16ª Reunión Anual
Sociedad Española de Investigación sobre Cannabinoides

Granada, 22-23 de septiembre de 2015

PROGRAMA CIENTÍFICO

Martes, 22 de septiembre

08:00-09:00 **Entrega de documentación**
Palacio de Congresos de Granada
Paseo del Violón, s/n
18006 - Granada

09:00-09:15 **Inauguración**
- Manuel Guzmán, Presidente de la SEIC
- José Luis Trejo, Vicepresidente de la SENC

9:15-10:30 **Sesión de comunicaciones orales**
"Señalización y neurodesarrollo"
(Moderadores: Izaskun Elezgarai y Francisco Carrillo)

09:15-09:30 Presentación (Izaskun Elezgarai)

09:30-09:45 O.1.1.

FUNCTIONAL SELECTIVITY OF CANNABINOID RECEPTORS SIGNALING IN MOUSE BRAIN CORTEX. I. Ibarra-Lecue, R. Díez-Alarcia, A.P. López-Cardona, L.F. Callado, E. Agirregoitia, L. Urigüen

09:45-10:00 O.1.2.

CIRCUIT-SPECIFIC SIGNALING IN ASTROCYTE-NEURON NETWORKS IN BASAL GANGLIA PATHWAYS. R. Martín, R. Bajo-Grañeras, R. Moratalla, G. Perea, A. Araque

10:00-10:15 O.1.3.

DEVELOPMENTAL LOSS OF CB1 CANNABINOID RECEPTOR BLOCKS NEURONAL MIGRATION AND LEADS TO PROFOUND LONG-LASTING CORTICAL MALFORMATIONS. J. Díaz-Alonso, A. de Salas-Quiroga, M. Parsons, C. Andradas, P. Garcez, J. Paraíso-Luna, D. García-Rincón, F. Guillemot, M. Guzmán, I. Galve-Roperh

10:15-10:30 O.1.4.

PRENATAL CANNABINOID ADMINISTRATION INDUCES LONG-LASTING ALTERATIONS IN THE OFFSPRING BY IMPAIRING CB1 RECEPTOR-DEPENDENT REGULATION OF PROJECTION NEURON DEVELOPMENT. A. de Salas Quiroga, J. Díaz Alonso, D. García Rincón, F. Remmers, D. Vega, M. Gómez Cañas, J. Paraíso Luna, B. Lutz, M. Guzmán, I. Galve Roperh

10:30-11:15 Café

11:15-12:45 Sesión de comunicaciones orales
"Aspectos psiquiátricos"
(Moderadores: Laura Cutando y Alejandro Higuera Matas)

11:15-11:30 Presentación (Laura Cutando)

11:30-11:45 O.2.1.

ALCOHOL BINGE DRINKING-INDUCED NEUROINFLAMMATION AND BRAIN DAMAGE IN FRONTAL CORTEX IS PREVENTED BY OLEOYLETHANOLAMIDE PRE-TREATMENT. M. Antón, T. Marbán, F. Alén, F.J. Pavón, J.C. Leza, F. Rodríguez de Fonseca, B. García-Bueno, R. Gómez de Heras, L. Orio

11:45-12:00 O.2.2.

A CHRONIC Δ^9-TETRAHYDROCANNABINOL TREATMENT DURING ADOLESCENCE INCREASES COCAINE SELF-ADMINISTRATION, COMPULSIVE DRUG SEEKING AND ESCALATION AT ADULTHOOD. J. Orihuel, R. Capellán, M. Ucha, D. Roura-Martínez, A. Contreras, M. Fernández-Cabrera, E. Ambrosio, A. Higuera-Matas

12:00-12:15 O.2.3.

CB_1 RECEPTOR AND $GABA_A$ SUBUNIT EXPRESSION IN THE MEDIAL AND LATERAL ORBITOFRONTAL CORTICES AND THEIR RELATIONSHIP TO DIFFERENT FORMS OF IMPULSIVE BEHAVIOR. A. Contreras, M. Ucha, D. Roura-Martínez, J. Orihuel, R. Capellán, E. Ambrosio, A. Higuera-Matas

12:15-12:30 O.2.4.

STRESS-INDUCED AMNESIA IS MEDIATED BY CENTRAL AND PERIPHERAL CB1 RECEPTORS. A. Busquets-Garcia, M. Gomis-González, R. K. Srivastava, L. Cutando, A. Ortega-Alvaro, S. Ruehle, F. Remmers, L. Bellochio, L. Bindila, G. Marsicano, B. Lutz, R. Maldonado, A. Ozaita

12:30-12:45 O.2.5.

CANNABINOID RECEPTOR 1 BLOCKADE RESTORES mTOR ACTIVITY AND COGNITIVE PERFORMANCE IN TWO MOUSE MODELS OF DOWN SYNDROME. A. Navarro-Romero, M. Gomis-González, A. Busquets-Garcia, M. Dierssen, R. Maldonado, A. Ozaita

13:00-14:30 Comida (Hotel Saray)

14:45-16:15 Sesión de comunicaciones orales
"Acciones periféricas"
(Moderadores: Eduardo Pérez Gómez y Ekaitz Agirregoitia)

14:45-15:00 Presentación (Eduardo Pérez Gómez)

15:00-15:15 O.3.1.

INVOLVEMENT OF THE CB_2 CANNABINOID RECEPTOR IN HUMAN COLON CANCER PROGRESSION. E. Martínez-Martínez, A. Martín-Ruíz, P. Martín, M. Provencio, J.M. García

15:15-15:30 O.3.2.
THC AS AN ANTI-HER2 THERAPY IN BREAST CANCER. S. Blasco-Benito, E. Pérez-Gómez, C. Andradas, M. Guzmán, C. Sánchez

15:30-15:45 O.3.3.

ACETAMINOPHEN (PARACETAMOL)-INDUCED LIVER INJURY ALTERS THE EXPRESSION OF THE ENDOGENOUS CANNABINOID SYSTEM. P. Rivera, S. Arrabal, A. Pastor, J.M. Decara, A. Vargas, L. Sánchez, E. Baixeras, M.I. Lucena, F. Rodríguez de Fonseca, J. Suárez

15:45-16:00 O.3.4.

EFFECTS OF THE NON-SELECTIVE CANNABINOID AGONIST WIN 55,212-2 ON VISCERAL SENSTITIVITY ALTERATIONS INDUCED BY THE ANTINEOPLASTIC DRUG 5-FLUOROURACIL IN THE RAT. H. Hernández Iriarte, G. Vera Pasamontes, R. Abalo Delgado

16:00-16:15 O.3.5.

INVOLVEMENT OF CANNABINOIDS DURING OOCYTE MATURATION IN BOVINE MODEL: IMPORTANCE ON FERTILIZATION AND EMBRYO PREIMPLANTATION DEVELOPMENT. A.P. López-Cardona, M.J. Sánchez-Calabuig, P. Beltrán-Breña, N. Agirregoitia, D. Rizos, A. Gutiérrez-Adán, E. Agirregoitia

16:15-17:15 Sesión de posters

17:15-19:00 Asamblea de la SEIC

21:00 **Cena del congreso**

Miércoles, 23 de septiembre

08:30-10:00 **Sesión de comunicaciones orales**
"Neuroprotección (I)"
(Moderadores: Concepción García y Leyre Mestre)

08:30-08:45 Presentación (Concepción García)

08:45-09:00 O.4.1.

HISTOLOGICAL AND BIOCHEMICAL BASIS OF THE CLINICAL NEUROPROTECTIVE EFFECTS OF CANNABIDIOL IN HYPOXIC-ISCHEMIC NEWBORN PIGS. A. Cabañas, L. Barata, M. Ceprián, H. Lafuente, L. Campa, M.R. Pazos, F.J. Alvarez, L. Jiménez-Sánchez, J. Martínez-Orgado

09:00-09:15 O.4.2.

CANNABIDIOL PREVENTS POST-HYPOXIC-ISCHEMIC HYPOMIELINATION BY RESTORING MATURE OLIGODENDROCYTE FUNCTION IN NEWBORN RATS. M. Ceprián, C. Vargas, S. Achicallende, L. García Toscano, X. Téllez Ribera, F. Penna, P. Grandes, I. Elezgarai, J. Fernández-Ruiz, L. Jiménez-Sánchez, M.R. Pazos, J. Martínez-Orgado

09:15-09:30 O.4.3.

CANNABIDIOL REDUCES PERI-INFARCT BRAIN DAMAGE AND RESTORES NEUROBEHAVIORAL FUNCTION IN A NEWBORN RAT MODEL OF ACUTE ISCHEMIC STROKE. M. Ceprián, C. Vargas, A. Cora, L. Barata, J. Martínez-Orgado, L. Jiménez-Sánchez

09:30-09:45 O.4.4.

CANNABIDIOL AND MESENCHYMAL STEM CELLS AS NEW THERAPIES IN ADOPTIVELY TRANSFERRED EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS. C. González-García, L. Campos-Ruíz, I. Moreno-Torres, M.J. Coronado Albí, A. Sánchez-López, J.A. García-Merino

09:45-10:00 O.4.5.

NEUROPROTECTION BY NON-PSYCHOTOPIC PHYTOCANNABINOIDS IN A CELLULAR MODEL OF STRIATAL DAMAGE RELEVANT FOR HUNTINGTON'S DISEASE: INVOLVEMENT OF NRF-2-MEDIATED MECHANISMS. S. Valdeolivas, O. Sagredo, J. Fernández-Ruiz

10:00-11:15 **Café y sesión de posters**

11:15-12:45 Sesión de comunicaciones orales
"Neuroprotección (II)"

11:15-11:30 O.4.6.

POTENTIAL NEUROPROTECTIVE ROLE OF SPECIFIC CB1 RECEPTOR SUBPOPULATIONS IN THE MOUSE CORTICOSTRIATAL CIRCUITRY. A. Ruiz-Calvo*, L. Bellocchio*, A. Chiarlone, R. Bajo-Grañeras, E. Resel, I. Galve-Roperh, M. Guzmán

11:30-11:45 O.4.7.

EFFECTS OF 2-AG IN ACUTE TMEV INFECTION: CNS IMMUNOMODULATION AND INHIBITION OF LEUKOCYTE EGRESS FROM THE SPLEEN THROUGH S1P1 RECEPTOR. M. Mecha, C. Cordero, A. Feliú, F.J. Carrillo-Salinas, G. Hernández-Torres, S. Ortega-Gutiérrez, D. Clemente, M.L. Lopez-Rodríguez, F. De Castro, C. Guaza

11:45-12:00 O.4.8.

CANNABINOID CB_2 RECEPTORS ARE SELECTIVELY EXPRESSED BY ACTIVATED MICROGLIAL CELLS IN ALZHEIMER'S DISEASE. A. López, C. Vázquez, N. Aparicio, R.M. Tolón, J. Romero, M.C. García, J. Fernández-Ruiz, B.N. Dittel, C.J. Hillard, Julián Romero

12:00-12:15 O.4.9.

NEW POTENTIAL TARGETS FOR CB_2 RECEPTOR IN CNS DISEASES WITH A NEUROINFLAMATORY COMPONENT. G. Navarro, I. Reyes, P. Morales, I. Etayo, E. Angelats, P. Goya, N. Jagerovic, R. Franco

12:15-12:30 O.4.10.

GPR55: A THERAPEUTIC TARGET FOR PD? M. Celorrio, E. Rojo-Bustamante, D. Fernández-Suárez, R. Franco, M.S. Aymerich

12:30-12:45 O.4.11.

BIOLOGICAL CHARACTERIZATION OF PM226, A CHROMENOISOXAZOLE, AS A SELECTIVE CB_2 RECEPTOR AGONIST WITH NEUROPROTECTIVE PROFILE. M. Gómez-Cañas, P. Morales, L. García-Toscano, C. Navarrete, P. Goya, M. García-Arencibia, E. Muñoz, J. Fernández-Ruiz, N. Jagerovic, M.R. Pazos

13:00-14:30 Inauguración Congreso SENC y aperitivo

14:30-17:00 Simposio SEIC-SENC
"Neurobiology of the Cannabinoid System"

14:30-15:30 Premios a las mejores publicaciones 2015
(Presentados por Carmen Guaza y Javier Fernández Ruiz)

15:30-16:15 Michelle Glass (Universidad de Auckland, Nueva Zelanda)

"Gs coupling of CB_1 cannabinoid receptors: ligand bias and the influence of receptor number"
(Presentada por Julián Romero)

16:15-17:00 Francis Chaouloff (INSERM-Universidad de Burdeos, Francia)
"Are you motivated to run? The role of the endocannabinoid system"
(Presentado por Pedro Grandes)

17:00-17:30 Entrega de premios y Clausura

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MATERNAL EXPOSURE TO EITHER FOOD RESTRICTION OR CAFETERIA DIET DURING PRE- AND GESTATIONAL PERIODS INDUCED SIMILAR EFFECTS ON THE ENDOCANNABINOID SYSTEM IN THE LIVER AND ADIPOSE TISSUE OF THEIR RAT OFFSPRINGS IN ADULTHOOD. R. Arco, J. Decara, M.T. Ramírez-López, M. Vázquez, R.N. Blanco, F. Alén, M. Antón, D. Ouro, L. Orio, J. Suarez, R. Gómez de Heras, F. Rodríguez de Fonseca

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ELECTRON MICROSCOPY LOCALIZATION OF MITOCHONDRIAL CB_1 IN CA1 HIPPOCAMPAL REGION IN CHRONIC STRESSED MICE. I.Bonilla, A. Gutiérrez, N. Puente, N. Royo, S. Peñasco, G. Marsicano, P. Grandes

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DELTA-9-TETRAHYDROCANNABINOL CAUSES SYNAPTIC AND MOTOR COORDINATION IMPAIRMENTS THROUGH A MECHANISM UNDERLYING COX-2 ACTIVATION AND MICROGLIAL REACTIVITY. L. Cutando, V. Salgado-Mendialdúa, R. Maldonado, A. Ozaita

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CSPGs MODULATION OF THE EXTRACELLULAR MATRIX AS A THERAPEUTICAL APPROACH IN A VIRAL MODEL OF MULTIPLE SCLEROSIS. A. Feliú, M. Mecha, F.J. Carrillo-Salinas, G. Hernández-Torres, S. Ortega-

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THE INHIBITORY EFFECT OF THE CANNABINOID AGONIST, CP55940 ON THE CYTOCHROME C OXIDASE ACTIVITY IS REDUCED IN A MODEL OF PARKINSON'S DISEASE. M.D. García-Fernández, T. Tolentino-Cortez, I. Manuel, R. Rodríguez-Puertas, E. Astigarraga, G. Barreda-Gómez

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CANNABIDIOL TREATMENT RESTORES THE IMPAIRED NEUROGENIC CONTRACTION OF BLADDER FROM RATS WITH NEONATAL HYPOXIC-ISCHEMIC BRAIN DAMAGE. L. García-Toscano, A. Martínez-Sáenz, M. Ceprián, V. Fernandes, L. Jiménez-Sánchez, X. Téllez, J. Fernández-Ruiz, S. Bustamante, M. Hernández, J. Martínez-Orgado, M. R. Pazos

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CANNABINOID CB2 RECEPTOR AS A POTENTIAL TARGET IN LRRK2-TRANSGENIC MICE: IS IT REALLY RELEVANT FOR PARKINSON'S DISEASE? C. Palomo-Garo, Y. Gómez-Gálvez, J. Ferrer, J.A. Ramos, J. Fernández-Ruiz, C. García

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LONG TERM SYNAPTIC PLASTICITY DEPENDENT ON CANNABINOID CB_1 RECEPTORS ACTIVATION IS ALTERED IN THE DENTATE GYRUS OF ADULT MICE EXPOSED TO ETHANOL DURING ADOLESCENCE. S. Peñasco, N. Puente, A. Ramos, N. Royo, A. Gutiérrez, I. Bonilla, L. Reguero, M.J. Canduela, J. Mendizabal-Zubiaga, F. Rodríguez de Fonseca, J. Suárez, I. Elezgarai, P. Grandes

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A MODERATE MATERNAL CALORIC RESTRICTION DURING PRECONCEPTIONAL AND GESTATIONAL PERIODS IMPAIRS HYPOTHALAMIC ENDOCANNABINOIDS AND N-ACYLETHANOLAMIDE LEVELS IN MALE OFFSPRING AT BIRTH. Ramírez-López, M.T., Vázquez, M., Bindila, L.,

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GPR55, A RECEPTOR THAT LIKES CANNABINOIDS. I. Reyes-Resina, I. Etayo-Labiano, E. Martínez-Pinilla, N.A. Balenga, G. Navarro-Brugal, R. Franco

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EXPRESSION OF ENDOCANNABINOID SYSTEM ELEMENTS IN THE BASAL GANGLIA OF LEVODOPA-TREATED PARKINSONIAN MONKEYS. E. Rojo-Bustamante, M.Á. Abellanas, M.R. Luquin, M.S. Aymerich

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CHRONIC STRESS IMPAIRS CANNABINOID 1 (CB_1) RECEPTOR-MEDIATED CONTROL OF GLUTAMATERGIC TRANSMISSION AND PLASTICITY IN YOUNG ADULT MICE DENTATE GYRUS. N. Royo, N. Puente, S. Peñasco, L. Reguero, A. Gutierrez, I. Bonilla, M.J. Canduela, J.L. Mendizabal-Zubiaga, I. Elezgarai, A. Ramos, P. Grandes

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PHARMACOLOGICAL BLOCKADE OF CANNABINOID CB1 RECEPTORS IN DIET-INDUCED OBESITY REGULATES DIHYDROLIPOAMIDE DEHYDROGENASE IN MUSCLE. S. Arrabal, M.A. Lucena, M.J. Canduela, A. Ramos-Uriarte, P. Rivera, A. Serrano, F.J. Pavón, J. Decara, E. Baixeras, M. Martín-Rufián, J. Márquez, P. Fernández-Llébrez, B. de Roos, P. Grandes, F. Rodríguez de Fonseca, J. Suárez

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DOWN-REGULATION INSTEAD UP-REGULATION OF CB2 RECEPTORS IN RESPONSE TO INFLAMMATION AND OXIDATIVE STRESS IN AN IN VIVO MODEL OF NEONATAL HYPOXIA-ISCHEMIA. X. Téllez, M. Ceprián, L. García-Toscano, L. Jiménez-Sánchez, J. González Rodrigo, J. Fernández-Ruiz, J. Martínez-Orgado, M.R. Pazos

O.1.1.

FUNCTIONAL SELECTIVITY OF CANNABINOID RECEPTORS SIGNALING IN MOUSE BRAIN CORTEX

I. Ibarra-Lecue[1], R. Díez-Alarcia[1,3], A.P. López-Cardona[4,5], L.F. Callado[1,3], E. Agirregoitia[2], L Urigüen[1,3]

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Evidence shows that, for most of G protein-coupled receptors (GPCRs), distinct agonists can differentially regulate several signaling pathways through the same receptor by a selective activation of different intracellular effectors, a mechanism known as functional selectivity. In the case of cannabinoid receptors, both the CB_1 and CB_2 receptors have been shown to preferentially couple to the $G_{i/o}$ family of heterotrimeric G-proteins. Furthermore, CB_1 receptor has been demonstrated to be capable of coupling to different families of G-proteins when activated by an agonist drug suggesting that different intracellular responses may be activated by the CB_1 receptor depending on the ligand.

The aim of the present study was to evaluate the functional coupling of both CB_1 and CB_2 receptors to different subtypes of G proteins ($G_{\alpha i1}$, $G_{\alpha i3}$, $G_{\alpha s}$, $G_{\alpha z}$, $G_{\alpha 12/13}$ and $G_{\alpha q/11}$) in mouse brain cortex membrane homogenates. For this purpose, stimulation of the [^{35}S]GTPγS binding by the CB_1/CB_2 cannabinoid agonists WIN55,212-2 and Δ^9THC (10^{-5} M) was determined by Scintillation Proximity Assay (SPA) technique.

WIN55,212-2 stimulated [^{35}S]GTPγS binding to all the G_α proteins evaluated (E_{max} range 117±2% to 131±3%). On the other hand, Δ^9THC induced a stimulation of the [^{35}S]GTPγS binding to $G_{\alpha i1}$, $G_{\alpha i3}$, $G_{\alpha s}$, and $G_{\alpha q/11}$ (E_{max} range 111±2% to 134±3%) but not to $G_{\alpha z}$ and $G_{\alpha 12/13}$ proteins. In all cases, activation of the G_α proteins by WIN55,212-2 and Δ^9THC was blocked by the antagonist O2050 (10^{-5} M). To elucidate the role of each cannabinoid receptor in this G protein activation, the same experiments were carried out with brain membranes from CB_1ko, CB_2ko and CB_1/CB_2 double ko mice. Results suggest that the stimulation of $G_{\alpha i1}$, $G_{\alpha i3}$, $G_{\alpha s}$, $G_{\alpha z}$, and $G_{\alpha q/11}$ G protein subtypes by WIN55,212-2 is CB_1 receptor-mediated. However, WIN55,212-2 seems to stimulate the coupling to $G_{\alpha 12/13}$ protein through a CB_2 receptor-dependant mechanism. In the same manner, Δ^9THC appears to activate $G_{\alpha i1}$, $G_{\alpha i3}$, $G_{\alpha s}$, $G_{\alpha z}$ and $G_{\alpha 12/13}$ through CB_1 receptors but stimulates [^{35}S]GTPγS binding to $G_{\alpha q/11}$ by activating CB_2 receptors.

Our results demonstrate that, in mice brain tissue, different exogenous cannabinoid ligands are able to selectively activate different G_α protein subtypes, inhibitory and non-inhibitory G_α protein subtypes, through the activation of both CB_1 and CB_2 receptors.

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Key words: functional selectivity, Scintillation Proximity Assay

O.1.2.

CIRCUIT-SPECIFIC SIGNALING IN ASTROCYTE-NEURON NETWORKS IN BASAL GANGLIA PATHWAYS

R. Martín[1]*, R. Bajo-Grañeras[1]*, R. Moratalla[1,2], G. Perea[1], A. Araque[3]

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Astrocytes are non-neuronal cells that are emerging as important regulatory elements in brain function by actively exchanging signals with neurons. They respond to neurotransmitters and release gliotransmitters that modulate synaptic transmission. However, the cell- and synapse- specificity of the functional relationship between astrocytes and neurons in particular brain circuits remains unknown. Here we show that in the dorsal striatum, which mainly comprises two subtypes of intermingled neurons (striatonigral and striatopallidal medium spiny neurons, MSNs) and synapses belonging two distinct neural circuits (the basal ganglia direct and indirect pathways), subpopulations of striatal astrocytes selectively respond to endocannabinoids released by the activity of specific MSN subtypes. In turn, these subpopulations of astrocytes release glutamate that selectively activates NMDA receptors in homotypic, but not heterotypic MSNs. Likewise, subpopulations of astrocytes lead to the selective regulation of homotypic synapses through activation of group I metabotropic glutamate receptors. Therefore, bidirectional astrocyte-neuron signaling selectively occurs between specific subpopulations of astrocytes, neurons and synapses, which establish circuit-specific functional astrocyte-neuronal networks.

Key words: astrocytic CB1 receptors, glial-neuronal interaction, tripartite synapse

O.1.3.

DEVELOPMENTAL LOSS OF CB1 CANNABINOID RECEPTOR BLOCKS NEURONAL MIGRATION AND LEADS TO PROFOUND LONG-LASTING CORTICAL MALFORMATIONS

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Besides its well-known neuromodulatory role in adult brain synapses, the cannabinoid CB1 receptor is an emergent important regulator of mammalian brain development. CB1 signaling has been proposed to regulate neuronal migration, but the precise molecular mechanisms involved remain obscure. In this study we demonstrate that the CB1 receptor is required for proper radial migration of cortical pyramidal neurons *in vivo*.

Transient CB1 knockdown by *in utero* electroporation of a siRNA-CB1 arrests radial migration of pyramidal neurons, resulting in long-lasting aberrant cortical layering and subcortical band heterotopias (SBH) that render the adult offspring more susceptible to pentylenetetrazol (PTZ)-induced seizures, pointing to an abnormal wiring. We found that a defined gradient of the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA), acting through CB1 receptors, direct migration of different classes of newborn pyramidal neurons in a postmitotic, cell-autonomous manner. We identified proteasomal degradation of the small G-protein RhoA as a key downstream event in the pro-migratory cascade triggered by CB1 activation. Hence, simultaneous RhoA knockdown rescued the migration arrest induced by CB1 loss of function and restores latency to PTZ-induced seizures. Our study demonstrates that the CB1 receptor drives radial migration of pyramidal neurons in the developing mouse cortex and indicates that abnormal endocannabinoid function during development might underlie some types of malformations of cortical development.

This work has been supported by FEDER and FIS (PI12-00919) funding.

Keywords: neuronal migration; endocannabinoids; CB1; RhoA; cortical development

O.1.4.

PRENATAL CANNABINOID ADMINISTRATION INDUCES LONG-LASTING ALTERATIONS IN THE OFFSPRING BY IMPAIRING CB1 RECEPTOR-DEPENDENT REGULATION OF PROJECTION NEURON DEVELOPMENT

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The cannabinoid CB_1 receptor, the main target of Δ^9-tetrahydrocannabinol (THC) and other psychoactive compounds of marijuana, plays a crucial regulatory role in brain development as evidenced by the neurodevelopmental consequences of its manipulation in animal models. Likewise, recreational cannabis abuse during pregnancy can affect structural and functional brain plasticity in the fetus. However, the precise neurobiological substrates underlying these actions are as yet unclear. Here we show that THC administration to pregnant mice in a restricted gestational time window reduced subcerebral projection neuron generation, altered corticospinal connectivity and, as a consequence, produced long-lasting alterations in the fine motor performance of the offspring. Neuronal traits upon THC exposure were reminiscent to those elicited by CB_1 receptor genetic ablation, and CB_1-null mice were resistant to THC-induced alterations. To determine the neuronal identity of THC-exposure actions we made use of a Cre-mediated, lineage-specific, CB_1-expression strategy in a CB_1-null background. Selective embryonic re-expression of CB_1 in dorsal telencephalic glutamatergic neurons, but not forebrain GABAergic neurons, rescued the deficits in corticospinal motor neuron development of CB_1-deficient mice and restored their susceptibility to THC-induced motor alterations. In addition, restricted embryonic THC administration induced an increase in seizure susceptibility that, in this case, was mediated by its ability to interfere with the CB_1-dependent regulation of glutamatergic neuron and inhibitory neuron development. Collectively, these findings show that some of the functional consequences of embryonic cannabinoid exposure that persist in the adulthood are solely mediated by the interference with the neurodevelopmental endogenous function of the CB_1 receptor, thus paving the way for identifying the precise neurobiological substrates that underlie the impact of cannabis abuse during pregnancy.

This work has been supported by FEDER and FIS (PI12-00919) funding
Keywords: cannabis, CB_1 cannabinoid receptor, pregnancy, corticospinal motor neuron, genetic rescue, motor behavior, epileptogenesis.

O.2.1.

ALCOHOL BINGE DRINKING-INDUCED NEUROINFLAMMATION AND BRAIN DAMAGE IN FRONTAL CORTEX IS PREVENTED BY OLEOYLETHANOLAMIDE PRE-TREATMENT

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Alcohol abuse is frequently characterized by a specific pattern of intake in binge drinking episodes, inducing neuroinflammation and brain damage. Here, we characterized the temporal profile of neuroinflammation in rats exposed to intragastric binge ethanol administrations and tested the anti-inflammatory/neuroprotectant properties of the cannabinoid analog oleoylethanolamide (OEA). Pre-treatment with OEA blocked the expression of High Mobility Group Box 1 (HMGB1) danger signal and the innate immunity Toll-like receptors 4 (TLR4), inhibiting the Nuclear factor-*kappa* B (NF-kB) proinflammatory cascade induced by alcohol binge in frontal cortex. OEA reduced the levels of Interleukin-1beta (IL-1β), the monocyte chemoattractant protein-1 (MCP-1), and the enzymes cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in ethanol binged animals. Elevations in plasma Tumor necrosis factor alpha (TNF-α) and IL-1β after ethanol were also inhibited by OEA. Additionally, OEA prevented ethanol-induced lipid peroxidation, caspase-8 and pro-apoptotic caspase-3 activation in frontal cortex. Finally, OEA blocked the rise in blood corticosterone levels after ethanol with no alteration in blood ethanol levels. Altogether, results highlight a beneficial profile of OEA as a potent anti-inflammatory/neuroprotectant compound to treat alcohol abuse.

Keywords: oleoylethanolamide, alcohol, neuroinflammation.

O.2.2.

A CHRONIC Δ9-TETRAHYDROCANNABINOL TREATMENT DURING ADOLESCENCE INCREASES COCAINE SELF-ADMINISTRATION, COMPULSIVE DRUG SEEKING AND ESCALATION AT ADULTHOOD.

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Cannabis continues to be the illegal drug most widely consumed by adolescents. There is epidemiological evidence suggesting that early marihuana consumption might act as a gateway to addiction to other drugs during adulthood. Using a preclinical model, we have previously shown that a chronic exposure to the synthetic cannabinoid CP 55,940 during adolescence facilitates the acquisition of cocaine self-administration in female rats. The aim of this study was to validate these previous results using the psychoactive component of the cannabis plant, THC, and to further explore the possible addictive phenotype induced by such treatment as well as some putative underlying psychological mechanism (such as alterations in Pavlovian to instrumental transfer –PIT- or in motor impulsivity).

Male and female adolescent Wistar rats (PND28-42) were injected with THC (3mg/kg i.p.) or vehicle (ethanol:chremophor:saline) every other day and left undisturbed until they reached adulthood (PND90). Subsequently, they underwent cocaine self-administration (0,5 mg/kg) under different conditions: fixed ratio, progressive ratio, punished cocaine intake (as a measure of compulsivity), extended access and cue-induced reinstatement after different withdrawal periods (incubation of seeking). In an additional sets of rats with the same adolescent treatment we studied the PIT and motor impulsivity using a 2-choice serial reaction time task.

THC-treated rats showed a facilitation of cocaine self-administration acquisition during the first seven sessions, but there were no clear differences during the progressive ratio phase. THC-treated males also displayed more compulsive cocaine seeking than the other groups. In addition, THC-exposed females exhibited higher cocaine intake during extended access, conversely they showed a decreased incubation phenomenon. During the PIT protocol, we observed that THC-exposed animals increased seeking behavior, anticipatory activity and higher sensitivity to appetitive conditioned stimuli. With regard to impulsive behavior, THC-exposed females also showed a trend to perform less premature responses than their controls.

These results provide evidence supporting the Gateway Hypothesis of Drug Addiction and the existence of an addictive phenotype in rats with a chronic THC treatment during adolescence.

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Key words: THC, Cocaine, Gateway hypothesis

O.2.3.

CB1 RECEPTOR AND GABAA SUBUNIT EXPRESSION IN THE MEDIAL AND LATERAL ORBITOFRONTAL CORTICES AND THEIR RELATIONSHIP TO DIFFERENT FORMS OF IMPULSIVE BEHAVIOR

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Impulsivity is a multifactorial construct involving a wide range of behaviors that occur without foresight and can be detrimental to the individual. The Orbitofrontal cortex (OFC) is a key region concerning decision making processes, and previous studies suggest that there is a functional dissociation between its medial and lateral subdivisions with regard to impulse control. In a previous SEIC meeting, we showed that medial but not lateral orbitofrontal CB_1 gene expression correlated with cognitive impulsivity as measured by the delay discounting task. In this study we wanted to verify if this same dissociation holds true in the case of motor impulsivity as measured in the 2-choice serial reaction time task. In addition, it has recently been proposed that impulsive behavior is under GABAergic control. Because GABA transmission is regulated by endocannabinoids and bearing in mind the previously mentioned dissociation at the level of CB_1 receptors we set out to analyze the gene expression of two important elements of the $GABA_A$ complex, the α_1 and γ_2 subunits in the two subdivisions of the OFC mentioned before.

We could not find any significant association between motor impulsivity indices and CB_1 gene expression in any of the divisions of the OFC. As regards the gene expression of the $GABA_A$ subunits, the γ_2/α_1 ratio was positively correlated to cognitive impulsivity in the medial but not lateral OFC. However, no significant correlations were found for the expression of the $\alpha 1$ or γ_2 subunits alone. Conversely, there we found a significant negative association between motor impulsivity and α_1 gene expression in the lateral but not medial OFC.

These results add to our knowledge on the neurochemical regulation of the different forms of impulsive behavior and the neural loci that govern them.

Key words: Impulsivity, Orbitofrontal Cortex

O.2.4.

STRESS-INDUCED AMNESIA IS MEDIATED BY CENTRAL AND PERIPHERAL CB1 RECEPTORS

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Memory consolidation is strongly influenced by emotional experiences. The endocannabinoid system modulates both emotions and memory, but the integration of these functions in memory consolidation has not been investigated. We have studied the involvement of cannabinoid type 1 (CB1) receptors in the stress-induced amnesia over a non-emotional memory. For this purpose, we used a model for non-emotional episodic memory in mice, the novel-object recognition test. We found that stress promotes an impairment in the consolidation of a long-term memory trace in this cognitive task. These effects were not observed after systemic rimonabant injection, or in knockout mice lacking CB1 receptors in all cell types, but also after rimonabant intra-hippocampal administration pointing to a central role of endocannabinoids. Interestingly, adrenalectomy and the peripherally-restricted CB1 receptor antagonist AM6545 also prevented the amnesic effects of stress, pointing to a concomitant peripheral component. Moreover, using several cell type-specific conditional CB_1 receptor knockout mouse lines, we found that CB1 receptors in dopamine β-hydroxylase (DBH) expressing adrenergic/noradrenergic cells are necessary and also sufficient for this stress-induced amnesia, whereas CB1 receptors in all other cell populations were not involved in such a response. In this regard, we describe the expression of CB1 receptors in the adrenal medulla, a tissue enriched in DBH-expressing chromaffin cells. Interestingly, both animals treated with AM6545 and animals lacking CB1 receptors in DBH-expressing cells (DBH-CB1-KO mice) showed an extended release of adrenaline after stress exposure, pointing to a crucial role of peripheral, most likely adrenal gland, CB1 receptors in the accurate control of the physiological responses to stress. In summary, central and peripheral adrenergic/noradrenergic transmission determine the consolidation of non-emotional memories, and this function is under the direct control of CB1 receptors expressed in DBH-positive cells. This interplay between peripheral and central processes, tightly controlled by CB1 receptors, is a solid basis for the development of novel approaches to treat both memory- and stress-related disorders.

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Key words: CB1 receptor, stress, amnesia, cognitive function.

O.2.5.

CANNABINOID RECEPTOR 1 BLOCKADE RESTORES MTOR ACTIVITY AND COGNITIVE PERFORMANCE IN TWO MOUSE MODELS OF DOWN SYNDROME

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Down syndrome (DS) is the most common genetic cause of intellectual disability and is caused by the complete or partial trisomy of the human chromosome 21. DS patients present alterations in multiple organs and systems, however, the most limiting phenotype is their cognitive impairment. Nowadays, there is no therapeutic approach available to treat this trait. The alteration of the mammalian target of rapamycin (mTOR), a key signaling pathway for synaptic plasticity, has been described on several intellectual disability disorders including DS. Specifically, it has been described that this pathway is hyperactive in the hippocampus of a DS mouse model, the Ts1Cje. On the other hand, our group has demonstrated that the pharmacological blockade of the type 1 cannabinoid (CB1) receptor normalizes the hyper-activation of mTOR. Therefore, we hypothesized that the blockade of the CB1 receptor may normalize the enhanced mTOR activity in DS models and, therefore, improve their cognitive impairment.
We used a transgenic mouse model that over-expresses the serine-threonine kinase dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A (TgDyrk1A), a kinase relevant for the behavioral symptoms and neuronal alterations of DS patients. Sub-chronic treatment with the CB1 receptor antagonist/inverse agonist rimonabant normalized the hyper-phosphorylation of mTOR and rictor, both components of the mTOR complex 2, in the hippocampus of TgDyrk1A model. This normalization was not related to specific alterations of the endocannabinoid system since no changes were observed in the levels of expression of the components of this neuromodulatory system. At the behavioral level, TgDyrk1A mice showed a significant impairment in three memory tasks; novel object recognition, novel place recognition and trace fear conditioning. Deficits in these tests were normalized after the sub-chronic administration of rimonabant. Interestingly, rimonabant also reversed the cognitive impairment of the most commonly used and best characterized model of DS, the Ts65Dn mouse line.
In conclusion, our results suggest that the blockade of the CB1 receptor may be a new potential therapeutic approach to treat intellectual disability in DS.

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Keywords: Intellectual disability, Down syndrome, endocannabinoid system.

O.3.1.

INVOLVEMENT OF THE CB2 CANNABINOID RECEPTOR IN HUMAN COLON CANCER PROGRESSION

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Colorectal cancer (CRC) is the third most common malignancy and the fourth cause of cancer mortality worldwide. A large amount of studies has demonstrated that during cancer progression the endocannabinoid system suffers alterations in different types of tumor. Particularly, in colon cancer is described down-regulation of CB_1 receptor, up-regulation of CB_2 receptor or increasing amounts of endocannabinoids levels. However, little is known about the clinical relevance of these alterations in patients with colon cancer, and a controversy whether this system prevents tumorigenesis or, if conversely, promotes tumor progression is still open. Recently we have demonstrated that CB_2 expression is a poor prognostic marker in colon cancer patients since its expression correlated with worse disease free survival (DFS, $p=0.014$) and overall survival (OS, $p<0.001$). Immunohistochemical analysis confirmed that CB_2 was expressed at high levels in tumor epithelial cells but it was not detected, or at very low levels, in normal epithelial cells, and the samples with high CB_2 expression were the ones with more Ki67 staining, indicating that those tumors with higher amounts of CB_2 have greater proliferation.

In the present study we investigated the molecular consequences of CB_2 activation with a wide range of CB_2 agonists' dosage on *in vitro* and *in vivo* models. MTT assays with colon cancer derived cell lines, HT29 and SW480, treated with CB_2 agonists, JWH-133 and HU-308, showed that sub-micromolar doses of agonist lead to an increase in proliferation, and cell cycle analysis revealed an increase of cells in G2-M phase. Both results work in line with the correlation found in tumor tissues between CB_2 and Ki67. Moreover we observed an increase in Akt phosphorylation in a time-dependent manner with 0,1 µM of JWH-133 followed by increase of phosphorylated levels of glycogen synthase kinase 3β (GSK3β), a well-known Akt substrate, which leads to its inactivation. Along we observed an increase in SNAIL1 levels, a zinc finger transcription factor involved in the epithelial-mesenchymal transition (EMT), which could be a consequence of GSK3β inactivation since the last one inactivates SNAIL1. Finally, preliminary studies in mice bearing HT29 xenograft, a 14-days treatment of 25µg/day of JWH-133 increased a 26% the rate of tumor growth compared to vehicle-treated mice. Further molecular studies with these mice are being performed.

Collectively, these results suggest that CB_2 receptor activation with doses not high enough to trigger cell death could collaborate with tumor progression. It is important if a CB_2 targeted therapy is being considered for those patients since tumor-delivery of cannabinoids could be complicated due to its chemical nature and the active dose could be crucial for the effect on the disease progression.

Key words: CB_2 receptor, Colon cancer, synthetic agonists.

O.3.2.
THC AS AN ANTI-HER2 THERAPY IN BREAST CANCER

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It has been widely demonstrated that pharmacological activation of cannabinoid receptor CB_2 by exogenous cannabinoids produces antitumoral responses in different models of breast and many other types of cancer. On the other hand, we have recently showed that cannabinoid receptor CB_2, in the absence of exogenously applied cannabinoids, forms heteromers with HER2, and confers pro-oncogenic properties on breast cancer cells by activating HER2-driven signaling via the non-receptor tyrosine kinase SRC (Pérez-Gómez et al. JNCI 2015).
In this context, we aimed at determining which the effect of Δ^9-tetrahydrocannabinol (THC) is on the CB_2-HER2-SRC pro-oncogenic signaling platform and its consequences on breast tumor progression.
Our results show that the pharmacological activation of CB_2 by THC in human HER2+ breast cancer cells produces antiproliferative responses that are accompanied by the disruption of the CB_2-HER2 heteromers. Moreover, we have found that THC decreases HER2 protein levels and SRC activation in cell cultures and in a xenograft model of this pathology. Finally, the combination of THC with standard HER2-targeted therapies results in an increased anti-tumoral action *in vitro* and *in vivo*.
Taken together, these results point to THC as an anti-HER2 therapy and provide preclinical evidence for the use of this cannabinoid, alone or in combination with anti-HER2 treatments, for the management of HER2+ breast cancer.

Key words: CB_2 cannabinoid receptor, breast cancer, THC, HER2

O.3.3.

ACETAMINOPHEN (PARACETAMOL)-INDUCED LIVER INJURY ALTERS THE EXPRESSION OF THE ENDOGENOUS CANNABINOIDE SYSTEM

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Drug-induced liver injury (DILI) is a multifactorial disease involving hepatotoxicity caused by medicines, recreational drugs and herbal products. Hepatotoxicity induced by acetaminophen (APAP), an analgesic and antipyretic agent commonly used in clinical practice, is the most frequent cause of DILI in humans when administered in an acute or cumulative overdose. Interestingly, it was described in brain that APAP can be catabolized to form AM404, a potent CB1/TRPV1 receptor activator. AM404 formation is FAAH-dependent and can inhibit COX1, COX2 and protaglandin synthesis. AM404 was also found in liver homogenates incubated with APAP. However, it is still unclear how APAP-induced liver injury can affect the endocannabinoid system (ECS), a key player in hepatic diseases.

To this aim, we performed: 1) an *in vitro* dose and time-response model of human HepG2 cells cultured with APAP (0.5, 5, 10, 20 mM) for 2, 6 and 24 hours; 2) a model of male mouse (Crl:CD1) sacrificed 6, 24 and 48 hours after one oral-gavage administration of APAP (750 mg/kg); 3) a model of male mouse (Crl:CD1) treated with APAP for 1, 3 and 4 days (750 mg/kg, a dose/day) and sacrificed 6 hours, 6 days or 15 days (recovery) after the last oral-gavage administration. Liver samples were collected for histopathological, biochemical and molecular analyses. Then, we analyzed the liver expression of PPARα receptor and several ECS components such as the cannabinoid CB_1 and CB_2 receptors and the synthesis/degradation enzymes DAGLα, DAGLβ, NAPE-PLD, MAGL and FAAH.

Histological (sinusoidal dilatation, ballooning, inflammation, haemorrhage and necrosis), biochemical (increased levels of the serum transaminases AST, ALT and GGT) and liver damage-markers (CYP2e1, αSMA, caspase-3) for genetic alterations indicated a reliable animal model of APAP-induced liver injury. Our results indicated an increased dose-response expression of all ECS components analyzed in the HepG2 cells after 24 hours of APAP treatment. We also observed an increased gene expression of several ECS components (*cnr2*, *daglα*, *nape-pld*, *faah*) in the mouse liver after 24-48 hours of the acute APAP administration. In contrast, a down-expression of several ECS components (DAGLβ, MAGL, FAAH), including PPARα, was found when APAP was repeatedly administrated up to 4 days of treatment. 15 days after the last 4-days APAP administration, livers were totally recovered showing a normal histology and gene expression of CYP2e1, αSMA and caspase-3. Moreover, the recovery period of 15 days also allowed a turnover to basal levels of the APAP-induced down-expression of PPARα, DAGLβ, MAGL and FAAH. The endocannabinoid system was affected by APAP in a dose and time-dependent manner resulting in a promising target to fight hepatotoxicity induced by acetaminophen.

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Key words: liver injury, paracetamol, DILI

O.3.4.

EFFECTS OF THE NON-SELECTIVE CANNABINOID AGONIST WIN 55,212-2 ON VISCERAL SENSTITIVITY ALTERATIONS INDUCED BY THE ANTINEOPLASTIC DRUG 5-FLUOROURACIL IN THE RAT.

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Background and aim. Side effects associated to cancer chemotherapy are extremely feared by patients and may interfere with optimal treatment. The antineoplastic drug 5-fluorouracil (5-FU) induces painful peripheral neuropathy and intestinal mucositis, which may cause severe diarrhoea, abdominal pain and secondary weight loss. In the search for new drugs that may alleviate chemotherapy-induced side effects, we have studied the effects of the cannabinoid agonist WIN 55,212-2 (WIN) on the alterations induced by 5-FU on visceral sensitivity in the rat.

Methods. Male Wistar rats received two intraperitoneal (i.p.) injections of 5-FU on two consecutive days (75 and 150 mg/kg, respectively, cumulative dose = 225 mg/kg). Six to ten days after the first 5-FU administration, we recorded weight changes and divided the rats into two groups, which received WIN (1 mg/kg, i.p.) or its vehicle, respectively. Twenty minutes after, we performed the behavioural experiments. First, we evaluated the presence of peripheral neuropathy using the Von Frey test (to detect mechanical allodynia). Then, we measured the immobilization time on the ring test (to detect catalepsy, as the most characteristic central sign of cannabinoids). Finally, we used an intracolonic balloon and inflated it at increasing pressures to induce visceral pain (at steps of 15 mmHg every 5 min, up to 75 mmHg and then return to 0 mmHg for additional 5 min). The mean duration and the number of abdominal contractions were recorded using an i-Pad located under the rat cage.

Results. As expected, compared to control values, 5-FU reduced weight gain and induced mechanical allodynia. WIN, at the dose tested, alleviated neuropathy and did not induce catalepsy. When rats were subjected to mechanical colonic stimulation, the number of contractions increased in a pressure-dependent manner in control rats. In 5-FU-treated rats, the number of abdominal contractions was significantly reduced, suggesting the presence of visceral hypoalgesia. Although differences were not statistically different, WIN tended to normalize visceral sensitivity to intracolonic mechanical response.

Conclusions. 5-FU-induced peripheral neuropathy may be alleviated by the use of cannabinoids at non-psychoactive doses. Visceral hypoalgesia induced by 5-FU, whose physiopathological consequences are unknown, might be due to the non-specific damage of the colonic mucosa as well as its intrinsic and extrinsic innervation. More studies are needed to definitely determine the effects of cannabinoids on the altered visceral sensitivity induced by 5-FU.

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Keywords: chemotherapy, 5-fluororuacil, cannabinoids, WIN 55,212-2, visceral pain, painful peripheral neuropathy, catalepsy.

O.3.5.

INVOLVEMENT OF CANNABINOIDS DURING OOCYTE MATURATION IN BOVINE MODEL: IMPORTANCE ON FERTILIZATION AND EMBRYO PREIMPLANTATION DEVELOPMENT

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Introduction: The oocyte is a complex cell and even today the whole biochemical and physiological processes necessary for successful oocyte maturation are not totally understood. There are some evidences indicating that endocannabinoids play an important role in cellular communication during the transport of embryos through the oviduct and also during implantation and placentation processes. However little is known about the role played in the maturation of the oocyte, a key process in achieving potentially fertilizable cells. Our aim was to analyze the involvement of cannabinoids in in vitro maturation (IVM) of bovine oocytes, specie with a similar embryo development to human.

Methods: Oocytes in germinal vesicle (GV), metaphase I (MI) and metaphase II (MII) were analyzed by immunofluorescence and RT-PCR to search the protein and mRNA from CB1 and CB2 receptors. During maturation, oocytes in GV stage were incubated with HU-210 and THC at 100 nM and the phosphorylation status of Akt and ERK1/2 was observed at 0h, 5 min and 24h. Besides, after 24h incubation, the oocytes were fertilized and cultured in vitro until blastocyst stage to observed the effect of cannabinoids in the amount of 2 cells-embryo and rate of blastocyst. Finally, the mRNA of blastocyst was extracted to analyze the expression of genes involved in apoptosis, oxidative stress, pluripotency and implantation by quantitative real time PCR

Results: 1) Cannabinoid receptors CB1 and CB2 were present in bovine oocytes during the different stages of in vitro oocyte maturation**. 2)** The presence of HU-210 and THC during IVM did not lead in a more efficient IVF or in a better in vitro development to blastocyst stage. **3)** When we observed the regulation of pAkt and pERK1/2, two kinases involved in different signaling pathways of meiosis, we noted that Akt phosphorylation increased in granulose cells of immature oocytes within 5 min of their exposure to 100 nM THC but not after HU-210 treatment in comparison of vehicle treatment. On the contrary, the phosphorylation of ERK1/2 kinase maintained its normal regulation in presence or absence of cannabinoids. **4)** The embryo production was not affect by cannabinoids, however, the relative mRNA expression in blastocyst stage of day 7 shows differences in some genes related with the embryo quality.

Conclusions: The identification of pathway(s) by which cannabinoids could modulate the IVM of bovine oocytes and/or the in vitro development of the fertilized egg to blastocyst stage, will provide an interesting target to improve in vitro production of embryos.

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Key words: oocyte maturation, bovine, blastocyst, THC, HU-210

O.4.1.

HISTOLOGICAL AND BIOCHEMICAL BASIS OF THE CLINICAL NEUROPROTECTIVE EFFECTS OF CANNABIDIOL IN HYPOXIC-ISCHEMIC NEWBORN PIGS

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Background and aim: Cannabidiol (CBD) has demonstrated neuroprotective effects with clinical significance in animal models of newborn hypoxic-ischemic encephalopathy (NHIE). We aimed to study the histological and biochemical basis of such effects in a survival model of NHIE in newborn pigs. **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 or CBD 1 mg/kg single dose or in 3 repeated doses u.i.d. Non-HI piglets served as controls. Every 24 h brain activity and function were assessed by amplitude-integrated EEG (aEEG) and a neurobehavioral score (NBS), respectively. Object-related and social playfulness activity was assessed by video recording, and anxiety was quantified as the restless time during holding. Finally, brain was harvested for histological (Nissl staining –necrosis-, and TUNEL –apoptosis-), immunohistochemical (density of mature neurons -NeuN-, developing neurons –NeuD-, activated astrocytes –Ki67+GFAP- and myelination –MBP-), H^+-MRS (Lac/NAA –metabolic derangement-) and neurotransmitter (Norepinephrine –NE-, 5-HT and Dopamine) studies. **Results:** Data obtained 72 h after the HI insult are summarized in the table. CBD administration preserved brain activity and restores neurobehavioral function 72 h after a HI insult. This was associated with the reduction of necrotic and apoptotic cell death as well as of glial activation. In addition, CBD modulated brain metabolic derangement as well as neurotransmitter release. CBD administration protected the developing neurons as well as myelination. CBD effects were similar no matter it was administered in 1 or 3 doses.

Item		***SHM (n=4)***	***HV (n=9)***	***HC1 (n=5)***	***HC3 (n=6)***
CLINICAL	*aEEG (mean amplitude) (μV)*	18 (0.1)	13.8 (1.4)*	22.0 (2.6)#	17.8 (2.1)#
	NBS (points)	35.5 (0.5)	29.0 (2.1)*	35.1 (0.7)#	34.7 (0.6)#
	Object playfulness (% time)	24.6 (13.2)	18.4 (8.9)*	24.4 (4.6)#	28.7 (8.9)#
	Social playfulness (% time)	54.9 (3.7)	24.9 (9.4)*	43.6 (8.2)#	41.6 (11.1)#
	Anxiety (sec)	3.5 (0.5)	6.7 (1.5)*	2.2 (0.9)#	2.5 (1.1)#
HISTOL	*% necrotic neurons (NIssl)*	1.85 (0.3)	10.7 (2.6)*	2.5 (0.7)#	2.4 (0.6)#
	Apoptotic cells (TUNEL)	2.8 (0.7)	187.3 (21.2)*	59.9 (16.3)*#	59.5 (7.5)*#
IHC	*NeuN+ cells/area*	4.1 (0.4)	2.7 (0.3)*	4.1 (0.5)#	4.0 (0.3)#
	Neu D+ cells/area	132.8 (17.6)	88.7 (9.5)*	130.6 (19.9)#	120.3 (18.2)#
	KI67-GFAP+ cells/area	24.2 (12.8)	62.7 (6.6)*	48.1 (5.1)*#	45.4 (4.8)*#
	MBP (mean intensity/pixel)	100 (18)	56 (9)*	66 (12)*	111 (13)#
BIOCHEM	*Lac/NAA (ratio)*	0.86 (0.1)	2.7 (0.9)*	0.9 (0.1)#	1.2 (0.1)#
	NE (fmol/100 mg)	3.01 (0.2)	4.41(1.0)	4.06 (0.3)	3.77 (0.4)
	5HT (fmol/100 mg)	35.6 (3.3)	69.5 (7.2)*	35.1 (7.2)#	47.6 (10.2)#
	Dopamine (fmol/100 mg)	0.8 (0.0)	1.53 (0.1)*	0.7 (9.2)#	0.9 (0.1)#

Mean (SEM). Kruskall-Wallis (Dunne's test). (*): p<0.,05 vs. SHM; (#) p<0.05 vs. HV

Conclusions: CBD shows a comprehensive neuroprotective profile preventing cell death and glial activation, modulating neurotransmitter release and supporting neuro-repair.

Supported by FIS PI09/01900, PI1200192, PS1301722 and GWCRI09119

Key words: Cannabidiol, neurotransmitters, neuroprotection, neuro-repair

O.4.2.

CANNABIDIOL PREVENTS POST-HYPOXIC-ISCHEMIC HYPOMIELINATION BY RESTORING MATURE OLIGODENDROCYTE FUNCTION 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 hypomyelination, a key role in the genesis of cerebral palsy. Cannabidiol (CBD) has demonstrated to protect neurons and glial cells (astrocytes) after a HI insult in newborn animals.
Aim: to determine if CBD protects OL cells after a HI insult in newborn rats.
Methods: unilateral HI brain damage was induced in newborn Wistar 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 immunohistochemical (IHC) studies on the subventricular zone (SVZ), parietooccipital cortex (Cx) and External Capsula (EC) were performed. In P8 and P14 rats, KI67 was used to detect proliferating cells, Olig2 for OPC and SOX10 for preOL. At P37 GSTπ and Myelin basic protein (MBP) fluorescence in CC were used to quantify mOL number and myelinisation, respectively. Expression of the pro-glial proliferative GDNF was quantified by Westernblot. Finally, electronic microscopy (EM) studies were done at P37 to determine the number of axons and myelin thickness per axon.
Results: In SVZ the number of proliferating cells increased in HI groups at P8 (SHM>HV=HC), falling then at P14 in HV but not in HC. Only preOL population was increased in HI animals at P14. In Cx there was a decrease of OPC and preOL in both HI groups at P8 (SHAM>HV=HC). In HV there was an increase of preOL at P14 but a decrease of mOL number and myelin content at P37. CBD administration potentiated OPCs population at P14 and preserved the number of mOL and myelin content at P37 (SHAM=HC>HV). In EC (White Matter) there were no differences among groups in the number of OPC or preOL at P8 and P14 or of mOL at P37 (SHAM=HV=HC). However, CBD prevented the fall of MBP signal at P37 due to the preservation of MBP signal per mOL, the preservation of myelin thickness (SHAM=HC>HV) and the increase of the number of axons (HC>HV). There were no differences in GDNF levels among the different groups.
Conclusions: HI insults do not reduce the number of OL cells but impairs the function of mOL, reducing axonogenesis and mature myelin production. CBD restores mOL function leading to normal myelination. This effect is likely due to the preservation of cellular environment rather than to a direct proliferative effect.

Supported by FPU13/05495, PS 1301722, PI1200192 and GWCRI09119.

Key words: hypoxia-ischemia, cannabidiol, myelin

O.4.3.

CANNABIDIOL REDUCES PERI-INFARCT BRAIN DAMAGE AND RESTORES NEUROBEHAVIORAL FUNCTION IN A NEWBORN RAT MODEL OF ACUTE ISCHEMIC STROKE

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Background: Acute arterial ischemic stroke affects 4/1000 life newborns, leading to long-lasting sequelae. There is no treatment available for patients up to now. Cannabidiol (CBD) has demonstrated neuroprotective effects in models of acute hypoxic-ischemcia brain damage in newborn animals.
Aim: To determine the possible beneficial effect of CBD in a model of stroke in newborn rats.
Methods: A nylon filament was introduced through the left carotid artery until occluding the left MCA for 3 h in 7 day-old Wistar rats. The occluder was then removed and 15 min after reperfusion CBD 5 mg/kg (SC, n= 10) or vehicle (SV, n=11) were administrated i.p. Similarly manipulated but non-stroke rats remained as controls (SHM, n=12). One month after the stroke, some behavioral tests were carried out: cylinder rear test (CRT, hemiparesis), beam walking (coordination), sticker removal (sensitive-motor), novel object recognition (memory) and social interaction. Then, a MRI study was carried out to quantify the volume of damage. .
Results: Volume of infarct was not modified by CBD (31.3(2.1) vs 30.9(2.8) % brain volume for SV and SC, respect., NS), but CBD reduced the volume of perilesional edema (13.5(1.9) vs 5.9(2.5) %BV, $p<0.05$). CBD administration restored neurobehavioral function, regarding hemiparesis (CRT -0.1(0.05), 0.23(0.07) and 0.02(0.1) for SHM, HSV and SC, $p<0.05$), coordination (time to cross the beam 9.7(2), 16.3(3) and 7.5(1.7) sec, $p<0.05$; number of fails 0.8(0.2), 2.6(0.8) and 1.3 (0.4), $p<0.05$), memory (NOR 15.4(2.4), 43.6(11) and 20.8(11), $p<0.05$) and social interaction (108(17), 76.5(9) and 94.8(10) sec, $p<0.05$). No differences were seen in the sensitive domain. The administration of CBD prevented MCAO-induced hypomyelination.
Conclusions: In a model of severe stroke in newborn rats, post-insult CBD administration reduces peri-infarct brain damage, restoring neurobehavioral function in the long term and restoring myelination.

Supported by FPU13/05495, PS 1301722, PI1200192 and GWCRI09119.

Key words: stroke, cannabidiol, neuroprotection.

O.4.4.

CANNABIDIOL AND MESENCHYMAL STEM CELLS AS NEW THERAPIES IN ADOPTIVELY TRANSFERRED EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS.

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Background: Cannabidiol (CBD), compound not acting on CB1 or CB2 receptors, seems to offer new tools to manipulate inflammation without psychotropic effects. It exerts a wide range of anti-inflammatory properties. CBD was shown to alleviate the pathological changes in the murine demyelinating disease, experimental autoimmune encephalomyelitis (EAE). Mesenchymal stem cells (MSC) are known for their important immunosuppressive properties over the entire immune system. Their therapeutic potential has been demonstrated in a variety of autoimmune disease models including EAE. Both CBD and MSC are potential therapies for MS. Neither CBD nor MSC, have been tested in the passive variety of EAE.
Objectives: To compare CBD and MSC efficacy as therapies for adoptively transferred EAE.
Methods: Mouse MSC (mMSC) were isolated from bone marrow of C57BL/6J mice, expanded *in vitro* and characterised by flow cytometry. Encephalitogenic T cells were obtained from lymph nodes and spleens of C57BL/6J mice inoculated with MOG_{35-55}, primed with MOG_{35-55} (25µg/ml) and IL-12 (25ng/ml) and transferred intraperitoneally (i.p) to naïve mice three days later. Three groups of 5-7 transferred mice were established: EAE+vehicle (solutol, ethanol and PBS (phosphate-buffered saline) (1:2:7)); EAE+CBD 50mg/kg/d (solutol, ethanol and CBD 10mg/ml) (1:2:7) (i.p) daily during 25 days); and EAE+0.5 $x10^6$ mMSC (intravenously twice a week for 3 weeks). Animals were examined for neurological signs up to day 28.
Magnetic resonance imaging (7-T Bruker Pharmascan) was performed after 15 days of i.p and i.v therapy with CBD and mMSC, respectively. Images were acquired using T1-weighted, T2-weighted and T2*-weighted techniques.
Results: Compared to control EAE mice, CBD induced a highly significant suppression of clinical signs without untoward effects. MMSC treatment had a mild but significant effectiveness in clinical evolution. On MRI, control mice had inflammatory brain lesions that were less frequent in mMSC treated mice and absent in the CBD treated group.
Conclusions: Adoptively induced EAE allows for a better delineation of the effector role of encephalitogenic cells, on the blood-brain barrier and the CNS, avoiding possible interferences that inflamed lymph nodes and spleen might have on MSC circulation. MMSC effectively suppressed EAE on clinical and imaging grounds, but to a lesser extent compared to the CBD treated group. CBD might be a suitable agent for MS considering its efficacy and the lack of psychoactive effects.

Key words: EAE, Cannabidiol, Mesenchymal Stem Cells.

O.4.5.

NEUROPROTECTION BY NON-PSYCHOTOPIC PHYTOCANNABINOIDS IN A CELLULAR MODEL OF STRIATAL DAMAGE RELEVANT FOR HUNTINGTON'S DISEASE: INVOLVEMENT OF NRF-2-MEDIATED MECHANISMS

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Different plant-derived and synthetic cannabinoid, alone or in combination, have provided neuroprotection in experimental models of Huntington's disease (HD), even the issue has extended to the clinical scenario with the development of a pilot trial, unfortunately failed, using the phytocannabinoid-based medicine Sativex® in HD patients. In the present study, we wanted to further investigate some of the molecular mechanisms underlying the positive effects of phytocannabinoids, using an *in vitro* strategy with striatal derived cell lines generated from a knock-in mouse containing homozygous huntingtin loci with a humanized exon 1 with either 7 (Q7/Q7, wild-type cells) or 111 (Q111/Q111, HD cells) polyglutamine repeats. We first investigated whether botanical extracts enriched in Δ^9-tetrahydrocannabinol (Δ^9-THC-BDS) or cannabidiol (CBD-BDS), as well as their 1:1 combination as in Sativex®, were effective against different cytotoxic insults in Q7/Q7 and Q111/Q111 cells. We used excitotoxins, mitochondrial toxins, or hydrogen peroxide (H_2O_2), which resemble those cytotoxic events occurring in HD brains, although our data were positive only in the case of cells treated with H_2O_2. Thus, in the case of Q7/Q7 cells, we found that CBD-BDS, alone or combined with Δ^9-THC-BDS as in Sativex®, but not Δ^9-THC-BDS, prevented cell death after H_2O_2-induced oxidative damage. We observed that this protection was accompanied by an increase in heme oxygenase-1 (HO-1) and NAD(P)H:quinone oxidoreductase-1 (NQO-1) expression, which are enzymes induced by the activation of the transcription factor Nrf-2. In addition, the expression of Keap-1, the cytosolic protein that regulates Nrf-2 availability, was found to be reduced after H_2O_2 in Q7/Q7 cells, but this response was reversed by the treatment (CBD-BDS alone or combined with Δ^9-THC-BDS). Our data also pointed to an activation of Nrf-2 translocation to the nucleus detected by immunofluorescence, and an increase of autophagy flux after the treatment with the phytocannabinoids in Q7/Q7 cells. In general, the responses in Q111/Q111 cells were relatively similar compared to Q7/Q7 cells, although some differences in the magnitude of these responses were evident. For example, the effects of phytocannabinoids by recovering the low expression levels of HO-1 and NQO-1 elicited by H_2O_2 were much more moderate in Q111/Q111 cells, and the same happened with the expression of Nrf-2 and, in particular with its translocation to the nucleus which was significantly lower in these cells. However, these differences were not completely translated to the capability of phytocannabinoids to prevent cell death after H_2O_2, which resulted to be relatively similar in both types of cells, thus indicating that other mechanisms of protection should work in the case of Q111/Q111 cells. In summary, all these data suggest that a Nrf-2-mediated mechanism may mediate the neuroprotective effects of CBD (and other antioxidant non-psychotropic phytocannabinoids), but not Δ^9-THC, against oxidative damage in striatal neurons, a mechanism that is relatively conserved in cells expressing mutated forms of huntingtin, thus supporting their relevance for the treatment of HD.

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O.4.6.

POTENTIAL NEUROPROTECTIVE ROLE OF SPECIFIC CB1 RECEPTOR SUBPOPULATIONS IN THE MOUSE CORTICOSTRIATAL CIRCUITRY

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The CB1 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 CB1 receptor in neurological diseases is hampered, at least in part, by the lack of knowledge of the cell-population specificity of CB1 receptor action.

Aim: In order to study the potential neuroprotective role of different CB1 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.

Methods: In a first series of experiments we expressed mutant huntingtin under minimal neuronal (CaMKII) or astroglial (GFAP) promoters in the dorsal striatum in order to achieve its expression in medium-sized spiny neurons (MSNs) or striatal astrocytes, respectively. One day after infection with mutant huntingtin, animals were treated daily i.p. for 2 weeks with 1 mg/Kg of THC or 8 mg/Kg of JZL-184, a potent and selective inhibitor of monoacylglycerol lipase.

Results: Selective mutant huntingtin expression in astrocytes, as well as in MSNs, led to impairments in motor coordination and alterations of striatal markers. When mutant huntingtin was expressed solely in MSNs, none of the pharmacological treatments prevented striatal damage. In contrast, when mutant huntingtin expression was restricted to astrocytes, THC and JZL-184 normalized motor ability and striatal integrity. We are currently investigating in further detail the possible neuroprotective role of CB1 receptors located on striatal astrocytes.

Conclusions: Glial restricted expression of mutant Huntingtin elicits motor impairment and striatal alterations that are prevented by use of cannabinoid coumpounds. Since astrocytes are crucial for neuronal physiology and their dysregulation occurs in many neurodegenerative diseases, this cell type may represent a relevant target to apply cannabinoid-based therapies.

Key words: CB1 receptor, huntingtin, corticostriatal circuitry

O.4.7.

EFFECTS OF 2-AG IN ACUTE TMEV INFECTION: CNS IMMUNOMODULATION AND INHIBITION OF LEUKOCYTE EGRESS FROM THE SPLEEN THROUGH S1P1 RECEPTOR

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The regulation of the brain-immune axis by cannabinoids provides promising therapeutic implications in a variety of neuroinflammatory conditions. 2-Arachidonoylglycerol (2-AG) has emerged as the chief endocannabinoid neuromodulator, but less is known about its central and peripheral immunomodulatory actions. We have used the intracerebral injection of the TMEV virus in SJL/J mice as a model of acute neuroinflammation. 2-AG and the reversible inhibitor of 2-AG degradation (UCM-03025) were administered in TMEV-infected mice. Immunohistochemistry against Iba1, Arg-1 and MHC-II was performed in coronal sections of the nervous parenchyma at the lesion site. RT-PCR analyses were done to evaluate the effects of 2-AG levels in pro-inflammatory & anti-inflammatory cytokines, chemokines, and ICAM-1. The immune infiltration in the CNS and the number of lymphocytes in lymph nodes and spleen were evaluated by flow cytometry. The results show that the direct administration of 2-AG, or the inhibition of its degradation by the compound UCM-03025, decreased the number of microglial cells at the infection site and polarized them towards a reparative M2 phenotype. Central immunomodulatory effects of 2-AG also included the decrease of iNOS, TNFα, IL-1β, CCL2, SOCS3 and ICAM-1, whereas it induced an increase of IL-10, CX3CR1 and CCR2/CCL5 expression. Peripheral immunomodulatory effects of 2-AG consisted of a decrease in the immune infiltration into the CNS, and the inhibition of leukocyte egress from the spleen through a mechanism that involves the sphingosine S1P1 receptors. In conclusion, the endocannabinoid 2-AG exerted dual actions in the brain-immune axis to control the inflammatory response in the CNS.

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Key words: microglia, 2-AG, neuroinflammation, spleen, sphingosine-1-phosphate.

O.4.8.

CANNABINOID CB2 RECEPTORS ARE SELECTIVELY EXPRESSED BY ACTIVATED MICROGLIAL CELLS IN ALZHEIMER'S DISEASE

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Cannabinoid CB_2 receptors are candidate targets for the development of novel therapies, mainly in the context of inflammation. However, our current knowledge of their pathophysiological roles has been limited by the lack of appropriate experimental tools. We recently reported 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. These mice were then crossed with transgenic mice bearing five mutations for familial Alzheimer's Disease (5xFAD mice; Oakley et al, J Neurosci 26; 10129-10140, 2006), resulting in $CB_2^{eGFP/f/f}$/5xFAD mice. These mice produce massive amounts of beta amyloid 1-42 peptide and exhibit neuritic plaques since they are 3 months old, progressively increasing with age. Intense neuroinflammation takes place in cortical, hippocampal and thalamic areas of the $CB_2^{eGFP/f/f}$/5xFAD mouse brain, triggering glial activation. In this context, we analyzed the pattern of expression of CB_2-controlled eGFP expression. We found intense GFP signal in plaque-associated cells with morphological features of microglia in cortex, hippocampus and thalamus, while negligible in other cell types throughout the CNS. This observation was further corroborated by co-localization studies with Iba1, confirming the restricted expression of GFP to activated microglial cells in the vicinity of amyloid plaques. These data confirm and expand previous observations in human AD brains regarding the inducible nature of cannabinoid CB_2 receptors in the context of neuroinflammation.

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Key words: CB_2 receptor, Microglia, Alzheimer's disease.

O.4.9.

NEW POTENTIAL TARGETS FOR CB2 RECEPTOR IN CNS DISEASES WITH A NEUROINFLAMATORY COMPONENT

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Endocannabinoids are neuromodulators that act on the specific cannabinoid CB_1 and CB_2 receptors, and represent potential therapeutic targets for pain and/or alterations to the central nervous system (CNS). Stress-exposure produces excitoxicity and neuroinflammation, contributing to the cell damage observed in stress-related neuropathologies. The endocannabinoid system is present in stress-responsive neural circuits and it is emerging as a homeostatic system. Aiming at elucidating a potential regulatory role of CB_2 receptor in stress-induced excitotoxicity and neuroinflammation, Zoppi et al., (2014) have recently shown that pharmacological manipulation of CB_2 receptor may result in therapeutic benefit for the treatment of stress-related pathologies with a neuroinflammatory component. Actually the involvement of CB_2 receptors in neuroinflammation has been known for long (Rom et al., 2013; Leleu-Chavain et al., 2012) however it is not providing the necessary answers to devise a consensuated treatment to combat neuroinflammation. In the present study it has been designed a new library of chromenopyrazoles, synthesized and characterized by cAMP at the cannabinoid CB_2 receptors. These new molecules exhibit a selective CB2 receptor ligand profile. By cAMP assays we have tested different chromenopyrazoles as potential agonists and/or antagonists for the cannabinoid CB_2 receptor. We have detected different compounds more potent than CB_2R agonists JWH133 or CP55490 and others that could act as potential antagonists. Our findings could indicate the real potential of CB_2 receptor as new target for the treatment of CNS diseases having a neuroinflammatory component.

Key words: CB_2R, cAMP, neuroinflamation

O.4.10.
GPR55: A THERAPEUTIC TARGET FOR PD?

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One of the main challenges for the development of new therapies for Parkinson´s disease (PD) is to find a strategy that protects the remaining dopaminergic neurons from the on-going neurodegenerative process or that even restores the nigrostriatal pathway or function. Given the role of the endocannabinoid system (ECS) in the control of movement and the changes in this system that occur in PD, the ECS represents a potentially important therapeutic target for this disease. GPR55 was considered the third cannabinoid receptor based on amino acid sequence homology in the binding region. It is unknown whether GPR55 is involved in the motor impairment observed in PD and whether it could represent and interesting pharmacological target for the disease. The aim of this project was to study the neuronal expression of GPR55 in the basal ganglia under physiological and pathological conditions and to determine whether it could be a potential therapeutic target for PD. Using *in situ* hybridization we have demonstrated the expression of GPR55 in the nigrostriatal system. The effect of Abn-CBD (5 mg/kg), a GPR55 agonist, and CBD (5 mg/Kg), a GPR55 antagonist was studied in the chronic MPTP mouse model. Abn-CBD induced a clear symptomatic improvement in parkinsonian motor behavior in the pole test and in the rotarod test, and protected dopaminergic neuron bodies of the SNpc from neurodegeneration. In contrast, neither improvement in motor performance nor neuroprotection were detected with CBD treatment. Both Abn-CBD and CBD decreased the microglial activation in the striatum, although this effect did not correlate with the performance in the motor test or the extent of dopaminergic degeneration. In conclusion, our results support that GPR55 receptor activation could be an interesting target the treatment of PD. Further experiments are necessary to ascertain expression of GPR55 receptors in microglia and to explore the symptomatic effect of GPR55 receptors activation.

Keywords: neuroprotection; Parkinson´s disease; cannabinoids; GPR55

O.4.11.

BIOLOGICAL CHARACTERIZATION OF PM226, A CHROMENOISOXAZOLE, AS A SELECTIVE CB2 RECEPTOR AGONIST WITH NEUROPROTECTIVE PROFILE

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Cannabinoids have emerged as promising neuroprotective agents due to their capability to activate specific endocannabinoid targets, which play a role in the control of neuronal homeostasis and survival. Specifically, those ligands that selectively target and activate the CB_2 receptor may be useful for their anti-inflammatory and neuroprotective properties in various neurological disorders, with the advantage of being devoid of psychotropic effects associated with the activation of CB_1 receptors. The aim of this work has been to investigate the pharmacological profile of 7-(1,1-dimethylheptyl)-4,4-dimethyl-9-methoxychromeno[3,4-d]isoxazole (PM226), a compound derived from a series of chromenoisoxazoles, which seems to have a promising profile related to the CB_2 receptor activity. This compound binds selectively to the CB_2 receptor with an affinity in the nanomolar range (K_i = 12.8 ± 2.4 nM) and negligible affinity at the CB_1 receptor (K_i > 40000 nM). PM226 was also evaluated in GTPγS binding assays specific to the CB_2 receptor showing agonist activity (EC_{50} = 38.67 ± 6.70 nM). *In silico* analysis of PM226 indicated that it has a good pharmacokinetic profile and the theoretical ability to cross the blood-brain barrier. The compound may be also a potential PPAR-γ ligand. PM226 was investigated in an *in vitro* model to explore its neuroprotective properties. Conditioned media were collected from LPS-stimulated cultures of BV2 microglial cell line in the absence or presence of different doses (0.1, 1 and 10 μM) of PM226, and then media were added to cultured M213-2O neuronal cells to record their influence on cell viability evaluated using MTT assays. As expected, cell viability was significantly reduced by the exposure to these conditioned media, while the addition of PM226 attenuated this reduction in a dose-dependent manner. This effect was reversed by co-incubating with the CB_2 antagonist SR144528. PM226 is being now studied in the *in vivo* model of Huntington´s disease generated by stereotaxic lesions with malonate. Preliminary MRI analysis shows that PM226 administration decreased the striatal lesion volume caused by malonate. Overall, PM226 has shown to have a promising neuroprotective profile derived from its ability to selectively activate CB_2 receptor, so that it could be a useful disease-modifying agent in those neurodegenerative pathologies in which the activation of these receptors may have therapeutic value.

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P.1.

MATERNAL EXPOSURE TO EITHER FOOD RESTRICTION OR CAFETERIA DIET DURING PRE- AND GESTATIONAL PERIODS INDUCED SIMILAR EFFECTS ON THE ENDOCANNABINOID SYSTEM IN THE LIVER AND ADIPOSE TISSUE OF THEIR RAT OFFSPRINGS IN ADULTHOOD

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The risk of developing metabolic and behavioral-related disorders in later life of offsprings is strongly associated with perinatal nutrition leading obesity. As obesity has been associated with an overactivation of the endocannabinoid system, a signaling system implicated in energy balance, appetite and food preference, we evaluated the effects of the maternal exposure to either food restriction or a highly caloric diet on the gene expression of PPARα, PPARγ and several ECS components (CB_1, CB_2, DAGLα, DAGLβ, NAPE-PLD, FAAH and MAGL) in the liver and epididimal white adipose tissue (eWAT) of the rat offsprings in adulthood. To this aim, female Wistar rats were fed with a standard chow (C, 2.9 Kcal/g), palatable-cafeteria (P, 4.88 Kcal/g) and restricted control (R, 20% of caloric reduction) diets during the perinatal period (premating, pregnancy and lactation). Food restriction was applied up to the 20th day of gestation. Offsprings fed with the standard chow diet were sacrificed at adulthood (22 weeks) to collect liver and eWAT samples for the ECS molecular analysis.

Results indicated that cafeteria diet induced an increase in body weight gain during pregestation and lactation, but not gestation. Food restriction decreased the body weight gain in all perinatal periods. Cafeteria diet and food restriction respectively increased or reduced caloric intake, but no effects on intake were observed during lactation. Regarding the rat offsprings in adulthood, maternal exposure of either food restriction or cafeteria diet induced a similar profile in the ECS mRNA levels in the liver and eWAT. Both food restriction and cafeteria diet induced a decrease in the gene expression of *Pparγ*, *Magl* and *Faah* in the liver of female offsprings, the gene expression of *Nape-pld* in the liver of male offsprings, and the gene expression of *Daglβ* and *Faah* in the eWAT of male offsprings. Food restriction specifically decreased the gene expression of *Pparα* in the liver of female offsprings and the gene expression of *Daglα*, *Magl* and *Nape-pld* in the eWAT of male offsprings. In contrast, we found an increased gene expression of *Cnr1* in the eWAT of female offsprings.

We propose a potential role of the endocannabinoid system in the maternal diet-induced responses that involve long-term metabolic adaptations in the liver and adipose tissue of offspring.

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Keywords: prenatal period, offspring, obesity.

P.2.

THE ENDOGENOUS CANNABINOID SYSTEM IS DIFFERENTIALLY EXPRESSED DURING MURINE C2C12 MYOGENESIS IN A TIME-DEPENDENT MANNER

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A large number of processes are implicated in the myotube formation. Recently, it has been discovered that the endocannabinoid system (ECS), a lipid-derived endogenous system, and the peroxisome proliferator-activated receptor (PPAR) isoforms β/δ are implicated in myogenesis. Using C_2C_{12} myoblasts, it was described that the endocananbinoid 2-arachidonoylglycerol (2-AG) regulates muscle cell differentiation via CB_1 receptor-dependent inhibition of K^+ channels. However, the endogenous cannabinoid tone (2-AG, 2-LG, 2-OG, AEA, DGLEA, DHEA, LEA, OEA, PEA, POEA and SEA) and the expression of PPARα receptor and several ECS components, including the cannabinoid CB_1 and CB_2 receptors, the biosynthesis enzymes DAGLα, DAGLβ and NAPE-PLD, and the degradation enzymes MAGL and FAAH, during myogenesis have not been characterized yet. Functional C_2C_{12} myotubes were obtained after 6 days of culture in a differentiation medium (DM) containing DMEM (25 mM glucose) supplemented with 20 mM HEPES, 2% horse serum, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 1% L-glutamine. During differentiation, the overall content of acylglycerol in DM was increased, specifically 2-LG. In contrast, the overall content of acylethanolamine in DM was decreased, being evident for OEA, PEA and SEA. The differentiated C_2C_{12} myotubes showed an increase in the gene expression of CB_1, CB_2, DAGLα, DAGLβ, NAPE-PLD, MAGL and FAAH. Moreover, increased protein levels of CB_1, CB_2, PPARα, DAGLα and FAAH were also observed, being mostly confirmed by immunocytochemistry. Interestingly, after 24 hours in refreshed DM, C_2C_{12} myotubes showed a decreased protein expression of CB_2 and PPARα. Furthermore, the PPARα agonist OEA increased the gene expression of the muscle differentiation marker myogenin. These data indicated that the endocannabinoid system, particularly PPARα, can play a crucial role in the time course of the muscle formation.

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Keywords: C_2C_{12}, differentiation, myogenesis

P.3.

ELECTRON MICROSCOPY LOCALIZATION OF MITOCHONDRIAL CB1 IN CA1 HIPPOCAMPAL REGION IN CHRONIC STRESSED MICE

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The seven-transmembrane G protein coupled cannabinoid receptor type-1 (CB_1) forms part of the brain endocannabinoid (eCB) system. This system is involved in many neural functions ranging from food intake to cognition. Because of the functional activity, mammalian brain is one of the organs with the highest energy demands and mitochondria are key determinants of its functions.

Stress responses are crucial for survival and normal functioning of the organism. However, an inadequate or long-lasting response causes physiological alterations. Increasing evidences suggest that the eCB system participates in stress. We have recently demonstrated in the hippocampus that CB_1 receptors are at neuronal mitochondria ($mtCB_1$) membranes, where they directly control cellular respiration and energy production and modulate synaptic plasticity. As hippocampal CB_1 expression and its binding capacity have been reported to be altered due to chronic stress, we wondered whether $mtCB_1$ is affected under stress conditions.

The aim of this study was to investigate the subcellular distribution of $mtCB_1$ in the CA1 hippocampal region of chronic stressed mice. For this purpose, young adult Swiss mice were subjected to chronic restraint stress for 1 hour/day during ten days. Thereafter, mice were sacrificed and perfusion-fixed through the heart. A preembeddding immunogold method for high resolution electron microscopy was applied to hippocampal sections of control and stressed mice. After analyzing the CB_1 distribution pattern, 29,34 ± 0,91% of total mitochondria were CB_1 immunopositive in chronic stressed mice while in controls about 17,49 ± 0,67% localized $mtCB_1$. The difference between both groups was statistically significant ($p < 0,0001^{***}$, Mann Whitney test).

These initial results indicate that exposure of young adult mice to chronic stress significantly increases CB_1 inmunopositive mitochondria, possibly reflecting a link between chronic stress and brain mitochondria activity through CB_1.

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P.4.

THE MICRORNA LET-7D IS A TARGET OF THE CB1 CANNABINOID RECEPTOR AND REGULATES CANNABINOID TOLERANCE

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The CB_1 cannabinoid receptor, the molecular target of endocannabinoids and cannabis active components, is one of the most abundant metabotropic receptors in the mammalian brain. Cannabis is widely used for both recreational and medicinal purposes, and readily leads to tolerance, which can progressively augment the amount of drug used by recreational consumers and decrease the therapeutic efficacy of cannabinoid-based medicines, especially upon use over long periods of time. Despite the ever-growing fundamental roles of microRNAs (miRNAs) in the CNS, including drug tolerance, the possible molecular connections between the CB_1 receptor and miRNAs remain surprisingly unknown. Here, by using reporter gene constructs that express interaction sequences for miRNAs in human human neuroblastoma SH-SY5Y cells, we show that CB_1 receptor activation enhances the expression of several miRNAs, including *hsa-let-7d*, that is known to have a widespread role in neuron pathophysiology. This was confirmed by measuring *hsa-let-7d* expression levels. The up-regulatory effect of the CB_1 receptor on *hsa-let-7d* seemed to rely on the modulation of the ERK and cAMP pathways. Knocking-down CB_1 receptor in zebrafish reduced *dre-let-7d* levels, and, likewise, knocking-out CB_1 receptor in mice decreased *mmu-let-7d* levels in the striatum and hippocampus. On the other hand, knocking-down *dre-let-7d* in zebrafish increased CB_1 receptor expression. Likewise, in SH-SY5Y cells chronically exposed to a cannabinoid or opioid agonist we found that *hsa-let-7d* enhanced not only cannabinoid tolerance but also cannabinoid-opioid cross-tolerance. Taken together, this study provides the first evidence that a miRNA, namely let-7d, is a target of the CB_1 receptor in the brain and participates in cannabinoid tolerance, therefore providing a potential new target for improving the therapeutic activity of cannabinoids.

Palabras clave: CB_1 receptor, microRNA, let-7d, tolerance

P.5.

DELTA-9-TETRAHYDROCANNABINOL CAUSES SYNAPTIC AND MOTOR COORDINATION IMPAIRMENTS THROUGH A MECHANISM UNDERLYING COX-2 ACTIVATION AND MICROGLIAL REACTIVITY

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Chronic use of cannabis has been associated to cerebellar dysfunction in humans. We previously demonstrated in mice that exposure to delta-9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis, triggers microglial reactivity in the cerebellar molecular layer, and enhances the expression of neuroinflammatory molecules, such as interleukin 1β (IL-1β) cytokine or cyclooxygenase-2 (COX-2) enzyme. In the cerebellum, COX-2 is found in Purkinje cells and its expression is generally up-regulated following brain insults, via glutamatergic and inflammatory mechanisms. COX-2 synthesizes prostaglandins, which modulate synaptic plasticity and microglial activation through their action on EP prostaglandin receptors, such as EP2 receptors, that are mainly expressed on activated microglia. We evaluated the role of COX-2 in the cerebellar deficits associated with cannabis consumption and withdrawal. THC administration in mice (5mg/kg; 5.5 days; twice per day) produced COX-2 and EP2 enhanced expression in the cerebellum, which were still increased 5 days after THC-treatment. THC withdrawal was characterized by fine motor coordination deficits in the footprint, beam walking and hanger test performance. We evaluated the expression of ionotropic glutamate receptor 2 (GluR2) and glutamate receptor delta 2 (GluRδ2), both involved in cerebellar long-term depression (LTD) performance. Both receptors were modified in the cerebellum of THC-withdrawn mice. Interestingly, the sub-chronic administration of the COX-2 inhibitor NS-398 (10mg/kg; 5 days; once per day) after THC exposure, prevented the alterations in fine motor coordination observed in the THC-withdrawn mice and normalized EP2, GluR2 and GluRδ2 expression levels. In addition, NS-398 also prevented microglial reactivity in the cerebellar molecular layer. These results suggest that COX-2 plays an important role in the control of microglial reactivity in the cerebellum during THC-withdrawal conditions, revealing COX-2 as crucial in the cerebellar deficits associated to repeated cannabis exposure.

Key words: COX-2, GluR2, motor coordination, THC

P.6.

ANTI-OBESITY EFFICACY OF GLUCAGON-LIKE PEPTIDE-1 RECEPTOR ACTIVATOR LIRAGLUTIDE: EFFECTS ON PPARα, PPARγ AND THE CANNABINOID CB1 AND CB2 RECEPTORS

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Among the new therapeutic targets against diet-induced obesity, the endocannabinoid system (ECS) remains a valuable alternative. In fact, increased concentrations of endogenous cannabinoid-like compounds can balance the stimulation of cannabinoid and/or PPAR receptors in a pathophysiological manner contributing to the (de)regulation of energy metabolism. For instance, the CB1 receptor activator arachidonoylethanolane (AEA) enhances food intake and energy storage, while oleoylethanolamine (OEA) binds to PPARα to reduce food intake and promote lipolysis. The long-acting GLP-1 receptor agonist liraglutide is another promising anti-obesity strategy with proven efficacious in the glycemic control of type-2 diabetes coupled to insulin-independent metabolic benefits for body weight, blood pressure and hepatic steatosis and dyslipidemia. The main objective of this study was to determine whether the endocannabinoid and GLP1 signalling systems are interconnected in the regulation of energy metabolism. To this aim, both lean (STD, 2.9 kcal/g) and high-fat diet (HFD, 5.24 kcal/g)-induced obesity (DIO) rats were used to compare the peripheral effects of the subcutaneous administration of liraglutide (25 µg/kg, 11 days) on the gene expression of PPARα, PPARγ and the cannabinoid CB_1 and CB_2 receptors in liver, abdominal skeletal muscle and epididymal white adipose tissue (eWAT).
After 12 weeks of *ad libitum* feeding, rats treated with liraglutide for 11 days showed a reduction in food intake and bogy weight as well as plasma levels of triglycerides and VLDL in a diet-independent manner. Interestingly, hepatic fat content was specifically decreased in HFD-fed liraglutide-treated rats. HFD increased the *Pparγ* expression in liver, muscle and eWAT as well as the *Pparα* expression in muscle. However, liraglutide only reversed the HFD-induced increase in *Pparγ* expression in liver and muscle. Our results showed that HFD specifically increased CB_1 expression in eWAT, but no effect of liraglutide on CB_1 and CB_2 expression in liver, muscle and eWAT of HFD-fed rats were detected. However, regarding the lean rats, liraglutide induced an increase in CB_1 expression in liver and a decrease in CB_2 expression in eWAT. These results indicated that the anti-obesity effect of GLP-1R activation could be mainly produced via the modulation of the PPARγ signalling system in liver and muscle. Further studies, such as the analysis of the endocannabinoid tone, will be required.

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Keywords: GLP-1, endocannabinoid system, obesity.

P.7.

CSPGS MODULATION OF THE EXTRACELLULAR MATRIX AS A THERAPEUTICAL APPROACH IN A VIRAL MODEL OF MULTIPLE SCLEROSIS

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The accumulation of extracellular matrix proteins (ECM) and chondroitin sulphate proteoglycans (CSPGs) that provokes scar formation are considered important factors for the failure of regeneration and remyelination in CNS injury and multiple sclerosis (MS). Previous results of our group showed that a combination of phytocannabinoids (CBD: THC) ameliorated symptomatology and modulated the accumulation of CSPGs in a viral model of MS, the Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD).

The aim of the present study was to address whether the pharmacological inhibition of the accumulation of CSPGs by xyloside treatment could affect the course evolution and symptomatology of TMEV-IDD. In addition, we investigated the effects of the endocannabinoid 2-arachidonoylglycerol (2AG) and its hydrolysis inhibition by UCM-03025 on i) the expression of CSPGs at the chronic phases of TMEV-IDD and ii) the production of CSPGs by astrocyte in culture.

Our results show that the upregulation of CSPGs that occurs at the chronic phases of TMEV-IDD were modulated by xyloside and UCM-03025 treatments leading to an amelioration of motor deficits in TMEV-infected mice. Moreover, *in vitro* results confirm that 2AG regulates the production of CSPGs in astrocytes.

As CSPGs are known to be involved in remyelination failure in human MS and in murine demyelinating models, it is suggested that 2-AG, by modulating CSPGs, would be involved in CNS reparative mechanisms.

Key words: Chondroitin sulphate proteoglycans, TMEV-IDD, UCM-03025.

P.8.

THE INHIBITORY EFFECT OF THE CANNABINOID AGONIST, CP55940 ON THE CYTOCHROME C OXIDASE ACTIVITY IS REDUCED IN A MODEL OF PARKINSON'S DISEASE

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The presence of CB1 receptors in the mitochondrial outer membrane together with the evidences that cannabinoids cause a decrease in the function of the electron transport chain indicate that this system can play an important role in some neurodegenerative diseases such as Parkinson´s disease (PD). In fact, it is known that mitochondrial dysfunction has been implicated in the pathogenesis of PD.
Thus, and considering the continuing increase of the aging population and neurodegenerative diseases prevalence, we have studied the effect produced by the cannabinoid agonist CP55940 in the heart cell membrane of control and MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treated non-human primate (Macaca fascicularis). This animal model clearly reproduces the main pathological and clinical features of PD.
For this purpose, we determined the cannabinoid density an activity in the heart using cell membrane microarrays, as well as the activity of cytochrome c oxidase.
We observed that cytochrome c oxidase activity decreases not only in parkinsonian monkeys, but also the CP55940 evoked-inhibition. However, no significant changes were observed neither in the [3H]-CP55940 binding nor in the [35S]GTP□S binding stimulated by WIN 55.212-2, indicating that the alterations are produced downstream of the cannabinoid receptors.

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Key words: Parkinson’s disease, mitochondria, cannabinoids

P.9.

CANNABIDIOL TREATMENT RESTORES THE IMPAIRED NEUROGENIC CONTRACTION OF BLADDER FROM RATS WITH NEONATAL HYPOXIC-ISCHEMIC BRAIN DAMAGE

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Damage of organs other than the brain often complicates neonatal hypoxic-ischemic (HI) encephalopathy (NHIE) after perinatal asphyxia. Multiorganic damage has a huge importance in the evolution of NHIE affected newborns as there is a proportional relation between the number of affected extracerebral organs and adverse evolution. Besides, the frequency and stretch of multiorganic damage are not predictable. Specifically, the urinary tract could be affected in asphyctic newborns; however, there are no studies about the secondary bladder damage or malfunction after HI brain damage. On other hand, non-psychoactive phytocannabinoid cannabidiol (CBD) has neuroprotective properties in different HI models. CBD treatment not only reduces brain damage but also reduces secondary damage in extracerebral organs such as lung. The aim of the current study is to investigate whether functional changes of endocannabinoid system are involved in the motor dysfunction of urinary bladder from rats with neonatal HI brain injury. To this aim, the Rice-Vanucci model in newborn Wistar rats (P7) was used. Briefly, unilateral HI brain damage was induced by exposure to hypoxia (10% FiO_2) for 112 min after left carotid artery electrocoagulation under anaesthesia. Ten minutes after the end of HI pups received s.c. vehicle (HV) or a single 1 mg/kg dose of CBD (HC). Other pups remained as controls (SHM). Seven days 7 (P14) after HI, rats were sacrificed and bladders strips from HV, HC and SH rats were mounted in myographs for isometric force recordings under a passive tension of 1 g during a normalization period of 60 min. The contractile effect induced by electrical field stimulation (EFS) and acetylcholine (ACh), was studied on basal tension of the samples pretreated with propranolol and N^G-nitro-L-arginine, beta-adrenergic receptor and nitric oxide synthase blockers. Our results show that EFS and ACh induced frequency- and concentration-dependent contractions. ACh responses were similar in both HV and SHM rats. However, neurogenic contractions, elicited by EFS, were greatly diminished in HV rat bladder versus that obtained in SHM rat, being these responses reverted as a consequence of treatment with CBD. In conclusion, the impaired bladder motor function related with HI brain damage may be restored by CBD treatment. Endocannabinoid system could be a promising therapy for urinary tract dysfunction secondary to neonatal HI.

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P.10.

CANNABINOID AND LYSOPHOSPHATIDIC ACID SYSTEMS IN THE TRIPLE TRANSGENIC MICE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common cause of dementia in aging populations. The triple transgenic mice (3xTg-AD) initiate the accumulation of senile plaques and neurofibrillary tangles at 6 months of postnatal development. The present study analyzes the involvement of neurolipids, such as endocannabinoids (eCB) and lysophosphatidic acid (LPA) in this mice model of familial AD using functional and receptor autoradiography combined with the identification of phospholipid (PL) precursors of the endogenous ligands by Imaging Mass Spectrometry (MALDI-IMS).

The autoradiographic distribution of functional CB_1 receptors in the mice model showed a decreased activity in cortex layer VI (WT 435.4 ± 58.2% over basal levels; 3xTg-AD 238.4 ± 22.9%) and posterior amygdala (WT 295.5 ± 41.7%; 3xTg-AD 112.8 ± 28.9%). On the contrary, LPA_1 activity was increased in transgenic mice at striatum (WT 3.3 ± 7.2%; 3xTg-AD 23.1 ± 3.8%), motor cortex (WT 6.2 ± 12.4%; 3xTg-AD 26.1 ± 7.6%), corpus callosum (WT 90.8 ± 12.3%; 3xTg-AD 189.6 ± 17.4%) and hippocampal CA1 area (WT -18.7 ± 7.8%; 3xTg-AD 22.7 ± 4.4%).

Quantitative densitometry showed a higher density of CB_1 receptors in 3xTg-AD mice than in WT at brain areas where intracellular Aβ has been build up, such as hippocampal CA1 area (WT 193.8 ± 33.8 fmol/mg t.e.; 3xTg-AD 284.6 ± 19.8 fmol/mg t.e.; $p<0.05$) and cingulate cortex (WT 166.8 ± 27.7 fmol/mg t.e.; 3xTg-AD 236.8 ± 17.1 fmol/mg t.e.; $p<0.05$).

The application of IMS to the transgenic mice model identified modification in different PL species and one of the possible LPA precursors, the PA (34:1), was up-regulated in the same brain areas where the LPA_1 activity was increased.

The eCB and LPA neurolipid systems would activate in familial AD at initial stages by increasing the synthesis of the endogenous ligands and the efficiency of their target receptors.

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P.11.

ASSESSMENT OF TYPE-I CANNABINOID RECEPTORS IN ASTROCYTES OF MUTANT MICE DENTATE GYRUS

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Type-1 cannabinoid (CB_1) receptor is widely expressed in the brain mediating the effects of (endo)cannabinoids. Evidences have shown its activation in astrocytes might be playing an important role in neuronal modulation of synaptic transmission and plasticity. To identify low levels of CB_1, avoiding their misinterpretation as background staining; "rescue" strategies are needed. However, it is necessary to evaluate if genetic re-expression maintains normal CB_1 expression in specific cell types of the "rescue" mutants.

We analyzed the subcellular CB_1 distribution in astrocytes of the dentate molecular layer. We used conditional "CB_1 rescue mice" which re-express CB_1 only in astrocytes (GFAP-*CB_1*-RS mice) and transgenic mice carrying GFP under the control of the GFAP promoter (GFAP-GFP mice). Specific CB_1, GFAP and GFP antibodies combined with a preembedding immunogold and an immunoperoxidase method for electron microscopy were applied to hippocampal sections of the mutants, as well as of *CB_1*-WT and of *CB_1*-KO mice that were used as controls.

The results showed that 40.69% ± 3.69% of astrocytic sections were CB_1 immunopositive in GFAP-*CB_1*-RS. No significant differences were observed comparing with *CB_1*-WT (44.81% ± 3.62%). Sparse unspecific particles were detected in a few astrocytic elements of *CB_1*-KO (1.77% ± 0.72%) and GFAP-*CB_1*-KO mice (3.08% ± 1.03%). In GFAP-GFP mice, 51.68% ± 2.70% of the GFP immunoreactive astrocytic processes were CB_1 positive (significant difference compared to *CB_1*-WT, *; $p< 0.05$).

To summarize, the proportion of CB_1 immunopositive astrocytic processes in *CB_1*-WT is maintained in GFAP-*CB_1*-RS mice, showing the great potential of these transgenic mice to study CB_1 in brain cell types where the CB_1 expression is low. Besides, more CB_1 positive astrocytic processes were observed in GFAP-GFP mice, suggesting that a better CB_1 detection in astrocytes can be achieved in these reporter mice. The expression of CB_1 in astrocytes might be higher than what was expected.

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Key words: astroglia, mutant mice, immuno-electron microscopy.

P.12.

ROLE OF CANNABINOID CB2 RECEPTORS IN BRAIN DAMAGE FOLLOWING HYPOXIA-ISCHEMIA IN ADULT MICE

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The endogenous cannabinoid system seems to play an important role in the neuropathology associated with brain ischemia. The aim of this study was to evaluate the neuroprotective effects of cannabinoid CB2 receptors in the behavioural and biochemical alterations induced following hypoxia-ischemia. Mice lacking CB2 receptors (KO) and control littermates (WT) were anesthetized, and the left common carotid artery was permanently ligated. Following recovery, mice were placed in a hypoxia chamber with 10% oxygen for 60 min. Behavioural measurements in the rotarod, beam walking, object recognition, open field, and Irwin tests were carried out 24 h, 72 h and 7 days after this procedure. After testing, brains were prepared for histological and immunohistochemistry analysis. Hypoxia-ischemia induced brain damage ipsilateral to the carotid ligation, although significant differences in lesion size were observed in both genotypes. WT mice showed small damage in the hippocampus and cortex, while KO mice exhibited larger lesions in hippocampus, striatum, cortex and amygdala. Behavioural alterations were observed in both genotypes. However, WT mice progressively recovered motor functionality, while KO mice showed persistent deficits in motor learning, coordination and balance. A significantly higher expression of astrocytes and microglia in the hippocampus, striatum and cortex was observed in KO with respect to WT mice, consistent with the greater lesion size observed in these animals. Memory deficits in the object recognition test were observed 72 h following hypoxia-ischemia in KO and WT mice. However, no recovery in this function was observed in either genotype, suggesting that CB2 receptors may not exert neuroprotective effects on memory dysfunction. Our results indicate that CB2 receptors may have a specific neuroprotective role in motor learning, coordination and balance deficits following hypoxia-ischemia insult, and strongly suggest that they may be important new targets to accelerate the recovery of these brain functions after brain damage occurs.

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Key words: CB2 receptors, Brain damage, Hypoxia-ischemia,

P.13.

ENDOCANNABINOID SIGNALLING IN THE BASOLATERAL AMYGDALA MODULATES THE ANXIOUS-LIKE BEHAVIOUR IN 3XTG-AD MICE

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Cholinergic degeneration and familial mutations have been identified in Alzheimer's disease patients affecting to the amyloid precursor processing and tau protein. The endocannabinoid system could be modulating the cholinergic system in learning and memory related areas. Therefore, we have used the AD-murine model, 3xTg-AD, to analyze this possible interaction using behavioural test and neurochemical analysis of the CB_1 receptors. The effects of the subchronic administration of the direct CB_1 agonist, WIN55,212-2 (1 mg/kg), and the indirect JZL-184 (8 mg/kg) were studied. Learning and memory latencies (s) were evaluated with the passive avoidance test. CB_1 density (fmol/mg prot) and activity (%) were measured by using [^{3}H]CP55,940 and functional [^{35}S]GTPγS autoradiography, respectively. The cellular localization of CB_1 on GABAergic and/or cholinergic terminals was also performed by immunofluorescence.

An increase in the learning latency can be considered as anxious-like behaviour. We observed higher learning latencies in 3xTg-AD mice compared to WT (26.24 ± 1.8 vs 12.28 ± 1.9; $p<0.001$).and lower retention latencies in 3xTG-AD ($p<0.05$). CB_1 density in the basolateral amygdala (BLA) was also higher in the transgenic mice (386 ± 12 vs 497 ± 20; $p<0.001$). However, CB_1 activation by direct or indirect agonists reduced both anxiety (15.7 ± 3.0 and 15.3 ± 2.4) and CB_1 density in BLA to WT levels (386 ± 32 and 247 ± 14) but had no effect in the retention latencies. The CB_1 activity was also modulated in a similar way. The learning latencies correlated with CB_1 density (Pearson $r = 0.7139$; $p = 0.0091$) but not with changes in CB_1 activity in BLA.

Histochemical studies revealed that CB_1 were localized on GABAergic terminals in BLA, where a significant decrease in $AChE^+$ fiber density was measured in 3xTg-AD mice (-12%).

The present work shows an increase on anxious-like behaviour in the 3xTg-AD model probably associated to the signalling by CB_1 in the BLA regulating the cholinergic input.

Key words: Alzheimer´s disease, cannabinoid, anxiety.

P.14.

EPIGENETIC AND PROTEOMIC EXPRESSION CHANGES PROMOTED BY EATING ADDICTIVE-LIKE BEHAVIOR

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An increasing perspective conceptualizes obesity and overeating as disorders related to addictive-like processes that could share common neurobiological mechanisms. In the present study, we aimed at validating an animal model of eating addictive-like behavior in mice, based on the DSM-5 substance use disorder criteria, using operant conditioning maintained by highly palatable chocolate-flavored pellets. For this purpose, we evaluated persistence of food-seeking during a period of non-availability of food, motivation for food and perseverance of responding when the reward was associated with a punishment. This model has allowed identifying extreme subpopulations of mice related to addictive-like behavior. We investigated in these subpopulations the epigenetic and proteomic changes. A significant decrease in DNA methylation of CB_1 gene promoter was revealed in the prefrontal cortex of addict-like mice, which was associated to an up-regulation of CB_1 protein expression in the same brain area. The pharmacological blockade (rimonabant 3mg/Kg; ip) of CB_1 receptor during the late training period reduced the percentage of mice that accomplished addiction criteria, which is in agreement with the reduced performance of CB_1 knockout mice in this operant training. Proteomic studies have identified proteins differentially expressed in mice vulnerable or not to addictive-like behavior in hippocampus, striatum and prefrontal cortex. These changes included proteins involved in impulsivity like-behavior, synaptic plasticity and cannabinoid signaling modulation, such as alpha-synuclein, phosphatase 1-alpha, doublecortin-like kinase 2 and diacylglycerol kinase zeta and were validated by immunoblotting. This model provides an excellent tool to investigate the neurobiological substrate underlying the vulnerability to develop eating addictive-like behavior.

P.15.

DIFFERENT APPROACHES TO DETECT OLIGOMERIZATION IN CANNABINOID SYSTEM

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The study of interactions between proteins and cellular molecules is fundamental to the understanding of biological systems. The classical pharmacological paradigm associates one ligand with one receptor and one receptor with one signaling pathway, so, traditionally it has been considered that G protein-coupled receptors (GPCR) signal exclusively as single monomeric entities. Today, homo- and heteromerization of GPCRs is becoming well accepted, so, they can combine among themselves to generate new and unique biochemical and functional characteristics. In fact, this is key for the integration of extracellular signals and the subsequent cell response via several mechanisms, including selective ligand-binding, trafficking and/or downstream signaling.

Standard technologies like co-immunoprecipitation (Co-IP) or biophysical and biochemical techniques such as resonance energy transfer (RET) and bimolecular fluorescence complementation (BiFC) have been key to demonstrating very close proximity of two receptors, which is indicative of direct intermolecular receptor-receptor interactions. The combination of BiFC and bioluminescence energy transfer techniques constitutes a powerful approach to detect the protein-protein interactions localized in the plane of the membrane, and is useful to detect heteromerization of three different GPCRs. The *in situ* proximity ligation assay (PLA) is a novel antibody-based technology that avoids the need of express receptor constructs considering that is capable to detect single protein events in tissue and cell samples prepared for microscopy.

Cannabinoid receptors (CB_1 and CB_2) are members of the class A GPCR subfamily and mediate many physiological effects exerted by endogenous cannabinoids, such as anandamide and 2-arachidonoyl-glycerol (2-AG). The signalling pathways and subsequent physiological actions of endocannabinoids are further diversified by the occurrence of dimers and oligomers of cannabinoid receptors with other GPCRs. Interestingly, the CB_1 receptor forms homomers and also heteromers with CB_2 receptors, demonstrated by biolumniscence resonance energy transfer (BRET) in transfected cells and PLA in rat brain derived primary cultures. Furthermore, different interactions with other receptor are described. A combination of BiFC and BRET techniques was used to identify the occurrence of D_2R-CB_1R-$A_{2A}R$ hetero-oligomer in living cells, and it can be described also by Co-IP and sequential resonance energy transfer (SRET). PLA was used also to reveal the expression of CB_2-GPR55 heteromers in human breast and glioblastoma cells.

Therefore, this knowledge can provide new potential targets to treat neurodegenerative diseases, pain, mental disorders and drug addiction, and besides, participate in neuroprotection process.

Key words: protein-protein interactions, cannabinoids, GPCR

P.16.

IDENTIFICATION OF A NEW GPR55 CHEMOTYPE BASED ON THE CHROMENOPYRAZOLE SCAFFOLD

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The orphan G protein-coupled receptor GPR55 has been proposed as a novel component of the endocannabinoid system. However, the validity of this categorization is still under debate mainly due to the lack of potent and selective agonists and antagonists of GPR55, which may facilitate the study of these receptors in relation with endocannabinoid-related physiological processes.[1] Nevertheless, recent evidence suggests that GPR55 represents a possible target for the treatment of various diseases due to its role in inflammation, neuropathic pain, bone physiology, diabetes and cancer. In this scenario, the design of potent and selective GPR55 ligands remains a major challenge for medicinal chemists.

There are only few selective GPR55 ligands discovered so far due to lack of receptor structural data and inconsistencies between GPR55 pharmacological functional outcomes. In this context, we have designed and synthesized novel GPR55 ligands based on a chromenopyrazole scaffold (figure 1). Appraisal of GPR55 activity of the new compounds was accomplished by using an innovative cell-impedance label-free based assay in GPR55-HEK293 cells. The real-time impedance responses provide an integrative assessment of the cellular consequence to GPR55 stimulation taking into account the different possible signaling pathways. The ability to antagonize the putative endogenous ligand LPI was evaluated as well. Dose-response experiments were also performed in normal HEK293 cells. To investigate the specific relation of GPR55 with the endocannabinoid system, the binding of these GPR55 ligands towards the classical cannabinoid receptors was also analyzed through CB_1 and CB_2 radioligand binding experiments. Analysis of the pharmacological data obtained allowed selective potent GPR55 partial agonists and GPR55 antagonists to be identified. In summary, we have discovered a novel GPR55 chemotype that may serve to develop appropriate pharmacological tools or novel drugs to continue with the challenging goal of the validation of this receptor.

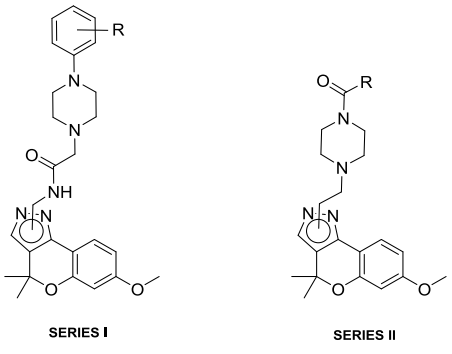

Figure 1: Proposed structures.
Series I: (Phenylpiperazinyl)acetamidomethyl-chromenopyrazole;
Series II: (Acylpiperazinyl)ethyl-chromenopyrazole.

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Key words: GPR55, synthesis, cell-impedance based assays.

P.17.

DIFFERENTIAL EXPRESSION OF CANNABINOID CB1-ADENOSINE A2A RECEPTOR HETEROMERS DURING HUNTINGTON'S DISEASE PROGRESSION

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Huntington's disease (HD) is a neurodegenerative disorder caused by expansion of a CAG repeat within the N-terminal region of huntingtin protein. Dysfunction and death of striatal medium-sized spiny neurons is the major neuropathological feature in HD. However, cognitive deficits and motor disturbances can be observed in HD patients and animal models at early disease stages. CB_1 cannabinoid receptors (CB_1R) play a pivotal role in the control of motor behaviour and we previously have found that receptor deletion aggravates the symptoms, neuropathology and molecular pathology of HD. Moreover, pharmacological administration of Δ^9-tetrahydrocannabinol to HD mice exerted a therapeutic effect and ameliorated those parameters, suggesting that activation of these receptors may attenuate disease progression. CB_1R belongs to the G-protein coupled receptor (GPCR) superfamily and protein-protein complexes, called heteromers, between GPCRs have been described. Heteromers are biochemically distinct entities from the single receptors behaving as novel signalling units with specific functional characteristics. Previously, heteromers between CB_1R and adenosine A_{2A} receptors ($A_{2A}R$) were described to be located in brain areas affected in HD. Here we set about to study if $A_{2A}R$-CB_1R heteromers can be a potential therapeutic target in HD, by identifying the expression of $A_{2A}R$-CB_1R heteromers in different brain regions during the progression of the disease and determining the specific biochemical characteristics of these heteromers. As HD models we used conditionally immortalized striatal neuronal progenitor cells expressing normal ($STHdH^{Q7}$) or mutant ($STHdH^{Q111}$, 111 glutamines) huntingtin and a widely accepted preclinical model of HD, the $Hdh^{Q7/Q111}$ mice expressing huntingtin allele with 111 glutamines and wild-type mice $Hdh^{Q7/Q7}$. By using the proximity ligation assay (PLA), we detected the expression of $A_{2A}R$-CB_1R heteromers in both $STHdH^{Q7}$ and $STHdH^{Q111}$ cells and in different brain areas of $Hdh^{Q7/Q111}$ mice in early but not in late stages of the illness. The loss of heteromer expression seems associated to the progression of HD since it was not observed in $Hdh^{Q7/Q7}$ mice. To know the physiological consequences of heteromer loss we investigated the biochemical specific characteristics of $A_{2A}R$-CB_1R heteromers. In label free dynamic mass redistribution assays using $STHdH^{Q7}$ and $STHdH^{Q111}$ cells CB_1R and $A_{2A}R$ agonist signalling was not sensitive to cholera or pertussis toxin but was inhibited by a Gq protein inhibitor. Moreover, both receptor agonists were unable to activate or inhibit adenilyl cyclase but were able to increase intracellular Ca^{+2}, indicating that, different from the single receptors, $A_{2A}R$-CB_1R heteromers are coupled to a Gq protein. Negative cross-talk in the global signalling was observed when $A_{2A}R$-CB_1R heteromers were co-activated. Also antagonists of each receptor were able to block both agonists' signalling in a cross-antagonism phenomenon. The negative cross-talk and cross-antagonism in ERK1/2 and Akt phosphorylation was also observed in brain slices where $A_{2A}R$-CB_1R heteromers are expressed. Our results demonstrate that $A_{2A}R$-CB_1R heteromers play a pivotal role in controlling cannabinoid signalling and indicate that these heteromers might be a target for treating HD in the early stages of the illness.

P.18.

THE BASAL FOREBRAIN CHOLINERGIC PATHWAY IS MODULATED BY CB1 RECEPTORS

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The selective vulnerability of the basal forebrain cholinergic pathway (BFCHOL) is responsible for most of the clinical alterations in learning and memory processes that are characteristic of the Alzheimer's disease (AD). The muscarinic receptor (MR) antagonism, e.g. using scopolamine, (Scop), is used as a memory impairment model in rodents. Biphasic effects induced by low or high doses of cannabinoids have been reported in relation to both cognitive functions and hippocampal acetylcholine (ACh) release.

The aim of the present study was to evaluate the effects on spatial and working memories induced by a subchronic treatment with WIN55,212-2 at a low dose in the Scop model of learning and memory deficit. The brains were dissected 48h after the last administration for [^{35}S]GTPγS autoradiographical evaluation of the functional activity of both CB_1 rand MR in the BFCHOL pathway.

The administration of WIN55,212-2 or the vehicle did not modify the learning and memory trials in the Barnes maze test, but a significant effect of WIN55,212-2 as memory-saver of impairment induced by Scop was recorded when the time spent in the target quadrant was measured (time in target quadrant: WIN + Scop: 78,7± 13 sec vs VEH + Scop: 45,6 ± 3 sec).

The *in vitro* experiments showed that the CB_1 activity was higher in the basal forebrain of WIN55,212-2-treated rats compared to vehicle (WIN: 553 ± 93 % stimulation over basal vs VEH: 273 ± 44 %) and at the layer VI of the motor cortex (WIN: 1201 ± 65 % vs VEH: 730 ± 86 % $p<0.05$, n=11). Moreover, the MR activity was also higher with the WIN treatment in the BFCHOL pathway. (*nbM*: VEH: 204 ± 26 % vs WIN: 366 ± 42 % of stimulation; *Hippocampus*: VEH: 112 ± 27. vs WIN: 219 ± 35 nCi/g t.e., $p<0.05$; n=11) A moderate stimulation of the eCB system at the BFCHOL pathway could have protective effects on the MR-mediated impairment in spatial memory.

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P.19.

CANNABINOID CB2 RECEPTOR AS A POTENTIAL TARGET IN LRRK2-TRANSGENIC MICE: IS IT REALLY RELEVANT FOR PARKINSON'S DISEASE?

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Most of cases of Parkinson's disease (PD) have a sporadic origin and are 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 is already available (Ramonet *et al*. PLoS One 2011) and apparently may represent a valuable experimental model for investigating PD pathogenesis and novel treatments. We designed a long-term study with these animals aimed at: (i) elucidating the changes experienced by the endocannabinoid signaling system in the basal ganglia during the progression of the disease in these mice, and (ii) evaluating the potential of cannabinoids, in particular compounds selectively targeting the CB_2 receptor, as disease-modifying agents in these mice. Our results unequivocally demonstrate that: (i) LRRK2 transgenic mice develop motor impairment consisting of anomalies in rotarod performance, reflecting a deficit in motor coordination and dystonia, and a strong deficiency in the hanger-wire test, reflecting muscle weakness, rather than hypokinesia which was difficult to be demonstrated in the actimeter; (ii) these behavioral responses occurred in absence of any evidence of reactive gliosis and neuronal injury, as well as synaptic deterioration in the basal ganglia, although these could be evident in other unexplored CNS structures; (iii) furthermore, there was no changes in the status of the CB_2 receptor, as well as in other elements of the endocannabinoid signaling, in the basal ganglia; (iv) paradoxically, the activation of this receptor by HU-308 or Δ^9-THCV partially reverses the poor response in the hanger-wire test of LRRK2 transgenic mice, but the neuronal substrates and CNS areas responsible for this improvement remain to be identified. In summary, our data support the interest of the CB_2 receptor as a potential pharmacological target in LRRK2 transgenic mice, although the neuronal substrates and CNS structures underlying these benefits do not appear to be related to the basal ganglia and to the presumed parkinsonian features of these mice. In our hands, these mice do appear to develop neurological anomalies in motor coordination and, in particular, in muscle strength, which support the interest to investigate potential alterations in the cerebellum and, in particular, in upper and lower motor neurons.

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P.20.

LONG TERM SYNAPTIC PLASTICITY DEPENDENT ON CANNABINOID CB1 RECEPTORS ACTIVATION IS ALTERED IN THE DENTATE GYRUS OF ADULT MICE EXPOSED TO ETHANOL DURING ADOLESCENCE

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Alcohol drinking, especially among adolescents and young adults, is a serious public health concern. Ethanol interacts with the endocannabinoid system (ECS) whose function may be altered in ethanol dependence. Here, we investigated the effect of ethanol consumption on excitatory synaptic transmission and plasticity mediated by the cannabinoid CB_1 receptor in dentate gyrus (DG).

Male C57BL6 mice were exposed to intermittent ethanol intake (20% (v/v) in tap water) using a 4 days drinking-in-the-dark procedure during adolescence (PD 30 to 54). Animals were given access to ethanol (or water) for 2h sessions during 3 days, and 4h session on the 4th day. At 18-21 days withdrawal from ethanol, adult mice were sacrificed. Electrophysiological, immunohistochemical, and molecular techniques were applied.

Excitatory postsynaptic potentials (fEPSPs) were evoked after stimulation of the medial perforant path and recorded in the supragranular zone of the dentate molecular layer (ML) in the presence of the $GABA_A$ antagonist picrotoxin. CB_1 activation by CP55,940 (10μM) inhibited fEPSPs in controls (26.43±2.77% of baseline) as already shown. However, this effect was not observed in ethanol-exposed mice (4.9±7.47% of baseline). Furthermore, ML synaptic stimulation (10min, 10Hz) triggered a long term depression (LTD) of the excitatory transmission that was absent in adult mice after ethanol consumption during adolescence (2.7±3.12% of inhibition; $p<0.0001^{***}$). This plasticity was CB_1 dependent as the AM251 antagonist (4μM) abolished LTD (8±6.6% of inhibition). CB_1 immunoreactivity decreased in ML of ethanol-exposed (87.47±0.58%) vs control (100±0.77%) mice. Also, the relative mRNA and CB_1 protein significantly decreased, while a significant increase in MAGL (mRNA and protein) was detected.

Altogether, repetitive exposure to ethanol during adolescence leads to a deficit of endocannabinoid-dependent LTD in adult DG excitatory synapses, probably due to a down-regulation of CB_1 receptors and a reduction of the endocannabinoid tone by an increase of MAGL.

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Key words: electrophysiology, electron microscopy, hippocampus.

P.21.

SYNTHESIS, PHARMACOLOGICAL EVALUATION AND DOCKING STUDIES OF PYRROLE STRUCTURE-BASED CB2 RECEPTOR ANTAGONISTS

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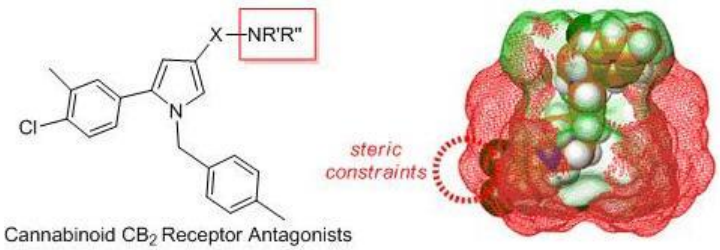
During the last years, there has been a continuous interest in the development of cannabinoid receptor ligands that may serve as therapeutic agents and/or as experimental tools. This prompted us to design and synthesize analogues of the CB_2 receptor antagonist *N*-fenchyl-5-(4-chloro-3-methyl-phenyl)-1-(4-methyl-benzyl)-1*H*-pyrazole-3-carboxamide (SR 144528). The structural modifications involved the bioisosteric replacement of the pyrazole ring by a pyrrole ring and variations on the amine carbamoyl substituents. Two of these compounds, the fenchyl pyrrole analogue GR16 and the myrtanyl derivative GR20, showed high affinity (K_i in the low nM range) and selectivity for the CB_2 receptor and both resulted to be antagonists/inverse agonists in an *in vitro* CB_2 receptor bioassay. Cannabinoid receptor binding data of the series allowed identifying steric constraints within the CB_2 binding pocket using a study of Van der Waals'volume maps. Docking of the myrtanyl derivative GR20 in the inactive state of the CB_2 receptor model confirmed the importance of the interaction between the amide hydrogen of GR20 and the aspartic acid D275 at the binding site.



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Keywords: bioisosterism, cannabinoid receptors, CB_2 antagonism, docking studies

P.22.

A MODERATE MATERNAL CALORIC RESTRICTION DURING PRECONCEPTIONAL AND GESTATIONAL PERIODS IMPAIRS HYPOTHALAMIC ENDOCANNABINOIDS AND N-ACYLETHANOLAMIDE LEVELS IN MALE OFFSPRING AT BIRTH

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Over the last few years, several investigations have focused on the effect of undernutrition during critical periods of life in the offspring and their risk of developing metabolic diseases later in life. Additionally, animal models have shown that hypothalamic neuronal circuitry could be altered by maternal undernutrition. On the other hand, the endocannabinoid system plays an important role in brain development and metabolic regulation. Although its activity in the brain can be modified by dietary manipulations, the impact of a maternal diet restriction on the hypothalamic endocannabinoids at birth has been unexplored. Here, we performed two experiments in female Wistar rats: in experiment 1 (preconceptional-gestational food restriction), control rat dams were given standard chow ad libitum throughout the perinatal period, whereas restricted dams (R1) were given a 20% calorie-restricted diet starting two weeks before mating to gestational day 20 (GD 20). In experiment 2 (gestational food restriction), control rats were given standard chow diet throughout the perinatal period as well. Meanwhile, restricted dams (R2) received a 20% calorie-restricted diet during the full pregnancy. In both experiments, weight gain of dams was monitored and birth outcomes were assessed. Hypothalamic endocannabinoids and N-acylethanolamides (NAEs) were also measured in male offspring. We found that R1 rat dams gained less weight than controls during preconception and pregnancy, although they showed a higher percentage of increase in body weight than controls when they were allowed to eat ad libitum. In contrast, R2 rat dams started to gain less weight at GD12 until the end of the pregnancy as compared to controls. Regarding neonatal outcomes, offspring from R1 had a normal birth weight but they tended to have a lower litter size. They also showed significantly decreased levels of anandamide (AEA), arachidonoylglycerol (2-AG), arachidonic acid (AA) and palmitoylethanolamide (PEA) at postnatal day zero. On the other hand, offspring from R2 were underweight at birth, but litter size was unaffected. Additionally, they displayed decreased levels of AA and oleoylethanolamide (OEA), but not different levels of AEA or 2-AG at birth. These results show that a maternal diet restriction alters weight gain during pregnancy, modifies neonatal outcomes and decreases the levels of hypothalamic endocannabinoids, their direct metabolite AA and other NAEs at birth. Furthermore, the timing of maternal caloric restriction could substantially impact these variables. Importantly, the data suggest that endocannabinoid signalling can be altered by maternal caloric restriction and this could lead to the modification of the hypothalamic circuit. This hypothesis could explain the metabolic long-term effects documented in previous studies using similar models of maternal undernutrition.

Key words: Endocannabinoid system, N-acylethanolamides, fetal programming, perinatal, undernutrition, hypothalamus

P.23.
GPR55, A RECEPTOR THAT LIKES CANNABINOIDS

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The endocannabinoid system includes two cannabinoid, CB_1 and CB_2, receptors. However, some evidence pointed to GPR55 as the third cannabinoid receptor. GPR55, the receptor for lysophosphatidylinositol that triggers intracellular calcium liberation via Gα13/RhoA, promotes proliferation through ERK1/2-MAPK activation, regulates cytoskeleton rearrangement and migration and shows a wide expression profile [1], has attracted attention, as it potentially explains some endocannabinioid effects that are non- CB_1/ CB_2 receptor mediated. Moreover, GPR55 has been reported to be activated by exogenous and endogenous cannabinoid compounds, for example the cannabinod receptor ligands, AM251 and SR141716A (rimonabant), act as GPR55 agonists, while the potent synthetic cannabinoid agonist, CP55,940, has been described to acts as a GPR55 antagonist/partial agonist [2]. It has also been described that GPR55 responds to the endogenous cannabinoid ligands anandamide and 2-arachidonylglycerol and the phytocannabinoids delta-9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol, in a cell-type and tissue-dependent way [4].
We have reported using bioluminescence resonance energy transfer (BRET) and *in situ* proximity ligation assays (PLA) that GPR55 forms heteromers with either CB_1 or CB_2 receptors [1, 3]. Also, the heteromer fingerprint for CB_1-GPR55 and CB_2-GPR55 has been identified in transfected cells and slices from striatum. CB_1-GPR55 receptor heteromer fingerprint consists of cross-antagonism (CB_1 receptors antagonists block the effect of GPR55 agonist) on ERK1/2 phosphorylation. The cross-antagonism was also observed on GPR55-mediated NFAT activation [3]. CB_2-GPR55 receptor heteromerization leads to a reduction in GPR55-mediated activation of transcription factors whereas ERK1/2-MAPK activation is potentiated in the presence of CB_2 receptors. CB_2 receptor-mediated signalling is also affected by co-expression with GPR55. Label-free assays confirmed the cross-talk between the two receptors [1].
Preliminary results show that cells transiently transfected with GPR55 respond to JWH133 and Δ^9-THC. LPI but also the cannabinoids are able to increase the signal of a sensor that becomes fluorescent in the presence of calcium, whereas no signal is detected in untransfected cells. In transiently transfected cells Δ^9-THC and LPI increases ß-arrestin recruitment to GPR55, while JWH133 and CP55940 do not produce any effect. These intriguing results merit further experimental work to understand cannabinoid-GPR55 interactions at a molecular level.

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Keywords: GPR55, endocannabinoid system, putative CB_3, heteromers

P.24.

ENVIRONMENTAL ENRICHMENT REVERSES COGNITIVE IMPAIRMENT ASSOCIATED WITH ALTERATION OF CB1-DEPENDENT LTD AFTER ETHANOL CONSUMPTION DURING ADOLESCENCE

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Alcohol consumption, especially during adolescence, is one of the main problems that concern our society. Studies in the last decade indicate that the endocannabinoid system (ECS) function may be altered in ethanol dependence. An enriched environment (EE) has been shown to significantly facilitate recovery from brain injury due to important anatomical, molecular and functional changes that occur along brain development. However, it is not known whether an EE has any effect on cognitive impairment associated with ethanol consumption. Our aim is to investigate this during the adolescence period and the role of an enriched environment to counteract ethanol administration effects on plasticity mediated by cannabinoid CB_1 receptors in the hippocampal dentate gyrus.

Male C57BL6 mice were exposed to a 4 days drinking-in-the-dark (DID) procedure during adolescence (PD 30 ± 2 to 54 ± 2). The animals were given free access to ethanol (20% (v/v) in tap water) or water for 2-h sessions during three consecutive days, and a 4-h session on the 4th day. Then, from postnatal 56 to 74 withdrawal days, the animals were reared under two different conditions: standard laboratory condition (SC) and enriched environment. Recognition memory was assessed by the novel object recognition test (NORT) in the last three days of the withdrawal period (from p70 to p73). Specifically, total exploration time, discrimination index % and recognition index were measured in each group (SC-H_2O, SC-OH, EE-H_2O and EE-OH). The next day (p74) adult mice were sacrificed.

Synaptic stimulation (10min, 10Hz) of the medial perforant path triggered a CB_1-dependent long term depression (LTD) of the excitatory transmission that was absent in adult mice after ethanol consumption during adolescence (2.7 ± 3.12% of inhibition; $p<0.0001$***). Furthermore, a significant lower recognition memory in the alcohol treated group (SC-OH) compared to the untreated control group (SC-H_2O) was observed. Interestingly, both enriched groups (EE-H_2O and EE-OH) showed higher values for each of the measured parameters in the novel object recognition test compared to the SC reared ethanol treated group (SC-OH). These results suggest that this experimental paradigm can reverse the effects of ethanol consumption on recognition memory that appears associated with an impairment of CB_1-long term synaptic plasticity.

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Key words: alcohol consumption, endocannabinoid system, enriched environment, memory.

P.25.

EXPRESSION OF ENDOCANNABINOID SYSTEM ELEMENTS IN THE BASAL GANGLIA OF LEVODOPA-TREATED PARKINSONIAN MONKEYS

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Parkinson's disease is a neurodegenerative disease caused by the progressive degeneration of dopaminergic neurons in the substantia nigra (SN). Levodopa is commonly used to alleviate the symptoms of the disease, but patients usually develop dyskinesias as a side-effect of the treatment. The endocannabinoid system regulates neurotransmission and its elements are abundant in the basal ganglia. The aim of this study was to describe how the elements of the endocannabinoid system are affected by the dopaminergic degeneration and by the administration of levodopa in parkinsonian monkeys. Parkinsonism was induced by the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and dyskinesias by chronic levodopa treatment of parkinsonian monkeys. The putamen, the globus pallidus pars externa (GPe) and pars interna (GPi), the subthalamic nuclei (STN) and the substantia nigra (SN) were dissected from coronal cryostat sections, RNA was extracted and analyzed by real-time PCR. Cannabinoid receptor type 1 (CB1R) mRNA and N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) expression were significantly decreased in the the STN of dyskinetic monkeys compared to untreated MPTP monkeys. The mRNA expression of CB1R in the putamen and in the STN, and NAPE-PLD in the STN correlated positively with the disability score of monkeys. These results suggest a relevant role of endocannabinoid system in the STN and motor coordination.

Key words: Parkinson’s disease, Neuroprotection, Endocannabinoid System, Parkinsonian Monkeys.

P.26.

CHRONIC STRESS IMPAIRS CANNABINOID 1 (CB1) RECEPTOR-MEDIATED CONTROL OF GLUTAMATERGIC TRANSMISSION AND PLASTICITY IN YOUNG ADULT MICE DENTATE GYRUS

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The endocannabinoid (eCB) system plays a role in stress responses, also hippocampal CB_1 receptor expression and binding capacity were shown to be altered under chronic stress. However, anatomical and physiological changes of CB_1 at synapses of the stress-involved dentate gyrus (DG) are poorly reported.

We have performed *ex vivo* electrophysiological and anatomical techniques in DG of young adult male mice subjected to either acute or chronic restraint stress. Upon dentate medial perforant path (MPP) stimulation, the CB_1R agonist CP55,940 (10 µM) reduced field excitatory postsynaptic potentials (fEPSP) amplitude to 20.51 ± 5.881% of baseline ($p < 0.001$; n=7) in the presence of the $GABA_A$ antagonist picrotoxin (100 µM) in non-stressed mice. After an acute restraint stress the effectiveness of CP55,940 was only slightly reduced (fEPSP amplitude: 15.68 ± 3.40% of baseline; $p < 0.001$, n=5). However, after repetitive stress exposure fEPSP amplitude was completely impaired (3.46 ± 5.91% of baseline; $p > 0.05$, n = 5). Furthermore, synaptic stimulation of the dentate molecular layer (10 min, 10 Hz) triggered a long term depression of the excitatory synaptic transmission (12.19 ± 1.31% inhibition; $p < 0.001$, n=3) in non-stressed mice, that was absent after chronic stress (0.1 ± 1.19% of inhibition, $p < 0.001$, n=4).

Anatomically, there was a slight significant decrease of CB_1 immunoparticle density in DG excitatory terminals after chronic stress (1.02 ± 0.08) versus non-stressed mice (1.21 ± 0.06 $p < 0.05$, n=2), in contrast with the high significant CB_1 increase in inhibitory terminals (stress: 23.71% ± 0.72%; non stress: 16.09% ± 0.89% $p < 0.001$, n=2).

In summary, chronic stress causes a neuronal excitatory/inhibitory imbalance with small CB_1 changes in excitatory synapses but a remarkable impairment of excitatory synaptic transmission and plasticity in the dentate molecular layer.

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Key Words: Stress, endocannabinoid system, perforant path, *Ex vivo* electrophysiology.
Type of comunication: Póster

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PHARMACOLOGICAL BLOCKADE OF CANNABINOID CB1 RECEPTORS IN DIET-INDUCED OBESITY REGULATES DIHYDROLIPOAMIDE DEHYDROGENASE IN MUSCLE

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Cannabinoid CB_1 receptors peripherally modulate energy expenditure and metabolism. Here we investigated the effects of the CB_1 receptor blockade on the expression of glucose/pyruvate/tricarboxylic acid metabolism in rat abdominal muscle. Dihydrolipoamide dehydrogenase (DLD), a flavoprotein component (E3) of α-ketoacid dehydrogenase complexes with diaphorase activity in mitochondria, was specifically analyzed. Through comprehensive proteomic approaches involving two-dimentional electrophoresis and MALDI-TOF/LC-ESI trap mass spectrometry, we identified seven key enzymes from either glycolytic pathway or tricarboxylic acid cycle - regulated by both diet and CB_1 receptor activity. These enzymes were glucose 6-phosphate isomerase (GPI), triosephosphate isomerase (TPI), enolase (Eno3), lactate dehydrogenase (LDHa), glyoxalase 1 (Glo1) and the mitochondrial DLD, whose expressions were modified by the CB_1 receptor antagonist AM251 in a hypercaloric diet-dependent manner. Specifically, AM251 (3 mg kg^{-1}, i.p., 14 days) blocked the high-carbohydrate diet (HCD)-induced increase of the expression of GPI, TPI, Eno3 and LDHa, suggesting a down-regulation of glucose/pyruvate/lactate pathways under glucose availability. AM251 also alleviated the HCD-induced decrease of Glo1 expression, suggesting a methylglyoxal pathway up-regulation. Both CB_1 receptor antagonism (AM251-treated rats) and deletion ($CB_1^{-/-}$ mice) increased the expression of DLD and the mitochondrial respiratory gene *Cox4i1*. Interestingly, we identified the presence of CB_1 receptors at the membrane of striate muscle mitochondria, as was described in brain. We also found that AM251 increased the DLD expression and NADH-related diaphorase/oxidative activity in mitochondria. These findings suggest that CB_1 receptors modulate mitochondrial metabolism by targeting DLD and reveal new cannabinoid-dependent modulatory mechanisms of energy expenditure through the muscle mitochondrial respiration.

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DOWN-REGULATION INSTEAD UP-REGULATION OF CB2 RECEPTORS IN RESPONSE TO INFLAMMATION AND OXIDATIVE STRESS IN AN IN VIVO MODEL OF NEONATAL HYPOXIA-ISCHEMIA

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Hypoxic-ischemic (HI) brain damage is the most frequent acquired cause of neonatal encephalopathy. Despite the dramatic consequences of perinatal HI, an effective treatment has still not been found. Previous studies have demonstrated that the phytocannabinoid cannabidiol (CBD) afforded neuroprotection in the immature brain following HI. Even though previous data showed that neuroprotective effects of CBD may be due in part to the activation of CB_2 receptors, further studies are needed to understand the specific role of CB_2 receptors in the physiopathology of neonatal HI. For example, in contrast to brain damage in adult subjects, which proved an up-regulatory response of CB_2 receptors located in glial elements that has been interpreted as an endogenous protective response, there is not any evidence of a similar response in the neonatal brain following HI. Therefore, our aim was to investigate the changes in CB_2 receptors in the neonatal brain in response to the inflammation and oxidative stress elicited by an HI insult. To this end, the Rice-Vannucci model was used to induce unilateral HI brain damage in newborn Wistar rats (P7), whose brains were analyzed at 24 hours (P8) or 1 week (P14) after injury. Western-bot and qPCR analyses were used to quantify the alterations in this receptor following HI damage in the brain. Our results proved an unexpected down-regulation of CB_2 receptors in the immature brain in response to the HI insult, which contrasts with the up-regulatory response found in the adult brain. This difference presumably indicates that the immaturity of the neonatal brain also includes the lack of a protective up-regulatory response of CB_2 receptors against degenerating conditions. This finding is supported by the fact that the decrease in CB_2 receptors runs in parallel to an intense proinflammatory and oxidative response characterized by microglial activation, generation of pro-inflammatory cytokines, and reduction in the production of neurotrophins. It is also supported by the fact that the treatment with CBD, which is well-known that reduces the proinflammatory response in the neonatal brain following the HI insult, also attenuated the magnitude of the CB_2 receptor down-regulation. In conclusion, the immaturity of the brain at the neonatal age also includes a deficit in its ability to elicit the up-regulatory response of CB_2 receptors against inflammatory conditions, frequently described in the adult and aged brain. This presumably explains the intensity of the proinflammatory and oxidative response observed in the neonatal brain following an HI insult and represents an important issue for the development of cannabinoid-based therapies in this disease.

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Keywords: neonatal hypoxia-ischemia, cannabidiol, inflammation, oxidative stress