

# XII REUNIÓN ANUAL



**Pamplona, 24 a 26 de Noviembre de 2011**



**cima**  
CENTER FOR APPLIED MEDICAL RESEARCH  
UNIVERSITY OF NAVARRA



**Gobierno de Navarra**



**Almirall**



**PHYTOPLANT RESEARCH**



Organiza



Colaboran



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**LUGAR DE CELEBRACIÓN:**

Campus de Ciencias de la Universidad de Navarra, Salón de Actos del Edificio de Bibliotecas



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# 12<sup>a</sup> Reunión Anual Sociedad Española de Investigación sobre Cannabinoides

Pamplona, 24 a 26 de noviembre de 2011

## Programa científico

### Jueves, 24 de noviembre

- 12:00-14:00** **Entrega de documentación** (Campus de Ciencias de la Universidad de Navarra, Salón de Actos del Edificio de Bibliotecas)
- 14:00-15:30** **Comida**
- 15:30-16:00** **Inauguración**
- Fernando de la Puente, Director de I+D de la Universidad de Navarra
  - Fermín Castiella, Exdirector del Programa Foral de Drogodependencias, Gobierno de Navarra
  - Rafael Franco, Presidente del Comité Organizador
  - Javier Fernández-Ruiz, Presidente de la SEIC
- 16:00-17:00** **Conferencia Inaugural** (presentada por Manuel Guzmán)  
Beat Lutz, Universidad de Maguncia, Alemania:  
"Cannabinoid CB<sub>1</sub> receptor functions in periphery-brain circuits"
- 17:00-18:30** **Mesa Redonda 1:** "Avances en el desarrollo de medicamentos cannabinoides" (moderadores: Julián Romero y Rafael Franco)
- Vanesa Fernández, Echo Pharmaceuticals
  - María Luz Bellido, VivaCell Biotechnology
  - Guillermo Velasco, Universidad Complutense de Madrid
  - Javier Fernández-Ruiz, Universidad Complutense de Madrid
- 18:30-19:00** **Café**
- 19:00-21:00** **1<sup>a</sup> Sesión de comunicaciones orales**  
"Sistema endocannabinoide: nuevos compuestos, localizaciones y receptores"  
(moderadores: María Gómez-Ruiz y María Javier Ramírez)

- 19:00 Presentación (María Gómez-Ruiz)
- 19:15 O-1.1  
CHARACTERIZATION OF SELECTIVE CB<sub>2</sub> RECEPTOR LIGANDS BASED ON DIARYL-PYRAZOLE AND TRIAZOLE STRUCTURES. M. Gómez-Cañas, P. Morales, L. Hernández-Folgado, M. Gómez-Ruiz, N. Jagerovic, J. Fernández-Ruiz, P. Goya
- 19:30 O-1.2  
CHROMENOPYRAZOLES: NON-PSYCHOACTIVE AND SELECTIVE CB<sub>1</sub> CANNABINOID AGONISTS WITH PERIPHERAL ANTINOCICEPTIVE PROPERTIES. P. Morales, L. Hernández-Folgado, R. Girón, E. Sánchez, J. Cumella, D.P. Hurst, M. Gómez-Cañas, M. Gómez-Ruiz, P. Goya, P.H. Reggio, M.I. Martin, J. Fernández-Ruiz, N. Jagerovic
- 19:45 O-1.3  
SYNTHESIS AND EVALUATION OF NOVEL CANNABINOID QUINONES AS PPAR-GAMMA LIGANDS. M.L. Bellido, F. Conde, C. Navarrete, A. González, P.M. Íñigo, L. Mestre, F. Carrillo, G. Appendino, C. Guaza, E. Muñoz
- 20:00 O-1.4  
ULTRASTRUCTURAL DISTRIBUTION OF THE CB<sub>1</sub> CANNABINOID RECEPTOR IN GABAERGIC AND CORTICAL AND SUBCORTICAL GLUTAMATERGIC TERMINALS IN THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS. L. Reguero, N. Puente, I. Elezgarai, J.L. Mendizabal-Zubiaga, M.J. Canduela, I. Buceta, A. Ramos, J. Suárez, F. Rodríguez De Fonseca, G. Marsicano, P. Grandes
- 20:15 O-1.5  
ANATOMICAL CHANGES IN PARALLEL FIBRE TERMINALS LACKING CB<sub>1</sub> IN ADULT RODENT CEREBELLAR CORTEX. I. Buceta, N. Puente, L. Reguero, J.L. Mendizabal-Zubiaga, M.J. Canduela, S. Gómez-Urquijo, G. Marsicano, P. Grandes, I. Elezgarai
- 20:15 O-1.6  
IMMUNOCYTOCHEMICAL LOCALIZATION OF CB<sub>1</sub> RECEPTORS IN HIPPOCAMPAL MITOCHONDRIA. N. Puente, G. Marsicano, P. Grandes
- 20:45 O-1.7  
A ROLE FOR GPR55 IN MULTISTAGE MOUSE SKIN CARCINOGENESIS. E. Pérez-Gómez, C. Andradas, M. Quintanilla, Juana M. Flores, J. Paramio, M. Guzmán, C. Sánchez

**Cena libre**

## Viernes, 25 de noviembre

- 9:00-10:00**     **2ª Sesión de comunicaciones orales**  
"Sistema endocannabinoide: efectos amnésicos y psiquiátricos"  
(moderadores: Andrés Ozaita y Marisol Aymerich)
- 9:00            Presentación (Andrés Ozaita)
- 9:15            O-2.1  
INVOLVEMENT OF PROTEIN KINASE C-GAMMA SIGNALING ON THE AMNESIC-LIKE EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL. M. Gomis-González, A. Busquets-Garcia, E. Puighermanal, A. Blázquez-Prunera, R. Maldonado, A. Ozaita
- 9:30            O-2.2  
QUANTIFICATION OF ENDOCANNABINOIDS IN POSTMORTEM HUMAN BRAIN OF SCHIZOPHRENIC SUBJECTS. C. Muguruza, M. Lehtonen, N. Aaltonen, J. Arrieta, C. Cubero, J.J. Meana, L.F. Callado
- 9:45            O-2.3  
RESIDUAL ATTENTIONAL DEFICIT CAUSED BY FREQUENT CONSUMPTION OF CANNABIS. L. Hernández-Bellido, J.L. Rubio-Gómez, O. López-Guarnido, E. Gómez-Milán, M. Ruiz-Veguilla
- 10:00-12:30**     **Café y sesión de pósters**
- 12:30-14:00**     **Mesa Redonda 2: "Aspectos psiquiátricos del consumo de cannabis"**  
(moderadores: Koldo Callado e Ismael Galve-Roperh)
- Ignacio Mata Pastor, Fundación Argibide de Pamplona
  - Ana González Pinto, Hospital Santiago Apóstol de Vitoria
  - Luis Alfonso Núñez Domínguez, Centro Médico de Pamplona
- 14:00-15:30**     **Comida**
- 15:30-18:00**     **3ª Sesión de comunicaciones orales**  
"Sistema endocannabinoide: efectos neuroprotectores y neuroproliferativos"  
(moderadores: Ester Aso y José Luís Lanciego)
- 15:30            Presentación (Ester Aso)
- 15:45            O-3.1  
THE CB<sub>1</sub> ANTAGONIST/CB<sub>2</sub> AGONIST URB447 CONFERS NEUROPROTECTION AFTER NEONATAL HYPOXIA-ISCHEMIA. D. Alonso-Alconada, S. Carloni, A. Alvarez, S. Girelli, A. Duranti, A. Tontini, O. Arteaga, I. Lara, D. Piomelli, W. Balduini, E. Hilario
- 16:00            O-3.2  
CANNABIDIOL INDUCES SUSTAINED MODULATION OF EXCITOTOXICITY AND OXIDATIVE STRESS IN IMMATURE BRAIN AFTER HYPOXIA-ISCHEMIA. N. Mohamed, M.R. Pazos, A. Gómez, R. Layunta, M. Ceprián,

H. Lafuente, M. Santos, F.J. Alvarez, J. Fernández-Ruiz, J. Martínez-Orgado

16:15 O-3.3  
INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM IN THE NEUROPROTECTIVE EFFECTS OF MINOCYCLINE AFTER TRAUMATIC BRAIN INJURY IN MICE. A.B. López-Rodríguez, E. Siopi, D.M. Kerr, G.K. Ford, C. Marchand-Leroux, D.P. Finn, M. Jafarian-Tehrani, M.P. Viveros

16:30 O-3.4  
PRECLINICAL EVALUATION OF SATIVEX® AS A DISEASE-MODIFYING AGENT IN HUNTINGTON'S DISEASE. S. Valdeolivas, O. Sagredo, V. Satta, J.A. Ramos, R.G. Pertwee, J. Fernández-Ruiz

16:45 O-3.5  
CB<sub>1</sub> CANNABINOID RECEPTORS LOCATED ON GLUTAMATERGIC TERMINALS CONFER NEUROPROTECTION IN HUNTINGTON'S DISEASE. A. Chiarlone, C. Blázquez, E. Resel, L. Bellocchio, J.J. Ferrero, E. Soria-Gómez, O. Sagredo, C. Benito, J.M. Flores, M. Sendtner, J. Romero, J. Sánchez-Prieto, B. Lutz, J. Fernández-Ruiz, G. Marsicano, I Galve-Roperh, M. Guzmán

17:00 O-3.6  
ENDOCANNABINOID SYSTEM BLOCKADE AS A NOVEL APPROACH TO TREAT FRAGIL X SYNDROME. A. Busquets-Garcia, M. Gomis-González, C. Agustín-Pavón, A. Pastor, R. De La Torre, M. Dierssen, R. Maldonado, A. Ozaita

17:15 O-3.7  
ENDOCANNABINOID-MEDIATED NEUROPROTECTION IN A MPTP MICE MODEL OF PARKINSON'S DISEASE. D. Fernández-Suárez, M. Celorrio, R. Franco, M. Aymerich

17:30 O-3.8  
CORRELATION BETWEEN CB<sub>2</sub> RECEPTOR EXPRESSION AND A-BETA LEVELS AND SENILE PLAQUE SCORES IN ALZHEIMER'S DISEASE FRONTAL CORTEX. M. Solas, M. J. Ramírez, R. Franco

17:45 O-3.9  
ROLE OF CB<sub>1</sub> CANNABINOID RECEPTORS IN THE CONTROL OF CEREBELLAR FUNCTION THROUGH THE MICROGLIAL ACTIVATION. L. Cutando, E. Puighermanal, M. Gomis-González, J.M. Delgado-García, A. Gruart, R. Maldonado, A. Ozaita

**18:00-19:00** **Café y sesión de pósters**

**19:00-20:30** **Asamblea de la SEIC**

**21:30** **Cena del congreso**

## Sábado, 26 de noviembre

- 9:00**                    **Presentación del Premio a la Mejor Publicación 2011** (presentado por Carmen Guaza)
- 10:00-11:15**        **3ª Sesión de comunicaciones orales (continuación)**
- 10:00                    O-3.10  
CANNABIDIOL PROTECTION OF OLIGODENDROCYTE PROGENITOR CELLS INVOLVES THE ATTENUATION OF ENDOPLASMIC RETICULUM STRESS. M. Mecha, A. Torrao, L. Mestre, F.J. Carrillo, P. Iñigo, R. Mechoulam, C. Guaza
- 10:15                    O-3.11  
HIGH EXPRESSION OF CANNABINOID RECEPTOR CB<sub>1</sub> DEFINES A NEW SUBTYPE OF CELLS IN THE SPINAL CORD EPENDYMA. D. Garcia-Ovejero, A. Arévalo-Martin, B. Paniagua-Torija, Y. Sierra-Palomares, E. Molina-Holgado
- 10:30                    O-3.12  
THE ENDOCANNABINOID SYSTEM MODULATES PROLIFERATION AND MIGRATION OF OLIGODENDROCYTE PROGENITOR CELLS. O. Gómez, M.A. Sanchez-Rodriguez, E. Molina-Holgado
- 10:45                    O-3.13  
CB<sub>2</sub> CANNABINOID RECEPTORS PROMOTE NEURAL PROGENITOR CELL PROLIFERATION VIA mTORC1 SIGNALING. J. Palazuelos, Z. Ortega, J. Díaz-Alonso, M. Guzmán, I. Galve-Roperh
- 11:15-12:00**        **Café**
- 12:00-13:00**        **Entrega de Premios a las Mejores Comunicaciones**  
**Clausura**

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ANALYZING THE ROLE OF CB<sub>2</sub> RECEPTORS DURING PRIMARY DEMYELINATION AND REMYELINATION. A. Bernal-Chico, F. Pérez-Cerdá, C. Matute, S. Mato

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INTRA-HIPPOCAMPAL INJECTION OF CANNABIDIOL INDUCES ANTIDEPRESSANT-LIKE EFFECT IN THE RAT FORCED SWIMMING TEST. C. Biojone, M. Silva, P.C. Casarotto, F.A. Moreira, F.S. Guimarães, S.R. Joca

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ULTRASTRUCTURAL LOCALIZATION OF THE CB<sub>1</sub> CANNABINOID RECEPTOR IN HIPPOCAMPAL ASTROGLIA. M.J. Canduela, J.L. Mendizabal-Zubiaga, L. Reguero, N. Puente, I. Buceta, I. Elezgarai, J. Suárez, F. Rodríguez de Fonseca, G. Marsicano, P. Grandes

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ADMINISTRATION OF THE CB<sub>2</sub> AGONIST HU308 AFTER BRAIN HYPOXIA-ISCHEMIA IS NEUROPROTECTIVE IN NEWBORN RATS. M. Ceprián, M.R. Pazos, A. Gómez, N. Mohammed, R. Layunta, M. Santos, R. Mechoulam, J. Martínez-Orgado

### P.6

CIRCADIAN RHYTHM AND CB<sub>1</sub> CANNABINOID RECEPTOR EXPRESSION IN THE PREFRONTAL CORTEX AND RELATED LIMBIC AREAS OF OBESE ZUCKER RATS. L. Echeazarra, S. Barrondo, G. García del Caño, I. González Burguera, M. López de Jesús, J. Sallés

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CHANGES ON METABOLIC PARAMETERS INDUCED BY ACUTE CANNABINOID ADMINISTRATION (CBD, THC) IN A RAT EXPERIMENTAL MODEL OF NUTRITIONAL VITAMIN A DEFICIENCY. L. El Amrani, J.M. Porres, A. Merzouki, A. Louktibi, P. Aranda, M.L. Jurado, G. Urbano

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IDENTIFICATION OF CB<sub>2</sub> RECEPTORS IN THE BRAIN OF PATIENTS AND EXPERIMENTAL MODELS OF PARKINSON'S DISEASE. M.C. García, V. Cinquina, C. Palomo-Garo, M. Moreno-Martet, J.A. Ramos, J. Fernández-Ruiz

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5HT<sub>1A</sub> RECEPTORS ARE INVOLVED IN CANNABIDIOL-INDUCED PROTECTION OF NEWBORN PIG BRAIN AFTER HYPOXIC-ISCHEMIC INSULTS. A. Gómez, M.R. Pazos, R. Layunta, N. Mohammed, M. Ceprián, M. Santos, F. Tendillo, J. Fernández-Ruiz J, J. Martínez-Orgado

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MICE LACKING THE PPAR-ALPHA GENE PRESENT REDUCED NUMBER OF DOPAMINE NEURONS IN THE SUBSTANTIA NIGRA WITHOUT ALTERING MOTOR BEHAVIOR OR DOPAMINE NEURON DECLINE OVER LIFE. R. González-Aparicio, J.A. Flores, E. Fernández-Espejo

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SEXUALLY DIMORPHIC EFFECTS OF MATERNAL DEPRIVATION AND/OR JUVENILE CANNABINOID EXPOSURE ON ADULT RATS: A BEHAVIOURAL, ENDOCRINE AND IMMUNOHISTOCHEMICAL STUDY. A. Llorente-Berzal, M. López-Gallardo, A.B. López-Rodríguez, D. Rotllant, K. Mackie, A. Armario, R. Nadal, M.P. Viveros

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CONSUMPTION OF CANNABIS IN A SAMPLE OF YOUNG PEOPLE FROM ANDALUSIA (SPAIN): DIFFERENTIAL VISION IN FUNCTION OF GENDER. O. López-Guarnido, L. Hernández-Bellido, H.C. Cataño, M. Ruiz-Veguilla

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PERIPHERAL MECHANISMS INVOLVED IN THE CONTROL OF MUSCULAR PAIN. M.I. Martín, A. Bagües, E. Sánchez

**P.14**

NEW APPROACHES FOR THE STUDY OF GPCR SIGNALLING PATHWAYS. E. Martínez\*, A. Ricobaraza\*, M. Zamarbide, J. Oyarzabal, R. Franco

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EFFECTS OF CHRONIC ADMINISTRATION OF CANNABINOID RECEPTOR INVERSE AGONIST (AM 251) ON THE FOOD INTAKE, BODY WEIGHT GAIN AND OXYGEN CONSUMPTION IN OBESE ZUCKER RATS. I. Merroun, J.M. Porres, G. Urbano, P. Aranda, J. Llopis, M. Errami, M. López-Jurado

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AN ULTRA-LOW DOSE OF RIMONABANT REDUCES GHRELIN-INDUCED OREXIGENIC ACTIONS. L. Orio, F. Alen, M.T. Ramírez-López, F.R. de Fonseca, R. Gómez de Heras

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IMMUNOLocalIZATION OF VARIOUS COMPONENTS OF THE ENDOCANNABINOID SYSTEM IN THE CEREBELLUM OF PATIENTS WITH SPINOCEREBELLAR ATAXIAS. C. Rodríguez-Cueto, C. Benito, F. Espejo-Porras, E. de Lago, J. Romero, J. Fernández-Ruiz, M.L. Hernández-Gálvez, M. Gómez-Ruiz

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THE ABSENCE OF A FUNCTIONAL PPAR-ALPHA GENE EXACERBATES NEUROPATHIC AND VISCERAL PAIN IN FEMALE MICE. J. Ruiz-Medina, J.A. Flores, I. Tasset, I. Tunez, O. Valverde, E. Fernández Espejo

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PARTICIPATION OF CYCLOOXYGENASE-2 IN THE TRANSFORMATION OF 2-ARACHIDONOYL-GLYCEROL TO PROSTAGLANDIN-GLYCERYL ESTERS IN EXPERIMENTAL MODELS OF HUNTINGTON'S DISEASE. O. Sagredo, R. Pazos, T. Bisogno, S. Valdeolivas, F. Piscitelli, S. Petrosino, V. Di Marzo, J. Fernández-Ruiz

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CB<sub>1</sub> CANNABINOID RECEPTOR-DRIVEN PRONEUROGENIC SIGNALLING CONTROLS CORTICOSPINAL NEURON DEVELOPMENT AND FUNCTION. J. Díaz-Alonso, T. Aguado, A. de Salas, C.S. Wu, J. Palazuelos, H.C. Lu, C. Hofmann, B. Lutz, M. Guzmán, I. Galve-Roperh

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EFFECT OF OPIOID AND CANNABINOID DRUGS IN TWO MODELS OF MUSCLE PAIN IN RATS. E. Sánchez, A. Bagües, M.I. Martín

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CO-EXPRESSION OF mRNAs CODING ENDOCANNABINOID RECEPTORS 1 AND 2 IN THE MONKEY BASAL GANGLIA OUTPUT NEURONS. S. Sierra, A.J. Rico, N. Luquin, V. Gómez-Bautista, E. Roda, R. Franco, J.L. Lanciego

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EXPRESSION OF CB<sub>2</sub> RECEPTOR AND MU-OPIOID RECEPTOR ON IMMUNE CELLS AFTER MORPHINE SELF-ADMINISTRATION IN LEWIS AND FISHER344 RATS. M. Ucha, S.M. Coria, A. Higuera-Matas, M.A. Assis, D. Roura

## CONFERENCIA INAUGURAL

### CANNABINOID CB<sub>1</sub> RECEPTOR FUNCTIONS IN PERIPHERY-BRAIN CIRCUITS

Beat Lutz

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The endocannabinoid system (ECS) constitutes a wide-spread signaling system in the organism, acting in a paracrine and autocrine manner. Insights into the “logic” of the ECS have greatly progressed in the recent years, by extensively using pharmacological and genetic tools in mice. The functional analysis of the CB<sub>1</sub> receptor in the context of the entire animal has been the focus of our research. While this receptor was originally annotated as the “brain-type” cannabinoid receptor, it has become evident during the recent few years that the CB<sub>1</sub> receptor comprises roles not only in the central nervous system, but also in a large variety of peripheral organs, including sympathetic neurons, adipocytes, liver and muscles. Using conditional mutagenesis in mice, cell-type and organ-specific CB<sub>1</sub> receptor deletions have been generated and analyzed in a variety of physiological and pathophysiological processes. Caused by the wide distribution of CB<sub>1</sub> receptors, it is not surprising that this receptor is involved in many different processes in various organ systems. While it is relevant and interesting to evaluate the functional importance of CB<sub>1</sub> receptors in a particular cell type, such a specific gene inactivation will consequently also influence other cell types and organ systems. Thus, it is important to understand these interactions and to integrate CB<sub>1</sub> receptor functions into the context of an intricate network of organ interactions. This will be exemplified with CB<sub>1</sub> receptor mutants with restricted mutations in CNS, adipocytes, and sympathetic neurons/adrenals, regarding metabolism, energy balance, and behaviour.

**Notes:**

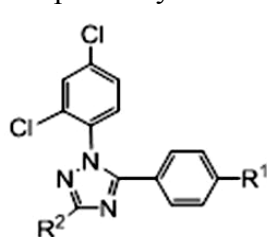
## Oral 1.1

### CHARACTERIZATION OF SELECTIVE CB<sub>2</sub> RECEPTOR LIGANDS BASED ON DIARYL-PYRAZOLE AND TRIAZOLE STRUCTURES

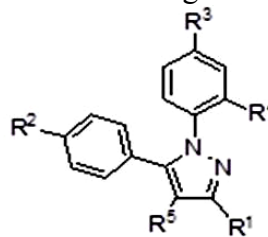
María Gómez-Cañas<sup>1-3</sup>, Paula Morales<sup>4</sup>, Laura Hernández-Folgado<sup>4</sup>, María Gómez-Ruiz<sup>1-3</sup>, Nadine Jagerovic<sup>4</sup>, Javier Fernández-Ruiz<sup>1-3</sup> and Pilar Goya<sup>4</sup>

<sup>1</sup>Departamento de Bioquímica y Biología Molecular, Instituto Universitario de Investigación en Neuroquímica, <sup>2</sup>Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), <sup>3</sup>Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Facultad de Medicina, Universidad Complutense, 28040-Madrid, Spain. <sup>4</sup>Instituto de Química Médica, CSIC, Juan de la Cierva 3, 28006-Madrid, Spain

An alternative way to avoid the unwanted psychoactive effects (mediated by CB<sub>1</sub> receptors) of cannabinoid agonists, when used for therapies, is to target the CB<sub>2</sub> receptor selectively. This receptor type has a more restricted distribution in the body than the CB<sub>1</sub> receptor, which is particularly notorious in the CNS. Ligands that selectively target this receptor may be used for their anti-inflammatory, anti-tumoral and neuroprotective properties (agonists) in various neurological disorders, and also as an experimental tool (antagonists) to identify physiological processes that are mediated by this receptor. Several selective CB<sub>2</sub> receptor ligands have been developed so far including agonists (i.e. HU-308, JWH-133, AM1421) or antagonists (i.e. SR144528, AM630). We are presently working with the following chemical structures:



Triazole structure



Diarylpyrazole structure

These structures are being used as a template to develop selective ligands that, once characterized, may be used to either activate or block this receptor. After the design, synthesis and characterization of numerous compounds derived from these key structures, we reached four compounds with affinity in the nanomolar range (a couple of them, in the low nanomolar range) for the CB<sub>2</sub> receptor and negligible binding at the CB<sub>1</sub> receptor:

Compound	LH29a	MA46	CFF5.31B	CFF5.59
Ki for CB <sub>1</sub>	>40 μM	>40 μM	>40 μM	>40 μM
Ki for CB <sub>2</sub>	149.8 nM	458.2 nM	17 nM	65 nM
Chemical structure				

We are presently studying the functional activity of these ligands as CB<sub>2</sub> receptor agonists, antagonists or inverse agonists using GTPγS binding analyses, and we plan to develop *in vitro* studies of their ability to regulate glial-mediated influences on neuronal survival.

***Acknowledgments:*** *These studies were supported by MICINN (SAF2009-12422-C02-02 and SAF2009-11847), RETICS (RTA RD06/001/0014) and Comunidad de Madrid (S-SAL-0261-2006). Authors are indebted to Yolanda García-Movellán for administrative support.*

**Notes:**

## Oral 1.2

### CHROMENOPYRAZOLES: NON-PSYCHOACTIVE AND SELECTIVE CB<sub>1</sub> CANNABINOID AGONISTS WITH PERIPHERAL ANTINOCICEPTIVE PROPERTIES

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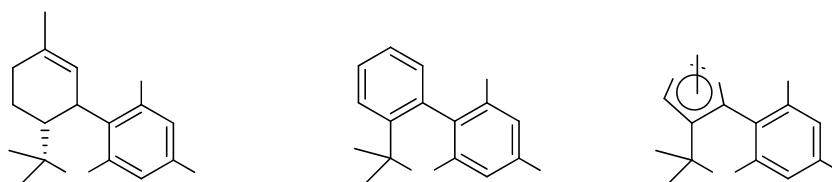
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The unwanted psychoactive effects of cannabinoid receptor agonists have limited their development as medicine. These CB<sub>1</sub> mediated side effects are due to the fact that CB<sub>1</sub> receptors are largely expressed in the central nervous system. Since it is known that CB<sub>1</sub> receptors are also located peripherally, there is a growing interest in targeting cannabinoid receptors located outside the brain.

A library of chromenopyrazoles designed in analogy to the classical cannabinoid cannabinal were synthesized, characterized and tested for cannabinoid activity. Within the chromenopyrazoles series, some of them showed to be selective CB<sub>1</sub> ligands in radiolabeled binding assays. The functional activities of selected chromenopyrazoles were evaluated in isolated tissues. Behavioral tests, *in vivo*, were then carried on the most effective CB<sub>1</sub> cannabinoid agonist. This chromenopyrazole did not induce modifications in any of the tested parameters on the mouse cannabinoid tetrad, discarding psychoactive effects. This lack of agonistic activity in the central nervous system suggests that it does not readily cross the blood-brain barrier. Moreover, the selected chromenopyrazole can induce antinociception in a peripheral model of orofacial pain in rat mediated through peripheral mechanisms.



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**Notes:**

## Oral 1.3

### SYNTHESIS AND EVALUATION OF NOVEL CANNABINOID QUINONES AS PPAR $\gamma$ LIGANDS

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Peroxisome proliferator-activated receptor-gamma (PPAR $\gamma$ ) has already been considered as an attractive molecular target for the treatment of neuroinflammatory disorders. Plant-derived cannabinoids such as  $\Delta$ -9-tetrahydrocannabinol (THC) and Cannabidiol (CBD), synthetic cannabinoids such as ajulemic acid, HU221 and WIN55212-2, and endocannabinoids such as anandamide, 2-arachidonoylglycerol and N-arachidonoyl-dopamine are endowed with anti-inflammatory properties mediated by PPAR $\gamma$ .

Using a fluorescence polarization-binding assay we have screened the PPAR $\gamma$  ligand activity of a library of natural phytocannabinoids including THC, CBD, CBG, CBDV, CBC, THCV, CNB and abnormal CBD and the CBD quinone derivative HU-331. Among the natural cannabinoids CBN, abnormal CBD and CBG showed the higher potency as PPAR $\gamma$  ligands. Interestingly HU-331 was at least 5 fold more potent than CBD indicating that the quinone pharmacophore enhances the PPAR $\gamma$  agonistic activity of natural phytocannabinoids. Inspired by this finding we have developed a series of new cannabinoid quinones derived from CBD (VCE-004) and CBG (VCE-003) that are potent PPAR $\gamma$  agonists. In contrast to HU-331 the new CBD and CBG quinones did not show cytotoxic activity against a panel of different cancer cell lines (AGS, HeLa, MDA-MB-231, T47D and HTC-116). In addition, VCE-003 protects neuronal cells from glutamate-induced cytotoxicity. Functional assay indicates that cannabinoid quinones activate the transcriptional activity of human PPAR $\gamma$  at pharmacological concentrations, showing that VCE-003 and VCE-004 induce adipocyte differentiation and modulate the expression of PPAR $\gamma$ -dependent genes, such as aP2, IL-6, resistin or adipoQ.

PPAR $\gamma$  agonists have been reported to be potential therapeutic targets in multiple sclerosis (MS). Here, we investigate the activity of VCE-003 in the murine model of MS induced by Theiler's virus (TMEV) infection. VCE-003 treatment (5 mg/Kg) clearly ameliorated the symptoms associated to the disease, decreased microglia reactivity and the expression of VCAM-1 in the spinal cord of treated animals. The changes in the expression of multiple genes associated with MS were investigated by qRT-PCR arrays in the brain and spinal cord. We have found that TMEV infection induced a different pattern of inflammation in both tissues, and some proinflammatory markers as well as key enzymes in oligodendrocyte function were significantly modified in VCE-003-treated animals.

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**Notes:**

## Oral 1.4

### ULTRASTRUCTURAL DISTRIBUTION OF THE CB<sub>1</sub> CANNABINOID RECEPTOR IN GABAERGIC AND CORTICAL AND SUBCORTICAL GLUTAMATERGIC TERMINALS IN THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS

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CB<sub>1</sub> cannabinoid receptors are enriched in the hypothalamus, particularly in the ventromedial nucleus (VMH), which participates in homeostatic functions including food intake. Although it is well established that CB<sub>1</sub> activation modulates excitatory and inhibitory synaptic transmission in the brain, CB<sub>1</sub> contribution to the molecular architecture of the excitatory and inhibitory synaptic terminals in the VMH is not known. Therefore, the aim of this study was to investigate the precise subcellular distribution of CB<sub>1</sub> in the VMH. Light and electron microscopy techniques were used as well as *CB<sub>1</sub>-WT*, *CB<sub>1</sub>-KO* and conditional mutant mice bearing a selective deletion of CB<sub>1</sub> in cortical glutamatergic (*Glu-CB<sub>1</sub>-KO*) or GABAergic neurons (*GABA-CB<sub>1</sub>-KO*).

At light microscopy, CB<sub>1</sub> was distributed in the VMH of *CB<sub>1</sub>-WT* and *Glu-CB<sub>1</sub>-KO*, being remarkably reduced in *GABA-CB<sub>1</sub>-KO*, particularly in the dorsomedial part, suggesting the presence of CB<sub>1</sub> in GABAergic profiles. The immunolabeling fully disappeared in *CB<sub>1</sub>-KO*. In the electron microscope, CB<sub>1</sub> was localized on preterminals/synaptic terminals making excitatory and inhibitory synapses. There were not significant differences in the percentage of CB<sub>1</sub> positive profiles and CB<sub>1</sub> density in terminals making asymmetric or symmetric synapses in *CB<sub>1</sub>-WT* mice. To define the contribution of cortical glutamatergic and GABAergic terminals to the intrinsic CB<sub>1</sub> pattern in the VMH, *Glu-CB<sub>1</sub>-KO* and *GABA-CB<sub>1</sub>-KO* mice were used. CB<sub>1</sub> was still observed in VMH terminals of both mutant strains. The statistical analysis revealed that the proportion of CB<sub>1</sub> positive terminals/preterminals in *CB<sub>1</sub>-WT* (20.4%) and *Glu-CB<sub>1</sub>-KO* (20.8%) was reduced in *GABA-CB<sub>1</sub>-KO* (12.4%). CB<sub>1</sub> density was similar (~0.5 immunoparticles/μm) in all animal conditions. Finally, CB<sub>1</sub> labeled boutons making clear asymmetric synapses slightly decreased in *Glu-CB<sub>1</sub>-KO* relative to *CB<sub>1</sub>-WT*, indicating that CB<sub>1</sub> was also distributed in cortical and subcortical excitatory synaptic terminals.

These anatomical data support the VMH as a hub candidate in the endocannabinoid-mediated modulation of the excitatory and inhibitory neurotransmission of cortical and subcortical pathways regulating essential hypothalamic functions such as feeding behavior.

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**Notes:**

## Oral 1.5

### ANATOMICAL CHANGES IN PARALLEL FIBRE TERMINALS LACKING CB<sub>1</sub> IN ADULT RODENT CEREBELLAR CORTEX

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We showed in our previous investigation that the topography of type-1 cannabinoid receptor (CB<sub>1</sub>) in the cerebellar granule cell axons, the parallel fibres, changes from the axon to the synaptic terminal from P5 to adult. These results suggest the implication of CB<sub>1</sub> in the maturation processes that take place in the cerebellar parallel fibres during the postnatal development. If this is true, the presence of CB<sub>1</sub> may also have an impact on the typical anatomical features of the granule cell axons in the cerebellar molecular layer. This hypothesis led us to analyze the morphology of the parallel fibre terminals in the absence of CB<sub>1</sub>. For this purpose, cerebellar vermis obtained from adult *CB<sub>1</sub>-WT* and *CB<sub>1</sub>-KO* mice were investigated by electron microscopy.

We first measured the area of the parallel fibre terminals (PFT) in the vermal spinocerebellar lobe 5 and in the vestibulocerebellar lobe 10 of *CB<sub>1</sub>-WT* and *CB<sub>1</sub>-KO* mice. The results indicated that parallel fibre terminals lacking CB<sub>1</sub> are around 30% larger than in *CB<sub>1</sub>-WT* (difference statistically significant;  $p < 0,001$ ). Furthermore, the density of the synaptic vesicles was analyzed. Parallel fibre terminals of *CB<sub>1</sub>-KO* mice contain 20% in lobe 5 and 13% in lobe 10 lower density of vesicles than in *CB<sub>1</sub>-WT*. Thus, the relationship between the area and the number of vesicles of the parallel fibre terminals is inversely proportional. Moreover, we studied the number of synapses that the PFT form with the Purkinje cell dendritic spines. The results pointed out that approximately 95% of these terminals make synapses only once in both animals. To assure that the ultrastructural changes occur specifically at the PFT we measured the area of the postsynaptic Purkinje cell dendritic spines. The results showed that this area remains unchanged.

Taken together, besides the functional deficiency of synaptic plasticity observed at the parallel fibre-Purkinje cell synapse in mice lacking selectively CB<sub>1</sub> in cerebellar granule cells (Carey et al., 2010), our findings strongly suggest that the absence of CB<sub>1</sub> also impairs the acquisition of at least some of the ultrastructural features of the parallel fibre terminals.

Funding: Dr. Pedro Grandes' laboratory is supported by GIC07/70-IT-432-07, SAF2009-07065 and RETICS RD07/0001/2001 (M.J. Canduela). L. Reguero is in receipt of a Predoctoral Fellowship from the Basque Country Government (BFI 07.286). I. Buceta is supported by a Predoctoral Fellowship from the Basque Country University.

**Notes:**

## Oral 1.6

### IMMUNOCYTOCHEMICAL LOCALIZATION OF CB<sub>1</sub> RECEPTORS IN HIPPOCAMPAL MITOCHONDRIA

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Type-1 cannabinoid (CB<sub>1</sub>) receptors are G protein-coupled receptors (GPCRs) highly enriched on neuronal plasma membranes, where they tightly control neuronal activity, metabolism and functions. Some studies suggested that  $\Delta^9$ -tetrahydrocannabinol could affect mitochondrial functions and also that CB<sub>1</sub> receptor signaling regulates mitochondrial biogenesis in peripheral tissues. The neuroanatomical observation that CB<sub>1</sub> immunogold particles are often detected on neuronal mitochondria in the electron microscope, prompted us to investigate the specificity of this staining by using mutant mice constitutively lacking the CB<sub>1</sub> receptor (*CB<sub>1</sub>-KO* mice). By immunogold electron microscopy, CB<sub>1</sub> receptor immunoreactivity was detected on mitochondrial membranes (mtCB<sub>1</sub>) of CA1 hippocampal neurons of wild-type, but not of *CB<sub>1</sub>-KO* mice. We observed that 28.4%±2.5% of CA1 mitochondria contain the receptor in wild-type animals, whereas only 2.9%±0.7% of CA1 mitochondria in *CB<sub>1</sub>-KO* mice show non-specific background staining. Mitochondria in axon terminals or dendrites displayed similar proportions of mtCB<sub>1</sub> receptor expression. The density of mtCB<sub>1</sub> immunogold staining was of 18.3±2.0 particles/μm<sup>2</sup> of mitochondria in wild-type mice and dropped to background values of 0.4±0.1 particles/μm<sup>2</sup> in *CB<sub>1</sub>-KO* mice. Comparing the number of mtCB<sub>1</sub> particles to the total amount of CB<sub>1</sub> labeling (mitochondrial and extra-mitochondrial) revealed that 15.5%±4.2% of neuronal CB<sub>1</sub> protein is localized in the organelle. Detailed inspection and semi-quantification of immunogold images revealed that approximately 95% of mtCB<sub>1</sub> is localized on the outer membrane of mitochondria.

We analyzed the anatomical distribution of mtCB<sub>1</sub> in the hippocampus of conditional CB<sub>1</sub> mutant mice, lacking the receptor either in cortical glutamatergic (*Glu-CB<sub>1</sub>-KO*) or in forebrain GABAergic neurons (*GABA-CB<sub>1</sub>-KO*), respectively. MtCB<sub>1</sub> was still present in CA1 hippocampal neurons of both mutant strains, with a similar reduction in the proportion of labeled mitochondria as compared to wild-type mice. In contrast, the density of mtCB<sub>1</sub> was reduced by 50.4% only in *GABA-CB<sub>1</sub>-KO*, but not in *Glu-CB<sub>1</sub>-KO* mice. No significant difference between these two genotypes was observed concerning the proportion of mtCB<sub>1</sub> versus total CB<sub>1</sub> expression. Thus, both neuronal populations contain mtCB<sub>1</sub> receptors, which are however more densely expressed in hippocampal GABAergic neurons as compared to glutamatergic ones.

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**Notes:**

## Oral 1.7

### A ROLE FOR GPR55 IN MULTISTAGE MOUSE SKIN CARCINOGENESIS

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Recent studies have shown a link between the putative cannabinoid receptor GPR55 and cancer. Specifically, it has been proposed that GPR55 modulates cancer cell proliferation and migration. To further understand the role of the receptor in cancer in general and in tumor initiation and progression in particular, we have used a classical mouse skin carcinogenesis model. The two-stage chemical protocol involves the treatment of mice with a single dose of a carcinogen [7,12-dimethylbenz(a)anthracene (DMBA)], followed by repeated applications of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). This treatment results in the outgrowth of highly differentiated benign papillomas. A small subset of papillomas eventually progresses to malignant squamous cell carcinomas (SCC), some of which undergo an epithelial–mesenchymal transition, generating aggressive and metastatic spindle cell carcinomas (SpCC).

Our results show that GPR55 is upregulated upon carcinogenic and proliferative stimuli. Thus, while GPR55 is hardly detected in normal mouse skin, the levels of the receptor are dramatically increased during chemically-induced skin carcinogenesis as well as after short-term TPA treatment. In line with these results, we observed that GPR55 is overexpressed in human skin tumors compared with the corresponding non-tumoral epithelium. GPR55 knock-out mice, unlike their corresponding wild type littermates, were resistant to TPA-induced epidermal hyperproliferation and dermal immune infiltration. Furthermore, GPR55 deficient mice showed reduced papilloma formation compared with their wildtype counterparts. Several *in vitro* approaches confirmed that GPR55 expression confers oncogenic properties on skin tumor cells.

Taken together, these findings suggest that GPR55 is required for tumor initiation in skin carcinomas.

**Notes:**

## Oral 2.1

### INVOLVEMENT OF PROTEIN KINASE C-GAMMA SIGNALING ON THE AMNESIC-LIKE EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL

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Delta9-tetrahydrocannabinol (THC), the main psychoactive component in *Cannabis sativa* preparations activates several intracellular signaling pathways in the mouse brain after acute administration, and some of these molecular events are related with its amnesic-like effect. A key step in the modulation of these signaling cascades involved in the memory deficit produced by cannabinoids is the activation of the glutamatergic N-methyl-D-aspartate receptors (NMDAR). Thus, pre-treatment of mice with the NMDAR antagonist MK801, prevents the memory deficit produced by cannabinoids in both short-term and long-term assays. We observed that the acute administration of THC (10 mg/kg, i.p.) promotes the phosphorylation of hippocampal protein kinase C (PKC) isoforms on their catalytic domain. This step was dependent of NMDAR activation since pre-treatment with MK801 significantly reduced the THC-mediated PKC phosphorylation. In this regard, THC administration also modulated the phosphorylation of hippocampal neurogranin, a PKC substrate abundantly expressed in brain regions involved in cognitive function. Neurogranin is the main postsynaptic protein regulating the availability of calmodulin in neurons by binding to calmodulin in the absence of calcium, and its phosphorylation is preferentially mediated by PKCgamma, a conventional PKC-type activated by  $Ca^{2+}$  and diacylglycerol. To assess the relevance of PKCgamma signaling on the amnesic-like effects of THC, we evaluated the cognitive effect produced by two doses of THC (3 and 10 mg/kg) in mice lacking this isoform of PKC (*Prkcc*<sup>-/-</sup>). For this purpose, we used two different memory tasks: the object recognition test and the context recognition test. *Prkcc*<sup>-/-</sup> mice performed both memory tests correctly showing no differences in comparison with wild-type mice. Interestingly, THC at the dose of 10 mg/kg showed amnesic-like effects, measured in the object recognition test, in control and *Prkcc*<sup>-/-</sup> mice, although this effect was less obvious in *Prkcc*<sup>-/-</sup> mice than in wild-type mice. Instead, the dose of THC 3 mg/kg did not produce amnesic-like effects in *Prkcc*<sup>-/-</sup> mice when measured in any of the behavioral tests. Thus, we hypothesize that signaling through PKCgamma plays an important role in the amnesic-like effects of THC as a downstream target of NMDAR activation after excitatory/inhibitory imbalance promoted by the cannabinoid agonist.

**Notes:**

## Oral 2.2

### QUANTIFICATION OF ENDOCANNABINOIDS IN *POSTMORTEM* HUMAN BRAIN OF SCHIZOPHRENIC SUBJECTS

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There is a wealth of evidence indicating the involvement of the endocannabinoid (EC) system in the pathophysiology of schizophrenia. Previous studies have described an increase in anandamide levels in the cerebrospinal fluid and in the blood of schizophrenic subjects. However, to our knowledge, there are no published data reporting EC levels in the brain tissue of schizophrenic subjects.

The aim of this study was to evaluate the EC levels in three *postmortem* brain regions of schizophrenic subjects and matched controls.

Human brain samples were collected from 19 patients with diagnosis of schizophrenia (DSM-IV), and 19 controls matched by age, gender and *postmortem* delay. After liquid-liquid extraction of the lipid fraction from homogenized brain samples, the ECs were determined by quantitative liquid chromatography with tandem mass spectrometric detection in cerebellum (CB), hippocampus (HC) and prefrontal cortex (PFC). This method was used to measure the levels of four ECs (2-arachidonoylglycerol (2-AG), arachidonylethanolamide (anandamide, AEA), dihomogamma-linolenylethanolamide (LEA), and docosahexaenylethanolamide (DHEA)) and two other cannabimimetic compounds (palmitoyl ethanolamide (PEA) and oleoyl ethanolamide (OEA)).

A significant increase in 2-AG levels was observed in the PFC of schizophrenic subjects compared to controls (+137±25%; p=0.009). The AEA and DHEA concentrations were significantly reduced in the HC of schizophrenic subjects compared to the control group (-34±8%; p=0.004 and -32±7%; p=0.004, respectively). There was also a significant decrease in DHEA levels in the CB of schizophrenic subjects compared to matched controls (-40±6%; p=0.001). No statistically significant differences were found in the levels of LEA, PEA and OEA between schizophrenic and control subjects in any of the studied brain areas.

The present results demonstrate that some ECs are specifically altered in some areas of the brain of schizophrenic subjects. Moreover, these data provide further evidence that the EC system may be involved in the pathophysiology of schizophrenia.

**Notes:**

## Oral 2.3

### RESIDUAL ATTENTIONAL DEFICIT CAUSED BY FREQUENT CONSUMPTION OF CANNABIS

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**Objectives:** To examine if there is residual attention deficit (after 12 hours of abstinence) and long term attention deficit (after more than one year of abstinence) associated with prolonged use of cannabis.

**Material and methods:** Subject recruitment was performed using the “snowball” method. In order to measure attention performance we used the *Attention Network Test* (ANT), which allows measuring independently three different attention components: alerting, orienting and executive control.

Two criteria were taken into account in order to divide the subject in four groups: Time of abstinence and frequency of cannabis usage.

- 1) Former users with less than daily consumption (N=6). More than a year of abstinence.
- 2) Current users with less than daily consumption (N=8). More than 12 hours of abstinence.
- 3) Current users with daily consumption (N=8). More than 12 hours of abstinence.
- 4) Former users with daily consumption (N=8). More than a year of abstinence.

**Results:** No significant differences in the percentage of correct answers were found. There was a group main effect for the average of the reaction time of all the trials,  $F(3,26)=4,79$ ;  $p<.008$ . There were also group main effects for the reaction times regarding the three attention components: alerting, orienting and executive control. These results translate into slower performance for the group of current daily users when compared with the other groups.

**Conclusions:** It seems that the effects in attention caused by the “cannabis hangover” are evident even 12 hours after the last consumption, although there does not seem to persist any deterioration after more than a year of abstinence. Nevertheless, due to the reduced sample size and other factors that may affect the results (such as the concomitant usage of other psychoactive substances), it is necessary to continue exploring the possibility of long term deficits associated with the usage of cannabis.

**Notes:**

## Oral 3.1

### THE CB<sub>1</sub> ANTAGONIST/CB<sub>2</sub> AGONIST URB447 CONFERS NEUROPROTECTION AFTER NEONATAL HYPOXIA-ISCHEMIA

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**Introduction:** Since there is still no specific treatment for perinatal brain lesions due to the complexity of neonatal hypoxic-ischemic pathophysiology, interest has grown in the neuroprotective potential of cannabinoids after perinatal asphyxia. The aim of the present study was to evaluate the *time-dependent* neuroprotective effect of the first mixed CB<sub>1</sub> antagonist/CB<sub>2</sub> agonist URB447 in a neonatal model of hypoxia-ischemia.

**Methods:** PN(post-natal)7 Wistar rats were randomly assigned to four hypoxic-ischemic groups: pups with the left common carotid artery ligated and then asphyxiated for 2 hours with 8% O<sub>2</sub> (HI, n=10), three of the ischemic groups receiving a single i.p. injection of URB447 (1 mg/kg), 1 hour before starting the ischemic procedure (HI+URB447 1h-PRE, n = 10), 30 minutes (HI+URB447 30min-POST, n = 10) or 3 hours after hypoxia (HI+URB447 3h-POST, n = 10). Pups without ischemia or asphyxia were used as controls (SHAM, n=8). Brain coronal sections were Nissl-stained to evaluate brain infarction and MBP-immunolabelled to determine oligodendroglial injury. Apoptosis was assessed by means of caspase-3 activation and the presence of TUNEL+ cells. One-factor ANOVA was performed and p<0.05 was considered statistically significant.

**Results:** URB447 led to a neuroprotective effect reducing brain infarction and apoptotic cell death in newborn rats when administered both before and after the end of the hypoxic-ischemic procedure. Moreover, URB447 restored MBP-immunostaining pattern, whose loss is considered a hallmark of white matter damage.

**Conclusion:** Our results suggest that treatment with URB447 strongly reduced HI-induced neurodegeneration in neonatal rats.

**Acknowledgements:** Grants from the Basque-Government (IT-287-07/GIC07/779) and Ministry of Health (FIS09/02326).

**Notes:**

## Oral 3.2

### CANNABIDIOL INDUCES SUSTAINED MODULATION OF EXCITOTOXICITY AND OXIDATIVE STRESS IN IMMATURE BRAIN AFTER HYPOXIA-ISCHEMIA

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**Background:** excitotoxicity (in particular, glutamate release) and oxidative stress are thought to be some of the more important factors involved in immature brain damage after hypoxia-ischemia (HI)

**Aim:** to evaluate the antiexcitotoxic and antioxidant effects of CBD in HI immature brain using two different experimental paradigms.

#### Methods:

- *Paradigm 1: short-term study ex vivo after global HI:* sedated and ventilated newborn pigs (1-2 day-old) underwent HI damage by adding hypoxia (FiO<sub>2</sub> 10%) to brain ischemia by bilateral carotid artery compression for 30 min. Half an hour after HI piglets received i.v. vehicle (HV, n=8) or CBD 1 mg/kg (HC, n=9). Samples from frozen brain, obtained 6 h after HI, were studied by proton magnetic spectroscopy (H-MRS) obtaining the following ratios: Glu/NAA (excitotoxicity) and GSH/Cr (oxidative stress). Similarly studied animals without HI insult served as controls (SH, n=6).

- *Paradigm 1: medium-term study in vivo after focal HI:* unilateral HI brain damage was induced in newborn Wistar rats (7-10 day-old: P7-P10) by exposure to hypoxia (10% FiO<sub>2</sub>) for 120 min after left carotid artery electrocoagulation under anaesthesia. Ten minutes after the end of HI pups were treated s.c. with vehicle (HV, n=6) or CBD 1 mg/kg (HC, n=8). Other pups remained as controls (n= 7). Seven days after HI rats were studied by H-MRS to quantify Glu/NAA and GSH/Cr ratios in selected areas of interest in the ipsilateral (HI) and in the contralateral (non-HI) hemispheres.

#### Results:

- *Paradigm 1:* As observed 6 h after the insult, HI led to an increase of excitotoxicity as well as of the oxidative stress; these effects were effectively modulated by CBD administration (Glu/NAA: 0.5±0.02, 0.62±0.03 y 0.5±0.03 for SH, HV y HC, p<0.05; GSH/Cr: 0.17±0.005, 0.11±0.01 y 0.17±0.01 for SH, HV y HC, p<0.05).

- *Paradigm 2:* Seven days after HI, both excitotoxicity (increased Glu release) and oxidative stress (GSH consumption) were still increased in the rat HI hemisphere; administration of CBD after the HI modulated such increases, the effect being still evident 7 days after HI (Glu/NAA: 0.12±0.01, 0.22±0.01 y 0.15±0.01 for SH, HV y HC, p<0.05; GSH/Cr: 0.77±0.05, 0.67±0.05 y 0.77±0.05 for SH, HV y HC, p<0.05). Interestingly, in the contralateral hemisphere there was no increase of excitotoxicity but oxidative stress was increased similarly to that observed in the ipsilateral hemisphere; CBD was effective to prevent that increase to occur too (Glu/NAA: 0.13±0.01, 0.14±0.01 y 0.14±0.01 for SH, HV y HC, NS; GSH/Cr: 0.77±0.05, 0.57±0.05 y 0.85±0.05 for SH, HV y HC, p<0.05).

**Conclusions:** excitotoxicity is a phenomenon occurring in the HI brain tissue, whereas oxidative stress is a global phenomenon. Both conditions are prolonged several days after HI. CBD effectively modulates excitotoxicity and has proven to be a potent antioxidant in this scenario. CBD protective effects are non dependent on the mechanism of HI damage nor on the species involved, and are covering the HI as long as excitotoxicity and oxidative stress prevailed.

*Supported by grants FIS PS09/01900 and GWCRI091190-2*

## Notes:

## Oral 3.3

### INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM IN THE NEUROPROTECTIVE EFFECTS OF MINOCYCLINE AFTER TRAUMATIC BRAIN INJURY IN MICE

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Traumatic brain injury (TBI) and its consequences are the primary cause of death in young individuals. This type of lesion leads to an increase in extracellular calcium that triggers many cascades and mediates glial activation and the release of cytokines and reactive oxygen species. It also causes local and diffuse brain oedema, axonal injury and functional impairment. Since glial activation plays a key role in the development of the secondary damage following TBI, it seems likely that controlling (decreasing) this step could be beneficial and could lead to neuroprotective effects. Recent studies show that minocycline, a highly lipophilic semi-synthetic derivative of the antibiotic tetracycline, is able to suppress microglial activation, reduce the lesion volume and decrease the TBI-induced locomotor hyperactivity from 48h up to 3 months. It is well known that the endocannabinoid system (ECS) plays a very important role in mediating compensatory and reparation mechanisms under pathological situations. Thus, the ECS controls inflammation by acting on glial and endothelial cells and by modifying calcium currents, p38-MAPK activation, caspase-3 expression or nitric oxide production. Some of these mechanisms are shared with minocycline neuroprotective pathways although the specific mechanism by which this drug inhibits glial activation is not well known. On the basis of these premises, we hypothesized that the ECS could be involved in the neuroprotective effects of minocycline. To address this hypothesis and by using a TBI model in mice, we first defined the kinetic profile of brain endocannabinoid levels. Then, at the time of the maximum peak, we evaluated the effects of minocycline on the levels of endocannabinoids. Finally, we studied the possible involvement of CB1 and CB2 receptors in the effects of minocycline on oedema and neurological impairment. The results show a maximum peak of oleoylethanolamine (OEA) and palmitoylethanolamide (PEA) at 6h post-TBI that is notably decreased with minocycline treatment. Furthermore, the neuroprotective effect of minocycline on brain oedema and neurological impairment was blocked by the use of CB1 and CB2 receptor antagonists. The present results suggest that the ECS is involved in the neuroprotective effects of minocycline in a TBI model.

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**Notes:**

## Oral 3.4

### PRECLINICAL EVALUATION OF SATIVEX® AS A DISEASE-MODIFYING AGENT IN HUNTINGTON'S DISEASE

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Several cannabinoid agonists afford neuroprotection in experimental models of Huntington's disease (HD), although the type of compound (i.e. CB<sub>1</sub> agonist, CB<sub>2</sub> agonist, antioxidant cannabinoid) most effective depends on the pathological characteristic(s) that mainly operate in each experimental model used. For example, CB<sub>1</sub> receptors become down-regulated, even in presymptomatic phases, in HD and their activation induces positive effects mainly in excitotoxic models (i.e. quinolinate-lesioned mice). CB<sub>2</sub> receptors, however, are up-regulated, mainly in glial elements, so that their pharmacological activation limits glial-derived events that aggravate striatal damage in animal models priming local inflammatory episodes (malonate-lesioned rats) and also excitotoxicity. In addition, antioxidant cannabinoids like cannabidiol (CBD) are effective against oxidant injury of striatal neurons as recapitulated in 3-nitropropionate-lesioned rats. Lastly, combinations of these effects have been found in the transgenic models that best reproduce HD pathogenesis (i.e. R6/2 mice). Therefore, these observations support the idea that the type of cannabinoid compound(s) that may be useful for a disease-modifying therapy in HD patients should be a multi-targeting cannabinoid or a combination of different selective compounds. We have proposed that the cannabis-based medicine Sativex®, which is a combination of botanical extracts enriched with either Δ<sup>9</sup>-tetrahydrocannabinol (Δ<sup>9</sup>-THC) or CBD, may serve this purpose in HD. Our proposal is based on evidence that a Sativex®-like combination of Δ<sup>9</sup>-THC- and CBD-enriched botanical extracts attenuated cytotoxic events and preserved striatal neurons in the above models of HD in which striatal damage depends predominantly on a specific cytotoxic mechanisms. Indeed, this Sativex®-like combination of Δ<sup>9</sup>-THC- and CBD-enriched botanical extracts removed the deficiency in endogenous antioxidant defenses and attenuated the up-regulation of calpain that occurs in 3-nitropropionate-lesioned rats. It reduced edema and normalized glutamate anomalies typical of quinolinate-lesioned mice, and it also reduced edema and inflammatory events (astrogliosis and microgliosis) predominantly associated with malonate toxicity in rats. The therapeutic potential of the combination of Δ<sup>9</sup>-THC- and CBD-enriched botanical extracts is presently being studied in R6/2 mice and will soon be evaluated at the clinical level, in a trial directed at assessing the efficacy of Sativex® as a disease-modifying agent in a population of early symptomatic HD patients.

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**Notes:**

## Oral 3.5

### CB<sub>1</sub> CANNABINOID RECEPTORS LOCATED ON GLUTAMATERGIC TERMINALS CONFER NEUROPROTECTION IN HUNTINGTON'S DISEASE

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Endocannabinoids prevent excessive synaptic activity by engaging CB<sub>1</sub> cannabinoid receptors, the same receptors targeted by  $\Delta^9$ -tetrahydrocannabinol, the major active component of marijuana. CB<sub>1</sub> receptors are the most abundant G protein-coupled receptors in the brain, and, in particular, are very highly expressed in GABAergic terminals of the forebrain, in which they inhibit GABA release. In addition, lower amounts of CB<sub>1</sub> receptors reside on terminals of principal neurons, where they control glutamatergic transmission. Despite the widely reported neuroprotective activity of CB<sub>1</sub> receptors, the precise relevance of these two different CB<sub>1</sub> receptor subpopulations in neurodegenerative processes is as yet unknown.

Here, we show that CB<sub>1</sub> receptors are severely down-regulated in striatal GABAergic neurons but remain unaffected in corticostriatal glutamatergic neurons in a transgenic mouse model of Huntington's disease (the R6/2 mouse). Administration of the NMDA receptor antagonist MK-801 prevented Huntington's disease-like neurodegeneration in R6/2 mice, while the GABA<sub>A</sub> receptor antagonist picrotoxin was ineffective. In addition, pharmacological delivery of MK-801, but not of picrotoxin, was able to compensate for the deleterious effects produced by CB<sub>1</sub> receptor genetic elimination in R6/2 mice. Currently, we are assessing the neurodegenerative response to striatal excitotoxicity induced by quinolinic acid administration in conditional mutant mice lacking CB<sub>1</sub> receptors in the GABAergic and/or the glutamatergic neuronal compartment of the forebrain. This approach may provide a more direct evidence of the role of CB<sub>1</sub> receptors activity located on glutamatergic neurons in the prevention of excitotoxicity in Huntington's disease, which might allow a therapeutic target to achieve neuroprotection.

**Notes:**

## Oral 3.6

### ENDOCANNABINOID SYSTEM BLOCKADE AS A NOVEL APPROACH TO TREAT FRAGIL X SYNDROME

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Fragile X syndrome (FXS), the most common form of inherited mental retardation, is caused by the loss of the Fmr1 gene product FMRP (fragile X mental retardation protein). Patients with FXS exhibit a wide range of neurological deficits, including cognitive impairment, seizure susceptibility, emotional liability, sleep disorders, attention deficits, pain alterations, self-injurious behaviour, and autism. Previous findings associated some of these conditions with an over-activation of type I metabotropic glutamate receptors, especially mGluR5. In this regard, enhanced endocannabinoid signalling has been pointed to be the cause of elevated neuronal excitability in Fmr1 knockout mice. The aim of this study was to test if the endocannabinoid system (ECS) could serve as a palliative pharmacological target using the Fmr1 knockout (KO) mice in an effort to mitigate some of the symptoms associated to FXS. We observed a clear memory impairment using the object recognition task, a decreased sensitization in inflammatory pain and a reduced-anxiety phenotype in the elevated plus-maze in the Fmr1 KO compared to wild-type (WT) mice. On the other hand, the expression of different components of the ECS and the basal levels of anandamide and 2-arachidonoylglycerol were not modified in Fmr1 KO mice, with respect to WT mice. Instead, an increased expression of mGluR5 and the over-activation of the mTOR signalling pathway were observed. Interestingly, the cognitive deficiency was sensitive to the acute administration of the CB1 receptor antagonists rimonabant and AM251, the mGluR5 antagonist MTEP, and the mTOR specific inhibitor temsirolimus. Moreover, a sub-chronic treatment with rimonabant and MTEP totally reversed the impairment in memory consolidation in Fmr1 KO mice and re-established the control activity of the mTOR signalling cascade in the hippocampus. Moreover, rimonabant also rescued the control level of sensitization in the inflammatory pain test. Finally, pharmacological blockade of CB2, but not CB1 cannabinoid receptors, partially reversed the reduced-anxiety phenotype observed in Fmr1 KO mice. Altogether these results point to the ECS as a potential palliative target in fragile X syndrome conditions.

**Notes:**

## Oral 3.7

### **ENDOCANNABINOID-MEDIATED NEUROPROTECTION IN A MPTP MICE MODEL OF PARKINSON'S DISEASE**

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of nigrostriatal dopaminergic neurons. Current treatments provide symptomatic relief, but fail to prevent or slow down the disease progression. Different elements of the endocannabinoid system (eCBs) are highly expressed within the basal ganglia nuclei, specially in the striatum and they are altered in PD patients and animal models of the disease. It has been proposed that this system may play a role in protection against neuronal damage. The current study examined the effect of cannabinoid system on the dopaminergic degeneration in the progressive MPTP/ probenecid (MPTPp) model of PD. C57B1/6J mice received MPTP (20 mg/kg) plus probenecid (250 mg/kg) twice per week for 5 weeks. At the same time, compound A, whose structure can not be disclosed, was given five days a week. Treatment with compound A reversed MPTP-associated motor deficits as revealed by the analysis of the time that animals turned nose down (inversion time) and the total time required to climb down a pole using the pole test. Furthermore, immunohistochemistry analysis of the substantia nigra pars compacta showed that the treatment protected against MPTP-induced loss of tyrosine hydroxylase-positive neurons. In conclusion, our data suggest that modulation of the endocannabinoid system by compound A protects against MPTP-induced nigrostriatal degeneration.

**Notes:**

## Oral 3.8

### **CORRELATION BETWEEN CB<sub>2</sub> RECEPTOR EXPRESSION AND A-BETA LEVELS AND SENILE PLAQUE SCORES IN ALZHEIMER'S DISEASE FRONTAL CORTEX**

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Alzheimer's disease (AD) is the most common form of dementia among older people. The illness is characterized by cognitive deficits and presence of neuropathological markers,  $\beta$ -amyloid ( $A\beta$ ) deposited in amyloid plaques and neurofibrillary tangles composed of hyperphosphorylated Tau fibrils. Currently, there is no effective treatment for AD. Cannabinoid CB<sub>2</sub> receptors (CB<sub>2</sub>R) were first linked to AD due to their selective expression in neuritic plaque-associated microglia. In the present study, postmortem brain tissues from a cohort of prospectively assessed, neuropathologically confirmed AD patients and aged controls were used to measure CB<sub>2</sub>R receptors by immunoblotting. Correlational analyses were then performed for the neurochemical and cognitive data. The level of CB<sub>2</sub>R was significantly higher (40%,  $p < 0.05$ ) in samples from patients ( $n=15$ ) when compared with controls ( $n=16$ ). As CB<sub>2</sub>R in the CNS is mainly expressed in glia the notable increase in the expression of CB<sub>2</sub>R in samples from patients may be related to a higher expression of glial markers. Increases of glial cells was confirmed using a specific marker, the glial fibrillar acidic protein (GFAP), which was significantly higher in samples from AD patients (250% of increase versus control samples,  $p < 0.001$ ). The variation in the amount of CB<sub>2</sub>R did not correlate with cognitive status assessed by the MMSE test. However, it should be noted that the expression levels of CB<sub>2</sub>R correlated with two relevant AD molecular markers  $A\beta_{42}$  levels and the senile plaque score. These results may constitute the basis of CB<sub>2</sub>R-based therapies and/or diagnostic approaches.

**Notes:**

## Oral 3.9

### ROLE OF CB<sub>1</sub> CANNABINOID RECEPTORS IN THE CONTROL OF CEREBELLAR FUNCTION THROUGH THE MICROGLIAL ACTIVATION

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The endocannabinoid system (ECS), composed by cannabinoid receptors, endocannabinoids (eCBs) and enzymes involved in the synthesis and degradation of these eCBs, controls several functions in the brain, where is mainly involved in synaptic homeostasis. Thus, eCBs mediate multiple forms of synaptic plasticity acting on presynaptic type 1 cannabinoid receptors (CB1Rs) through the inhibition of neurotransmitter release. We observed that microglial reactivity was readily detected in the molecular layer of the cerebellum in young adult mice lacking the CB1R (Cnr1<sup>-/-</sup>). In this brain area CB1Rs are highly expressed and they mainly regulate at the presynaptic level the release of glutamate from parallel fibers. The microgliosis was characterized by local changes in the expression of the microglial activation marker CD11b and changes in the microglia morphology, as well as by the increased expression of pro-inflammatory cytokines. A flaw on glutamate homeostasis at the cerebellar cortex on the Cnr1<sup>-/-</sup> was pointed as a possible cause for the local activation of microglia. Indeed, AMPA type glutamate receptor 1, a subunit exclusively expressed in Bergmann glia and related with neuron to glia signaling, was found heavily phosphorylated in Cnr1<sup>-/-</sup> mice. Interestingly, these mice present subtle but significant impairments in their cerebellar function that are only revealed in young adult mice after using a demanding test for coordination, such as the coat-hanger test. In addition, this cerebellar impairment can be clearly revealed in a conditioned paradigm of cerebellar learning, the delayed eyed blink conditioning task. Interestingly, microglial inhibition by minocycline sub-chronic treatment during five days reduced the expression of microglial activation markers and attenuated the motor coordination deficits observed in the coat-hanger test and in the eyeblink conditioning performance. These results point to CB1Rs as key players in cerebellar homeostasis, and to the direct participation of microglial cells in the modulation of cerebellar functions to a degree not previously foreseen.

**Notes:**

## Oral 3.10

### **CANNABIDIOL PROTECTION OF OLIGODENDROCYTE PROGENITOR CELLS INVOLVES THE ATTENUATION OF ENDOPLASMIC RETICULUM STRESS**

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Oligodendrocytes are the myelinating cells of the Central Nervous System (CNS). Damage to oligodendrocytes and to the myelin sheath is considered responsible for many of the functional abnormalities observed in human diseases such as Multiple Sclerosis (MS). Among recent therapeutic agents for MS, cannabinoids raised considerable interest, as they potentially modulate the inflammatory, neurodegenerative, immunological and demyelinating components of MS.

In the present study, we have investigated the effect of Cannabidiol (CBD), a non psychotropic component of *Cannabis sativa*, in primary cell cultures of oligodendrocyte progenitor cells (OPCs). In previous studies, dose response experiments showed that CBD at doses of 1µM directly protects OPCs from oxidative stress cell death and from LPS/IFN $\gamma$ -induced cell death. In the present work, we show that tunicamycine treatment induced OPCs death that was reduced by CBD through a mechanism that involved the decrease in phosphorylation and activation of the protein eIF2 $\alpha$ , one of the initiators of the ER stress pathway. Moreover, this effect was also seen in inflammatory conditions (LPS/IFN $\gamma$ ), accompanied by a decrease in other ER stress markers (CHOP) and in the apoptotic effectors (Bax, Caspase12), and by an increase in the anti-apoptotic effector Bcl-2. These actions could explain some of the “oligoprotective” effects of CBD promoting the survival of OPCs by attenuating the ER stress pathway. Further experiments are in progress in order to identify the intracellular pathways and the mechanisms involved in this protective actions of CBD in cells of oligodendroglial lineage.

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**Notes:**

## Oral 3.11

### HIGH EXPRESSION OF CANNABINOID RECEPTOR CB<sub>1</sub> DEFINES A NEW SUBTYPE OF CELLS IN THE SPINAL CORD EPENDYMA

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Cell replacement is a promising field in the search for spinal cord repair after injury. Cells surrounding the spinal cord central canal may provide a source of stem/precursor cells that give rise to neurons, astrocytes or oligodendrocytes. However, there is an heterogeneity of cells inside the ependyma that is still far from being understood. Here we describe a subtype of cells mostly located in the lateral and dorsal central canal that comprise less than 1% of the total ependymal cells, and are present at all levels of the spinal cord. These cells are readily identified by its very high expression of the cannabinoid receptor CB<sub>1</sub>. They are characterized by an oval/round soma, apical nucleus, a variable number of cilia (0, 1 or 2) and one short and sometimes ramified basal process. They are closely related with basal lamina labyrinths or fractones derived from supraependymal microglia. In addition, they express markers of stem/precursor cells such as vimentin, nestin, Sox2, Sox9 and GLAST but not others such as LeX or GFAP. These cells are not proliferating in the normal adult spinal cord (they are not labeled by short-time injections of BrdU and they do not express Ki67). However, during early postnatal life and after a spinal contusion, 30-50% of these cells enter the cell cycle and are express Ki67. The presence of these cells is not limited to rats, but can also be detected in mouse central canal.

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**Notes:**

## Oral 3.12

### THE ENDOCANNABINOID SYSTEM MODULATES PROLIFERATION AND MIGRATION OF OLIGODENDROCYTE PROGENITOR CELLS

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During CNS development oligodendrocyte progenitor cells (OPCs) migrate from germinal regions to myelinate neuronal axons that form white matter tracts. In the adult brain, OPCs repopulate demyelinated axons to restore functional nerve transmission. Our recent studies reported the expression of a whole endocannabinoid system in oligodendroglial cells that participates in cell differentiation. The present study examines the involvement of the endocannabinoid system in the *in vitro* proliferation and migration of OPCs. Experiments using OPCs primary cultures showed that CB1 and CB2 receptor blockade with AM281 and AM630, respectively, during 24 hours, decreases OPC proliferation assessed by BrdU incorporation and phospho-H3 or Ki67 immunolabeling. Likewise, the inhibition of diacylglycerol lipases (DAGL), the enzymes responsible for the synthesis of 2-AG, with RHC-80267 or THL, reduces cell proliferation. Levels of proteins involved in the progression of cell cycle (cyclin-D, cyclin-E, cdk-2, cdk-4, p-cdc2, p27 and p21) were assessed by Western blot. Our data demonstrated that cyclins, cdks and p-cdc2 were reduced when the endocannabinoid system was inhibited whereas p27 and p21 were up-regulated. In addition, we studied the migration of OPCs by three different assays; agarose drop, Boyden chamber (chemotaxis assay) and scratch wound assay. AM281, AM630 or RHC-80267 reduced the number and velocity of migrating cells. Migration of OPCs requires an active reorganization of the actin cytoskeleton, and one of the proteins participating in this process is cofilin, whose phosphorylation inhibits its actin-severing activity. Our data show that blockade of endocannabinoid system increases levels of phospho-cofilin which parallels a decreased migration capacity, possibly by modulation of actin dynamics. Overall, our data suggest a novel role of endocannabinoids in oligodendrocyte development such an activation of cannabinoid receptors by 2-AG facilitate the proliferation and migration of OPCs.

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**Notes:**

## Oral 3.13

### **CB<sub>2</sub> CANNABINOID RECEPTORS PROMOTE NEURAL PROGENITOR CELL PROLIFERATION VIA mTORC1 SIGNALING**

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The endocannabinoid (eCB) system is known to regulate neural progenitor (NP) cell proliferation and neurogenesis. In particular, CB<sub>2</sub> cannabinoid receptors have been shown to promote NP proliferation. As CB<sub>2</sub> receptors are not expressed in differentiated neurons, CB<sub>2</sub>-selective agonists are promising candidates to manipulate NP proliferation, and indirectly neurogenesis, overcoming the undesired psychoactive effects of neuronal CB<sub>1</sub> cannabinoid receptor activation. Here, by using NP cells, brain organotypic cultures and in vivo animal models, we investigated the signal transduction mechanism involved in CB<sub>2</sub> receptor-induced NP cell proliferation and neurogenesis. Exposure of hippocampal HiB5 NP cells to the CB<sub>2</sub> receptor-selective agonist HU-308 led to the activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin complex 1 (mTORC1) pathway, which, by inhibiting its downstream target p27Kip1, induced NP proliferation. Experiments conducted with the CB<sub>2</sub> receptor-selective antagonist SR144528, inhibitors of the PI3K/Akt/mTORC1 axis and CB<sub>2</sub> receptor transient-transfection vectors further supported that CB<sub>2</sub> receptors control NP cell proliferation via activation of mTORC1 signaling. Likewise, CB<sub>2</sub> receptor engagement induced cell proliferation in an mTORC1-dependent manner both in embryonic cortical slices and in adult hippocampal NPs. Thus, HU-308 increased ribosomal protein S6 phosphorylation and 5-bromo-2'-deoxyuridine incorporation in wild-type but not CB<sub>2</sub> receptor-deficient NPs of the mouse subgranular zone. Moreover, hippocampal NP proliferation induced by HU-308 and excitotoxicity was blocked by the mTORC1 inhibitor rapamycin. Altogether, these findings provide a mechanism of action and a rationale for the use of non-psychomimetic CB<sub>2</sub> receptor-selective ligands as a novel strategy for the control of NP cell proliferation and neurogenesis.

**Notes:**



## Poster 1

### CORRELATION OF TEMPORAL EXPRESSION OF ENDOCANNABINOID RECEPTORS AND DEGRADATION ENZYMES DURING THE MEIOTIC RESUMPTION IN HUMAN OOCYTES

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**Introduction:** It is known that cannabinoids have a role in the mammalian reproduction via receptors and degradation enzymes, but up to date, there is not any study about the role of the cannabinoids in the maturation of the female gametes.

**Objective:** The purpose of the present study is to compare the differential expression and distribution of CB<sub>1</sub>, CB<sub>2</sub>, FAAH and MAGL in the maturation of human oocytes, to hypothesise a possible role of the endocannabinoid system in the meiotic resumption of human oocyte.

**Methods:** A total of 750 human oocytes from patients of the assisted reproduction program (IVF/ICSI) of the Cruces Hospital were analyzed, 187 at germinal vesicle (GV) stage, 128 at metaphase I (MI) and 435 at metaphase II (MII). RT-PCR and Immunocytochemistry techniques were used to detect the presence of transcripts and proteins.

**Results:** The immunostaining pattern of analyzed proteins changed during the different stages of maturation. The localization of CB<sub>1</sub> was peripheral at GV, homogeneous over the entire oocyte at MI and peripheral again at mature MII stage. The CB<sub>2</sub> receptor localization was peripheral at GV and MI stages but homogeneously spread in the cell at MII. The localization of FAAH stayed peripheral throughout the three stages. Finally, MAGL was weakly detected. The PCR results showed the existence of CB<sub>1</sub> and MAGL but no CB<sub>2</sub> and FAAH transcripts in human oocytes.

**Conclusion:** The immunostaining comparison suggests a possible coordinated action of the cannabinoids via receptors and regulation enzymes in the maturation of the gamete. Therefore, they could be involved in the process of fertilization.

## Poster 2

### ANALYZING THE ROLE OF CB<sub>2</sub> RECEPTORS DURING PRIMARY DEMYELINATION AND REMYELINATION

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Multiple sclerosis (MS) is a complex, inflammatory demyelinating disease whose etiology remains unknown. The profound heterogeneity in the pattern of MS lesions has led to the hypothesis that although demyelination may be initiated by autoreactive T cells migrating from the periphery, lymphocyte recruitment and activation can also follow primary oligodendroglial death. Demyelination during MS is accompanied by a remyelination process that ultimately fails, contributing to the axonal damage and subsequent disability that characterizes this disease. Administration of the copper chelator cuprizone is an accepted model for studying MS pattern III lesions, characterized by primary degeneration of mature oligodendroglia, without T cell involvement or blood-brain barrier disruption. Brain endocannabinoid system has been reported to be dysregulated in MS lesions and autoimmune MS models, suggesting that its pharmacological modulation may be useful for the treatment of this demyelinating disease. We have previously reported that cuprizone-induced demyelination is accompanied by a time-dependent increase in the gene expression of CB<sub>2</sub> receptors that normalizes upon withdrawal of the toxin. To further analyze the role of CB<sub>2</sub> receptors during primary demyelination we studied the effect of cuprizone administration in CB<sub>2</sub> knockout mice. Adult C57BL6 wild-type and CB<sub>2</sub> knockout mice were treated with 0.3% cuprizone in the diet for 3 and 6 weeks (demyelination), or fed cuprizone for 6 weeks and allowed to recover for 2 weeks (remyelination). We analyzed the extent of demyelination, the inflammatory reaction (astroglial and microglial reactivity) and the proliferation of oligodendrocyte precursors (OPCs) in coronal sections from both genotypes containing the corpus callosum (CC). We detected no major differences in the degree of myelin staining between wild-type and CB<sub>2</sub> knockout mice during cuprizone administration and withdrawal. In the same sense, the number of microglial cells (CD11b positive) and OPCs (NG2 positive) in the CC (cells/mm<sup>2</sup>) were similar between both genotypes, although we observed a tendency to an increased astroglial reactivity (GFAP staining) in CB<sub>2</sub> knockout mice treated with cuprizone for 6 weeks. These data suggest that endogenous activation of CB<sub>2</sub> receptors during cuprizone administration does not play a crucial role in the cellular events contributing to myelin damage and repair in this model of primary demyelination.

**Acknowledgements:** Funded by grants from Fundación de Investigación Médica Mutua Madrileña, MICINN (SAF2010-21547) and CIBERNED. Susana Mato and Ana Bernal are recipients of a Ramón y Cajal contract and a fellowship from the University of the Basque Country, respectively.

## Poster 3

### INTRA-HIPPOCAMPAL INJECTION OF CANNABIDIOL INDUCES ANTIDEPRESSANT-LIKE EFFECT IN THE RAT FORCED SWIMMING TEST

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Cannabidiol (CBD) is a non-psychotomimetic compound from *Cannabis sativa* that induces anxiolytic- and antipsychotic-like effects in animal models. Recently, we reported [Zanelati *et al.*, *Br J Pharmacol*, 159(1), p.122, 2010] antidepressant-like effect of systemically injected CBD, an effect mediated by 5-HT<sub>1A</sub> receptors. However, the brain sites involved in this effect have not been described so far. Considering the involvement of the dorsal hippocampus (DH) in the neurobiology of depression and that it has a high expression of 5-HT<sub>1A</sub> receptors, we aimed at investigating the involvement of this brain region in CBD-induced antidepressant-like effects. Male Wistar rats (240g) with guide cannulas aimed at the dorsal hippocampal were submitted to the forced swimming pretest session (15 min), which was followed by the test session (5 min of forced swimming), 24h later. CBD (15, 30, 60 nmol/0.5 ul), 8-OH-DPAT 10 nmol or vehicle (grape seed oil) were microinjected in the DH 10 min before the test session. The latency for the first immobility and the total immobility time in the test session were scored by a trained and blinded observer. An independent group of rats received the same drug treatments and was tested in the open field test, where the total distance moved was recorded during 5 min, in order to investigate possible unspecific locomotor effects induced by the treatments. Data was analyzed by one-way ANOVA followed by Dunnett's test. CBD (15, 30 nmol) and 8-OH-DPAT decreased the total immobility time [ $F_{4,44}=4.93$ ,  $p<0.05$ ] and increased the latency to present the first episode of immobility [ $F_{4,44}=3.48$ ,  $p<0.05$ ]. In the open field test, no difference was observed between CBD 30, 60 nmol and vehicle group [ $F_{2,12}=0.087$ ,  $p>0.05$ ]. Intra-hippocampal CBD induces antidepressant-like effects similar to the 5-HT<sub>1A</sub> agonist 8-OH-DPAT, an effect that is dissociated from unspecific locomotor effects. Therefore, the hippocampus seems to be an important brain site mediating CBD-induced antidepressant-like effects.

**Financial support:** FAPESP, CNPq, CAPES.

## Poster 4

### ULTRASTRUCTURAL LOCALIZATION OF THE CB<sub>1</sub> CANNABINOID RECEPTOR IN HIPPOCAMPAL ASTROGLIA

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Glial cells are the most abundant cell population in the central nervous system. It is well established that type 1 cannabinoid receptors (CB<sub>1</sub>) are present in astrocytes where they exert relevant physiological roles. However, the localization of CB<sub>1</sub> in specific glial compartments has remained elusive greatly due to tissue processing conditions and the CB<sub>1</sub> antibodies used.

In this study, GFAP-CB<sub>1</sub>-WT mice, conditional mutant mice with a selective deletion of CB<sub>1</sub> from astrocytes (GFAP-CB<sub>1</sub>-KO) and CB<sub>1</sub>-KO animals were transcardially perfused with Zamboni's fixative (2% formaldehyde and 15% picric acid in PBS). Brains were removed and hippocampal sections were incubated in primary polyclonal rabbit anti-CB<sub>1</sub> (1:1,000) and monoclonal mouse anti-GFAP (1:1,000) antibodies. A pre-embedding silver-intensified immunogold method and an immunoperoxidase method for electron microscopy were used for the localization of CB<sub>1</sub> and GFAP, respectively, in the hippocampal CA1 region.

Under the particular fixation conditions used, abundant silver-intensified gold particles decorating many synaptic terminals were observed. Furthermore, CB<sub>1</sub> immunoparticles were seen in GFAP immunoreactive astrocytes distributed in the neuropil around synaptic and non-synaptic neuronal compartments. The statistical analysis revealed that 52.8% of astrocytes were CB<sub>1</sub> immunopositive in GFAP-CB<sub>1</sub>-WT. In comparison, only 3% of astrocytes had CB<sub>1</sub> immunoparticles in GFAP-CB<sub>1</sub>-KO mice (79% reduction in the number of astrocytes labelled). We also analyzed CB<sub>1</sub> in synaptic terminals of wild-type and mutant mice. There was no significant difference of CA1 axons/terminals labelled with the same CB<sub>1</sub> antibody between wild-type (40%) and GFAP-CB<sub>1</sub>-KO (37.5%) mice. Moreover, the CB<sub>1</sub> antibody was specific as only labelled 6% of total astrocytes and axons/terminals of CB<sub>1</sub>-KO mice.

As a conclusion, the precise subcellular localization of CB<sub>1</sub> in glia by using particular tissue conditions for immunocytochemistry as shown here, paves the way for a better understanding of how CB<sub>1</sub> in identified astrocytic compartments affects these cells' function in health and disease.

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## Poster 5

### ADMINISTRATION OF THE CB<sub>2</sub> AGONIST HU308 AFTER BRAIN HYPOXIA-ISCHEMIA IS NEUROPROTECTIVE IN NEWBORN RATS

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**Background:** Several data emerged lately pointing to a role for CB<sub>2</sub> receptors in hypoxic-ischemic (HI) brain damage, thus suggesting that CB<sub>2</sub> agonists may protect brain from acute ischemic brain damage in adult animals.

**Aim:** to demonstrate that a CB<sub>2</sub> agonist is protective for the immature brain after a HI insult.

**Methods:** unilateral HI brain damage was induced in newborn Wistar rats (7-10 day-old: P7-P10) by exposure to hypoxia (10% FiO<sub>2</sub>) for 110 min after left carotid artery electrocoagulation under anaesthesia. Ten minutes after the end of HI pups were treated s.c. with vehicle (HV, n=8) or with the CB<sub>2</sub> agonist HU308 1 mg/kg single dose (HU, n=10). Other pups remained as controls (n=6). Seven days after HI rats were sacrificed, transcardially perfused with paraformaldehyde (PFH) 4% and their brains removed. Then, a histological study by Nissl staining was carried out in brain frontoparietal cortex and CA1 area of hippocampus of the ipsilateral hemisphere, scoring the tissue damage from 0 (normal) to 5 (massive destruction).

**Results:** HU reduced brain damage, as observed by the reduction in the neuropathological score in cortex (score: 0.5±0.2, 3.2±0.3 and 1.4±0.2 points for control, HV and HU, respectively) and hippocampus (score: 0.5±0.2, 2.0±0.5 and 1.0±0.3 points for control, HV and HU, respectively). No side effects were observed in pups treated with HU.

**Conclusions:** Treatment with the CB<sub>2</sub> agonist HU308 reduced brain damage in the short term without apparent side effects.

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## Poster 6

### CIRCADIAN RHYTHM AND CB1 CANNABINOID RECEPTOR EXPRESSION IN THE PREFRONTAL CORTEX AND RELATED LIMBIC AREAS OF OBESE ZUCKER RATS

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In the present study, we report on the application of a specific polyclonal antibody to the intracellular domain of the CB1 cannabinoid receptor at the histochemical level in prefrontal cortex and related limbic areas, hippocampus and amygdala, of both hemispheres of lean and obese Zucker rats. Furthermore, the potential changes in the expression of CB1 cannabinoid receptor by the circadian rhythm in obese Zucker rats *versus* their lean littermates was assessed.

**Materials and methods:** A series of antibodies, designed against the extreme amino-terminus and the carboxy-terminus of CB1 receptor, to detect endogenous CB1 receptors were immunocytochemically tested against Sprague-Dawley rat brain CB1 receptors and then, screened by Western blotting in subcellular fractions enriched in nuclear elements (P1), plasmatic membrane (P2) and cytosol. We tried out different fixation protocols and antibody dilutions to optimize the histological method for immunodetection. The validation of the specificity of the antibodies was done through the observation of lack of staining after the primary antibody blockage with a specific blocking peptide or through the observation of lack of staining in CB1<sup>-/-</sup> mice. Zucker lean and obese rats were sacrificed at different moments of the circadian activity rhythm (light/dark cycle inverted) in order to study the possible implication of the circadian rhythm in the regulation of CB1 receptor in both lean and obese rats.

**Results:** Higher levels of CB1 receptor expression in prefrontal cortex (infralimbic, prelimbic and anterior cingulate cortex) were observed in obese Zucker rats when compared with their lean littermates. We found an increase of 26% in II/III layer, 34% in V(a) layer and 30% in VI layer. However, no differences were found in hippocampus (CA1, CA2, CA3 and dentate gyrus) and amygdala (lateral, basolateral and central areas). On the other hand, we found no interhemispheric differences in both lean and obese Zucker rats in all studied regions. Finally, we observed an increase of 41% in lean Zucker rats when light/dark cycle was inverted, that was not observed in the obese type.

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## Poster 7

### CHANGES ON METABOLIC PARAMETERS INDUCED BY ACUTE CANNABINOID ADMINISTRATION (CBD, THC) IN A RAT EXPERIMENTAL MODEL OF NUTRITIONAL VITAMIN A DEFICIENCY

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The effect of vitamin A deficiency (VAD) and acute administration of cannabidiol (CBD) or tetrahydrocannabinol (THC) on the levels of retinol in plasma and the liver, plasmatic retinol binding protein, nutritive utilization of protein, and different hematic and biochemical parameters was studied. Vitamin A deficiency was developed during a 50-day experimental period in which rats consumed a vitamin A-free diet. Cannabidiol (10 mg/kg body weight) or tetrahydrocannabinol (5 mg/kg body weight) were administered intraperitoneally 2 hours prior to sacrifice of the animals. The nutritional deficiency caused a significant decrease in plasmatic and liver contents of retinol and biochemical parameters of glycemic, lipidic, and mineral metabolism. Acute intraperitoneal administration of Cannabidiol and tetrahydrocannabinol did not improve the indices of vitamin A status in either control or vitamin A-deficient rats. However, it had a significant effect on specific biochemical parameters such as glucose, triglycerides, and cholesterol.

In conclusion, under our experimental conditions, the reported effects of cannabinoid administration on certain signs of nutritional vitamin A deficiency appeared to be mediated through mechanisms other than changes in retinol metabolism or its mobilization after the acute administration of such compounds.

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## Poster 8

### IDENTIFICATION OF CB<sub>2</sub> RECEPTORS IN THE BRAIN OF PATIENTS AND EXPERIMENTAL MODELS OF PARKINSON'S DISEASE

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Cannabinoid CB<sub>2</sub> receptors have been identified in glial cells recruited at lesioned sites in various neurodegenerative disorders, including Alzheimer's disease, Huntington's chorea, amyotrophic lateral sclerosis, multiple sclerosis, and others, in which this receptor seems to play a neuroprotective role (Fernández-Ruiz et al., Trends Pharmacol. Sci. 28, 39-45, 2007). However, the issue has remained controversial in Parkinson's disease (PD) with only a recent study describing CB<sub>2</sub> receptor up-regulation in glial elements and beneficial effects of CB<sub>2</sub> agonists in MPTP-lesioned mice (Price et al., Eur. J. Neurosci., 2009). By contrast, CB<sub>2</sub> receptor up-regulation was very poor in 6-hydroxydopamine-lesioned rats (García et al., Brit. J. Pharmacol., 2011) whereas its activation with compounds targeting selectively this receptor did not reduce nigrostriatal damage (García-Arencibia et al., Brain Res., 2007). In this work, we wanted to re-examine the issue by performing CB<sub>2</sub> receptor immunostainings in the basal ganglia of different experimental models of parkinsonism (i.e. mice lesioned with 6-hydroxydopamine, MPTP or LPS). Our data confirmed that CB<sub>2</sub> receptors are up-regulated in the substantia nigra and/or striatum in experimental parkinsonism, in particular in those cases in which the lesion is provoked by a pro-inflammatory neurotoxin like LPS. Lower responses were found in those cases, such as 6-hydroxydopamine, in which inflammatory responses are modest. Using double-staining analyses (and, in some cases, by analyzing the morphological characteristics of positive cells), we could identify the cellular substrates in which CB<sub>2</sub> receptors are located in lesioned structures, that corresponded to glial elements, in particular microglial cells and also possibly astrocytes. We also studied this issue in post-mortem tissues from patients affected by PD provided by different biobanks. In most of cases examined, CB<sub>2</sub> receptor immunostaining was found also in glial elements and the signal exhibited a marked increase compared to control subjects. However, in contrast with experimental models developed in mice, immunostaining for the CB<sub>2</sub> receptor was also found in neuromelanin-containing cells that, in the substantia nigra, correspond to tyrosine hydroxylase-positive neurons. The signal was found in both control subjects and PD patients, and, in this case, the labelling for the CB<sub>2</sub> receptor was significantly lower in the patients compared to controls. Therefore, these data show an up-regulatory response of CB<sub>2</sub> receptors in glial elements located in lesioned structures of PD patients and also in various models of experimental parkinsonism in mice, in particular in those models showing greater inflammation and glial activation. However, they also show for the first time a neuronal location for the CB<sub>2</sub> receptor in the human substantia nigra in healthy conditions, likely in tyrosine hydroxylase-containing neurons that degenerate in PD, which explain the significant reduction in CB<sub>2</sub> receptor immunostaining found in these neurons in the samples coming from PD patients.

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## Poster 9

### 5HT<sub>1A</sub> RECEPTORS ARE INVOLVED IN CANNABIDIOL-INDUCED PROTECTION OF NEWBORN PIG BRAIN AFTER HYPOXIC-ISCHEMIC INSULTS

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**Background:** cannabidiol (CBD) is known to increase serotonin 5HT<sub>1A</sub> receptors activity. This effect is thought to be involved in the protective effects of CBD in brain and other tissues.

**Aim:** to determine whether or not 5HT<sub>1A</sub> receptors are involved in CBD neuroprotection in hypoxic-ischemic (HI) newborn brain.

**Methods:** sedated and ventilated newborn pigs (1-2 day-old) underwent HI damage by adding hypoxia (FiO<sub>2</sub> 10%) to brain ischemia by bilateral carotid artery compression for 30 min. Half an hour after HI piglets received i.v. vehicle (HV, n=8) or CBD 1 mg/kg (HC, n=9), alone or with the 5HT<sub>1A</sub> antagonist WAY100630 (0.1 mg/kg; HCW, n=5). Hemodynamic parameters and brain function (continuous amplitude-integrated EEG record) were monitored up to six hours post-HI. At the end of experiment, piglets were euthanized and their brains removed; left brain hemisphere was frozen in isopentane and stored at -80°C for biochemical studies (cytokine microarrays), whereas right hemisphere was preserved in 4% paraformaldehyde for histological studies (density of dead neurons by Nissl staining). Samples from the frozen hemisphere were studied by proton magnetic spectroscopy (H-MRS) obtaining the following ratios: NAA/Cho (neuronal death) and Lac/Cr (metabolic impairment). Similarly studied animals without HI insult served as controls (SH, n=6).

**Results:** CBD reduced brain damage as observed by the histological (5±1, 26±3 and 9±3% dead neurons for SH, HV and HC, p<0.05), functional (final aEEG amplitude: 85±7, 18±3 and 65.5±9% baseline for SH, HV and HC, respectively, p<0.05) and H-MRS studies (NAA/Cho: 7.1±0.6, 4.7±0.5 and 7.8±0.3; Lac/Cr: 2.7±0.3, 5.7±0.9 and 3.4±0.3, for SH, HV and HC, respect., all p<0.05). CBD neuroprotection was related with the reduction of inflammation (IL-1: 116.7±7, 138±9 and 121±4 pg/mL; IL-6: 23±2, 28±3 and 22±2 pg/mL, for SH, HV and HC, p<0.05). Coadministration of WAY abolished the neuroprotective effects of CBD (HCW histology: 18±3%; aEEG: 17±2%; NAA/Cho: 5.5±0.8; Lac/Cr: 6.7±0.7; all p<0.05 vs. CBD). The deleterious effect of WAY was not due to the abolishment of CBD anti-inflammatory effect (HCW IL-1: 117±8 pg/mL; IL-6: 21±2 pg/mL; all NS vs CBD). Interestingly, none of SH and HC animals needed treatment with inotropic drug because of hypotension, but such treatment was needed in 4/8 HV and 4/5 HCW (p<0.05).

**Conclusions:** 5HT<sub>1A</sub> receptors are involved in CBD neuroprotection in newborn pig brain after HI. 5HT<sub>1A</sub> receptors are not involved in CBD anti-inflammatory effect but seem to be involved in the vascular effects of CBD.

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## Poster 10

### **MICE LACKING THE PPAR-ALPHA GENE PRESENT REDUCED NUMBER OF DOPAMINE NEURONS IN THE SUBSTANTIA NIGRA WITHOUT ALTERING MOTOR BEHAVIOR OR DOPAMINE NEURON DECLINE OVER LIFE**

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Peroxisome proliferator-activated receptor alpha (PPAR- $\alpha$ ), which are expressed by neurons of the nigrostriatal circuit, plays a prominent role in oxidative stress and neuroinflammation. The objectives were: i) to discern if levels of antioxidant molecules and pro-inflammatory cytokines, along with PPAR- $\gamma$  expression are modified in the nigrostriatal region of null PPAR- $\alpha$  mice, ii) to discern whether dopaminergic neuronal features of the substantia nigra pars compacta (SNpc) and dorsal striatum are affected in null mice, and iii) to establish if aging-induced decline of nigral neurons is different in null PPAR- $\alpha$  mice relative to wild-type littermates. A substantial decrease in anti-oxidant molecules was found in SNpc of null mice, by using ELISA. The pro-inflammatory factors TNF- $\alpha$  and IL-3 were found to be reduced in the substantia nigra, suggesting dual and opposite effects of PPAR- $\alpha$  deficiency on oxidative and pro-inflammatory molecules. Immunohistological and stereological studies revealed that young null mice present a smaller SNpc (-19.8%; TH downregulation was discarded). Normal locomotion in an open-field was not affected in null mice. Dopamine cell death could be caused by reduced protection against oxidative stress. Old null mice showed a percentage reduction of nigral dopamine neurons similar to that of young null animals, with a rate of decline over life of around 44%, the same value than that of wild-type littermates. These findings suggest that nuclear PPAR- $\alpha$  is necessary for the normal development of the substantia nigra along with normal levels of anti-oxidant molecules. Lack of PPAR- $\alpha$  does not modify the normal motor behavior of mice or decline of nigral dopamine neurons throughout life.

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## Poster 11

### SEXUALLY DIMORPHIC EFFECTS OF MATERNAL DEPRIVATION AND/OR JUVENILE CANNABINOID EXPOSURE ON ADULT RATS: A BEHAVIOURAL, ENDOCRINE AND IMMUNOHISTOCHEMICAL STUDY

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We have recently reported that early maternal deprivation (MD) [24h at postnatal day (PND) 9] and/or an adolescent chronic treatment with the cannabinoid agonist CP-55,940 [(CP); 0.4 mg/kg/day, PND 28-42] in Wistar rats induced, in adulthood, diverse sex-dependent long-term behavioural and physiological alterations. Here we report a summary of the behavioural [prepulse inhibition (PPI), elevated plus maze (EPM) and holeboard (HB)] and endocrine (adrenocortical reactivity and leptin) results, as well as the effects of both treatments on hippocampal GFAP+ cells, CB1 receptors and BDNF (immunohistochemical analysis). Maternally deprived males showed an increased exploration of the open arms in the EPM which, according to previous data from our group, might be interpreted as increased risk-taking behavior. Adolescent exposure to the cannabinoid agonist induced an impairment of the PPI and an increased frequency of head-dipping and entries in the inner zone in the HB in females whereas in males it induced an increased adrenocortical responsiveness. Both, MD and adolescent cannabinoid exposure also induced sex-dependent changes in plasma leptin levels. As for the immunohistochemical analysis, we found that MD induced, in males, a significant increase in the number of GFAP+ cells in CA1 and CA3 areas and in the polymorphic layer of the Dentate Gyrus (DG), an effect that was attenuated by CP in the two latter regions. In turn, adolescent cannabinoid exposure induced, in control non deprived males, a significant increase in the number of GFAP+ cells in the polymorphic layer of the DG. MD induced a decrease in CB1 expression in both sexes and this effect was reversed in males by the cannabinoid treatment. The drug, "per se", induced a general decrease of CB1 immunoreactivity in males and the opposite effect was observed in females. Cannabinoid exposure tended to reduce BDNF expression in CA1 and CA3 hippocampal areas of females, whereas MD counteracted this trend and induced an increase of BDNF in females. As a whole, the present results show sex-dependent long-term effects of MD and juvenile cannabinoid exposure as well as functional interactions between the two treatments.

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## Poster 12

### CONSUMPTION OF CANNABIS IN A SAMPLE OF YOUNG PEOPLE FROM ANDALUSIA (SPAIN): DIFFERENTIAL VISION IN FUNCTION OF GENDER

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The studied sample is of 204 young people from Andalusia (Spain). The cases in this study were daily cannabis users for at least one month in lifetime, and the control consisted of those who did not smoke cannabis daily. Both the case and the control populations were identified by snowball sampling. The chain started in two areas of Andalusia (Almeria and Granada) with three daily cannabis users and four non-daily cannabis users in each area. The subjects were initially identified with the help of associations of cannabis plant growth. All of them agreed to participate in the study and each one helped to identify two friends: one daily cannabis user (new case) and another non-daily cannabis user (control).

We found that there are significant differences in the distribution between daily and non-daily consumers depending on the gender: in the group of daily cannabis consumers 53.9% are women and 46.1% are men, while among non daily cannabis consumers 24.8% are women and 75.2% are men. Men have 3.5 times more risk of being daily consumers than women ( $p=0,002$ ). Nevertheless, the average age of starting cannabis consumption is 15.59 years old (12-27), with no differences regarding gender. There are also no significant differences in the average age of beginning a more intense consumption, which is of 18.13 years old (13-35). No significant gender differences were found in the frequency of cannabis consumption during the period of maximum consumption. In the group of people that does not use cannabis anymore there are significant differences regarding gender when considering the age in which they stop consuming cannabis: men are 3.9 years older than women when they do it.

## Poster 13

### PERIPHERAL MECHANISMS INVOLVED IN THE CONTROL OF MUSCULAR PAIN

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Muscular pain is a frequent symptom of many clinically defined conditions, despite this, there are few pharmacological options for its treatment. Opioids have been extensively used whilst cannabinoids are an emerging option in the treatment of this kind of pain; recently a cannabinoid extract, Sativex®, has been commercialized for the treatment of certain muscular pains.

Centrally acting opioids and cannabinoids have shown to have a good antinociceptive effect in several pain models but sometimes their use can be hampered by their central effects, so peripheral targets could be an interesting alternative. Also, physiologic and pharmacologic differences have been reported between the trigeminal and spinal innervated areas.

Our aim is to compare the role of peripheral cannabinoid and opioid receptors in two models of acute muscle pain: masseter (trigeminal innervation) and gastrocnemius (spinal innervation).

Hypertonic saline (HS) injected into the masseter evokes a hindpaw shaking behaviour and in the gastrocnemius it causes withdrawal of the affected paw, these are considered as indexes of nociception. Loperamide (a peripherally acting opioid) was administered 30 minutes i.p. and WIN55,212 (WIN) (a non selective cannabinoid agonist) i.m. 5 min before HS injection to determine the implication of these receptors in both pain models. The effect of loperamide was antagonised with naloxone (an opioid antagonist that crosses the blood-brain barrier) and naloxone methiodide (an opioid antagonist which acts peripherally) twenty minutes before the agonist, WIN was antagonised by AM251 (a selective CB<sub>1</sub> receptor antagonist) and AM630 (a selective CB<sub>2</sub> receptor antagonist) five minutes before its administration.

In the masseter pain model, both, loperamide (0.6-2.5 mg/kg) and WIN (0.0125-0.025 mg/kg) had a dose dependent antinociceptive effect. Loperamide was reversed by both opioid antagonists (0.5-1 mg/kg) also WIN was reversed by the two cannabinoid antagonists AM251 (0.04 mg/kg) and AM630 (0.02 mg/kg). However, loperamide (5-10 mg/kg) did not reduce the nociceptive behaviour in the gastrocnemius model whilst WIN (0.0125-0.1 mg/kg) did induce an antinociceptive effect which was reversed by both antagonists AM251 (0.02 mg/kg) and AM630 (0.04 mg/kg).

Our results suggest that peripheral opioid and cannabinoid receptors participate in the control of pain induced by HS in the masseter (trigeminally innervated) whilst in the gastrocnemius (spinally innervated) only peripheral cannabinoids have an antinociceptive effect.

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## Poster 14

### NEW APPROACHES FOR THE STUDY OF GPCR SIGNALLING PATHWAYS

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During the last decade, G protein-coupled receptor (GPCR) oligomers have been proposed to play critical roles in cell signalling and several diseases, opening new avenues for drug discovery. The existence of these receptor-receptor interactions and their signalling pathways has been demonstrated by several approaches including immobilized protein-protein interaction assays, radioactive-ligand binding studies,  $\text{Ca}^{+2}$  mobilization event, cAMP assays or western-blot. However, despite extensive evidence regarding the formation and the dynamics of GPCR heterodimers, none of the approaches used up to now has revealed the function of these signalling complexes in living cells. In the present work, we describe the combined use of three different techniques: bioluminescence resonance energy transfer (BRET), time-resolved fluorescence resonance energy transfer (TR-FRET) and impedance-based label free technology (CellKey), as a tool to identify GPCR heterodimers and study their cell signalling pathways in living cells.

## Poster 15

### EFFECTS OF CHRONIC ADMINISTRATION OF CANNABINOID RECEPTOR INVERSE AGONIST (AM 251) ON THE FOOD INTAKE, BODY WEIGHT GAIN AND OXYGEN CONSUMPTION IN OBESE ZUCKER RATS

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Obesity is characterized by an increase in white fat mass, which results from an excess in food intake relative to energy expenditure. It is often associated with insulin resistance, dyslipidemia and hypertension, a cluster of conditions referred to as the metabolic syndrome. Several data indicates the endocannabinoids and CB1 receptors as important modulators of body weight and appetite. Based on these observations, blockade of this system has been used as an approach for the treatment of obesity and related metabolic disorders, reason why it is of great interest to elucidate the mechanisms through which the cannabinoid receptor inverse agonist AM 251 has been widely employed as anti obesity agent in animal models.

**Methods:** In all the experiments we have used presatiated lean (fa/+) and obese (fa/fa) Zucker rats (6weeks old). On group of obese rats were treated with AM 251 (3mg/kg, ip, once daily), while a second group was injected with vehicle. A third group of lean rats was injected with vehicle for 21 days. Consumption of O<sub>2</sub> of individual animals was measured in an open-circuit indirect calorimetric system.

**Results:** A significant decrease in food intake throughout the experimental period was observed on the AM 251-injected when compared to the vehicle-injected obese rats. Reductions in food intake brought about by AM 251 were accompanied by significant reductions in body weight gain of these animals. A significant decrease in food intake and body weight gain was observed in vehicle-administered lean rats in comparison to obese rats injected with AM 251 and vehicle. Administration of AM 251 to dose of 3mg/kg also led to a significant increase in the consumption oxygen and the energy expenditure after 21 days of injection in comparison with vehicle-injected group.

**Conclusions:** The results from this study demonstrated that the cannabinoid receptor inverse agonist AM 251 activates thermogenesis, suggesting that its antiobesity property is due to the increase in energy expenditure and oxygen consumption in addition to the initial decrease in food intake. Our results represent a potentially useful approach for the treatment of obesity and metabolic disorders.

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## Poster 16

### AN ULTRA-LOW DOSE OF RIMONABANT REDUCES GHRELIN-INDUCED OREXIGENIC ACTIONS

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The role of the endocannabinoid system modulating food intake and energy balance is widely described, as well as the interaction of such system with other peptides and hormones involved in appetite control. Ghrelin is an endogenous regulator of energy homeostasis that stimulates appetite and a positive energy balance. This anabolic molecule is produced by the stomach and the hypothalamus and its receptor co-localizes with CB1 receptor in the hypothalamus. We aimed to study: 1) the effect of central ghrelin administration on food intake in rats with negative and equilibrate energy balance; 2) the effect of an ultra-low and subanorectic dose of the CB1 antagonist Rimonabant in ghrelin-induced orexigenic effects. We found that: 1) actions of ghrelin depends on the feeding status of the animal, increasing food intake only in free feeding rats versus 24h food-deprived or chronic food-restricted animals; 2) an ultra-low dose (30 ug) of Rimonabant blocks ghrelin-induced hyperphagia in free feeding animals. Our results suggest that the endocannabinoid system may interact with ghrelin in the control of meal initiation and has important implications for the clinic due to the dose of rimonabant needed for the blockage of ghrelin-induced hyperphagia is extremely low, avoiding side potential undesirable central effects of CB1 antagonism treatment.

## Poster 17

### IMMUNOLOCALIZATION OF VARIOUS COMPONENTS OF THE ENDOCANNABINOID SYSTEM IN THE CEREBELLUM OF PATIENTS WITH SPINOCEREBELLAR ATAXIAS

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Spinocerebellar ataxias (SCAs) are a group of neurodegenerative diseases, clinically and genetically heterogeneous, characterized by loss of balance and motor incoordination due to dysfunction/degeneration of the cerebellum and its afferent and efferent connections. SCAs share some characteristics with other motor diseases and, given that certain cannabinoids improve motor symptoms and attenuate the progression of brain damage in concordance with the changes found in the endocannabinoid system in these disorders, we decided to study the status of this signaling system in SCAs. We used postmortem samples from patients with different types of ataxias (obtained from two brain banks: Hospital Clinic, Barcelona, Spain, and Netherlands Brain Bank, Amsterdam, The Netherlands). We found a different immunostaining for the CB<sub>1</sub> receptor, the CB<sub>2</sub> receptor and the endocannabinoid-degrading enzyme FAAH in the cerebellum of patients compared with the control subjects (these results were presented in the SEIC meeting in 2010). Now, we have conducted double-staining analyses in order to establish the cellular location of those elements of the endocannabinoid system that appear altered in the cerebellum of patients. For example, our former analyses showed an increase in the immunoreactivity for the CB<sub>1</sub> receptor in the cerebellum of patients that was evident in granular and Purkinje layers but also in the dentate nucleus and areas of white matter. The double-staining experiments showed that CB<sub>1</sub> receptors are located in Purkinje cells and also in astrocytes, reactive microglia and macrophages of the cerebellar white matter. Last year, we also reported an increase in the immunostaining for the CB<sub>2</sub> receptor in Purkinje and granular layers and in areas of white matter of patients. The double-staining experiments demonstrated that this receptor is located in Bergmann glia, as well as in astrocytes and microglia of the cerebellar white matter. Lastly, we also presented evidence of FAAH immunostaining in different parts of the cerebellum, but changes between patients and control subjects for this marker were only evident in the Purkinje layer and in areas of the white matter. The double-staining experiments conducted this year showed that FAAH is located mainly in Purkinje cells. In summary, our study demonstrates that the endocannabinoid system is significantly altered in the cerebellum of SCA patients. The identification of various elements of the endocannabinoid system in the Purkinje neurons, which are the main cells affected in SCAs, as well as the changes observed in these elements in SCA patients, suggest that alterations in this neuromodulatory system may be related to the pathogenesis of SCAs. This also supports the idea that the endocannabinoid system could be a potential therapeutic target for treating ataxias.

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## Poster 18

### THE ABSENCE OF A FUNCTIONAL PPAR-ALPHA GENE EXACERBATES NEUROPATHIC AND VISCERAL PAIN IN FEMALE MICE

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Increasing evidence suggests that PPARs participate in the control of chronic nociceptive responses such as neuropathic and inflammatory pain, and these receptors could play a role on acute pain. In the present study we used null (*PPAR-α* <sup>-/-</sup>) and wild-type female mice as well as the selective *PPAR-α* antagonist GW6471 to evaluate; i) the role of *PPAR-α* in the development and expression of neuropathic pain; ii) the involvement of *PPAR-α* on visceral and acute thermal cutaneous nociception evaluated through writhing, hot-plate, and tail-immersion tests, and iii) tissue levels of cutaneous pro-inflammatory factors such as nitric oxide, TNF- $\alpha$ , and interleukins-1 $\beta$  and -3.

Regarding neuropathic pain, higher basal sensitivity to thermal and mechanical non-noxious and noxious stimuli was observed in mice lacking *PPAR-α*. Cold and mechanical allodynia as well as heat hyperalgesia was augmented in null mice. Respect to visceral nociception, writhes after acetic acid were enhanced in mutant mice, but GW6417 was devoid of effects on number of writhes. Additionally, though basal thermal sensitivity was enhanced in *PPAR-α* <sup>-/-</sup> mice, cutaneous thermal nociception did not differ between genotypes, and blockade of *PPAR-α* was also devoid of effects on thermal nociception. Finally, sciatic nerve ligation enhanced pro-inflammatory factors in plantar tissue of all injured mice. However, these levels were higher in null mice. No changes in pro-inflammatory factors were observed in the hot-plate test. The findings of the present study indicate that the deletion of *PPAR-α* could enhance the cytokine-mediated inflammation implicated in the pathogenesis of neuroinflammatory processes such as neuropathic. Lack of *PPAR-α* but not acute blockade affects basal sensitivity to non-noxious thermal stimuli, a phenomenon that seems not to be related with changes in pro-inflammatory factors.

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## Poster 19

### PARTICIPATION OF CYCLOOXYGENASE-2 IN THE TRANSFORMATION OF 2-ARACHIDONOYL-GLYCEROL TO PROSTAGLANDIN-GLYCERYL ESTERS IN EXPERIMENTAL MODELS OF HUNTINGTON'S DISEASE

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Endocannabinoids, acting preferentially through CB<sub>1</sub> and/or CB<sub>2</sub> receptors, have demonstrated to serve as endogenous neuroprotective molecules against a variety of cytotoxic stimuli that operate in most neurodegenerative disorders. However, emerging evidence indicate that endocannabinoids, in particular 2-arachidonoyl-glycerol (2-AG), may also exert cytotoxic effects as a consequence of their transformation in eicosanoid-related compounds by eicosanoid-related enzymes such as cyclooxygenase-2 (COX-2). We have studied this phenomenon in an experimental model of Huntington's disease, malonate-lesioned rats. For example, we reduced the levels of 2-AG by inhibition of diacylglycerol lipases (DAGLs) with O-3841 and found that, rather than enhancing malonate toxicity in the striatum, the reduction in 2-AG levels was accompanied by an attenuation of malonate-induced GABA and BDNF deficits, by an increase in the number of Nissl-stained cells, and by a reduction in the magnitude of malonate-induced astrogliosis (GFAP immunostaining). By contrast, the administration of OMDM169, an inhibitor of monoacylglycerol lipase (MAGL), which elevated 2-AG levels caused exactly the opposite effect. We hypothesized that both responses are related to 2-AG availability for COX-2-mediated biotransformation into prostaglandin glyceryl esters (PG-Gs). Indeed, we found that COX-2 is induced *in vivo* in the striatum 24 hours after the lesion, in parallel to equivalent responses in other proinflammatory enzymes like inducible NOS, and to opposite responses in anti-inflammatory mediators like nuclear receptors of the PPAR family. The increase in COX-2 was also reproduced *in vitro* in cultured M-213 cells exposed to malonate. *In vivo*, using a sensitive ESI-IT-ToF LC-MS technique, we could not detect the major 2-AG oxygenated metabolite, PGE<sub>2</sub>-Gs, presumably because the generation of this compound is strictly localised to the lesioned areas, thus reaching levels (<0.1 pmol/g tissue) that cannot be detected in the whole striatum with our method. However, levels of this metabolite (4.2 ± 1.0 pmol/mg lipid extract) could be detected in cultured M-213 cells after the addition of malonate and OMDM169, in parallel to an enhancement in cell death compared to cells exposed to malonate alone, as in the *in vivo* experiments. The addition of 2-AG *in vitro* also enhanced malonate effects and the same was found after the blockade of MAGL with JZL184, whereas the inhibition of DAGL with O-3841 produced the opposite effects. Lastly, the enhancing effect found *in vitro* with JZL184 could be reversed by the COX-2 inhibitor celecoxib or the antagonist of PGE<sub>2</sub>-Gs AGN220675. In summary, the availability of 2-AG for COX-2-mediated transformation into PG-Gs may represent a novel mechanism for this endocannabinoid to control neuronal survival that would add to its classic

neuroprotective effects mediated by CB<sub>1</sub> and/or CB<sub>2</sub> receptors. We have studied this novel mechanism in an experimental model of Huntington's disease.

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## Poster 20

### CB1 CANNABINOID RECEPTOR-DRIVEN PRONEUROGENIC SIGNALLING CONTROLS CORTICOSPINAL NEURON DEVELOPMENT AND FUNCTION

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The generation and laminar fate specification of pyramidal neurons during neocortical development is regulated by a network of transcription factors that is starting to be understood. Here we investigated whether CB1 cannabinoid receptors regulate pyramidal neuron specification and cortical lamination. We found that CB1 receptor deletion reduced Pax6- and Tbr2-positive progenitor cell number in the ventricular and subventricular zone, and induced an expansion of premature post-mitotic Tbr1- and Satb2-positive cells as a consequence of precocious progenitor cell cycle exit. Conditional CB1 receptor ablation in the glutamatergic lineage, but not in the GABAergic lineage, reduced Ctip2-positive neuronal generation, which supports a pyramidal lineage-autonomous role of CB1 receptors in deep layer specification. Defective proneurogenic signalling in CB1 receptor deficient mice was ensued by defects in axonal fasciculation and corticospinal connectivity, resulting in impaired skilled motor function of adult mice. These findings show that CB1 cannabinoid receptor ablation, by impairing the transcriptional regulatory network responsible of pyramidal progenitor regulation, impacts progenitor cell cycle maintenance and laminar specification, thereby interfering with adult corticospinal function.

## Poster 21

### EFFECT OF OPIOID AND CANNABINOID DRUGS IN TWO MODELS OF MUSCLE PAIN IN RATS

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Muscle pain is a major clinical problem and animal models and new pharmacological options, to better investigate acute and chronic muscle pain and its treatment, are being studied.

Our aim is to compare the effect of systemical administration of opioid and cannabinoid agonists in two models of muscle acute pain: masseter and gastrocnemius in rat, induced by hypertonic saline (HS).

Effects of intraperitoneal (i.p.) administration of morphine and cannabinoids: WIN55,212-2 (CB<sub>1</sub>-CB<sub>2</sub> agonist), ACEA and JWH 015 (CB<sub>1</sub> and CB<sub>2</sub> agonists, respectively) were examined. These effects were antagonised with the corresponding antagonists (naloxone, naloxone methiodide, AM 251 or AM630).

In the masseter model, HS injected into the muscle evokes a paw shaking behaviour that is considered as an index of nociception<sup>1,2</sup>. In the gastrocnemius model<sup>2</sup>, after HS injection, the time that rat spent with lifted or retracted paw was recorded. Modification of these behavioural parameters, after pharmacological treatment, was studied.

In the masseter model, morphine and the CB<sub>1</sub> and CB<sub>2</sub> agonists reduced HS-induced nociceptive behaviours. The antinociceptive effect of morphine was blocked by both naloxone and naloxone methiodide. The effect of the cannabinoid agonists was antagonised by their respective antagonists.

In the gastrocnemius model, morphine was able to avoid the nociceptive behaviour induced by HS and this effect was only antagonized by naloxone. WIN and ACEA induced an antinociceptive effect while JWH 015 did not modify the nociceptive behaviour.

Our results suggest that central and peripheral opioid receptors and CB<sub>1</sub> and CB<sub>2</sub> receptors could be implicated in the control of masseter pain, induced by HS injection, whereas on gastrocnemius pain, central opioid receptors and only CB<sub>1</sub> receptors are involved in the antinociceptive effect.

Opioids and cannabinoid drugs could be an interesting therapeutic option to treat muscle pain.

<sup>1</sup> Ro *et al.*, *Pain*, 2003; 104: 179-185.

<sup>2</sup> Sánchez *et al.*, *Pharmacol Biochem Behav*, 2010; 96: 488-495.

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## Poster 22

### CO-EXPRESSION OF mRNAs CODING ENDOCANNABINOID RECEPTORS 1 AND 2 IN THE MONKEY BASAL GANGLIA OUTPUT NEURONS

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The endocannabinoid receptors 1 and 2 (CB1-R and CB2-R) are expressed in the central and peripheral nervous systems, respectively. However, we have recently demonstrated that neurons in both the external and internal segments of the globus pallidus (GPe and GPi) contain transcripts for CB2-R in monkeys. By using dual in situ hybridization techniques combined with retrograde tract-tracing, here we show that CB1 and CB2 mRNAs are co-expressed within pallidothalamic-projecting neurons. Furthermore, CB1 and CB2 mRNAs co-expression was maintained across control, MPTP-treated and dyskinetic monkeys. Most importantly, CB1 and CB2 transcripts share an identical subcellular distribution, therefore suggesting that CB2-Rs might play a similar role than CB1-Rs at the pre-synaptic level. The unequivocal demonstration that both types of endocannabinoid receptors are expressed in basal ganglia output neurons (which are known to be hyperactive in parkinsonian conditions) suggests that these receptors (particularly CB2-Rs) could be further considered as potential pharmacological targets for the treatment of movement disorders of basal ganglia origin.

## Poster 23

### EXPRESSION OF CB<sub>2</sub> RECEPTOR AND MU-OPIOID RECEPTOR ON IMMUNE CELLS AFTER MORPHINE SELF-ADMINISTRATION IN LEWIS AND FISHER344 RATS

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Opiate abuse compromises the central nervous system but it also affects immune function, increasing the vulnerability to neoplasia and infectious diseases. Opiate drugs (morphine and heroin) modulate the immune response by suppressing different functions. Considering that the immune cells express CB<sub>2</sub> cannabinoid receptors and mu-opioid receptors which have been associated with immunosuppression, the aim of the current work was to analyze the expression of these receptors on T- and B-cells and on monocytes/macrophages after morphine self-administration in Lewis (Lew) and Fisher344 (F344) male rats. Lew and F344 rats have been used to study the role of genetic factors in the proneness to addiction. Lewis animals show higher rates of self-administration of several drugs of abuse than those displayed by F344 rats, their histocompatible control. Lew and F344 rats were trained to self-administer morphine (1 mg/kg/infusion, i.v.) or vehicle for 15 days. 24hs after the last self-administration session, rats were decapitated and the spleens were removed. Spleen cell suspensions were obtained by gently grinding tissue into RPMI 1640 culture medium and mononuclear cells were separated by Ficoll-Hypaque density gradient (1.083 g/ml) centrifugation, counted and suspended at a final concentration of  $2 \times 10^6$  cells/ml. Four-colour immunofluorescence staining was performed and the intensity of stained cells was analysed by flow cytometry. 100  $\mu$ L of cells suspension were incubated 30 min with rabbit anti-rat CB<sub>2</sub> receptor or rabbit anti-rat mu-opioid receptor, washed and incubated 30 min with secondary antibody AlexaFluor647-conjugated mouse anti-rabbit IgG, and with FITC-conjugated mouse anti-rat CD3, PE-conjugated mouse anti-rat CD11b/c, and PECy5-conjugated mouse anti-rat CD45RA in order to determine T-cells, monocytes/macrophages, and B-cells, respectively. After that, cells were fixed prior to flow cytometry. Lymphocytes and monocytes were gated based on their characteristic light-scatter and fluorescence intensity was depicted on a three-decade logarithmic scale and in single-parameter analysis as histograms in order to calculate CB<sub>2</sub> and mu-opioid receptors expression on the different cell populations. We found a trend for morphine- and strain-related modulation in the immune expression of CB<sub>2</sub> and mu-opioid receptors. The results will be discussed in terms of the involvement of CB<sub>2</sub> and mu receptor in immune modulation and the well-known immunosuppressive actions of opiate drugs.

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