Sociedad Española de Investigación sobre Cannabinoides

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Sociedad Española de Investigacion sobre Cannabinoides

XV Reunión Anual

CUENCA

27-29 Noviembre 2014

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15ª Reunión Anual
Sociedad Española de Investigación sobre Cannabinoides

Cuenca, 27-29 de noviembre de 2014

PROGRAMA CIENTÍFICO

Jueves, 27 de noviembre

11:30-12:30 **Entrega de documentación**
Universidad Internacional Menéndez Pelayo
C/ Palafox, 1

12:30-12:45 **Inauguración**
- Representante UIMP
- José Martínez Orgado, Comité Organizador
- Manuel Guzmán, Presidente de la SEIC
- Autoridades locales

12:45-14:00 **Conferencia inaugural**
(presentada por José Martínez Orgado)
Ethan Russo, ICRS Past President
"Therapeutic uses of cannabinoids: the path from bench to bedside"

14:00-16:00 **Comida**

16:00-18:00 **Mesa Redonda**
"Uso clínico del cannabidiol: historia de una realidad"
(moderadores: José Martínez Orgado y Eduardo Muñoz)
- Ethan Russo, ICRS Past President
- Julián Isla, Fundación Dravet
- Javier Fernández Ruiz, Universidad Complutense
- Colin Stott, GW Pharma Ltd
- Arno Hazekamp, Bedrocan

18:00-18:30 **Café**

18:30-19:15 **Sesión de comunicaciones orales**
"Fitocannabinoides distintos del THC"
(moderadores: José Martínez Orgado y Eduardo Muñoz)

18:30-18:45 O.1.1.

CANNABIDIOL SHOWS A TEMPORARY THERAPEUTIC WINDOW LONGER THAN 12 HOURS IN HYPOXIC-ISCHEMIC NEWBORN MICE. N. Mohammed, M.R. Pazos, M. Ceprián, L. Jiménez, O.D. Saugstad, J. Martínez-Orgado

18:45-19:00 O.1.2.

NOVEL CANNABIDIOL DERIVATIVES FOR THE TREATMENT OF FIBROTIC DISEASES. I. Cantarero, C. del Río, C. Navarrete, G. Appendino, M. Gómez-Cañas, R. Pazos, J. Fernández-Ruiz, M.L. Bellido, E. Muñoz

19:00-19:15 O.1.3.

NOVEL CANNABIGEROL DERIVATIVES FOR THE TREATMENT OF NEURODEGENERATIVE AND NEUROINFLAMMATORY DISEASES. C. del Río, C. Navarrete, I. Cantarero, G. Appendino, F.J. Carrillo-Salinas, C. Guaza, C. Palomo-Garo, C. García, J. Fernández-Ruiz, J. Díaz-Alonso, J. Paraíso Luna, I. Galve-Roperh, M.L. Bellido, E. Muñoz

Cena libre

Viernes, 28 de noviembre

9:00-10:15 **Sesión de comunicaciones orales**
"Cannabinoides: desarrollo y metabolismo"
(Moderadores Javier Díaz Alonso y Juan Suárez)

9:00-9:15 Presentación (Javier Díaz Alonso)

9:15-9:30 O.2.1.

FUNCTION OF CB1 AND CB2 DURING OOCYTE MATURATION: IMPORTANCE ON FERTILIZATION AND EMBRYO DEVELOPMENT IN MOUSE. A.P. López-Cardona, N. Agirregoitia, A. Gutiérrez-Adán, E. Agirregoitia

9:30-9:45 O.2.2.

THE CB1 CANNABINOID RECEPTOR SIGNALS RADIAL MIGRATION OF PYRAMIDAL NEURONS IN THE DEVELOPING MOUSE CORTEX THROUGH THE MODULATION OF RHOA. J. Díaz-Alonso, A. de Salas-Quiroga, M. Parsons, C. Andradas, P. Garcez, D. García-Rincón, F. Guillemot, M. Guzmán, I. Galve-Roperh

9:45-10:00 O.2.3.

CORTICAL DEVELOPMENT ALTERATIONS INDUCED BY EMBRYONIC ∆9-TETRAHYDROCANNABINOL EXPOSURE IMPAIR SKILLED MOTOR ACTIVITY AND DISRUPT EXCITATORY-INHIBITORY BALANCE IN ADULT MICE. A. de Salas-Quiroga, J. Díaz-Alonso, D. García-Rincón, B. Lutz, L.F. Callado, M. Guzmán, I. Galve-Roperh

10:00-10:15 O.2.4.

DIHYDROLIPOAMIDE DEHYDROGENASE IS REGULATED BY THE BLOCKADE OF THE CANNABINOID CB1 RECEPTOR IN THE SKELETAL MUSCLE. S. Arrabal, M.Á. Lucena, M.J. Canduela, A. Serrano, F.J. Pavón, B. de Roos, P. Grandes, F. Rodríguez de Fonseca, J. Suárez

10:15-12:15 **Café y sesión de pósters**

12:15-13:30 **Sesión de comunicaciones orales**
"Cannabinoides: aspectos psiquiátricos (I)"
(moderadores: Alejandro Higuera Matas y Leyre Urigüen)

12:15-12:30 Presentación (Alejandro Higuera Matas)

12:30-12:45 O.3.1.

ROLE OF AKT SIGNALING PATHWAY IN CANNABIS-INDUCED PSYCHOSIS. I. Ibarra-Lecue, L.F. Callado, L.Urigüen

12:45-13:00 O.3.2.

CEREBELLAR CYCLOOXYGENASE-2 (COX-2) AND INTERLEUKIN 1β (IL-1β) MEDIATE THE CEREBELLAR FUNCTIONAL DEFICITS ASSOCIATED TO REPEATED CANNABIS EXPOSURE. L. Cutando, R. Maldonado, A. Ozaita

13:00-13:15 O.3.3.

CHRONIC STRESS IMPAIRS CANNABINOID 1 (CB_1) RECEPTOR-MEDIATED CONTROL OF GLUTAMATERGIC TRANSMISSION IN THE VENTRAL HIPPOCAMPUS OF YOUNG ADULT MICE. N. Royo, N. Puente, S. Peñasco, L. Reguero, M.J. Canduela, J.L. Mendizábal-Zubiaga, I. Elezgarai, A. Ramos, P. Grandes

13:15-13:30 O.3.4.

REPEATED THC ADMINISTRATION AFFECTS STRUCTURAL PLASTICITY IN THE HIPPOCAMPUS. V. Salgado-Mendialdúa, M. Gomis-González, R. Maldonado, A. Ozaita

13:30-15:15 Comida

15:15-16:15 Sesión de comunicaciones orales
"Cannabinoides: aspectos psiquiátricos (II)"

15:15-15:30 O.3.5.

A CHRONIC TREATMENT WITH Δ9 TETRAHYDROCANNABINOL ACCELERATES HABIT FORMATION IN MICE. M. Fernández-Cabrera, M. Ucha, E. Ambrosio, M. Miguéns, A. Higuera-Matas

15:30-15:45 O.3.6.

MODELLING EATING-ADDICTION IN MICE. S. Mancino, A. Burokas, J. Gutiérrez-Cuesta, M. Gutiérrez-Martos, E. Martín-García, M. Pucci, C. D'Addario, M. Maccarrone, R. Maldonado

15:45-16:00 O.3.7.

CB1 CANNABINOID RECEPTOR MEDIATES SPINE MORPHOLOGY CHANGES RELATED TO THE COGNITIVE DEFICTS OF THE NICOTINE WITHDRAWAL. R. Saravia, A. Flores, A. Plaza-Zabala, A. Busquets-García, A. Ozaita, R. Maldonado, F. Berrendero

16:00-16:15 O.3.8.

COGNITIVE DEFICITS IN AN ANIMAL MODEL OF DOWN SYNDROME RESPOND TO LOW DOSES OF CB1 RECEPTOR ANTAGONISTS. A. Navarro-Romero, M. Gomis-González, A. Busquets-García, M. Dierssen, R. Maldonado, A. Ozaita

16:15-17:45 Café y sesión de pósters

17:45-19:00 **Sesión de comunicaciones orales**
"Cannabinoides: aspectos neuroprotectores (I)"
(moderadores: Ruth Pazos y Nagore Puente)

17:45-18:00 Presentación (Ruth Pazos)

18:00-18:15 O.4.1.

CB_1 AND CB_2 SPECIFIC CONTRIBUTION TO THE PROGRESSION OF THE ALZHEIMER-LIKE PATHOLOGY IN APP/PS1 MICE. E. Aso, P. Andrés-Benito, M. Carmona, I. Ferrer

18:15-18:30 O.4.2.

THE MONOACYLGLYCEROL LIPASE INHIBITOR JZL184 IS NEUROPROTECTIVE AND ALTERS GLIAL CELL PHENOTYPE IN THE CHRONIC MPTP MOUSE MODEL. M. Celorrio, D. Fernández-Suárez, J.I. Riezu-Boj, A. Ugarte, R. Pacheco, H. González, J. Oyarzabal, C.J. Hillard, R. Franco, M.S. Aymerich

18:30-18:45 O.4.3.

CHARACTERIZATION OF A LRRK2-TRANSGENIC MOUSE MODEL FOR BIOCHEMICAL AND PHARMACOLOGICAL STUDIES AIMED AT DEVELOPING A CANNABINOID-BASED THERAPY IN PARKINSON'S DISEASE. C. Palomo-Garo, Y. Gómez-Gálvez, J. Fernández-Ruiz, C. García

18:45-19:00 O.4.4.

POTENTIAL NEUROPROTECTIVE ROLE OF SPECIFIC CB_1 RECEPTOR SUBPOPULATIONS IN THE MOUSE CORTICOSTRIATAL CIRCUITRY. A. Ruiz-Calvo, L. Bellocchio, A. Chiarlone, R. Bajo-Grañeras, E. Resel, I. Galve-Roperh, M. Guzmán

19:00 **Asamblea de la SEIC**

21:30 **Cena del congreso**

Sábado, 29 de noviembre

9:00-10:15 **Premios a las Mejores Publicaciones 2013**
(presentados por Ester Aso y Koldo Callado)
Mejor Publicación Predoctoral
Mejor Publicación Posdoctoral

10:15-11:30 **Sesión de comunicaciones orales**
"Cannabinoides: aspectos neuroprotectores (II)"

10:15-10:30 O.4.5.

CHANGES IN THE ENDOCANNABINOID SIGNALING SYSTEM IN CNS STRUCTURES OF TDP-43 TRANSGENIC MICE: RELEVANCE FOR A NEUROPROTECTIVE THERAPY IN TDP-43-RELATED DISORDERS. F. Espejo-Porras, M. Moreno-Martet, J.A. Ramos, J. Fernández-Ruiz, E. de Lago

10:30-10:45 O.4.6.

CHARACTERIZATION OF THE ENDOCANNABINOID SIGNALING SYSTEM IN A TRANSGENIC MOUSE MODEL OF SPINOCEREBELLAR ATAXIA TYPE-3 (SCA-3). C. Rodríguez-Cueto, M. Hernández-Gálvez, E. Moreno, A. Chiarlone, C.J. Hillard, P. Maciel, C. Lluis, M. Guzmán, P.J. McCormick, M. Gómez-Ruiz, J. Fernández-Ruiz

10:45-11:00 O.4.7.

PHARMACOLOGICAL AND GENETIC APPROACHES INVOLVING THE ENDOCANNABINOID SYSTEM IN THE TREATMENT OF FRAGILE X SYNDROME. M. Gomis-González, C. La Porta, A. Busquets-García, R. Maldonado, A. Ozaita

11:00-11:15 O.4.8.

DIFFERENTIAL BINDING OF LIGANDS TO CANNABINOID CB_2 RECEPTORS DETECTED BY TIME-RESOLVED FRET. I. Reyes-Resina, E. Martínez-Pinilla, J. Oyarzábal, R. Franco

11:15-11:30 O.4.9.

NEW MOUSE MODEL FOR THE STUDY OF CB_2 CANNABINOID RECEPTORS. C. Vázquez, M.C. García, R.M. Tolón, J. Fernández-Ruiz, B.N. Dittel, C.J. Hillard, J. Romero

11:30-12:00 **Pausa**

12:00-12:30 **Entrega de premios y Clausura**

12:30-13:45 **Visita guiada Cuenca**

13:45 **Aperitivo**

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PHARMACOKINETICS OF SATIVEX® IN DOGS: TOWARDS A POTENTIAL THERAPY IN CANINE DEGENERATIVE MYELOPATHY. M. Fernández-Trapero, F. Espejo-Porras, P.G. Urrutia-Cid, M. Moreno-Martet, E. de Lago, C. Pérez-Díaz, J. Fernández-Ruiz

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EVALUATING THE THERAPEUTIC POTENTIAL OF THE 2-AG HYDROLASE ABHD6 IN DEMYELINATION. A. Manterola, A. Bernal-Chico, M. Victoria Sánchez-Gómez, M. Canedo, K. Hsu, B. Cravatt, C. Matute, S. Mato

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HOMOBIVALENT LIGANDS FULLY SELECTIVE FOR CB_2 CANNABINOID RECEPTORS. P. Morales, L. Redondo-Gallego, M. Gómez-Cañas, M.R. Pazos, P. Goya, J. Fernández-Ruiz, N. Jagerovic

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P.18.
DIFFERENT MODULATION OF SPATIAL OR FEAR-CONDITIONED MEMORIES BY CB_1 AGONIST TREATMENT. M. Moreno, A. Llorente, I. Manuel, M.T. Giralt, R. Rodríguez-Puertas

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A NOVEL CANNABIDIOL DERIVATIVE INDUCES POLARIZATION OF M2 MACROPHAGES, HIF-1α STABILIZATION AND ALLEVIATES NEURO-INFLAMMATION IN DIFERENT MODELS OF MULTIPLE SCLEROSIS. C. Navarrete, F.J. Carrillo-Salinas, C. del Río, A. Feliú, M. Mecha, M.L. Bellido, I. Cantarero, B.L. Fiebich, C. Guaza, E. Muñoz

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CB_1 RECEPTOR SIGNALING IN EMBRYONIC STEM CELL DIFFERENTIATION INTO CORTICAL NEURONS. J. Paraíso-Luna, J. Díaz-Alonso, A. de Salas-Quiroga, D. García-Rincón, M. Guzmán, I. Liste, I. Galve-Roperh

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ETHANOL CONSUMPTION DURING ADOLESCENCE ALTERS EXCITATORY SYNAPTIC TRANSMISSION IN THE MOUSE VENTRAL HIPPOCAMPUS ACTING THROUGH CB_1 RECEPTORS. S. Peñasco, N. Puente, N. Royo, L. Reguero, M.J. Canduela, J. Mendizábal-Zubiaga, I. Elezgarai, A. Ramos, F. Rodríguez de Fonseca, P. Grandes

P.22.
THE ROLE OF ENDOCANNABINOID SYSTEM IN THE PATHOGENESIS OF ACETAMINOPHEN-INDUCED LIVER INJURY. P. Rivera, A. Vargas, J. Decara, J. Suárez, L. Sánchez, M.I. Lucena, F. Rodríguez de Fonseca

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NEUROPROTECTIVE EFFECT OF JZL184 IN MPP^+ TREATED SH-SY5Y CELLS THROUGH CB2 RECEPTORS. E. Rojo, C. Molina, M. Celorrio, R. Franco, M.S. Aymerich

P.24.
UP-REGULATION OF CB2 RECEPTORS IN LYMPHOCYTES OF PATIENTS WITH DRAVET SYNDROME. M. Rubio, S. Valdeolivas, E. Barroso, M. Montolio, A. Mingorance-Le Meur, J. Isla, L.M. Aras, O. Sagredo, J. Fernández-Ruiz

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BOTH, ANANDAMIDE AND OLEOYLETHANOLAMIDE INDUCE CELL PROLIFERATION AND APOPTOSIS IN THE BRAIN OF ALCOHOLIC RATS. P. Rivera, E. Blanco, L. Bindila, B. Lutz, F. Rodríguez de Fonseca, J. Suárez

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EVALUATION OF PHYTOCANNABINOIDS AS A DISEASE-MODIFYING THERAPY IN R6/2 MICE, A GENETIC MODEL OF HUNTINGTON'S DISEASE. S. Valdeolivas, O. Sagredo, J.A. Ramos, J. Fernández-Ruiz

P.27.
DIFFERENTIAL EFFECTS OF REPEATED ETHANOL BINGES ON THE ENDOCANNABINOID SYSTEM IN THE RAT LIVER VERSUS JEJUNUM. M. Vázquez, J. Suárez, Calleja-Conde, J.V. Echeverry-Alzate, P. Rivera, E. Giné, K.M. Bühler, A. Serrano, J.A. López-Moreno, F. Rodríguez de Fonseca

O.1.1.

CANNABIDIOL SHOWS A TEMPORARY THERAPEUTIC WINDOW LONGER THAN 12 HOURS IN HYPOXIC-ISCHEMIC NEWBORN MICE

N. Mohammed[1,2], M.R. Pazos[1,3], M. Ceprián[1,3], L. Jiménez[1], O.D. Saugstad[2], J. Martínez-Orgado[1]

[1]Instituto de Investigación Puerta De Hierro Majadahonda (Madrid), Spain, [2]Department of Pediatric Research, Oslo University Hospital. University of Oslo, Norway, [3]Departamento Bioquímica y Biología Molecular, CIBERNED, IRICYS, Facultad de Medicina, Universidad Complutense de Madrid, Spain

Background: cannabidiol (CBD) administered to newborn rodents 15 min after a hypoxic-ischemic (HI) insult leads to significant and long-term sustained neuroprotection.

Aim: to determine the temporary therapeutic window (TTW) of CBD, that is how long CBD administration can be delayed after HI without losing its neuroprotective effect. Such TTW is established in 6 h for the standard therapy, hypothermia.

Methods: 9-day old C57BL6 mice underwent a HI insult by being exposed to 10% oxygen for 90 min after electrocoagulation under anesthesia of the left carotid artery. Then, 0.1 mL of vehicle (ethanol:solutol:saline 2:1:17) (HV, n=25) or CBD (1 mg/kg) was administered s.c. 15 min, or 1, 3, 6, 12 or 24 h after the end of the HI insult (HC0.15 n=10; HC1, n=10; HC3, n=10; HC6, n=10; HC12, n=10; HC24, n=9, respectively). Seven days later pups were sacrificed, transcardially perfused with formaline 10% and their brains removed and stored in formaline. Then, the ipsilateral hemisphere volume (IHV) loss was calculated from T2W sequences of brain MRI scan. Then, the brains were processed for conventional (Nissl) staining to assess necrotic by a neuropathological score (NPS, from 0=no damage to 5=massive tissue damage). In addition the penumbral peri-lesional area (parieto-occipital cortex) was studied using TUNEL staining to assess apoptotic damage and GFAP immunohistochemistry to assess astrocyte viability. Non-HI mice served as controls (SHM, n=15).

Results: post-HI administration of CBD induced a neuroprotective effect as observed in all items (Table). CBD neuroprotection was apparent when CBD administration was delayed up to 12 h after HI. When CBD was administered 24 h after HI, some protective effect was still apparent regarding NPS and TUNEL, but not regarding IHV or astrocyte protection.

Item	***SHM***	***HV***	***HC0.15***	***HC1***	***HC3***	***HC6***	***HC12***	***HC24***
IHV (%)	0	*13.1(1.3)*	*5.1 (1)*	*6.4(1.8)*	*6.6(1.8)*	*6.4(0.9)*	*7.5(1.2)*	***10.0(1.7)***
NPS (pts)	0.5(0.2)	*2.9(0.2)*	1.0(0.5)	1.2(0.5)	*1.7(0.4)*	*1.6(0.3)*	*1.6(0.2)*	***2.0(0.4)***
TUNEL (n/field)	1 (0.9)	*12.3(0.7)*	0.7(0.2)	***1.7(0.5)***	***1.4(0.1)***	***2.8(1.3)***	***1.9(0.5)***	***7.4(1.2)***
GFAP (n/pixel)	.25(0.04)	*.75(.1)*	*.50(.06)*	*.48(.09)*	*.53(.09)*	*.42(.11)*	*.53(.08)*	***.59(.13)***

Italic: p<0.05 vs SHM. Underlined: p<0.05 vs. HV. **Bold: p<0.05 vs HC0.15**

Conclusions: CBD shows a TTW longer than usual for hypothermia. TTW for CBD seems to be between 12 and 24 h after the end of the HI insult.

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O.1.2.

NOVEL CANNABIDIOL DERIVATIVES FOR THE TREATMENT OF FIBROTIC DISEASES

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Scleroderma is a group of rare diseases that involve the hardening and tightening of the skin and connective tissues. There are two major forms of scleroderma: localized scleroderma and systemic sclerosis (SSc) that affect up to 30% of the patients. Scleroderma is associated with early and transient inflammation and vascular injury, followed by progressive fibrosis affecting both the skin and multiple internal organs. In fibroblasts, TGFβ stimulates collagen synthesis and myofibroblast transdifferentiation through the Smad signal transduction pathway. TGFβ-mediated fibroblast activation is the hallmark of scleroderma and related fibrotic conditions, and disrupting the intracellular TGFβ/Smad signaling may provide a novel approach to controlling fibrosis. Because of its potential role in modulating inflammatory and fibrotic responses both PPARγ and CB2 receptors represent atractive targets for the development of cannabinoid-based therapies.

We have previously found that resorcinyl-to-paraquinol oxidation of CBG (VCE-003) and CBD (HU-331) increases their PPARγ agonistic activities. However both VCE-003 and HU-331 are unstable electrophilic compounds, with limited prospects further development. As part of our study on the SAR of CBD we have generated novel non-electrophilic CBD quinol derivatives that activate PPARγ. From this series we have selected compound CDB-Q-8 for further characterization and to study efficacy on skin fibrotic animal models. CDB-Q-8 is a dual agonist of PPARγ and CB2, a combination of pharmacodynamic properties exceptionally interesting for disorders with elevated inflammation. CDB-Q-8 inhibits TGFβ-induced Col1A2 gene transcription and collagen synthesis through a pathway that is independent of Smad2 phosphorylation and prevents myofibroblast differentiation induced by TGFβ. To study the anti-fibrotic efficacy *in vivo* we used a bleomycin-induced murine model of scleroderma. Our results show a key role of this compound as a reducing agent of both dermal thickness and blood vessels associated collagen, and this effect was prevented in the presence of either T0070907 (PPARγ antagonits) or AM630 (CB2 antagonist). Using a fibrosis specific RT² Profiler PCR Array we found that CDB-Q-8 downregulates the expression of several genes associated with the fibrotic processs. In conclusion, this study highlights the therapeutic potential of CBD quinone derivatives for the treatment of fibrotic diseases such as SSc.

O.1.3.

NOVEL CANNABIGEROL DERIVATIVES FOR THE TREATMENT OF NEURODEGENERATIVE AND NEUROINFLAMMATORY DISEASES

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Different plant-derived and synthetic cannabinoids have shown to be neuroprotective in different experimental animal models of neurodegeneration and neuroinflammation through cannabinoid receptors (CBs)-dependent and -independent mechanisms. We have previously found that resorcinyl-to-paraquinol oxidation of CBG (VCE-003) increases its PPARγ agonistic activities and acts as immunosuppressor. VCE-003 alleviates neuroinflammation in EAE and TMEV infection models of MS. However VCE-003 is an unstable electrophilic compound, with limited prospects further development. As part of our study on the SAR of CBG we have generated novel non-electrophilic VCE-003 derivatives that activate PPARγ. From this series we have selected the CBG-Q-2 for further characterization and to study efficacy on neurodegenerative and neuroinflammatory murine models.

CBG-Q-2 is a PPARγ agonist with neuroprotective activity in vitro and in vivo. We have found that this compound enhances neural stem HiB5 cell survival during their neuronal differentiation and protects them against excitoxicity induced cell death by quinolinic acid treatment. In addition CBG-Q-2 prevents quinolinic acid-induced inhibition of mitochondrial activity and caspase-3 activation that paralleled with an increase in the percentage of $PCNA^+$ cells. This compound also protect from glutamate-, H_2O_2-, and mut-Huntinting-induced cytotoxicity in N2A, NSC34 and ST cells respectively. This compound was extremely active as neuroprotectant in mice intoxicated with 3-nitropropionate (3NP), improving motor deficits and preserving striatal neurons against 3NP toxicity. In addition, CBG-Q-2 attenuated the reactive microgliosis and the upregulation of proinflammatory markers induced by 3NP, and improved the levels of antioxidant defenses that were also significantly reduced by 3NP. We also found that CBG-Q-2 alleviates the motor akinetic symptomatology produced by a unilateral injection of 6-OH-DA into the striatum, and prevents also the reduction of tyrosine hydroxylase immunoreactivity in the affected substantia nigra. Finally we have also investigated the effect of this compound in two different models of MS, namely EAE and Theiler's murine encephalomyelitis virus (TMEV) model. In both models this compound alleviates symptomatology being more effective in EAE that in TMEV. In conclusion, this study highlights the therapeutic potential of non-electrophilic CBG-quinone derivatives for the treatment of neurodegenerative and neuroinflammatory diseases.

O.2.1.

FUNCTION OF CB1 AND CB2 DURING OOCYTE MATURATION: IMPORTANCE ON FERTILIZATION AND EMBRYO DEVELOPMENT IN MOUSE

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Many countries have begun to use immature oocytes in human assistant reproduction. But the results on pregnancies rate and deliveries are very low. The reason of those poor results could be that the oocyte is a complex cell and even today the whole biochemical and physiological process necessary for successful oocyte maturation is not totally understood. There are some evidences indicating that endocannabinoids plays an important role in cellular communication during the embryo implantation, but nothing is known about the role played by this system in the maturation of oocyte, a key process in achieving potentially fertilizable cells. Our study aims at analysing the involvement of cannabinoids in in vitro maturation IVM of mice oocytes to verify if the presence of cannabinoids in the culture media improves in vitro fertilization (IVF) rates and the subsequent in vitro development into blastocyst stage.

Oocytes in germinal vesicle (GV), metaphase I and metaphase II were analyzed by immunofluorescence and real time PCR to search the protein and mRNA from both receptors. Wild type oocytes in GV stage were incubated with CB1 agonist (HU-210) and antagonist (SR141716) and CB2 agonist (JHW-015) and antagonist (SR144528) during maturation in medium TCM-199 with EGF and 10% FCS, then we fertilized and cultured in vitro until blastocyst stage. Finally, KOCB1 and KOCB2 oocytes from in vivo and in vitro maturation were fertilized and we analyzed the embryo development to blastocyst.

According to our results, cannabinoid receptors CB1 and CB2 are present in mice oocytes during the different stages of both in vitro and in vivo oocyte maturation. In fact, the dose-dependence pharmacological activation of CB1 receptor during IVM using HU-210 synthetic cannabinoid, demonstrated that this activation is involved in a more efficient IVF and in a better in vitro development of the blastocyst coming from IVM oocytes since this effect was reverted by the antagonist SR141716. Moreover, the kinases PKB/Akt and ERK1/2, involved in different signaling pathways of meiosis, were differently regulated after cannabinoid treatment of oocytes at 1 hour and 17 hours of IVM, compared with vehicle treatment. On the other hand, the activation of CB2 with the agonist JHW-015 did not show difference in both the embryo development and the modulation of the studied kinases.

When we used in vivo maturation oocyte from knockout models (CB1-/- and CB2-/-) we observed that the absence of CB1 decreased the rate of achieved blastocyst, whereas the lack of CB2 did not have any effect comparing with wild type mouse. Nevertheless, these differences did not appear when we used the in vitro maturation model.

The identification of pathway(s) by which cannabinoids mediate the IVM of oocytes and/or the in vitro development of the fertilized egg, will provide an interesting target to test if the poor quality of human oocytes obtained by IVM could be improved using new culture media supplemented with cannabinoids.

O.2.2.

THE CB_1 CANNABINOID RECEPTOR SIGNALS RADIAL MIGRATION OF PYRAMIDAL NEURONS IN THE DEVELOPING MOUSE CORTEX THROUGH THE MODULATION OF RHOA

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The appropriate formation of the stereotypical 6-layered mature mammalian neocortex critically depends on the proper migration of newborn pyramidal cells. Our knowledge about the mechanisms that link extrinsic signals to the activity of the cell-autonomous factors that ultimately trigger the pro-migratory molecular cascade is still incomplete. Endocannabinoids, acting via their CB_1 receptors tune several cortical developmental processes such as progenitor cell proliferation and identity, neural specification and morphogenesis. In addition, they have been suggested to regulate neuronal migration, but the precise molecular mechanisms remain unclear.

In this study we first investigated the potential chemoattractant profile of the endocannabinoids for newborn pyramidal cells. Using embryonic cortical explants we show that neuronal migration is favored towards a source of endocannabinoids compared with the corresponding control. To explore the cell-autonomous role of CB_1 receptors in the control of radial migration we performed *in utero* electroporation-mediated CB_1 knockdown. Delayed radial migration of siRNA-CB_1 electroporated cells was consistently observed when compared to siControl electroporated cells. Interestingly, CB_1 signaling was found to promote radial migration in different pyramidal neurogenic waves, independently of CB_1-mediated progenitor cell proliferation. Finally, the migration deficits induced by CB_1 loss of function were rescued by concomitant silencing the small G protein RhoA or coexpression of a non-phosphorylatable form of cofilin, a downstream effector of RhoA. Overall, our results show that endocannabinoid signaling through CB_1 receptors drives radial migration in the developing mouse cortex, and identify the involvement of RhoA and downstream regulation of actin cytoskeleton dynamics as key mediators of CB_1 receptor pro-migratory signaling.

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O.2.3.

CORTICAL DEVELOPMENT ALTERATIONS INDUCED BY EMBRYONIC Δ^9-TETRAHYDROCANNABINOL EXPOSURE IMPAIR SKILLED MOTOR ACTIVITY AND DISRUPT EXCITATORY-INHIBITORY BALANCE IN ADULT MICE

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Developmental exposure to plant-derived cannabinoids induces long-lasting behavioral alterations in mice and humans. The neuropathogenic consequences elicited by developmental exposure to phytocannabinoids range from impulsive behavior, cognitive impairment or social deficits that influence the risk of neuropsychiatric disorders. However, the developmental mechanisms of Δ^9-tetrahydrocannabinol (THC) action remain obscure. In this study we investigated the functional consequences of CB_1 signaling alterations evoked by embryonic THC exposure (daily i.p. 3 mg/Kg injection to pregnant females from E12.5 to E16.5) in wild-type (WT) and CB_1 cannabinoid receptor-deficient ($CB_1^{-/-}$) mice. Immunofluorescence analysis showed that embryonic cannabinoid administration induced corticofugal projection neuron alterations. In addition, evaluation of retrogradely-labeled corticospinal motor neurons (CSMNs) further supported these findings. Subsequent evaluation of motor activity in the adult offspring revealed that embryonic THC exposure impairs skilled motor function in WT, but not $CB_1^{-/-}$ mice. These findings suggest that subchronic *in utero* THC exposure leads to reduced functional CB_1 signaling, as further demonstrated by reduced CB_1 protein levels in THC-exposed animals. We sought to investigate other plausible cellular and behavioral consequences of embryonic THC exposure, as the excitatory/inhibitory balance of neuronal activity. The latency to pentylenetetrazol-induced seizures was decreased in THC-exposed WT, but not $CB_1^{-/-}$ mice, which were sensitized irrespective of the treatment. Subsequent analysis revealed a reduction in total number and a shift in the distribution of hippocampal GABAergic cholecystokinin-containing interneurons in THC-treated animals. To elucidate the differential contribution of the two main neuronal populations, excitatory and inhibitory, in THC-induced development alterations, ongoing experiments using newly developed transgenic mice allowing the conditional rescue of CB_1 receptor expression in both populations are under analysis. Overall, the findings above support that prenatal cannabinoid exposure has a profound impact on the embryonic brain that persists in the adulthood, owing to alterations in the control exerted by the CB_1 cannabinoid receptor in the differentiation of corticofugal projection neurons and the appropriate integration of excitatory and inhibitory neuronal populations.

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O.2.4.

DIHYDROLIPOAMIDE DEHYDROGENASE IS REGULATED BY THE BLOCKADE OF THE CANNABINOID CB1 RECEPTOR IN THE SKELETAL MUSCLE

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Background and aims: Cannabinoid CB1 receptors directly modulate energy metabolism in the brain and peripheral tissues. Here we analyzed the presence of CB1 receptors in the skeletal muscle and the effects of the CB1 blockade on the muscle metabolism.

Methods and Results: Through a proteomic approach involving 2-D gel electrophoresis and MALDI-TOF mass spectrometry, we identified seven key glucose/pyruvate/citric acid-metabolizing enzymes, such as glucose 6-phosphate isomerase (GPI), the triosephosphate isomerase (TPI), the enolase (Eno3), the lactate dehydrogenase (LDHa), the glyoxalase 1 (Glo1) and the mitochondrial dihydrolipoamide dehydrogenase (DLD), which expressions were modified by the CB1 receptor antagonist AM251 in a diet-dependent fashion. Specifically, the systemic and repeated (14 days) administration of AM251 (3 mg/kg) blocked the high-carbohydrate diet (HCD)-induced increase of the protein expression of GPI, TPI, Eno3 and LDHa, suggesting a down-regulation of glucose/pyruvate/lactate pathway in a highly-glucose context. In contrasts, AM251 alleviated the HCD-induced decrease of the protein expression of Glo1, suggesting an upregulation of the methylglyoxal pathway in a probably highly-cytotoxic context. Interestingly, both the antagonism and deletion of the CB1 receptors displayed changes in the expression of the muscle DLD, an E3 component of the mitochondrial pyruvate/α-ketoglutarate dehydrogenase complexes. These data could be associated by the specific presence of CB1 receptors in the skeletal muscle mitochondria.

Conclusions: The outstanding results indicated that the overexpression of DLD by the CB1 receptor blockade under a highly-carbohydrate context can reveal a new mechanism of action on energy expenditure through the muscle mitochondria.

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O.3.1.

ROLE OF AKT SIGNALING PATHWAY IN CANNABIS-INDUCED PSYCHOSIS

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Cannabis is one of the most commonly used illicit drugs in the world. There is increasing evidence suggesting that cannabis use may be a risk factor for the development of psychosis and/or schizophrenia. However, the relationship between cannabis abuse and psychotic illness remains unclear.

Alterations in intracellular signaling pathways are important key targets for treating complex neuropsychiatric disorders. Akt signaling has emerged as the focal point for many signal-transduction pathways, regulating multiple cellular processes including transcription, apoptosis, endoplasmic reticulum stress response and cell proliferation. There is converging evidence indicating that genetic polymorphisms affecting Akt signaling may be a risk factor for mental illness. Thus, it has been described that genetic variations in *AKT1* may somehow mediate the psychotic effects associated with the use of cannabis, possibly through a mechanism involving cannabinoid-regulated Akt signaling. Importantly, it has been described that cannabinoids are able to activate the Akt pathway by acting on CB1 and CB2 receptors. Moreover, THC administration in mice activates Akt in several brain areas.

The objective of the present work was to evaluate the effects of THC chronic administration on psychotic symptoms and Akt signaling cascade. For that purpose, young mice (PN35) were chronically treated with THC (10 mg/kg) for 30 days. Psychotic-like behavior and protein expression of Akt, ribosomal protein s6 and their phosphorylated forms were evaluated in these mice. Control mice received a saline solution for the same period.

Mice were subjected to the prepulse inhibition (PPI) test in order to evaluate sensorimotor gating processes. Chronic THC (10 mg/kg) induced a significant decrease in the PPI response at 85 and 90 db when comparing to saline controls ($p<0.05$).

The immunoreactivity of phosphorylated forms of Akt and S6 proteins was significantly increased in THC treated mice when comparing to controls ($p<0.05$) while no changes were observed in their total forms.

Our findings demonstrate that chronic exposure to THC in young mice induces a psychotic-like behavior, and support the proposal that Akt signaling may contribute somehow to the cannabis-induced psychosis in mice.

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O.3.2.

CEREBELLAR CYCLOOXYGENASE-2 (COX-2) AND INTERLEUKIN 1β (IL-1β) MEDIATE THE CEREBELLAR FUNCTIONAL DEFICITS ASSOCIATED TO REPEATED CANNABIS EXPOSURE

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Chronic use of marijuana has been associated to cerebellar dysfunction in humans. We previously demonstrated that cannabis withdrawal triggers microglial reactivity in the cerebellar molecular layer, as well as an enhancement in the expression of some neuroinflammatory molecules such as interleukin 1β (IL-1β) cytokine or cyclooxygenase-2 (COX-2) enzyme. It has been described that neuronal COX-2 is expressed in cerebellar Purkinje cells and its expression is up-regulated following brain insults, via glutamatergic and inflammatory mechanisms. Furthermore, it has been shown that IL-1β acts on IL-1 receptor expressed in Purkinje cells in order to modulate their excitability. Moreover, repeated exposure to delta9-tetrahydrocannabinol (THC), as well as cannabis withdrawal, produce cerebellar functional deficits when fine motor coordination is assessed using footprint, beam walking and hanger tests. To establish the molecular mechanisms underlying these cerebellar deficits in fine motor coordination tasks, we assessed the expression of ionotropic glutamate receptor 2 (GluR2) and glutamate receptor delta 2 (GluRδ2) two receptors involved in cerebellar long-term depression (LTD), a cellular mechanism underlying cerebellar function. The expression of both receptors was found enhanced in the cerebellum under conditions of chronic THC exposure.The sub-chronic COX-2 enzyme inhibitor administration NS-398 (10mg/kg; 5 days; once per day), as well as, the administration of an IL-1R antagonist (100mg/kg; 3 days; once per day) could prevent the motor coordination alterations observed in the THC-withdrawn mice. Interestingly, the IL-1R antagonist administration also modifies GluR2 expression pointing to this receptor as a possible mechanism to explain the functional alterations associated with cannabis consumption. These results suggest the important role of COX-2 enzyme and the IL-1 receptor in the performance of cerebellar-dependent tasks, revealing that both mechanisms are crucial in the cerebellar deficits associated to repeated cannabis exposure.

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O.3.3.

CHRONIC STRESS IMPAIRS CANNABINOID 1 (CB_1) RECEPTOR-MEDIATED CONTROL OF GLUTAMATERGIC TRANSMISSION IN THE VENTRAL HIPPOCAMPUS OF YOUNG ADULT MICE

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Increasing evidences suggest that the endocannabinoid (eCB) system plays a role in stress responses. Genetic disruption of the endocannabinoid signaling by knocking out the cannabinoid 1 receptor (CB_1R) increases activity of the hypothalamic-pituitary-adrenal (HPA) axis, sensitizing animals to stress. Furthermore, hippocampal CB_1 receptor expression and CB_1 binding capacity have been reported to be altered due to chronic stress. In addition, several neurophysiological studies reveal changes in CB_1 receptor activity following stress. Despite these findings, little is known about how chronic and acute stress affects neuronal function and, in particular, the eCB-mediated synaptic transmission in the dentate gyrus of the ventral hippocampus, a brain region highly involved in stress responses.

To investigate this, we have performed *ex vivo* electrophysiological recordings in slices containing the ventral hippocampal dentate gyrus of young adult male mice subjected to either acute or chronic stress. A restraint model of stress was used (Patel *et al*., 2009).

Extracellular field recordings were performed in the supragranular zone of the dentate molecular layer of control, acute and chronic stressed mice to measure the effects of the CB_1R agonist CP 55,940 (10 μM) on field excitatory postsynaptic potentials (fEPSP). These potentials were evoked in the presence of the $GABA_A$ antagonist picrotoxin (100 μM), by stimulating the medial dentate perforant path. In non stressed mice, CP55,940 reduced fEPSP amplitude to 20.51 ± 7.06% of baseline ($p < 0.001$; n=5), illustrating that CB_1 receptor activation decreases glutamatergic synaptic transmission, as expected. After an acute restraint stress the effectiveness of CP55, 940 was only slightly reduced (fEPSP amplitude: 12.86 ± 3.96% of baseline; $p < 0.05$, $n = 5$). However, after repetitive exposure to stress (chronic stress) the excitatory synaptic transmission modulated by presynaptic CB_1 receptors was completely impaired (fEPSP amplitude: 3.46 ± 5.91% of baseline; $p < 0.05$, $n = 5$).

The results suggest that exposure of young adult mice to chronic stress impairs CB_1 receptor-mediated excitatory neurotransmission at the entorhino-dentate pathway. This synaptic alteration is not observed under conditions of acute stress.

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O.3.4.

REPEATED THC ADMINISTRATION AFFECTS STRUCTURAL PLASTICITY IN THE HIPPOCAMPUS

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Δ9-tetrahydrocannabinol (THC), the main psychoactive component of *Cannabis sativa* plant, causes memory impairment through the activation of CB1 cannabinoid receptors. These amnesic-like effects do not show tolerance after repeated exposure to THC, and are persistent for a few days after THC withdrawal. Memory formation and consolidation trigger alterations at excitatory synapses leading to dendritic spine restructuration in terms of shape and density, a phenomenon called structural plasticity. In this regard, structural plasticity depends on local synaptic regulation of protein synthesis and cytoskeleton re-arrangements where actin and its related proteins play an important role. The aim of this study is to analyse whether repeated exposure to THC has any effect on structural plasticity in the hippocampus, a brain area involved in memory formation, by using Thy1-EGFP transgenic mice that express enhanced green fluorescent protein (EGFP) in the principal neurons of the brain. This approach allows monitoring the analysis of dendritic spine shape and density to analyse possible changes after a pharmacological treatment. THC sub-chronic administration (10mg/kg i.p., 7 days) decreased the number of mushroom dendritic spine shape (mature form) and increased the thin and stubby shapes (immature forms). Interestingly, proteins involved in actin cytoskeleton modulations such as myristoylated alanine-rich C-kinase substrate (MARCKS), neurogranin, cofilin and profilin changed their expression and/or phosphorylation state after THC treatment. Mature forms of dendritic spines have been correlated to consolidated memory while immature forms were related to learning process. Therefore, our results support the hypothesis that a chronic THC treatment impairs memory consolidation and point to the key role played by actin cytoskeleton and its related proteins in this unwanted side-effect produced by THC.

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O.3.5.

A CHRONIC TREATMENT WITH Δ-9 TETRAHYDROCANNABINOL ACCELERATES HABIT FORMATION IN MICE

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Classic studies on instrumental conditioning have shown that two different processes control the acquisition and performance of actions that lead to rewards. During the early stages of acquisition, instrumental performance is normally goal-directed and sensitive to post-conditioning changes in reward value but as training proceeds, response control is ceded to the habit system and as a consequence becomes less sensitive to changes in reward value. These two modes of action seem to be controlled by separate neural systems. Indeed, current theories in the field state that initial goal-oriented behaviour would be mediated by the posterior dorsomedial striatum in concert with the prelimbic cortex while the control of behaviour by habits would rely on the integrity of the dorsolateral striatum and the infralimbic cortex. Given that CB_1 receptors are present in the dorsal striatum in a lateral-to-medial gradient of expression and that CB_1 KO mice show a decreased predisposition for habit formation, we hypothesized that a chronic treatment with Δ-9 tetrahydrocannabinol (THC) might accelerate the formation of habits in mice. In order to test this hypothesis, we treated adult male C57BL6/J mice with THC (10mg/ml/kg i.p.) or vehicle for fourteen days and submitted them to a minimal instrumental acquisition protocol that has been shown to induce goal-directed behaviour. We show that while vehicle-treated animals display the expected goal-directed behaviour (their lever presses decreased during the devaluation test), THC animals are insensitive to the decreased value of the expected consequence, a compelling evidence for habit-like behaviour. These results might be of relevance to conditions where abnormal habit formation has a central role such as addiction or obsessive-compulsive disorder.

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O.3.6.

MODELLING EATING-ADDICTION IN MICE

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An increasing perspective conceptualizes obesity and overeating as disorders related to addictive processes that are associated with specific brain changes. Certain commonalities exist between eating and drug use, such as mood alteration, external cue-control of appetite or motivation for reinforcement. Addiction is a chronic brain disorder characterized by an impaired ability to regulate the drive to obtain and use the drug, the onset of relapse; at present, it is discussed whether or not specific food eating disorders should be viewed as addictive processes. To explore the behavioural hallmarks and the neurobiological basis of eating-addiction, it is essential to validate a reliable animal model of addictive-like behaviour to palatable food. An animal model of drug-addiction has been recently developed in rats, based on the DSM-V criteria of substance dependence. In the present study, an animal model of eating addictive-like behaviour was validated in mice under operant conditioning, maintained by chocolate-flavoured pellets. Persistence of food seeking during a period of signalled no availability of food, and motivation for drug seeking and perseverance of the mouse's responding when the reward was associated with a punishment were evaluated. This model was used with the purpose of identifying two different and extreme populations of mice related to addiction score: addict population from non-addict population. We investigated the epigenetic changes in these two different populations. DNA methylation at CB_1 and CB_2 gene promoters were analyzed by real-time methylation-specific PCR. Preliminary results revealed that DNA methylation of CB_1 gene promoter region was different between addict and non-addict animals in prefrontal cortex and nucleus accumbens. Indeed, addicted mice trained with chocolate-flavoured pellets showed decreased DNA methylation of CB_1 gene promoter, which could correspond to a gene up-regulation expression of CB_1 receptor in prefrontal cortex. Furthermore, a significant down-regulation of CB_1 gene expression in the nucleus accumbens with a consistent increase in DNA methylation at gene promoter region of CB_1 receptor was observed. Furthermore, no significant differences were observed for DNA methylation at CB_2 gene promoters in prefrontal cortex and striatum. This study provides new evidence for a better understanding of the neurobiological mechanisms that may lead to addictive-like behaviour related to food intake.

O.3.7.

CB1 CANNABINOID RECEPTOR MEDIATES SPINE MORPHOLOGY CHANGES RELATED TO THE COGNITIVE DEFICTS OF THE NICOTINE WITHDRAWAL

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Withdrawal from nicotine causes somatic, affective and cognitive symptoms, including impaired attention and memory. Indeed, these cognitive deficits are gaining attention, since relapse to tobacco use after a period of abstinence may occur to ameliorate this cognitive impairment. The objective of this study was to investigate the possible neurobiological mechanisms underlying this nicotine effect. Nicotine was administered by using Alzet osmotic minipumps (25 mg/kg/day/14days) and withdrawal was precipitated by the administration of the nicotinic receptor antagonist, mecamylamine (2 mg/kg). A deficit in memory consolidation was observed when animals performed the object recognition task 24 hours after the precipitation of withdrawal, and this cognitive deficit was still present at least during 4 days. Interestingly, memory impairment was abolished by the administration of the CB1 cannabinoid receptor antagonist rimonabant (1 mg/kg), and in CB_1 knockout mice. Memory impairment associated with nicotine abstinence was also prevented by the administration of mGluR5 antagonist MTEP (1 mg/kg), the mTOR inhibitor temsirolimus (1 mg/kg) and the inhibitor of protein synthesis anisomycin (8 mg/kg). Moreover, chronic administration of rimonabant (1 mg/kg/day) during 4 days blocked the memory deficits 4 days after the precipitation of withdrawal. Synaptic plasticity was evaluated by measuring changes in dendritic spine density in neurons of hippocampus (CA1) and prefrontal cortex 4 days after the precipitation of nicotine abstinence. A decrease in the density of mushroom spines was found in the hippocampus of abstinent animals to nicotine. Interestingly, this decrease in mushroom spines was blocked by the chronic administration of rimonabant. These results suggest that the activation of the CB1 cannabinoid receptor could be, at least in part, responsible for the cognitive deficits and changes in synaptic plasticity observed during nicotine abstinence.

O.3.8.

COGNITIVE DEFICITS IN AN ANIMAL MODEL OF DOWN SYNDROME RESPOND TO LOW DOSES OF CB1 RECEPTOR ANTAGONISTS

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Down syndrome is the most common form of intellectual disability in humans affecting around 1 in 1,000 live births worldwide. It is caused by the complete or partial trisomy of chromosome 21 leading to the affection of multiple organs and systems. Nowadays, there is no therapeutic approach available to treat cognitive impairment, the most limiting phenotype of this syndrome. CB1 cannabinoid receptors have been proposed as a target to improve cognitive performance in fragile X syndrome. This genetic disorder shares common features with Down syndrome, such as the intellectual disability, an unbalance between excitatory/inhibitory inputs and an anomalous dendritic arborization. Therefore, we hypothesized that CB1 cannabinoid receptor blockade could also improve the cognitive performance in Down syndrome. To test this hypothesis we used a mouse model of Down syndrome that over-expresses Dyrk1A (TgDyrk1A), one of the genes which expression is enhanced in the trisomic condition and that has a relevant signaling activity in the central nervous system. These mice showed a clear impairment in the novel object recognition memory test compared to their wild-type littermates. This impairment was not normalized by a single administration of rimonabant (1 mg/kg). However, it was prevented after a sub-chronic treatment (7 days). Interestingly, a dose of rimonabant as low as 0.1 mg/kg (7 days) was able to normalize the cognitive performance of TgDyrk1A mice in this test. The expression of the components of the endocannabinoid system (CB1 cannabinoid receptors, diacylglycerol lipase, monoacylglycerol lipase, N-acyl phosphatidylethanolamine phospholipase D or fatty acid amide hydrolase) assessed in this transgenic model of Down syndrome did not show specific differences compared to wild-type littermates. However, similar to other models of pathologies displaying cognitive deficits associated to dendritic spine abnormality, we observed that the TgDyrk1A model presents an enhanced activation of the mTOR signaling pathway according to immunoblot data from hippocampal tissue. Therefore, we hypothesize that mTOR over-activity may contribute to the cognitive deficit in TgDyrk1A, and that antagonizing CB1 receptors may normalize the mTOR signaling, an important mechanism in dendritic spine turnover, supporting the normalization of cognitive performance. These data suggest that the modulation of the endocannabinoid system have potential therapeutic interest to treat cognitive deficits in Down syndrome.

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O.4.1.

CB_1 AND CB_2 SPECIFIC CONTRIBUTION TO THE PROGRESSION OF THE ALZHEIMER-LIKE PATHOLOGY IN APP/PS1 MICE

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Growing evidence indicates that the pharmacological stimulation of the endogenous cannabinoid system represents a promising therapy to curb several neurodegenerative processes associated to Alzheimer's disease (AD). The aim of our study was to compare the specific contribution of CB_1 and CB_2 receptors, the main targets of the cannabinoid-based therapies, to the progression of AD-like pathology in an animal model.

Two new mouse strains were generated by crossing APP/PS1 mice, a transgenic model of AD, with CB_1 and CB_2 knockout mice. The cognitive performance and some pathological hallmarks were evaluated at different ages (3 and 6 months) in both colonies. The neonatal viability of APP/PS1/Cnr1$^{-/-}$ was drastically reduced and the few mutants that were born died before 2 months of age. APP/PS1/Cnr1$^{+/-}$ were viable but exhibited an accelerated cognitive impairment from 3 months of age. APP/PS1/Cnr2$^{-/-}$ developed normally and exhibited similar cognitive performance than CB_2 sufficient transgenic mice. Moreover, a cannabis-based medicine was equally effective in CB_2 mutant transgenic mice than in their corresponding controls. However, higher Aβ burden and gliosis were observed in the cortex of non-treated APP/PS1/Cnr2$^{-/-}$ mice.

In conclusion, our results indicate that CB_1 receptor plays a prominent role in the progression of AD pathology since its deficiency compromises the viability of APP/PS1 mice and accelerates their cognitive impairment. In contrast, CB_2 receptor deficiency does not alter APP/PS1 mice cognitive performance or cannabis-based medicine therapeutic properties but exacerbates the Aβ-related pathology. Thus, a potential cannabinoid-based therapy against AD should target both CB_1 and CB_2 receptors in parallel in order to take advantage of the differential neuroprotective properties of these endogenous cannabinoid system components.

O.4.2.

THE MONOACYLGLYCEROL LIPASE INHIBITOR JZL184 IS NEUROPROTECTIVE AND ALTERS GLIAL CELL PHENOTYPE IN THE CHRONIC MPTP MOUSE MODEL

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Changes in cannabinoid receptor expression and concentration of endocannabinoids have been described in Parkinson's disease; however, it remains unclear whether they contribute to, or result from, the disease process. To evaluate whether targeting the endocannabinoid system could provide potential benefits in the treatment of the disease, the effect of a monoacylglycerol lipase inhibitor that prevents degradation of 2-arachidonyl-glycerol was tested in mice treated chronically with probenecid and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTPp). Chronic administration of the compound, JZL184 (8 mg/kg), prevented MPTPp-induced motor impairment and preserved the nigrostriatal pathway. Furthermore, none of the hypokinetic effects associated with cannabinoid receptor agonism were observed. In the striatum and substantia nigra pars compacta, MPTPp animals treated with JZL184 exhibited astroglial and microglial phenotypic changes that were accompanied by increases in TGFβ messenger RNA expression and in glial cell-derived neurotrophic factor messenger RNA and protein levels. JZL184 induced an increase in β-catenin translocation to the nucleus, implicating the Wnt/catenin pathway. Together, these results demonstrate a potent neuroprotective effect of JZL184 on the nigrostriatal pathway of parkinsonian animals, likely involving restorative astroglia and microglia activation and the release of neuroprotective and antiinflammatory molecules.

O.4.3.

CHARACTERIZATION OF A LRRK2-TRANSGENIC MOUSE MODEL FOR BIOCHEMICAL AND PHARMACOLOGICAL STUDIES AIMED AT DEVELOPING A CANNABINOID-BASED THERAPY IN PARKINSON'S DISEASE

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Most of cases of Parkinson's disease (PD) are of sporadic origin and caused predominantly by overexposure to some environmental factors, e.g. pesticides. However, some forms of parkinsonism are the consequence of dominant or recessive mutations in specific genes, e.g. α-synuclein, parkin and, more recently, leucine-rich repeat kinase 2 (LRRK2), whose G2019S mutation represents the most prevalent form of late-onset, autosomal dominant familial PD. LRRK2 gene encodes a complex protein with several protein interaction motifs and two enzymatically-active domains. This protein has been related to glial-derived inflammatory responses, but physiological substrates of LRRK2 are still under investigation. A transgenic mouse model expressing the G2019S mutation of LRRK2 has been recently developed (Ramonet *et al*. PLoS One 2011). It likely represents a valuable experimental model for studying PD, as the pathological phenotype (e.g. motor impairment and degeneration of nigrostriatal dopaminergic neurons) in these animals does not appear up to animals have 18 months of age, which translated to humans represents approximately 60-65 years, the age at which the disease appears in patients. We have designed a long-term study with these animals aimed: (i) at elucidating the changes experienced by the endocannabinoid signaling system in the basal ganglia during the progression of the disease in these mice, and (ii) at evaluating the potential of cannabinoids, in particular compounds selectively targeting the CB_2 receptor, as disease-modifying agents in these mice. The study is still in progress but some first data may be already presented. First, we followed the progression of motor impairment in these mice using different behavioral tests, e.g. actimeter, rotarod, hanging-wire and pole tests. We were able to find some alterations, e.g. worse rotarod performance, subtle hypokinesia evident in actimeter responses and, in particular, a strong deficiency in the hanger-wire test reflecting dystonia, that were already evident at ages (8 months) earlier than those previously expected (Ramonet *et al*. PLoS One 2011). Second, we collected the basal ganglia of LRRK2-transgenic and wild-type mice at the ages of 6 and 12 months to compare the status of some endocannabinoid elements. Our data indicate that the expression of CB_2 receptors and FAAH and MAGL enzymes is, in general, similar in the basal ganglia of LRRK2-transgenic and wild-type mice at these two ages. In parallel, we did not detect any evidence of microgliosis (Iba-1 immunostaining) and of injury in tyrosine hydroxylase-containing nigral neurons, but we expect this occurs when animals become 18 month-old, the age at which Ramonet *et al*. (PLoS One 2011) situated the signs of nigral degeneration, and that this represents important changes in the endocannabinoid signaling. Lastly, we are also conducting experiments with compounds targeting the CB_2 receptor, e.g. HU-308, to obtain any evidence that the signs of nigral degeneration may be attenuated with the activation of this cannabinoid receptor type.

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O.4.4.

POTENTIAL NEUROPROTECTIVE ROLE OF SPECIFIC CB_1 RECEPTOR SUBPOPULATIONS IN THE MOUSE CORTICOSTRIATAL CIRCUITRY

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The CB_1 receptor exerts a protective role in many different animal models of acute brain damage and chronic neurodegeneration, which has raised hope about the possible clinical use of cannabinoids as neuroprotective drugs. However, the assessment of the physiological relevance and therapeutic potential of the CB_1 receptor in neurological diseases is hampered, at least in part, by the lack of knowledge of the cell-population specificity of CB_1 receptor action. In order to study the potential neuroprotective role of different CB_1 receptor pools in the cortico-striatal circuitry we used an adenoviral-vector delivery strategy based on the expression of CFP-tagged mutant huntingtin harboring a pathogenic polyQ repeat of 94 residues under the control of specific promoters.

In a first series of experiments we have expressed mutant huntingtin under minimal neuronal (CaMKII) or astroglial (GFAP) promoters in the motor cortex or in the dorso-lateral striatum in order to achieve its expression in cortical principal neurons or cortical astrocytes (cortical injections), or in medium-sized spiny neurons (MSNs) or striatal astrocytes (striatal injections). These experiments have allowed us generating a phenotype of MSN damage upon the expression of mutant huntingtin in specific cell compartments of the cortico-striatal circuitry. We are currently evaluating the role of specific CB_1 receptor pools in these systems by using pharmacological approaches (e.g., THC injection) and genetic tools (e.g., conditional CB_1 receptor knockouts).

On the other hand, D_2R/indirect pathway-MSNs are believed to be more vulnerable to mutant huntingtin-induced toxicity than D_1R/direct pathway-MSNs. Hence, we will also combine the use of bacterial artificial chromosome (BAC) transgenic mice (BAC-D_1R-tomato and BAC-D_2R-GFP) together with the aforementioned viral gene transfer strategy to explore the cell-selective effects of mutant huntingtin and the CB_1 receptor in those two MSN populations.

O.4.5.

CHANGES IN THE ENDOCANNABINOID SIGNALING SYSTEM IN CNS STRUCTURES OF TDP-43 TRANSGENIC MICE: RELEVANCE FOR A NEUROPROTECTIVE THERAPY IN TDP-43-RELATED DISORDERS

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Amyotrophic lateral sclerosis (ALS) is a degenerative disease produced by the damage of the upper and lower motor neurons leading to muscle denervation, atrophy and paralysis. The disease may be sporadic (the most abundant cases) or associated with some gene mutations (familial ALS). In familial cases and depending on the mutated gene, ALS could be accompanied by features of frontotemporal lobar dementia (FTLD), which supports the idea that, instead a unique disorder, ALS belongs to a spectrum of disorders having motor but also cognitive deficits. One of these dual genes is *TARDBP*, encoding TDP-43 protein, which represents a new type of proteinopathy characterized by the presence of TDP-43 in the cytosol in the form of protein aggregates. ALS has been studied in relation with the neuroprotective potential of cannabinoids, but all studies have been conducted in the transgenic mouse model with mutations in superoxide dismutase-1 (SOD-1), the first gene that was identified in relation with ALS. The present study represents the first attempt to investigate the endocannabinoid system in an alternative model, TDP-43 transgenic mice. First, we used these mice at the onset of the disease (70 day-old) and at a post-symptomatic stage (110 day-old) for behavioral analyses. TDP-43 transgenic mice exhibited motor deficits in the rotarod test at both disease stages, but we were unable to reveal any change in cognitive tests (e.g., Water Morris test and T-test). Animals were euthanized at the post-symptomatic stage and their spinal cord and cerebral cortex collected for biochemical and histological analysis. We found a lower number of Nissl-stained cells in the spinal cord of TDP-43 transgenic mice compared with wild-type animals. In parallel, we also detected an important increase in mRNA (measured by qRT-PCR) and protein (measured by western blot) levels for the CB_2 receptor in the spinal cord of these animals, with no changes in the expression of other elements of endocannabinoid system. We also found an increase of microglial recruitment and activity as revealed the elevated Iba-1 immunolabeling. We assume that the up-regulation of CB_2 receptors presumably occurs in these microglial cells, but double-staining studies are required to confirm this. Finally, we also analyzed the cerebral cortex of TDP-43 transgenic mice but we did not obtain any significant change. In conclusion, our data support the idea that the endocannabinoid signaling system, in particular the CB_2 receptor, may serve for the development of a neuroprotective therapy in TDP-43-related disorders, in particular for TDP-43-related ALS. We are presently engaged in pharmacological experiments to investigate this possibility.

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O.4.6.

CHARACTERIZATION OF THE ENDOCANNABINOID SIGNALING SYSTEM IN A TRANSGENIC MOUSE MODEL OF SPINOCEREBELLAR ATAXIA TYPE-3 (SCA-3)

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Spinocerebellar ataxia type-3 (SCA-3) is an autosomal dominant inherited disease that, together with Huntington's disease and at least seven other diseases, comprises the so-called polyglutaminopathies. They are caused by a CAG triplet repeat expansion in the coding region of specific genes which produces pathogenic proteins that contain a critically expanded tract of glutamines. The presence of mutated proteins causes the death of specific neuronal subpopulations in specific brain regions; in the case of SCA-3, cell death occurs in cerebellum and brainstem, among others. Clinically, SCA-3 patients present progressive loss of motor coordination, accompanied by peripheral amyotrophy, rigidity, spasticity and dystonia. SCA-3 has no cure and also lacks an effective treatment to alleviate major symptoms and, in particular, to modify disease progression. Our earlier studies suggest that targeting the endocannabinoid signaling system may be a promising option in SCA-3 and other autosomal-dominant hereditary ataxias. These studies showed the existence of important changes in elements of this signaling system in the cerebellum of patients affected by SCA-3 and other types of ataxias. In the present study, we wanted to investigate the status of the endocannabinoid signaling in a transgenic mouse model of SCA-3, with emphasis in two brain regions particularly affected in this disease, the cerebellum and the brainstem. First, we measured the levels of endocannabinoids, and related *N*-acylethanolamines and 2-acylglycerols, and we found reductions in anandamide and oleoylethanolamide in the brainstem, but not in the cerebellum. These changes correlated with a parallel increase in the expression of the hydrolyzing enzyme FAAH, whereas other hydrolyzing (e.g. MAGL) and synthesizing (e.g. NAPE-PLD, DAGL) enzymes were not altered. Second, we measured the cannabinoid receptors in SCA-3 mice and found an increase in CB_1 receptors in the cerebellum with no changes in CB_2 receptors. In this structure, the high immunoreactivity for the CB_1 receptor was detected in the granular and molecular layers and in terminals of basket cells too. Lastly, we investigated the possibility of changes in the formation of heteromers of CB_1 receptors with other receptors. We found CB_1 receptors forming heteromers with CB_2 or adenosine A_{2A} receptors in the molecular and granular layers and in the pontine nucleus of wild-type animals, but both CB_1-CB_2 and CB_1-A_{2A} receptor heteromers were strongly reduced in SCA-3 mice which may have a key influence in future pharmacological studies. In summary, our results in SCA-3 mutant mice confirm a significant alteration in the endocannabinoid signaling system in the most important brain structures affected in this type of ataxia, suggesting that a pharmacological manipulation addressed to correct the changes in the endocannabinoid signaling could be a promising option in SCA-3. We are presently conducting pharmacological experiments to investigate this hypothesis.

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O.4.7.

PHARMACOLOGICAL AND GENETIC APPROACHES INVOLVING THE ENDOCANNABINOID SYSTEM IN THE TREATMENT OF FRAGILE X SYNDROME

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The fragile X syndrome (FXS) is the most common form of inherited intellectual disability and the leading monogenic cause of autism spectrum disorders. This syndrome courses with some clinical manifestations such as memory deficits, higher susceptibility to suffer epileptic seizures, low nociceptive sensibility or autistic behaviors among others. CB1 cannabinoid receptors (CB1R) have been proposed as an alternative target for therapies. The antagonist/inverse agonist rimonabant was appointed as a possible treatment on the bases of the effects described in the animal model of the disease, the Fmr1 knockout mouse. We have used pharmacological and genetic approaches to better characterize CB1Rs as a potential target therapy for different fragile X syndrome traits. We found that rimonabant at low doses (0.03-0.1 mg/kg) was effective in reducing the memory impairment in Fmr1 knockout mice. Moreover, alternate-day administration of low doses of rimonabant was equally useful in resolving the memory deficit. In addition, a CB1R neutral antagonist, NESS-0327, also prevented this cognitive deficit. On the other hand, we assessed whether the genetic attenuation of CB1R expression in the Fmr1 knockout mouse model would normalize the nociceptive responses of this mouse model or its altered sociability characteristics. Using the partial sciatic nerve ligation model of neuropathic pain, we found that Fmr1 knockout mice heterozygous for CB1R, similarly to their wild-type littermates, displayed mechanical allodynia and thermal hyperalgesia at earlier time points after the nerve injury than the Fmr1 knockout mice. Instead, the social phenotype in Fmr1 knockout mice, showing reduced exploration in the social preference test, was not modified by reducing CB1R expression. These data expands the pharmacological approaches available for potential treatment of certain traits in fragile X syndrome.

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O.4.8.

DIFFERENTIAL BINDING OF LIGANDS TO CANNABINOID CB_2 RECEPTORS DETECTED BY TIME-RESOLVED FRET

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Radioligand binding to cannabinoid type 2 (CB_2) receptors in membranes, from cells or tissues, has two serious drawbacks: the hydrophobic nature of many selective and non-selective ligands and the relatively low specific binding. The novel Time-Resolved FRET technology (HTRF®, Tag-lite®, CisBio) allows binding to G-protein-coupled receptors in living cells using non-radiolabelled compounds. It is based on receptors fused to Snap or Halo tags to which a terbium cryptate-containing donor may be specifically and covalently attached. The ligand/acceptor has been designed to be able to both bind with high affinity to the CB_2 receptor and to accommodate an extra moiety without losing its primary activity or selectivity *vs* CB1 receptors; after synthesis of the molecule (CM157), CisBio labeled it with its proprietary fluorescent dye. Energy transfer from the donor in the receptor surface and the acceptor in the fluorescent ligand is determined by HTRF. Saturation assays with standard agonists/antagonists provide affinities in the range of those described in the literature.

On the one hand, the use of a variety of natural and synthetic cannabinoids in competition assays in CB_2 receptor-transfected cells shows differential binding that requires interpretation from a functional point of view. On the other hand, cells expressing CB_2-receptor-containing heteromers display alterations in the binding to the cannabinoid receptor. The designed fluorescent probe and the HTRF-based binding procedure are useful tools to better understand CB_2 receptor biology.

O.4.9.

NEW MOUSE MODEL FOR THE STUDY OF CB_2 CANNABINOID RECEPTORS

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The study of cannabinoid CB_2 receptors has been limited by several factors. Among others, the questions on the specificity of anti-CB_2 antibodies and the expression of a truncated form of the protein in CB_2-knockout mice have been of most relevance. We here report the design and generation of a new transgenic mouse line that may help to unveil the precise pathophysiological roles of cannabinoid CB_2 receptors. The mouse model was generated by inserting an eGFP reporter gene preceded by an IRES sequence in the 3' UTR of the *Cb2* mouse gene. This approach results in the expression of the reporter gene under the control of the endogenous mouse *Cb2* promoter and transcript from the same bicistronic mRNA as the CB_2 protein. In addition, the whole exon 3, including the 3' UTR and the knocked-in reporter was flanked by *loxP* sites, allowing the conditional inactivation of the *Cb2* gene. The mouse model ($CB_2^{eGFP/f/f}$) was generated by homologous recombination in embryonic stem cells, in the C57BL/6J genetic background. The presence of GFP was determined in circulating cells by flow cytometry, revealing a relative abundance concordant with that expected for CB_2 receptors (CD4<CD8<macrophages<B cells). In addition, GFP was detected in cortical areas of the spleen and in the epithelial layer of the bladder. In the intact, healthy CNS, the expression of GFP was undetectable. We performed a hemilateral administration of lipopolysaccharide (LPS) in the striatum of $CB_2^{eGFP/f/f}$ mice. After one week of survival, mice were anesthetized, perfused and their brains used for morphohistological analysis. A dramatic increase in GFP expression was evident in the lesioned cortex, corpus callosum and striatum, but was unchanged in the non-lesioned side. Co-localization studies revealed that some, but not all, Iba-1 positive cells were also positive for GFP, while astrocytes did not express the fluorescent protein. We present a novel mouse model that may be useful to characterize the role of cannabinoid CB_2 receptors in a wide variety of pathophysiological processes.

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P.1.

FEEDING PATTERNS IN CHILDHOOD MODIFY FUTURE FOOD INTAKE AND FOOD PREFERENCE PATTERNS. HYPOTHESIS OF LONG-TERM CANNABINOID SYSTEM ALTERATION

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At the present time, children obesity is a preoccupant problem in our society because it has been related to an increased risk of health problems such as cardiovascular disease or diabetes in adulthood. The endocannabinoid system is involved in feeding behavior and has been found to be upregulated in obesity. Early dietary habits could impact future metabolic status, partly by inducing enduring alterations in the cannabinoid system. To test this hypothesis we submitted 4 groups of Wistar male rats to 4 feeding protocols based on two available diets: C (ad libitum standard chow) or P (free choice between ad libitum chow and a palatable food), that were made available to the animals in two different periods: PND 22 to PND 43 (corresponding to the childhood period), and PND 43 to die (corresponding to the adolescence and adulthood period). Thus we divided the animals in CC, CP, PC or PP, depending on whether they received the C or P diets in the first and second of those periods. We studied the preference for palatable food and behavior patterns of intake using the Compulsive Feeding Test. Additionally we tested the response to an acute i.p. dose (3mg/Kg) of AM251 which has been shown to effectively block CB1 receptors. Results show that the CC group shows a higher preference for palatable food and exhibits a more flexible intake behavior; since this group does not restrict their standard food intake when palatable food is not available. CP and PP groups show differences in preference for the palatable food, but not in weight gain. We then assessed the effect of the administration of AM251 on the intake of the two types of food (Standard and Palatable) observing that, while AM251 was able to reduce the palatable food intake in animals exposed only to standard chow, this effect was not observed in those animals that had been exposed to palatable food in their development and vice versa AM251 was able to reduce the standard food intake in animals exposed to palatable food in all their development. Here we show that early exposure to palatable food can have long-term consequences in behaviors like food intake flexibility and preference for palatable food. Additionally, our results would indicate that palatable food intake both in childhood and adult period can alter the normal functioning of the endocannabinoid system.

P.2.

ANATOMY AND FUNCTIONAL ROLE OF THE CB_1 RECEPTOR IN THE DENTATE GYRUS AFTER KAINATE-INDUCED SEIZURES IN WILD TYPE AND TRPV1-KO MICE

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We have recently demonstrated that the transient receptor potential vanilloid type 1 (TRPV1) is present at excitatory (Puente et al., 2014) and inhibitory synapses in the dentate molecular layer (ML). Recent studies suggest that cannabinoids play an important protective role in the modulation of circuits over-activated by epileptic seizures. Also, TRPV1 is up-regulated under these circumstances probably contributing to an alteration of the neuronal circuits balance in epilepsy (Fei-Ji Sun et al., 2012). The goal of this study was to investigate whether dentate TRPV1 has a role in the pathological state induced by the intrahippocampal injection of kainic acid, a model of medial temporal lobe epilepsy (MTLE).

Before kainic acid injection, a significant increase of cannabinoid 1 (CB_1) receptor immunoreactivity (optical density) was detected in the dentate gyrus of TRPV1-KO versus WT mice. Furthermore, the behavioral score after the intrahippocampal injection of kainic acid (50nl of 20mM) revealed that seizures were milder in TRPV1-KO than in WT during kainate-induced status epilepticus. Specific CB_1 antibodies combined with a very sensitive pre-embedding immunogold method for electron microscopy revealed that about 44% of the synaptic terminals were CB_1 immunopositive in the dentate molecular innermost zone of WT mice. Interestingly, about 62% of the synaptic boutons localized CB_1 immunoparticles in the inner 1/3 ML of TRPV1-KO. In accordance with these findings, the CB_1 agonist CP55,940 (10μM) induced a stronger inhibition of the excitatory synaptic transmission in the dentate inner 1/3 ML of TRPV1-KO (31,33±8,10%) than in WT (12,78±1,76%) under normal conditions. Finally, kainic acid injection yielded a decrease of CB_1 immunoreactivity in both WT and TRPV1-KO mice.

These results suggest that the absence of TRPV1 triggers some adaptative changes of the CB_1 expression in the dentate gyrus that may be beneficial in the control of epileptic seizures.

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P.3.

CANNABIDIOL PROMOTES OLIGODENDROCYTE SURVIVAL AFTER HYPOXIA-ISCHEMIA IN NEWBORN RATS

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Background: Hypoxic-ischemic (HI) insult disrupts the maturation of early (OPC) and late (preOL) oligodendrocyte precursors to mature oligodendrocyte (mOL) which eventually leads to hypomyelinization, a key role in the genesis of cerebral palsy. In the present work we aimed to determine how CBD treatment affects OL survival and maturation.

Methods: unilateral HI brain damage was induced in newborn Wister rats (7 day-old: P7) by exposure to hypoxia (10% FiO2) for 112 min after left carotid artery electrocoagulation under anaesthesia. Ten minutes after the end of HI pups received s.c. vehicle (HV, n=18) or CBD 1 mg/kg single dose (HC, n=24). Other pups remained as controls (SHM, n= 16). One (P8), 7 (P14) or 30 (P37) days after HI rats were sacrificed, transcardially perfused with formalin 4% and their brains cut off into coronal slices for immunohistochemical (IHC) study on the subventricular zone (SVZ), corpus callosum (CC) and cortex. In P8 and P14 rats: KI67 was used to detect proliferating cells, Olig-2 for glial precursors and SOX-10 for preOL. In P37 GSTπ IHC and Myelin basic protein (MBP) fluorescence in the CC and cortex were used to quantify the presence of mature OL.

Results: HI insult was associated with a decrease of OPC and preOL in cortex and white matter (WM) in P8, followed by an increment of these cells in P14. In cortex, CBD potentiated OPC population (HC>HV>SHM) but declined the number of preOL (HV>HC>SHM). However, the population of mOL in P37 was equal to SHAM in treated animal in contrast to vehicle group (SHM =HC>HV). Moreover, one month after HI in white matter vehicle group had fewer myelin/mOL ratio than SHAM and CBD groups (SHAM=HC>HV).

Conclusions: CBD administration preserves neuroproliferation, activating preOL and preserving OL maturation and myelin synthesis after a HI insult, thus preventing HI-induced hypomyelinization.

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P.4.

IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF CB_1-A_{2A} HETEROMERS IN THE MOUSE CORTICOSTRIATAL CIRCUITRY

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The CB_1 cannabinoid receptor, the main molecular target of endocannabinoids and the active components of cannabis, is the most abundant GPCR in the mammalian brain, where it is expressed in various neuronal populations, especially on GABAergic terminals. Several studies have shown that, depending on its location on one neuronal type or another, CB_1 exerts different physiological functions, thus suggesting the existence of neuron-type specific biological responses elicited by different receptor subpopulations. In support of this, we have recently shown that CB_1 receptors located on glutamatergic corticostriatal terminals, rather than on GABAergic medium-sized spiny neurons, play a crucial role in protecting the striatum from excitotoxic stimuli. The precise molecular mechanisms underlying this differential action of distinct CB_1 receptor pools remain still uncertain, but a growing body of evidence points to the heteromerization process as one possible explanation. In fact, a large number of studies supports that CB_1 can interact with other GPCRs to form heterodimers or higher-order oligomers. These interactions influence several aspects of CB_1 function, including the precise downstream signaling pathways that are activated by cannabinoid agonists.

To evaluate whether CB_1 may interact with other GPCRs in the mouse brain and whether these putative interactions may be responsible for the differential activity of distinct CB_1 receptor pools, we analyzed the formation of heteromers in the cortex and striatum of conditional mutant mice bearing a CB_1 genetic deletion in GABAergic (GABA-$CB_1^{-/-}$) or glutamatergic (Glu-$CB_1^{-/-}$) neurons. Our data show a significant reduction of CB_1-A_{2A} complexes in the cortex and striatum of GABA-$CB_1^{-/-}$ mice, while no differences were observed in Glu-$CB_1^{-/-}$ mice, thus suggesting that CB_1-A_{2A} heteromers are located in GABAergic but not glutamatergic neurons. None of the other CB_1 receptor heteromers analyzed changed remarkably in either line of CB_1 knockout mice. We are currently trying to identify the precise populations of GABAergic neurons that express the CB_1-A_{2A} heteromer, its exact subcellular location, and how its formation may alter the signaling pathways modulated by these receptors. In addition, we are also studying how the properties of the heteromer may change in a pathological setting such as Huntington's disease, in which we have already found a decrease of CB_1-A_{2A} complexes in mouse models (R6/1, R6/2 and $Hdh^{Q111/Q111}$) as well as in patients.

P.5.

PHARMACOKINETICS OF SATIVEX® IN DOGS: TOWARDS A POTENTIAL THERAPY IN CANINE DEGENERATIVE MYELOPATHY

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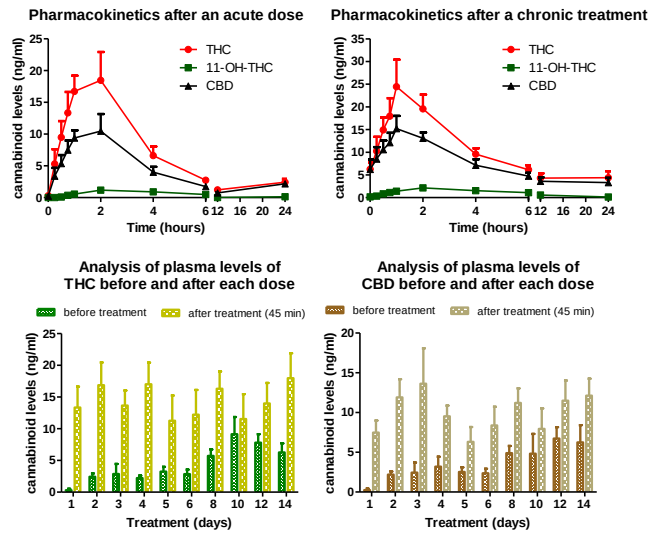
Degenerative myelopathy (DM), a disorder described in dogs in 1973, shares pathogenic mechanisms with some forms of human amyotrophic lateral sclerosis (ALS), including mutations in superoxide dismutase type 1 as one of the causes of the disease. An important aspect in studies of human pathologies is to may use big mammals which, in the phylogeny, are closest to humans, as well as to may investigate disorders that occur spontaneously, instead the classic experimentally-induced models in small mammals such as laboratory rodents. Therefore, this canine pathology represents a unique opportunity to investigate ALS in a context much more close to the human pathology. Our intention is to recruit a cohort of affected dogs and to conduct a pharmacological study with Sativex® to evaluate whether this phytocannabinoid-based medicine has beneficial effects on the disease progression in these dogs. Before to conduct such study and with the purpose of determining the best dosage and timing for using Sativex® in DM-affected dogs, we wanted to investigate its pharmacokinetics when administered to naïve dogs via sublingual delivery. We used Beagle dogs with a weight in the range 11-13 kg, 3 males and 3 females, which were treated with 3 consecutive puffs of Sativex® and immediately used for blood collection at different times during the following 24 hours (acute condition). In a second set of animals, dogs received 3 consecutive puffs daily at the same time during 14 days, and the last day they were used immediately after treatment for blood collection (chronic condition). Some respiratory and cardiovascular parameters were also measured in both conditions, but no changes were observed. Blood was used to obtain the plasma and to determine the levels of CBD, Δ^9-THC and its metabolite 11-hydroxy-Δ^9-THC by LC-MS/MS (see main results in the attached figure). Maximal levels of both Δ^9-THC and CBD were reached at 2 hours after administration in the acute condition and at 1 hour in the chronic condition (top panels). 11-Hydroxy-Δ^9-THC, which is mainly formed in the liver from Δ^9-THC, was not almost detected in concordance with the use of a sublingual delivery. Lastly, we recorded a progressive accumulation of both cannabinoids following the repeated exposure in subsequent days with a maximum in the day 14 (bottom panels).



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P.6.

HETEROMERIZATION OF GPR55 AND CANNABINOID CB_2 RECEPTORS MODULATES SIGNALING

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Heteromerization of G protein-coupled receptors is key on the integration of extracellular signals and the subsequent cell response via several mechanisms including heteromer-selective ligand binding, trafficking and/or downstream signaling. Considering the modulatory impact of the lysophosphatidylinositol G protein-coupled receptor 55 (GPR55) on the function of cannabinoid receptor subtype 2 (CB_2R) in human neutrophils, a potential heteromerization of CB_2R and GPR55 was hypothesized. Direct interaction of human GPR55 and CB_2R heterologously expressed in HEK293 cells was assessed by co-immunoprecipitation and bioluminescence resonance energy transfer (BRET) assays. Moreover, the cross-talk on signaling was investigated at downstream levels by label-free real-time methods (Epic dynamic mass redistribution and CellKey impedance assays), ERK1/2-MAP kinase activation and gene reporter assays. GPR55 and CB_2R colocalize on the surface of human embryonic HEK293 cells, co-precipitate in membrane extracts and form heteromers in living HEK293 cells. Whereas heteromerization leads to a reduction of GPR55-mediated activation of transcription factors (NFAT, NF-□B and CRE), the ERK1/2-MAP kinase activation is potentiated in the presence of CB_2R. CB_2R-mediated signaling is also affected by co-expression with GPR55. Moreover, label-free assays confirmed a cross-talk between the two receptors. In HEK293 cells expressing GPR55 and cannabinoid CB_2R receptors, heteromers that are unique signaling units are formed. The signaling by agonists of either receptor is governed i) by the presence or absence of the partner receptors (with the consequent formation of heteromers) and ii) by the activation state of the partner receptor.

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P.7.

ACTIVITY AND DENSITY DETERMINATION OF THE CB_1 CANNABINOID RECEPTOR IN ANIMAL MODELS USING CELL MEMBRANE MICROARRAYS

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The components of endocannabinoid system are widely distributed through the Central Nervous System (CNS). Type-1 cannabinoid receptors (CB_1) are highly expressed in CNS areas related with memory and movement where they modulate the activity of several neurotransmitter systems including dopaminergic or cholinergic. CB_1 receptors may play an important role in neurodegenerative disorders in which the integrity of these systems is committed, as it happens in Parkinson's disease (PD). In this context and considering the continuing increase of the aging population and neurodegenerative diseases prevalence, it is decisive to develop new techniques to study the relation between endocannabinoid system and PD. For this reason, we have developed cell membrane microarrays from different animal models consisted of cell membranes isolated from rats and non human primates. Moreover, a microarray, made of different brain regions of control and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treated non-human primate (*Macaca fascicularis*), was also created. This animal model has been well characterized in the past and clearly reproduces the main pathological and clinical features of PD.

First, we have performed some tests to study both distribution and activity of CB_1 receptors in rodent cell membrane microarray. The parkinsonian monkeys showed a decrease in the binding of [^{3}H]-CP55940 in cerebellum and putamen areas, whereas an increase was detected in globus pallidus, substantia nigra, thalamus and olfactory bulb. Regarding to CB_1 activity, the [^{35}S]GTPγS binding stimulated by WIN 55.212-2 was increased in the frontal cortex whilst it was decreased in the olfactory bulb.

Our results indicate that cell membrane microarrays should be considered a new useful tool for identifying and validating new therapeutic targets in neurodegenerative disorders.

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P.8.

STUDY OF THE CONTRIBUTION OF ORMDLS - A NOVEL FAMILY OF SPHINGOLIPID BIOSYNTHESIS REGULATORS - IN CANNABINOID ANTITUMORAL ACTION

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Cannabinoids - the active components of marijuana and their derivatives - are a novel family of antitumoral agents. A mandatory step in the mechanisms by which cannabinoid promote cancer cell death involves the stimulation of de novo synthesis of sphingolipids, an event that leads to the induction of autophagy and the subsequent activation of apoptosis. The biosynthesis of sphingolipids is a tightly regulated process. The main regulatory step of this metabolic pathway is the reaction catalized by serine palmitoyltransferase (SPT). The mechanism whereby cannabinoids and other antitumoral agents act on SPT is not yet established. However, a new family of proteins, the Orosomucoid-like proteins family (ORMDLs), has been shown to act as endogenous SPT regulators. In this context a GWAS study on glioma risk reported a SNP in the ORMDL3 regulatory expression region. Thus, it is likely that the involvement of ORMDL3 in cancer is related to the production of ceramides. The overall aim of this work is to study the mechanism behind the stimulation of de novo synthesis of ceramides by cannabinoids in cancer cells paying special attention to ORMDL proteins, the novel regulators of SPT. We have measured the expression levels of different members of the SPT complex by quantitative PCR and western blot in an astrocitoma cell line treated with Delta9-tetrahydrocannabinol (THC). We have studied by coimmunopretipitation studies the interaction between the different subunits and finally we have analyzed the contribution of ORMDL subunits to the serine palmitoyl transferase activity. At this point, we have data supporting that part of the increase in de novo ceramide synthesis by cannabinoids is due to changes in the expression of the components of the SPT complex. Further experiments must be done to fully understand the mechanism and the contribution of ORMDL family.

P.9.

BIOLOGICAL CHARACTERIZATION OF A 6-CYCLOPROPYL-1,4-DIHYDROINDENO[1,2-C]PYRAZOLE-3-CARBOXAMIDE AS A POTENTIAL CB2-/PPAR-α-DEPENDENT ANTI-INFLAMMATORY/NEUROPROTECTIVE AGENT

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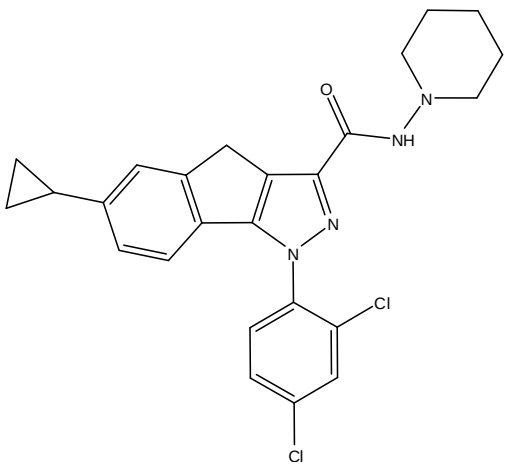
Cannabinoids have emerged as promising neuroprotective agents because their capability: (i) to share in the same molecule activity at different endocannabinoid targets, e.g. CB_1 and CB_2 receptors, and at cannabinoid receptor-independent cellular processes, e.g. antioxidant effects; or (ii) to be highly selective for cannabinoid receptors but allowing to get effects of broad-spectrum by the combination of different and complementary cannabinoids. Numerous compounds with natural (e.g. plant-derived cannabinoids or endogenous lipids) semi-synthetic (e.g. phytocannabinoid derivatives) or fully-synthetic origins have been investigated so far and they represent valuable tools for pharmaceutically exploiting the neuroprotective potential of this class of compounds. Here, we describe the biological characterization of a 6-cyclopropyl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide derivative (VAL168), whose chemical structure is shown below. The compound has affinity at the nanomolar range for the CB_2 receptor (Ki = 69.4 ± 2.8 nM) with weak affinity at the CB_1 receptor (Ki > 1800 nM). It did not reverse the activity of WIN55,212-2 in a CB_2 receptor-specific bioassay using stimulation of BV-2 cells with LPS, but it produced equivalent responses that were completely reversed by the selective CB_2 receptor antagonist SR144528, thus confirming its activity as a CB_2 receptor agonist. VAL168 was also examined for its activity at the PPAR receptors (α, δ and γ) using a Gal4/PPARs chimera system showing to be an specific PPAR-α activator. This profile as a CB_2 receptor agonist and PPAR-α ligand is extremely interesting for being used against neuroinflammatory conditions. This is being presently investigated in an in vitro model of microglial toxicity on neurons and, in the case of positive results, VAL168 will be evaluated in preclinical models of neuroinflammatory disorders, e.g. multiple sclerosis, with the purpose of a future interest for the clinical research.



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P.10.

POTENTIAL OF COMPOUNDS TARGETING THE CB_2 RECEPTOR AGAINST INFLAMMATION IN AN EXPERIMENTAL MODEL OF PARKINSON'S DISEASE

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Inflammation is an important pathogenic factor in Parkinson's disease (PD), so that it can contribute to kill dopaminergic neurons of the *substantia nigra pars compacta* and to enhance the dopaminergic denervation of the striatum. This can be reproduced experimentally in rodents by local injections of lipopolysaccharide (LPS) which, through an intense inflammatory response and activation of glial elements, provokes a rapid neurodegeneration. Using this experimental model, we have published previous data supporting a potential beneficial role for the CB_2 receptor in the control of these inflammatory responses. For example, the loss of tyrosine hydroxylase-containing nigral neurons provoked by LPS injections into the striatum was more intense in CB_2 receptor-deficient mice than in wild-type animals (García et al., BJP 2011). We also found that CB_2 receptor immunoreactivity was up-regulated, presumably in glial elements, after the LPS insult and compounds targeting this receptor, e.g. Δ^9-tetrahydrocannabivarin (Δ^9-THCV), reduced the glial activation and the death of dopaminergic neurons of the *substantia nigra pars compacta* (García et al., BJP 2011). In this study, we wanted to further explore the effects of CB_2 receptor activation or the genetic ablation of CB_2 receptor on the inflammatory response elicited by local injections of LPS into the striatum of mice. First, we confirmed the up-regulation of CB_2 receptors in the striatum and the substantia nigra of wild-type mice in response to LPS described previously with immunostaining (García et al., BJP 2011), in this occasion by measuring CB_2 receptor gene expression with qRT-PCR. Second, we analyzed CD68 immunostaining, which serve to identify activated microglia and infiltrated peripheral macrophages, and we found a significant increase in the basal ganglia in response to LPS insult, which, in the case of the substantia nigra, was much more intense in CB_2 receptor-deficient mice. Next, we observed that LPS-induced elevation of CD68 immunostaining was significantly reversed by activation of CB_2 receptors with HU-308 (5 mg/kg daily during 14 days) and, to a lesser extent, with Δ^9-THCV (2 mg/kg daily during 14 days) in wild-type mice, and these responses were completely lost in CB_2 receptor-deficient mice. Lastly, we measured gene expression for different pro-inflammatory mediators, e.g. TNF-α, IL-1β and iNOS, which resulted to be significantly elevated in response to LPS. The activation of CB_2 receptors apparently reduced these elevations, although the effects were certainly small. In conclusion, we have provided new evidence on the up-regulation of CB_2 receptors in an inflammatory model of PD, as well as on the benefits derived from their activation in relation with the activation of microglial cells, the infiltration of macrophages and the capability of these cells to generate proinflammatory factors.

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P.11.

ANATOMICAL ANALYSIS OF MUTANT MICE EXPRESSING TYPE-I CANNABINOID RECEPTORS (CB_1R) IN SPECIFIC CELL POPULATIONS OF THE HIPPOCAMPUS

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CB_1 receptors (CB_1R) are well known to be abundantly present at GABAergic terminals and at lower levels in other brain cell populations. However, due to the hugely different levels of expression among different cell types, anatomical analyses of wild-type mice often failed in the past, and low levels of expression have often been misinterpreted as background staining. For instance, we recently showed that, despite previous negative findings, CB_1R are present and functionally important in cortical glutamatergic neurons, in brain astrocytes and, at subcellular level, in brain mitochondria. Therefore, to reveal the low, but functionally very important, presence of CB_1R in specific cell types, the use of sophisticated tools such as "rescue" animals is necessary, which maintain normal expression of CB_1R exclusively in specific cell types.

The aim of this study was to investigate the subcellular distribution of CB_1 in the CA1 hippocampus of different types of mutant mice. Immunocytochemical techniques for high resolution electron microscopy were applied to hippocampal sections of *CB_1*-WT, *CB_1*-KO, conditional mutant mice bearing a selective deletion of CB_1 in cortical glutamatergic (Glu-*CB_1*-KO) or GABAergic neurons (GABA-*CB_1*-KO) as well as conditional "CB_1 rescue mice" re-expressing CB_1R exclusively in cortical glutamatergic neurons (Glu-*CB_1*-RS) or in astrocytes (GFAP-*CB_1*-RS).

Around 26% of the excitatory asymmetric synaptic terminals unequivocally identified by ultrastructural features were CB_1 immunopositive in Glu-*CB_1*-RS mice, decreasing to 1% in Glu-*CB_1*-KO mice and virtually disappearing in *CB_1*-KO mice. In addition, the statistical analysis revealed that the proportion of CB_1 positive excitatory terminals in Glu-*CB_1*-RS mice was not significantly different from the one observed in *CB_1*-WT (31%) and GABA-*CB_1*-KO mice (26%).

We also analyzed the CB_1 distribution pattern in CA1 astrocytes. In this case, around 37% of the astrocytic sections were CB_1 immunopositive in GFAP-*CB_1*-RS. No significant differences were observed when comparing the proportion of CB_1 immunopositive astrocytic elements with that in *CB_1*-WT mice (41%). Only scarce CB_1R gold particles were detected in astrocytes of *CB_1*-KO (2%) and GFAP-*CB_1*-KO (4%) mice.

To summarize, the proportion of CB_1 immunopositive presynaptic excitatory terminals and astrocytic processes in the CA1 hippocampi of *CB_1*-WT mice is maintained in "rescue" mice (Glu-*CB_1*-RS and GFAP-*CB_1*-RS). The results demonstrate the great potential of these transgenic mice to study CB_1 in brain regions, pathways and nerve cells where the localization of this cannabinoid receptor is particularly sparse.

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P.12.

INVOLVEMENT OF 5HT1A RECEPTORS IN THE NEUROPROTECTIVE EFFECTS OF CANNABIDIOL IN HYPOXIC-ISCHEMIC NEWBORN PIGS

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Background and aim: Cannabidiol (CBD) is an agonist of 5HT1A receptors. Previous studies have demonstrated post-hypoxia-ischemia (HI) administration of CBD has functional and a neurobehavioral beneficial effect, in which 5HT1AR activation plays a role. In the present work we aimed to get some insights into the mechanism of those CBD-induced beneficial effects.

Methods: 1 day-old piglets were studied for 72 h after a HI insult (carotid clamp and FiO2 10% for 20 min). Thirty min after HI piglets received vehicle (HV, n=8) or CBD 1 mg/kg single dose u.i.d (HC1, n=5), alone or with the 5HT1AR antagonist WAY100630 1 mg/kg/12 h (HC1W, n=4). Non-HI piglets served as controls (SHM, n=4). Then, the brains were processed for conventional (Nissl) staining to assess necrotic score and TUNEL staining to assess the apoptotic damage. In addition, GFAP and Iba-1 immunohistochemistry were performed to determine the astrocityc viability and microglial activation, respectively. Finally, H^+-MRS was made on frozen brain samples to assess neuronal and astroglial damage by Lac/NAA and mI/Cr ratios, respectively.

Results: CBD administration prevented neuronal necrosis and apoptosis, protects astrocytes and modulated microglial activation (Table). WAY100630 blocked the effects of CBD on neuronal necrosis, as observed by Nissl and Lac/NAA, but not on apoptosis. WAY100630 did not modify the protective effects of CBD on astrocytes nor its modulating effect on microglia.

Item	***SHM***	***HV***	***HC***	***HCW***
Death neurons (%)	4.3(0.3)	*13.7(1.3)*	*6.1 (1)*	***14.8(1.8)***
TUNEL (n/field)	1 (0.9)	*231.9(90)*	*73.1(20)*	*72.9(50)*
GFAP (size)	425 .4(50)	*688.9 (70)*	505.6(50)	481.1(40)
IBA-1 (branches length)	5.1(1)	*2.6(0.2)*	4.2(0.9)	*3.2(0.1)*
Lac/NAA	0.86(0.03)	*2.18(0.1)*	1.1(0.09)	**2.2(0.1)**
mI/Cr	1.6(0.1)	*1.2(0.08)*	1.4(0.09)	1.4(0.09)

Italic: p<0.05 vs SHM. Underlined: p<0.05 vs. HV. **Bold: p<0.05 vs HC**

Conclusions: CBD administration prevents necrotic neuronal death by some mechanism mediated by 5HT1AR. However antiapoptotic, glioprotective and immunomodulating effects of CBD were independent from 5HT1AR activation.

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P.13.

CANNABIDIOL ADMINISTRATION LEADS TO FUNCTIONAL RECOVERY IN A MCAO MODEL IN NEWBORN RATS

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Background: The neonatal period is a time of particular susceptibility and risk; two to four out of 1000 life newborns suffer from perinatal AIS (PAIS) although just 25% are diagnosed at that time. Typically, stroke affects cortex in the Middle Cerebral Artery (MCA) territory and in the left side - likely because of the patency of the Ductus Arteriosus. There is no treatment available for patients up to now. The antioxidant and the anti-inflammatory role of CBD as well as previous results in hypoxia-ischemia in newborns, makes this drugs as an excellent candidate in the treatment of PAIS.

Goal: Optimization of the MCAO model in newborn rats and evaluation of the CBD treatment, using behavioral and histological studies.

Methods: The left carotid artery will be dissected up to the internal and external branches division, to introduce a nylon filament that will be progressed through the internal carotid artery until occluding the left MCA. The filament is secured by a periarterial knot. The occlusion will be maintained for 3 h. After the occlusion period the pups will be anesthetized again to carefully remove the filament. Then, the carotid wound is sealed by an absorbable hemostat. Finally, the skin wound sealed by 5-0 silk stitches and the pup returned to the dam. 10 minutes after the reperfusion, CBD 1mg/kg and VEH were administrated i.p. randomized. 7 days after the stroke, several behavioral tests were performed in those animals. Neurobehavioral tests were chosen to study the natural reflex of the newborn rats such as negative geotaxis test, grasp reflex, posture tail reflex and the forepaw-grip test. Additional, weight was measured as a damage control. Then, pups were perfused intracardiacally and the brains were removed for MRI and histological studies.

Results: MRI studies didn´t show any reduction in the lesion volume in CBD treated animals, probably because of the strong severity of brain damage. However, vehicle animals showed a poorly performance in the different behavioral tests that was improved by CBD treatment.

Conclusions: In a model of severe stroke in newborn rats, post-insult CBD administration leads to functional benefits despite a lack of significant protection against tissue damage.

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P.14.

ROLE OF THE MIDKINE / ANAPLASIC LYMPHOMA KINASE AXIS IN THE RESISTANCE TO CANNABINOID ANTITUMORAL ACTION IN GLIOMA INITIATING CELLS

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Introduction: Glioblastoma multiforme (GBM) or grade IV astrocytoma is the most common and lethal form of primary brain tumors, as a consequence median survival after diagnosis is usually just 12 to 15 months. These tumors exhibit a high resistance to standard chemotherapy and radiotherapy which could be attributed, at least in part, to a cell population within the tumor that possesses stem-like characteristics, named here as glioma initiating cells (GICs). Elimination of these cells may be therefore crucial for achieving therapeutic efficacy.

In line with this idea, identification of the signalling mechanisms (including changes in the levels of growth factors or stimulation of their receptors) that regulate GICs proliferation and survival could help to devise more efficacious therapies to fight GBM.

Although cannabinoid administration has been shown to inhibit tumor growth of glioma xenografts in mice, Midkine (Mdk)/ Anaplasic Lymphoma Kinase (ALK) axis seems to be critically involved in glioma resistance to cannabinoid antitumoral action. Previously, Mdk has also been involved in resistance to anti-cancer therapies and in stem cell regulation, so targeting the Mdk/ALK axis in GSCs could be a good therapeutic strategy to enhance the efficacy of antitumoral action of cannabinoids.

Objectives: The main objective of this work is to analyse the role of the Mdk/ALK axis in the resistance to cannabinoid antitumoral action, as well as to develop novel therapeutic strategies based on combination of cannabinoids with inhibitors of the Mdk/ALK via or with temozolomide (TMZ), the benchmark agent used for the treatment of gliomas.

Results: Genetic (using inducible lentiviral particles able to express a shRNA antiMdk) or pharmacological (using specific anti-MDK antibodies or tyrosin-kinase inhibitor Crizotinib) inhibition of Mdk/ALK axis sensitizes GICs to cannabinoid antitumoral action.

Conclusion: Blockade of Mdk/ALK axis with tyrosine kinase inhibitors like crizotinib sensitizes GICs to treatment with cannabinoids. This combined treatment could be a potential therapeutic strategy to eliminate the GIC population in GBM patients and it may set the bases for the development clinical trials based on the use of crizotinib and cannabinoids.

P.15.

EVALUATING THE THERAPEUTIC POTENTIAL OF THE 2-AG HYDROLASE ABHD6 IN DEMYELINATION

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Multiple sclerosis (MS) is a chronic disease of the human central nervous system that is characterized by focal lesions with inflammation, infiltration of immune cells, demyelination and axonal damage. Activation of cannabinoid CB_1/CB_2 receptors is considered a potential therapeutic strategy for the treatment of MS based on the evidence that exogenous cannabinoid agonists exert neuroprotective and immunosuppressive effects in experimental models of the disease. Nevertheless, the therapeutic use of exogenous cannabinoids is limited by the possible adverse responses related to memory and learning impairment. An alternative therapeutic strategy consists of enhancing the concentration of the endocannabinoids anandamide and/or 2-arachidonoylglycerol (2-AG) by decreasing their enzymatic metabolism. In this context, we have investigated the potential of targeting the recently characterized 2-AG hydrolytic enzyme ABHD6 (α/β hydrolase domain lipase 6) as novel therapeutic strategy in MS. With this aim, we have studied the activity of peripherally restricted and/or centrally acting ABHD6 selective inhibitors KT182 and KT203 in 2 complementary *in vivo* models of myelin damage, namely the experimental autoimmune encephalomyelitis (EAE) model of MS and the cuprizone model of immune-independent demyelination. Chronic EAE was induced in C57BL/6 mice by immunization with MOG in Freund's adjuvant supplemented with *Mycobacterium tuberculosis*. Primary demyelination was induced by feeding mice with a 0.3% cuprizone containing diet for 3 weeks. Animals received daily intraperitoneal injections of KT182 or KT203 starting at the day of immunization in the EAE model or at the first day of cuprizone feeding.

Administration of ABHD6 inhibitors attenuated disease progression in EAE mice. Comparison of the motor score curves indicated that although both KT182 and KT203 ameliorated the deficits observed in vehicle-treated mice during the disease course, the brain permeable compound was more effective than the peripherally acting one. The ability of both compounds to attenuate the gene expression of proinflammatory cytokines in the brain and spinal cord of EAE mice is currently under investigation. Concerning the effect of KT182 in the cuprizone model, we measured a significant attenuation of weight loss associated to toxin administration in mice treated with the ABHD6 inhibitor. Altogether, our findings suggest that chronic administration of ABHD6 inhibitors may be a promising strategy for the treatment of demyelinating disorders.

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P.16.

HOMOBIVALENT LIGANDS FULLY SELECTIVE FOR CB_2 CANNABINOID RECEPTORS

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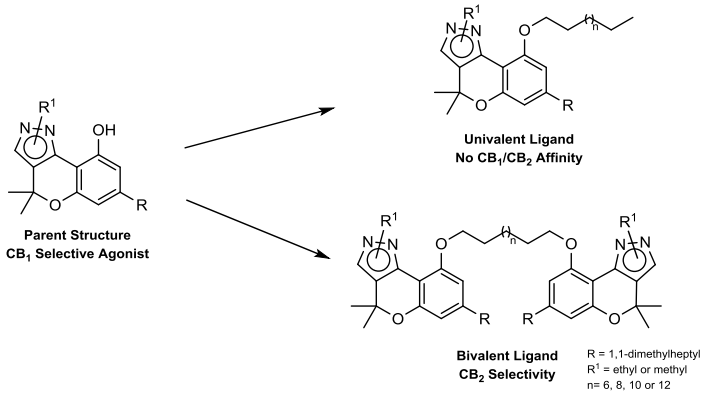
Growing evidence suggests that numerous GPCR receptors, cannabinoid among them, may form hetero- and homodimers.[1] This oligomerization interferes with the receptor function, activation and signal transduction. In this scenario, bivalent ligands, which consist of two pharmacophores connected by a spacer, have emerged in the last years as promising pharmacological entities and potential tools for the biological study of their respective dimeric receptors.[2]

Few homobivalent ligands have been described for the cannabinoid receptors, most of them target CB_1 and are based on the rimonabant scaffold. Heterobivalent ligands targeting CB_1 and opioid receptors have previously been reported by us.[2] Herein we report the design, synthesis and evaluation of CB_2 selective bivalent ligands based on a chromenopyrazole scaffold previously described by us as cannabinoid ligand.[3]

A series of homobivalent chromenopyrazoles containing alkyl chains as spacers and their respective univalent 9-alkoxychromenopyrazole analogs have been synthesized. Different alkyl chains (10 to 16 methylenes) were introduced in order to investigate the influence of the spacer length on affinity and potency. Their ability to bind to the cannabinoid receptors was measured through radioligand assays. They show significant CB_2 affinity with total CB_2 selectivity over CB_1 receptor. It is worthy to note that their corresponding univalent ligands did not display any affinity for CB_1 or CB_2 receptors, suggesting a possible interaction of both pharmacophores with CB_2 receptors. To our knowledge, this is the first time that fully CB_2 selective bivalent ligands are described.



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P.17.

TARGETING CB_2-GPR55 RECEPTOR HETEROMERS MODULATES CANCER CELL SIGNALING

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It has been previously described that cannabinoids, via CB_1 and/or CB_2 receptors, control cell fate, directing cells towards proliferation, differentiation or death depending on the cell type and its specific context. In tumor cells, these compounds usually produce proliferation-inhibiting and death-inducing effects both *in vitro* and *in vivo*, which makes them promising therapeutic options for the management of cancer. More recently, another G protein-coupled receptor (GPR55) has been related to cannabinoids, although its pharmacology is still quite controversial. Since both CB_2R and GPR55 are overexpressed in cancer cells and human tumors, they both control cell fate, and modulation of GPR55 activity by cannabinoids has been reported, we analyzed whether GPR55 participates in CB_2R-mediated cannabinoid effects on cancer cells. Our results demonstrate the existence and unique signaling properties of CB_2R-GPR55 heteromers in cancer cells. In addition, they show that the expression of these receptor heteromers has a major impact on cannabinoid signaling in these cells.

Together, these findings unveil the existence of previously unknown signaling platforms that may help to explain the complex behavior of cannabinoids and may constitute new targets for therapeutic intervention in oncology.

P.18.

DIFFERENT MODULATION OF SPATIAL OR FEAR-CONDITIONED MEMORIES BY CB_1 AGONIST TREATMENT

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The cholinergic system controls learning and memory processes that are affected in Alzheimer's disease (AD). The muscarinic receptor (MR) antagonist, scopolamine (Scop), causes memory impairment in the rat. Controversial data have been reported in relation to cognitive functions induced by cannabinoids in AD. In addition, cannabinoids exert different effects in learning and memory processes depending on the behavioural test, dose or via of administration used.

The aim of the present study was to evaluate the effects on behaviour induced by subcronic treatment with WIN55,212-2 (0,5 mg/kg; i.p.) in the rodent model of learning and memory deficit of acute Scop injection (2 mg/kg; i.p.). To evaluate the spatial memory and working memory Barnes maze test was used. Latency and number of errors to enter in the target hole were recorded during the learning phase. During the probe phase, measures of time spent per quadrant and first latency were recorded. Later, the short memory was evaluated by using the passive avoidance test.

The administration of WIN55,212-2 or the vehicle did not modify the learning and memory trials in Barnes maze test. But the WIN55,212-2-treated group showed shorter latencies to find the target hole during the first trial (WIN55,212-2: 63,1 ± 17 sec vs Vehicle: 126,6 ± 21 sec). At the "probe day" when both drugs were administered, a slight protection from the memory impairment induced by Scop was recorded in the first latency and the time spent in each quadrant. (1^{rst} latency: WIN55,212-2 + Scop: 14,2 ± 3 sec vs Vehicle + Scop: 47,5 ± 17 sec. Time per quadrant: WIN55,212-2 + Scop: 78,7± 13 sec vs Vehicle + Scop: 45,6 ± 3 sec). On the contrary, the passive avoidance test reported no differences between groups. These results support the existence of different interactions between cholinergic and cannabinoid systems depending on the brain pathways controlling different types of memory, spatial or fear-conditioned. Further behavioural and neurochemical studies are necessary to analyse the modulation of MR-mediated activity by cannabinoid receptors.

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P.19.

A NOVEL CANNABIDIOL DERIVATIVE INDUCES POLARIZATION OF M2 MACROPHAGES, HIF-1α STABILIZATION AND ALLEVIATES NEURO-INFLAMMATION IN DIFERENT MODELS OF MULTIPLE SCLEROSIS

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The use of cannabinoids in medicine is severely limited by their psychoactive effects. As the unwanted psychotropic effects of cannabinoids are mediated largely or entirely by the CB_1 receptor, a conceivable possibility would be to use cannabinoids that do not target that receptor, such as cannabidiol and its quinone derivatives. One of the first CBD quinone derivatives was HU-331, a non-psychotropic semi-synthetic cannabinoid with antitumoral activity. The counterpart of HU-331 in CBG is VCE-003, a compound with potent anti-inflammatory and immunosuppressive activities. However, both HU-331 and VCE-003 are unstable electrophilic compounds, with limited prospects further development. As part of our study on the SAR of CBD we have generated novel non-electrophilic CBD quinol derivatives that activate PPARγ. From this series we have selected the compound CBD-Q-8 for further characterization and efficacy in two different murine models of MS.

CBD-Q-8 is a dual PPARγ and CB_2 agonist that blunts IL-17-induced M1 polarization and enhances basal and IL-4-induced M2 polarization, especially $Arg1^+$ macrophages. We also show that CBD-Q-8 stabilizes HIF-1α, induces the transcriptional activity of the erythropoietin promoter, and protects NSC34 cells from glutamate- and H_2O_2-induced cytotoxicity. CBD-Q-8 also inhibits COX-2 expression and PGE_2 release in LPS-stimulated primary microglia cells. In addition, we found that CBD-Q-8 greatly ameliorates neurological deficits in two different models of MS, namely EAE and Theiler's murine encephalomyelitis virus (TMEV) model. Using MS specific RT² Profiler PCR Arrays we found a differential inflammatory profile between EAE and TMEV-induced encephalomyelitis. In both models CDB-Q-8 downregulated the expression of several genes including chemokines, cytokines and adhesion molecules that are closely associated with MS physiopathology. Histopathological analysis, in both models, reveal that CBD-Q-8 treatment affect demyelination and axonal damage. Expression of Iba-1 and Arg1 (microglia/macrophages) is also modified by the compound treatment.

In conclusion, this study highlights the therapeutic potential of CBD-Q-8 for the treatment of neuroinflammatory diseases such as MS.

P.20.

CB_1 RECEPTOR SIGNALING IN EMBRYONIC STEM CELL DIFFERENTIATION INTO CORTICAL NEURONS

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Pluripotent embryonic stem (ES) cell cultures constitute a powerful tool to investigate key aspects of nervous system development and particularly on the regulatory mechanisms involved in neuronal generation and differentiation. The endocannabinoid system exerts a regulatory role of neurodevelopment that influences neural progenitor proliferation, identity and neuronal differentiation. In particular CB_1 receptor signalling controls the differentiation of corticofugal deep layer neurons owing to their ability to regulate the transcription factor switch Ctip2/Satb2. We have now developed an ES default neuronal differentiation paradigm aimed to the generation of cortical projection neurons. ES-derived neuronal differentiation generates mainly excitatory glutamate-producing neurons that express distinctive markers characteristic of upper and deep layer cortical neurons. In addition, glial cells with astrocytic features are also generated although at a lesser extent. Proliferating murine ES cells and their differentiated neuronal progeny express CB_1 receptors and therefore constitute a robust tool to investigate the role of CB_1 signaling in the transition of pluripotent ES to multipotent neural stem cells (NSC), and secondly in their neuronal differentiation program. Results derived from ongoing experiments using murine ES cells with floxed Cnr1 gene and the human NSC line hNS1 will be presented. In summary, we have developed an ES-derived neuronal differentiation protocol allowing the efficient generation of cortical neurons that constitute a reliable indefinite source of cortical neurons suitable for the study of the neurodevelopmental role of the endocannabinoid system and for the assessment of the neuroprotective efficacy of new pharmacological drugs.

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P.21.

ETHANOL CONSUMPTION DURING ADOLESCENCE ALTERS EXCITATORY SYNAPTIC TRANSMISSION IN THE MOUSE VENTRAL HIPPOCAMPUS ACTING THROUGH CB_1 RECEPTORS

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Alcohol drinking, especially among adolescents and young adults, is a serious public health concern. Research within the past decade has made it clear that ethanol interacts with the endocannabinoid system (ECS) and that the function of the ECS may be altered in ethanol dependence. However, how ethanol affects neuronal function and, in particular, synaptic neurotransmission, needs further studies.

An intermittent ethanol intake (20% in drinking water), using a 4 days drinking-in-the-dark procedure, during the adolescence period (postnatal days, pnd, 28 ± 4 to 52 ± 4) was used to investigated the effect of ethanol consumption on the excitatory synaptic transmission modulated by the cannabinoid CB_1 receptor. For this purpose, an *ex vivo* electrophysiological approach was applied in the ventral hippocampal dentate gyrus, a brain region involved in addictive behaviors, of male adolescent mice.

Excitatory postsynaptic potentials (fEPSPs) were evoked after stimulation of the medial perforant path and recordings were made in the supragranular zone of the dentate molecular layer in the presence of the $GABA_A$ antagonist picrotoxin. Activation of CB_1 receptors by the exogenous cannabinoid agonist CP55,940 (10μM) inhibited fEPSPs in controls (21.32 ± 0.24% of baseline, $p < 0.01$), as previously described by other authors. However, this effect was not observed in alcoholic mice (0.38 ± 1.35% of baseline, ns). In support of this electrophysiological data, optical density analysis revealed a significant decrease of CB_1 immunoreactivity in the outer molecular layer of the ventral hippocampal dentate gyrus of alcoholic mice (86.65 ± 1.41%) with respect to control mice (100 ± 1.52%).

These results suggest that repetitive exposure to ethanol during adolescence leads to a down-regulation and functional silencing of CB_1 receptors in excitatory synapses of the ventral hippocampal dentate gyrus.

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P.22.

THE ROLE OF ENDOCANNABINOID SYSTEM IN THE PATHOGENESIS OF ACETAMINOPHEN-INDUCED LIVER INJURY

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Background & aims: Drug induced liver injury (DILI) is a common disease but difficult to diagnose and its pathogenesis is poorly understood. One of the most commonly used analgesic/antipyretic drugs is the acetaminophen or paracetamol (APAP) which, at high doses, causes liver injury in humans and rodents.

Endocannabinoids (EC) are lipid-derived signalling molecules modulating a number of central and peripheral functions that are mainly mediated by the CB1 and CB2 receptors. It was demonstrated that the endocannabinoid system (ECS) is implicated in the pathogenesis of several liver diseases, including fatty liver diseases, fibrosis, cirrhosis, etc. However, it was not described the participation of the ECS in the pathogenesis of DILI, and whether it is implicated in the mechanisms responsible for cell death (apoptosis and necrosis) and inflammation. Consequently, we designed this study to evaluate the role of the SEC in a model of mice with APAP-induced liver injury.

Methods: Thirty male Crl:CD1 (ICR) mice were divided into five groups: vehicle, APAPx1 (single APAP dose), APAPx3 (three APAP doses, one per day), APAPx4 (four APAP doses, one per day) and APAP-recovery (four APAP doses and 14 recovery-days). In the APAP groups, liver injury was induced by oral-gavage administration of APAP (750 mg/kg). Six hours after last APAP administration, all animals were sacrificed. Blood samples and liver tissues were collected for biochemical, molecular and histopathological analyses.

Results: Increase of serum transaminases (AST, ALT and GGT), a high score of liver histopathology (necrosis, haemorrhage, inflammation…) and increased expression of several damage-markers like CYP2e1, TNFα and αSMA demonstrated APAP-induced liver injury.

Our results indicated that the expression of several ECS/PPAR-like components is altered as a consequence of liver damage by APAP. We observed a decreased expression of PPARα, DAGL, MAGL, NAPE-PLD and FAAH, and an increased expression of CB2, PPARγ, GPR55 in the liver of mice treated with repeated administration of APAP (APAPx3 and APAPx4).

The recovery of the APAP damage, characterized by a decrease of CYP2e1, TNFα and αSMA expression, is associated with a normalization of PPARα, DAGLβ, MAGL and FAAH expression.

Conclusions: Our results indicate that active APA-induced liver injury is associated with profound changes in the expression of the ECS elements, suggesting a role for these lipids signalling system in DILI.

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P.23.

NEUROPROTECTIVE EFFECT OF JZL184 IN MPP$^+$ TREATED SH-SY5Y CELLS THROUGH CB2 RECEPTORS

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Growing evidence suggests that the endocannabinoid system plays a role in neuroprotection in Parkinson´s disease. Recently, we have shown the neuroprotective effect of monoacylglycerol lipase (MAGL) inhibition with JZL184 in the chronic MPTP mouse model. However, further investigation is needed to determine the neuroprotective mechanisms of the endocannabinoid system on the nigrostriatal pathway. The aim of this work was to investigate whether the neuroprotective effect of JZL184 in mice could be extended to an *in vitro* cellular model to further understand the mechanism of action of the drug. The SH-SY5Y cell line was selected based on its dopaminergic-like phenotype and its susceptibility to MPP$^+$. Furthermore, SH-SY5Y cells express both cannabinoid receptors, CB1 and CB2. The present study describes the neuroprotective effect of MAGL inhibition with JZL184 in SH-SY5Y treated with MPP$^+$. The effect of JZL184 in cell survival was blocked by AM630 and it was mimicked with JWH133 treatment. Rimonabant did not affect JZL184-induced cell survival. These results demonstrate that the neuroprotective effect of MAGL inhibition with JZL184 described in animal models of Parkinson´s disease could be extended to *in vitro* models such as SH-SY5Y cells treated with MPP$^+$. This represents a useful tool to study mechanisms of neuroprotection mediated by MAGL inhibition and we provide evidence for the involvement of CB2 receptors in the improvement of cell survival.

P.24.

UP-REGULATION OF CB2 RECEPTORS IN LYMPHOCYTES OF PATIENTS WITH DRAVET SYNDROME

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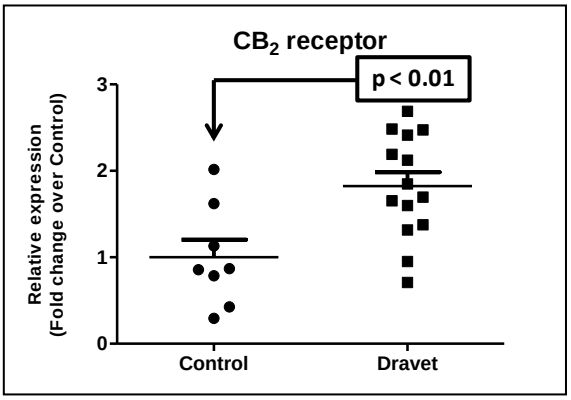
There is recent anecdotal evidence supporting that cannabidiol (CBD) is able to reduce epileptic seizures in Dravet syndrome (DS), even a clinical trial with CBD in patients with DS and related childhood epilepsy syndromes is presently in progress derived from the orphan designation by the US-FDA of a formulation of CBD (Epidiolex®; GW Pharmaceuticals) for clinical studies in these syndromes. The anticonvulsant properties of CBD and other cannabinoids are well-demonstrated in both preclinical and clinical studies but always investigated in adult epilepsy, so that these anecdotal data are the first evidence of a possible efficacy of CBD against infantile epileptic syndromes. We have hypothesized whether this efficacy may be associated with a pharmacological correction of a dysregulation in the endocannabinoid signaling system, which may contain potential targets for CBD. This dysregulation may be caused by impairments in endocannabinoid levels and/or in the activity of their receptors and enzymes. To provide an indirect proof of this dysregulation, we have investigated the status of the endocannabinoid signaling system in patients affected by DS (recruited with the help of the Spanish Dravet Foundation) through the analysis of gene expression of different endocannabinoid elements in their lymphocytes, which, in other neurological disorders, has been used as an indirect marker of possible changes occurring in the CNS. We have found that CB2 receptor gene expression is significantly elevated in DS patients compared to control subjects of similar age and gender (see attached figure), with no changes in the remaining endocannabinoid genes, e.g. CB1, TRPV1, TRPV2, GPR55 and GPR18 receptors, NAPE-PLD, DAGL, FAAH and MAGL enzymes. The expression of other related genes, e.g. PPAR-γ receptors, IL-1β, TNF-α, 5HT1A receptors, was also similar in DS patients compared to control subjects. In summary, our data proved the existence of an up-regulation of CB2 receptors in DS patients similar to the responses found with this receptor in neuroinflammatory disorders. Given that the only studies that showed an action of CBD mediated by CB2 receptors were conducted in immature animals, we assume that our data may provide a first proof to explain the efficacy of CBD in DS.



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P.25.

BOTH, ANANDAMIDE AND OLEOYLETHANOLAMIDE INDUCE CELL PROLIFERATION AND APOPTOSIS IN THE BRAIN OF ALCOHOLIC RATS

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Background & aims: Chronic alcohol exposure leads to disruption in adult neurogenesis and cell survival, an effect that might underlie structural, functional and behavioral alterations described in alcoholics. Endocannabinoids are regulated by alcohol and participate in the control of the cell proliferation and death.

Methods: We investigated the pharmacological effect of the repeated administration (5 days) of anandamide (AEA), oleoylethanolamide (OEA) and the FAAH inhibitor URB597, which limits the degradation of endocannabinoids, on the cell proliferation (phospho-H3 and BrdU) and apoptosis (cleaved caspase-3) response in the main neurogenic zones of the brain (SGZ, SVZ and hypothalamus). Studies were performed in adult rats with a voluntary consumption of alcohol or equicaloric carbohydrate diet for two weeks.

Results: Alcohol consumption reduced the number of BrdU+ cells and increased the number of apoptotic cells in the brain zones analyzed, suggesting a lower cell survival. These central effects of alcohol are accompanied with increased levels of plasmatic AEA, but not OEA. AEA and OEA treatment increased the subgranular cell proliferation (phospho-H3 and BrdU) as well as the hippocampal cell apoptosis in the alcoholic rats, suggesting an augmented cell turnover. A similar finding was observed in the SVZ/striatum of OEA-treated alcoholic rats where an increased number of proliferative and apoptotic cells was measured. However, in the hypothalamus, the effects of both acylethanolamides were minimal. URB597 treated-alcoholic rats showed an increased number of BrdU+ cells in the SGZ and SVZ, suggesting a higher cell proliferation. URB597 effects were accompanied with plasmatic increases of both AEA and OEA in all experimental groups. These changes in proliferation were accompanied by behavioral effects. Thus, AEA and OEA increased the familiar context habituation and OEA reduced voluntary ethanol consumption.

Conclusions: These results indicated that the endocannabinoids can positively modulate cell turnover, preferentially in the SGZ/hippocampus and SVZ/striatum of alcoholic rats, an effect of potential behavioral consequences.

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P.26.

EVALUATION OF PHYTOCANNABINOIDS AS A DISEASE-MODIFYING THERAPY IN R6/2 MICE, A GENETIC MODEL OF HUNTINGTON'S DISEASE

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Different cannabinoid compounds, used alone or in combination, have provided neuroprotection in experimental models of Huntington's disease (HD). In the present study, we first investigated whether a 1:1 combination of botanical extracts enriched in either Δ^9-tetrahydrocannabinol (Δ^9-THC-BDS) or cannabidiol (CBD-BDS), which are the main constituents of the cannabis-based medicine Sativex®, is neuroprotective in R6/2 mice, as it did in neurotoxin-based models of HD (Sagredo *et al*., J Neurosci Res, 2011; Valdeolivas *et al*., ACS Chem Neurosci, 2012). We recorded the progression of neurological deficits (e.g. rotarod performance) and the extent of the striatal damage, using different histological (immunostaining for neuronal markers) and biochemical (CB_1 and CB_2 receptors, neurotrophins, glutamate transporters, cytokines) markers, in R6/2 mice daily treated, starting at the 4 weeks after birth, with Sativex®-like combination of phytocannabinoids (5 mg/kg weight of Δ^9-THC-BDS and 5 mg/kg weight of CBD-BDS) or vehicle, and their corresponding wild-type animals. We observed strong alterations in these neurological, histological and biochemical parameters in R6/2 compared to wild-type mice. However, none of these changes was reversed by the treatment with the Sativex®-like combination of phytocannabinoids. Given that the treatment with Δ^9-THC alone had been effective in R6/2 mice (Blázquez *et al*., Brain, 2011), we assumed that its lack of efficacy in the Sativex®-like combination of phytocannabinoids might be related to the presence of CBD which, in some cases, may antagonize some positive effects of Δ^9-THC. This prompted us to evaluate next the effects of Δ^9-THC-BDS in absence of combination with CBD-BDS. In this second experiment, the treatment with Δ^9-THC-BDS (10 mg/kg) ameliorated the deteriorated rotarod performance in R6/2 mice during 2-3 weeks after initiated the treatment, but these benefits disappeared after these 3 weeks and this prolonged for 2 weeks more up to animals were euthanized. The analysis of their postmortem striatal tissue supported this lack of neurological benefits, as we were unable to find any improvement in the alterations found in these animals in biochemical and histological markers representing the striatal function. In conclusion, we were unable to find positive effects on the progression of striatal damage in R6/2 mice with the Sativex®-like combination of phytocannabinoids, despite the promising expectations generated by its beneficial effects in neurotoxin-based models of HD. By contrast, we observed a transient neurological improvement after the treatment with Δ^9-THC-BDS alone, which occurred at early phases but disappeared at advanced stages. This might indicate that: (i) in this model the combination with CBD could be antagonizing, instead of complementing, the beneficial effects of Δ^9-THC; and (ii) the need to use alternative phytocannabinoid combinations or a dose escalation paradigm, a fact that is presently under investigation.

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P.27.

DIFFERENTIAL EFFECTS OF REPEATED ETHANOL BINGES ON THE ENDOCANNABINOID SYSTEM IN THE RAT LIVER VERSUS JEJUNUM

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Background & aims: Binge drinking is a behavioral pattern, characterized by repeatedly drinking to intoxication, which may lead to alcohol dependence. Although the behavioral and physiological effects of ethanol in acute and chronic models have been studied, the relationship between the endocannabinoid system (ECS) and ethanol in a model with an acutely damaging event like a 4-day or 8-day binge model has received less attention. Indeed, barely any information is available concerning the influence of repeated binge pattern administration on the ECS in peripheral tissues and its role in the modulation of binge-like ethanol consumption. This study aimed to evaluate how binge-like ethanol consumption alters endocannabinoid levels in peripheral tissues (liver and jejunum) by investigating the consequence of binge-like consumption of alcohol on the expression of genes related to ECS.

Methods: 32 male Wistar rats were used for the binge-like drinking protocol. The animals were divided in four groups of eight rats each. In order to habituate the animals to the intragastric (i.g.) protocol and reduce nonspecific stress responses before alcohol administration, rats were administered tap water (i.g.) once/week twice. Then, rats were assigned into one of the following groups: water-control; one-alcohol binge; four-alcohol binges; and eight-alcohol binges. Every binge alcohol treatment was carried out once/week. Alcohol was orally administered (i.g.) at a dose of 3g/kg using a 25% alcohol solution at a volume of 15 mL/kg. Control animals received the same volume of liquid but were given tap water alone. The rats were deprived of food 12 h prior to the i.g. treatment in order to normalize the absorption of alcohol among the animals avoiding the presence/absence of food into their stomach. All the groups of animals were sacrificed by decapitation 120 min after the binge alcohol. After the sacrifice, the gene expression of relevant components of the ECS was analyzed in the liver and jejunum tissues using quantitative RT-PCR.

Results: Binge-like ethanol consumption was associated with significant changes in ECS gene expression in the liver and the jejunum. We found that mRNA expression of the ECS components analyzed (*Cnr1*, *Cnr2*, *Pparα*, *Nape-pld*, *Faah*, *Daglα/β*, *Mgll*) decreased in the liver of binge-4 and binge-8 groups. These changes correlate with the decreased expression of the inflammatory marker *Cd36*. However, the jejunum showed increased mRNA levels of *Cnr2*, *Pparα*, *Daglα* and *Mgll* mainly observed in binge-8 group, which also correlate with the increased expression of *Cd36*.

Conclusion: These results suggest that binge-like ethanol consumption alters the expression of relevant components of the endocannabinoid signaling system in a tissue-selective manner. These opposed effects in liver and jejunum may influence changes in pathways involved in alcohol abuse and may be important as future therapy targets in treating binge drinking, which increases the risk of alcoholism.

Grants: PNSD2010/143, RD12/0028/0001, RD12/0028/0009

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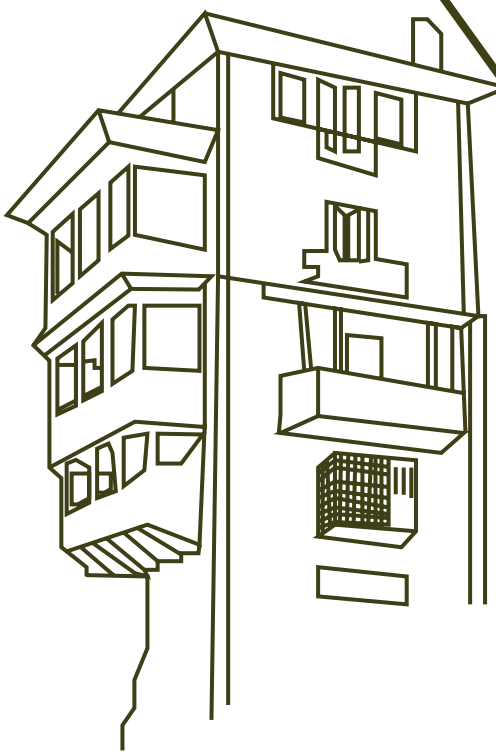

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