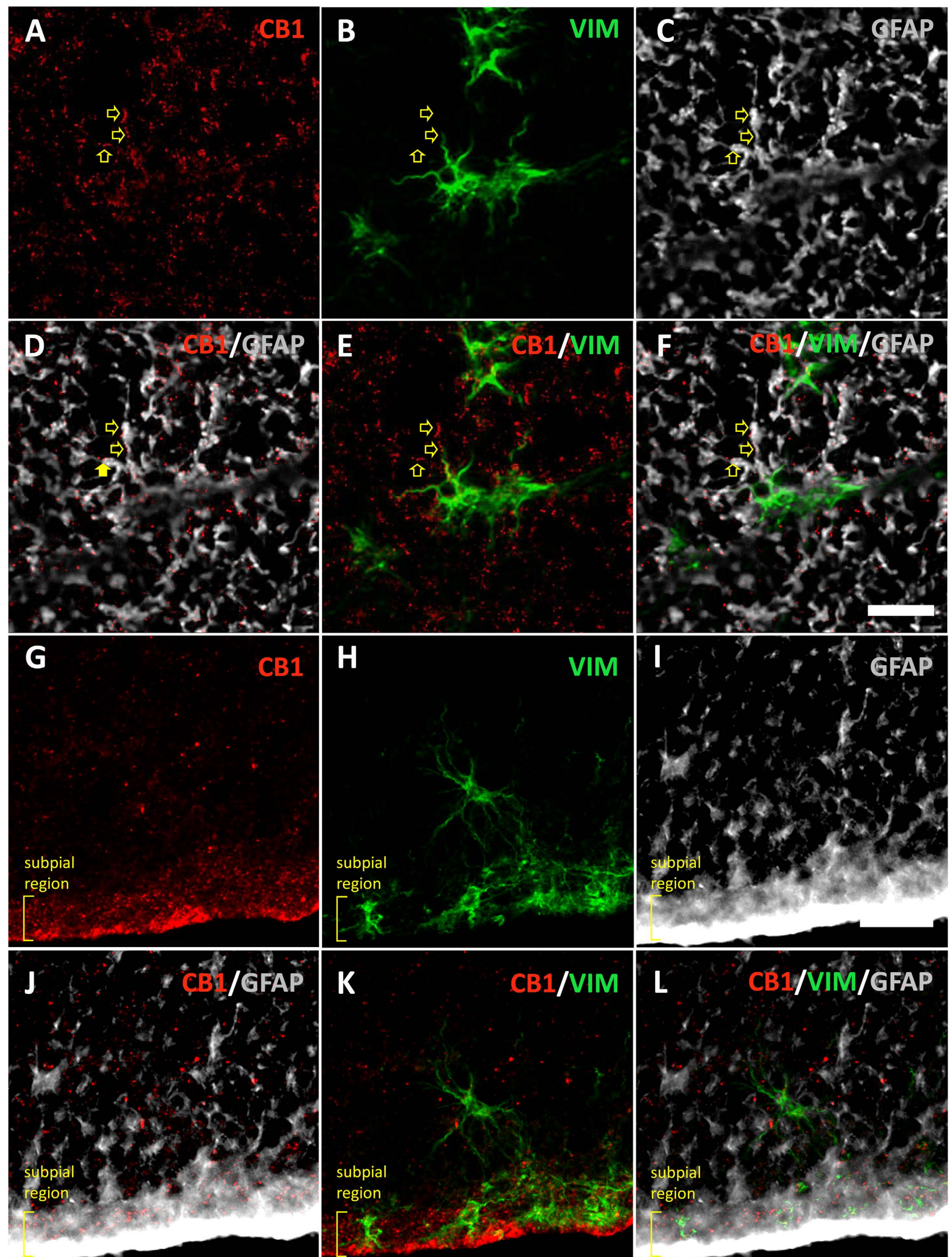


**Figure 2. $CB_1$ immunoreactivity can be found in astrocytes of other regions in the spinal cord.** (**A–F**) $CB_1$ is expressed in the processes of $GFAP^+$ and $Vim^+$ astrocytes (arrows) at the dorsolateral white matter. (**G–I**) A strong $CB_1$ expression can be found in astrocytic processes at the subpial region. *Scale bars: 25 μm.*

**Gene expression in human ependymal region.** All procedures were performed according to our published protocol[9]. Briefly, fresh frozen spinal cord blocks were cut in 25 μm thick sections and the ependymal region microdissected with a Laser Dissection Microscope. RNA extraction, amplification and reverse transcription were performed as previously described[9]. We also collected microdissected portions of ventral horn, which we used as a non-neurogenic, non-ependymal reference for gene expression.

Gene expression was studied with Taqman PCR Assays (Life Technologies, Madrid, Spain) either incorporated in Taqman Low Density Arrays (*DAGLA, #Hs00391374_m1; DAGLB, #Hs00373700_m1; MGLL, #Hs00200752_m1; NAPEPLD, #Hs00419593_m1*) or in individual assays (*ABHD6, #Hs00977889_m1; ABHD12, #Hs01018047_m1; CNR1, #Hs01038522_s1; CNR2, #Hs00361490_m1;*

| Autopsy number | Cause of Death | Gender | Age | Coded as | Postmortem delay | Used for |
|---|---|---|---|---|---|---|
| BC01015 | Unknown. No significant neuropathological alterations in the spinal cord | Male | 60 | Control | Unknown | IHC |
| BC01684 | Acute Hypoxia-ischemia | Male | 27 | Control | Unknown | IHC |
| A07/00044 | Cardiopulmonary arrest | Male | 39 | Control | 3 h 30 min | IHC |
| A07/00067 | Refractary septic shock and cardiac arrest. Ischemic cardiopathy | Male | 47 | Control | 4 h 55 min | IHC |
| A10/00017 | Hepatic metastasis. Probable pancreatic neoplasia | Male | 52 | Control | 03 h | IHC |
| A07/00041 | Multiorganic failure. Gastric tumour | Male | 43 | Control | 5 h 55 min | IHC, LCMD |
| A07/00084 | Refractary septic shock | Male | 46 | Control | 15 h | IHC, LCMD |
| A10/00026 | Multiorganic failure. Severe broncopathy | Male | 61 | Control | 3 h 55 min | LCMD |
| A05/00134 | Carcinoma and metastasis. With brain but not spinal cord metastasis. | Female | 32 | Control | 11 h 45 m | LCMD |
| A11/00052 | Endocarditis. No neuropathological features | Male | 76 | Control | 06 h 30 m | LCMD |
| A12/00046 | Cardiac arrest. No neuropathological features | Female | 75 | Control | 06 h10 m | LCMD |

**Table 2. Postmortem Spinal Cord tissue samples used for immunohistochemistry (IHC) and/or Laser Capture Microdissection (LCMD).**

*FAAH, #Hs01038660_m1; GPR55, #Hs00271662_s1*). We used 18S gene as an endogenous control (*18S, #Hs03003631_g1*). For assays incorporated on TLDAs, we added 1.25 ng cDNA/well. For assays performed individually, we added 1.5 ng cDNA/well. Assays were run on an Applied Biosystems® 7900HT Fast Real-Time PCR System. Data were analysed as described[9] using automatic detection of Ct, normalized with the endogenous gene ($\Delta$Ct vs 18S). Only genes expressed in at least three out of four samples were considered as consistently expressed and included in statistics. Enrichment was defined as higher and statistically significant expression in ependymal region vs ventral horn (Student's t-test with $\Delta$Cts, $p < 0.05$). To obtain folds of enrichment, we used Relative quantity formula, $RQ = 2^{-\Delta\Delta Ct}$.

**Immunohistochemistry.** To improve signal to noise ratio and avoid autofluorescence, we amplified $CB_1$ immunoreactivity using Tyramide Signal Amplification System (TSA Plus Cyanine 3 System #NEL744001KT, Perkin Elmer, USA). Free floating vibratome sections (40 μm) were rinsed on 0.1 M phosphate-buffered saline containing 0.5% bovine serum albumin +0.3% Triton X-100. Endogenous peroxidase inhibition and antigen demasking were performed as described[9]. Sections were then blocked with TSA Blocking Solution (45′) and incubated for 2 days with primary antibodies diluted in rinse solution +10% Normal Donkey Serum: guinea pig anti-$CB_1$ (1:2000, #CB1-GP-Af530-1, FSI, Japan), rabbit anti-GFAP (1:2000, #Z0334, DAKO, Spain) and mouse anti-Vimentin (1:300, #M0725, DAKO, Spain). Immunoreactivity was visualized by incubating sections with Alexa 488-, Alexa 555- and Alexa 633- secondary antibodies (1:1000, Invitrogen, Spain) or horseradish peroxidase donkey anti-guinea pig antibody (1:300, Jackson Immunoresearch, UK) followed by Tyramide-Cy3 diluted in TSA Amplification Buffer (1:50). Samples were analyzed with a LEICA SP5 confocal microscope. We ruled out the interference of nonspecific staining by omitting primary antibodies. We set the confocal parameters at a point where no signal was observed in these primary antibody controls and those settings were used for all the image acquisitions (Supplementary Figure 1A–F). Furthermore, as discussed in several reports, there is a variety of antibodies against $CB_1$ receptor, and some of them may show non-specific staining[21–23]. The specificity of $CB_1$ antibody used for this report has been extensively validated by other laboratories and ourselves in previous works[6,24,25]. We show here an additional validation in the Supplementary Figure 1 by using immunohistochemistry and TSA amplification on wild type (C57BL/6N) and $CB_1$ knockout mice tissue (kindly donated by Dr. Galve-Roperh[26]). Using restrictive confocal parameters (as we did for humans), we got rid out of autofluorescence, background staining and most of the non-specific staining observed in the knockout mice that, in these conditions, is limited to a dim intracellular neuronal staining, largely different from that observed in the wild type mice (Supplementary Figure 1L-Q). All post-capture image modifications were identically performed for controls, including cropping, noise reduction and minor adjustments to optimize contrast and brightness.

To quantitatively support $CB_1$ enrichment in the astrocytic area, we calculated the fraction of $CB_1$ found in $GFAP^+$ vs $GFAP^-$ areas on confocal planes (image size 190 μm × 190 μm) using Fiji (http://pacific.mpi-cbg.de). For this, we outlined GFAP borders using manual Threshold with Otsu Filter and used this ROI on the $CB_1$ image corresponding to the same confocal plane. We measured $CB_1^+$ Area inside and outside the selection ($GFAP^+$ and $GFAP^-$ areas, respectively) and expressed them as % of total $CB_1$ staining (Fig. 1K). We used Student T-test for statistical comparisons.

## References


1. Pertwee, R. G. *et al.* International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB(1) and CB(2). *Pharmacol Rev* **62,** 588–631, doi: 10.1124/pr.110.00300462/4/588 (2010).
2. Maccarrone, M., Guzman, M., Mackie, K., Doherty, P. & Harkany, T. Programming of neural cells by (endo)cannabinoids: from physiological rules to emerging therapies. *Nat Rev Neurosci* **15,** 786–801, doi: 10.1038/nrn3846nrn3846 (2014).
3. Garcia-Ovejero, D. *et al.* Neuroimmmune interactions of cannabinoids in neurogenesis: focus on interleukin-1beta (IL-1beta) signalling. *Biochem Soc Trans* **41,** 1577–1582, doi: 10.1042/BST20130198BST20130198 (2013).
4. Sabourin, J.-C. *et al.* A Mesenchymal-Like ZEB1+Niche Harbors Dorsal Radial Glial Fibrillary Acidic Protein-Positive Stem Cells in the Spinal Cord. *Stem Cells* **27,** 2722–2733, doi: 10.1002/stem.226 (2009).
5. Weiss, S. *et al.* Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. *J Neurosci* **16,** 7599–7609 (1996).
6. Garcia-Ovejero, D., Arevalo-Martin, A., Paniagua-Torija, B., Sierra-Palomares, Y. & Molina-Holgado, E. A cell population that strongly expresses the CB1 cannabinoid receptor in the ependyma of the rat spinal cord. *J Comp Neurol* **521,** 233–251, doi: 10.1002/cne.23184 (2013).
7. Milhorat, T. H., Kotzen, R. M. & Anzil, A. P. Stenosis of central canal of spinal cord in man: incidence and pathological findings in 232 autopsy cases. *J Neurosurg* **80,** 716–722, doi: 10.3171/jns.1994.80.4.0716 (1994).
8. Yasui, K., Hashizume, Y., Yoshida, M., Kameyama, T. & Sobue, G. Age-related morphologic changes of the central canal of the human spinal cord. *Acta Neuropathol* **97,** 253–259 (1999).
9. Garcia-Ovejero, D. *et al.* The ependymal region of the adult human spinal cord differs from other species and shows ependymoma-like features. *Brain* **138,** 1583–1597, doi: 10.1093/brain/awv089awv089 (2015).
10. Alfaro-Cervello, C. *et al.* The adult macaque spinal cord central canal zone contains proliferative cells and closely resembles the human. *J Comp Neurol* **522,** 1800–1817, doi: 10.1002/cne.23501 (2014).
11. Rodriguez-Cueto, C. *et al.* Changes in CB(1) and CB(2) receptors in the post-mortem cerebellum of humans affected by spinocerebellar ataxias. *Br J Pharmacol* **171,** 1472–1489, doi: 10.1111/bph.12283 (2014).
12. Meng, X. D. *et al.* Astrocytic expression of cannabinoid type 1 receptor in rat and human sclerotic hippocampi. *Int J Clin Exp Pathol* **7,** 2825–2837 (2014).
13. Carracedo, A. *et al.* Ceramide sensitizes astrocytes to oxidative stress: protective role of cannabinoids. *Biochem J* **380,** 435–440, doi: 10.1042/BJ20031714BJ20031714 (2004).
14. Sanchez, C., Galve-Roperh, I., Rueda, D. & Guzman, M. Involvement of sphingomyelin hydrolysis and the mitogen-activated protein kinase cascade in the Delta9-tetrahydrocannabinol-induced stimulation of glucose metabolism in primary astrocytes. *Mol Pharmacol* **54,** 834–843 (1998).
15. Molina-Holgado, F., Molina-Holgado, E. & Guaza, C. The endogenous cannabinoid anandamide potentiates interleukin-6 production by astrocytes infected with Theiler's murine encephalomyelitis virus by a receptor-mediated pathway. *FEBS Lett* **433,** 139–142, doi: S0014-5793(98)00851-5 (1998).
16. Molina-Holgado, F., Molina-Holgado, E., Guaza, C. & Rothwell, N. J. Role of CB1 and CB2 receptors in the inhibitory effects of cannabinoids on lipopolysaccharide-induced nitric oxide release in astrocyte cultures. *J Neurosci Res* **67,** 829–836, doi: 10.1002/jnr.10165 (2002).
17. Molina-Holgado, F. *et al.* Endogenous interleukin-1 receptor antagonist mediates anti-inflammatory and neuroprotective actions of cannabinoids in neurons and glia. *J Neurosci* **23,** 6470–6474, doi: 23/16/6470 (2003).
18. Shivachar, A. C. Cannabinoids inhibit sodium-dependent, high-affinity excitatory amino acid transport in cultured rat cortical astrocytes. *Biochem Pharmacol* **73,** 2004–2011, doi: S0006-2952(07)00185-2 (2007).
19. Navarrete, M. & Araque, A. Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. *Neuron* **68,** 113–126, doi: 10.1016/j.neuron.2010.08.043S0896-6273(10)00686-0 (2010).
20. Rasooli-Nejad, S., Palygin, O., Lalo, U. & Pankratov, Y. Cannabinoid receptors contribute to astroglial Ca(2)(+)-signalling and control of synaptic plasticity in the neocortex. *Philos Trans R Soc Lond B Biol Sci* **369,** 20140077, doi: 10.1098/rstb.2014.007720140077 (2014).
21. Grimsey, N. L. *et al.* Specific detection of CB1 receptors; cannabinoid CB1 receptor antibodies are not all created equal! *J Neurosci Methods* **171,** 78–86, doi: 10.1016/j.jneumeth.2008.02.014S0165-0270(08)00114-3 (2008).
22. Morozov, Y. M. *et al.* Antibodies to cannabinoid type 1 receptor co-react with stomatin-like protein 2 in mouse brain mitochondria. *Eur J Neurosci* **38,** 2341–2348, doi: 10.1111/ejn.12237 (2013).
23. Hebert-Chatelain, E. *et al.* Cannabinoid control of brain bioenergetics: Exploring the subcellular localization of the CB1 receptor. *Mol Metab* **3,** 495–504, doi: 10.1016/j.molmet.2014.03.007S2212-8778(14)00074-X (2014).
24. Uchigashima, M. *et al.* Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. *J Neurosci* **27,** 3663–3676, doi: 27/14/3663 (2007).
25. Yoshida, T. *et al.* Localization of diacylglycerol lipase-alpha around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid CB1 receptor. *J Neurosci* **26,** 4740–4751, doi: 26/18/4740 (2006).
26. Aguado, T. *et al.* The endocannabinoid system promotes astroglial differentiation by acting on neural progenitor cells. *J Neurosci* **26,** 1551–1561, doi: 26/5/1551 (2006).


## Acknowledgements

This work was funded by Wings For Life Foundation (Salzburg, Austria) and Fundacion Mutua Madrileña (Madrid, Spain). Laboratory of Neuroinflammation is also supported by Instituto de Salud Carlos III (Ministry of Economy and Competitiveness of Spain). We are grateful to Concepción Sanchez-Caro for technical help and Javier Mazarío and José Ángel Rodriguez-Alfaro at the Microscopy Facilities at the National Hospital for Paraplegics.

## Author Contributions

B.P.-T. and D.G.-O. Collection and assembly of data; IF: Provision of study material or patients; A.A.-M., E.M.-H. and D.G.-O. Conception and design, data analysis and interpretation; E.M.-H. and D.G.-O. Financial support; D.G.-O. Manuscript writing. All authors gave their final approval to the manuscript.

## Additional Information

**Supplementary information** accompanies this paper at http://www.nature.com/srep

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article**: Paniagua-Torija, B. *et al.* $CB_1$ cannabinoid receptor enrichment in the ependymal region of the adult human spinal cord. *Sci. Rep.* **5**, 17745; doi: 10.1038/srep17745 (2015).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/