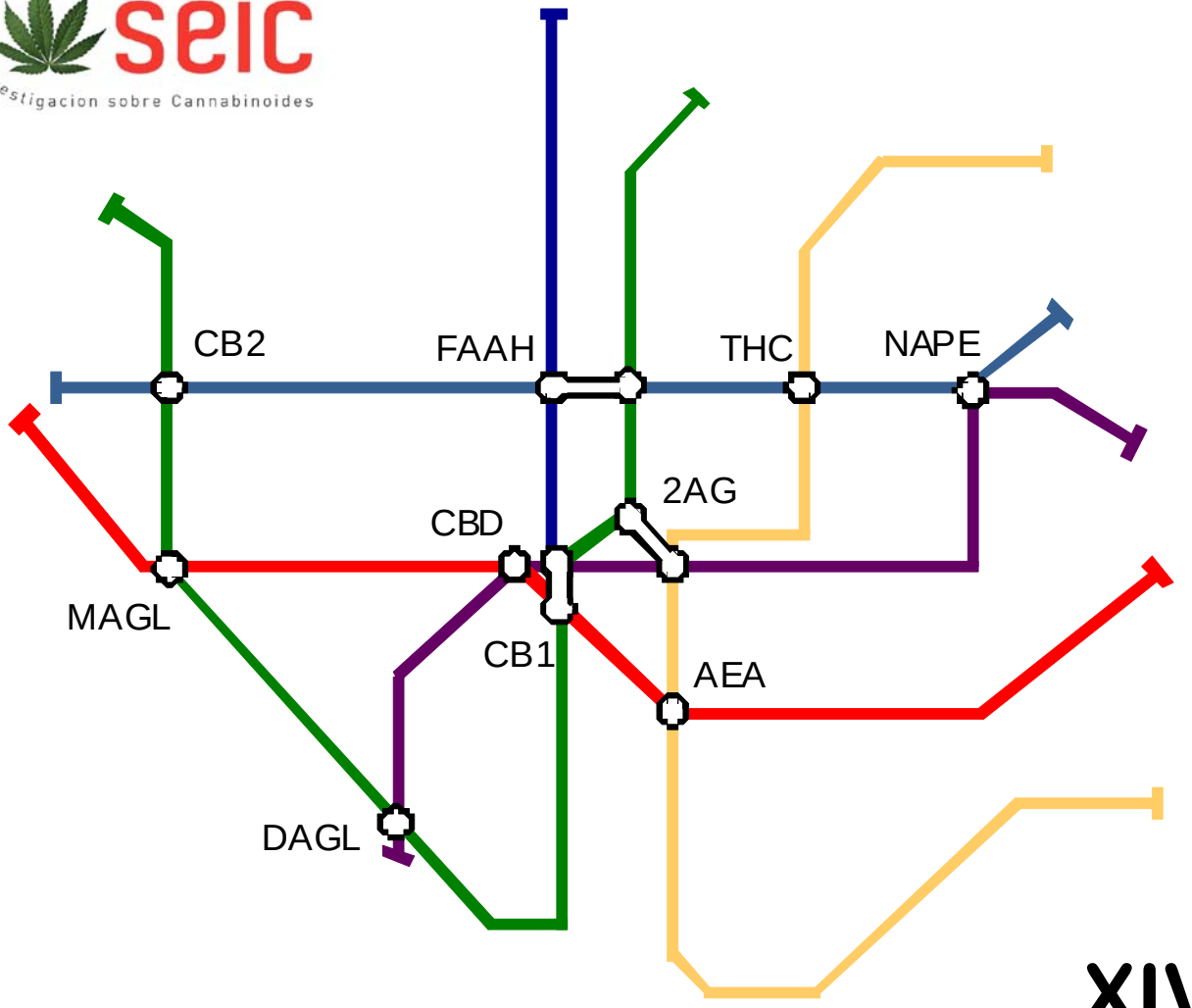
Sociedad Española Investigación Cannabinoides



XIV Reunión Anual

Barcelona

28-30 Noviembre 2013

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14ª Reunión Anual
Sociedad Española de Investigación sobre Cannabinoides

Barcelona, 28-30 de noviembre de 2013

PROGRAMA CIENTÍFICO

Jueves, 28 de noviembre

11:30-12:30 **Entrega de documentación**
Centro de Cultura Contemporánea de Barcelona, CCCB
C/ Montealegre, 5

12:30-12:45 **Inauguración**
- Ester Aso, Comité Organizador
- Manuel Guzmán, Presidente de la SEIC

12:45-13:45 **Conferencia inaugural**
(presentada por Rafael Maldonado)
Andreas Zimmer, Universidad de Bonn
"Cannabinoid signaling and aging"

13:45-15:30 **Comida**

15:30-17:45 **Mesa Redonda**
"Cannabinoides y terapia: del laboratorio a la clínica"
(moderadores: Javier Fernández Ruiz y Manuel Guzmán)
- Xavier Nadal, Phytoplant Research, Córdoba
- Tony Levi, Tikun Olam, Israel
- Javier Pedraza, Hospital Espírito Santo, Évora, Portugal
- Guillermo Velasco , Universidad Complutense de Madrid
- Juan García Caldentey, Hospital Fundación Jiménez Díaz, Madrid
- Yolanda Blanco, Hospital Clinic, Barcelona

17:45-18:00 **Descanso**

18:00-19:30 **Sesión de comunicaciones orales**
"Sistema endocannabinoide: efectos comportamentales y psiquiátricos (I)"
(moderadores: Fernando Berrendero y Óscar Prospero-García)

18:00-18:15 Presentación (Fernando Berrendero)

18:15-18:30 O-1.1
BASOLATERAL/BASOMEDIAL AMYGDALA CB1 EXPRESSION IN A MODEL OF DRUG SEEKING INCUBATION. D. Roura-Martínez, R. Santos-Toscano, M. Miguéns, A. Higuera-Matas, E. Ambrosio

18:30-18:45 O-1.2
BLOCKADE OF CANNABINOID CB1 AND CB2 RECEPTORS PREVENTS COCAINE-INDUCED REDUCTION OF CELL PROLIFERATION AND COCAINE-INDUCED INCREASE OF GLIOSIS IN ADULT MALE RATS**.** J. Suárez , P. Rivera, S. Arrabal, A. Vargas, F.J. Pavón, A. Serrano, E. Castilla-Ortega, P. Galeano, E. Blanco-Calvo, F. Rodríguez de Fonseca

18:45-19:00 O-1.3
INVOLVEMENT OF CANNABINOID RECEPTORS IN THE COGNITIVE DEFICTS ASSOCIATED WITH NICOTINE WITHDRAWAL. R. Saravia, A. Flores, A. Plaza-Zabala, A. Busquets-García, A. Ozaita, R. Maldonado, F. Berrendero

19:00-19:15 O-1.4
CEREBELLAR CYCLOOXYGENASE-2 (COX-2) PLAYS A CRUCIAL ROLE IN THE CEREBELLAR FUNCTIONAL DEFICITS ASSOCIATED TO REPEATED CANNABIS EXPOSURE. L. Cutando, R. Maldonado, A. Ozaita

19:15-19:30 O-1.5
COGNITIVE IMPAIRMENT INDUCED BY DELTA-9-TETRAHYDROCANNABINOL OCCURS THROUGH HETEROMERS BETWEEN CANNABINOID CB1 AND SEROTONIN 2A RECEPTORS. X. Viñals, E. Moreno, L. Lanfumey, T. Pastor, R. de la Torre, P.Gasperini, C. Lluís, E.I. Canela, P.J. McCormick, R. Maldonado, P. Robledo

Cena libre

Viernes, 29 de noviembre

9:00-10:30 **Sesión de comunicaciones orales**
"Sistema endocannabinoide: efectos comportamentales y psiquiátricos (II)"

9:00-9:15 O-1.6
MEDIAL BUT NOT LATERAL ORBITOFRONTAL CB1 GENE EXPRESSION PREDICTS IMPULSIVE CHOICE IN RATS. D. Roura-Martínez, M. Ucha-Tortuero, S.M. Coria, J. Ibias, E. Ambrosio, A. Higuera-Matas

9:15-9:30 O-1.7
ANANDAMIDE AND 2-AG MODIFY THE EXPRESSION OF CONDITIONED FEAR IN OPPOSITE DIRECTIONS. A. Llorente-Berzal, A.L. B. Terzian, V. di Marzo, M.P. Viveros, C.T Wotjak

9:30-9:45 O-1.8
ENDOCANNABINOIDS, MATERNAL CARE AND SLEEP. O. Prospero-García, A. Romano López, A.E. Ruiz Contreras, M. Méndez Díaz

9:45-10:00 O-1.9
A PERIPHERAL ENDOCANNABINOID MECHANISM FOR STRESS-INDUCED AMNESIA. A. Busquets-Garcia, M. Gomis-González, R. Srivastava, A. Ortega-Álvaro, L. Bellochio, G. Marsicano, B Lutz, R Maldonado, A. Ozaita

10:00-10:15 O-1.10
EVALUATION OF THE ENDOCANNABINOID SYSTEM IN POSTMORTEM BRAIN OF SUBJECTS WITH MAJOR DEPRESSION. C. Muguruza, M. Lehtonen, N. Aaltonen, S.P.H. Alexander, L.F. Callado

10:15-10:30 O-1.11
SYNTHETIC CANNABINOIDS. L.A. Núñez-Domínguez

10:30-12:15 **Café y sesión de pósters**

12:15-13:45 **Sesión de comunicaciones orales**
"Sistema endocannabinoide: efectos neuroprotectores y neuroproliferativos (I)"
(moderadores: Peter McCormick y Susana Mato)

12:15-12:30 Presentación (Peter McCormick)

12:30-12:45 O-2.1
ADDITIVE NEUROPROTECTIVE EFFECT OF CANNABIDIOL AND HYPOTHERMIA IN HYPOXIC-ISCHEMIC PIGLETS. H. Lafuente, M.R. Pazos, N. Mohamed, M. Ceprian, M. Santos, L. Arruza, F.J. Alvarez Diaz, J. Martínez-Orgado

12:45-13:00 O-2.2
ROLE OF 5HT1A RECEPTORS ON THE NEUROPROTECTIVE AND NEUROBEHAVIORAL EFFECTS OF CANNABIDIOL IN HYPOXIC-ISCHEMIC NEWBORN PIGS. M.R. Pazos, H. Lafuente, L. Barata, M. Ceprián, M. Santos, F.J. Alvarez, J. Martínez-Orgado

13:00-13:15 O-2.3
CANNABIDIOL PROMOTES OLIGODENDROCYTE SURVIVAL AFTER HYPOXIA-ISCHEMIA IN NEWBORN RATS. M. Ceprián, M.R. Pazos, F. Penna, M. Santos, J. Martínez-Orgado.

13:15-13:30 O-2.4
OLEOYLETHANOLAMIDE AND PALMITOYLETHANOLAMIDE EXERT NEUROPROTECTION OF CULTURE CORTICAL NEURONS SUBJECTED TO HYPOXIA. M.V. López, F. Rodríguez de Fonseca, P. Robledo, R. Maldonado, E. Fernández Espejo

13:30-13:45 O-2.5
SEXUAL DIMORPHISMS IN TIME-RELATED SEQUELAE AND RECOVERY COURSE AFTER TRAUMATIC BRAIN INJURY IN MICE. A FOCUS ON CB1 AND CB2 CANNABINOID RECEPTORS. A.B. López-Rodriguez, E. Acaz-Fonseca, L.M. Garcia-Segura, M.P. Viveros

13:45-15:30 Comida

15:30-17:00 Sesión de comunicaciones orales
"Sistema endocannabinoide: efectos neuroprotectores y neuroproliferativos (II)"

15:30-15:45 O-2.6
A RESTRICTED POPULATION OF CB1 CANNABINOID RECEPTORS WITH NEUROPROTECTIVE ACTIVITY. A. Chiarlone, L. Bellocchio, C. Blázquez, E. Resel, E. Soria-Gómez, J.J. Ferrero, O. Sagredo, C. Benito, J. Romero, J. Sánchez-Prieto, B. Lutz, J. Fernández-Ruiz, G. Marsicano, I. Galve-Roperh, M. Guzmán

15:45-16:00 O-2.7
NEUROPROTECTION WITH CANNABIGEROL IN HUNTINGTON'S DISEASE: STUDIES IN 3-NITROPROPIONATE-LESIONED OR R6/2 TRANSGENIC MICE. S. Valdeolivas, C. Navarrete, I. Cantarero, J. Fernández-Ruiz, E. Muñoz, O. Sagredo

16:00-16:15 O-2.8
POTENTIAL OF CANNABINOID CB1 AND CB2 RECEPTORS IN A ZEBRAFISH MODEL OF AMYOTROPHIC LATERAL SCLEROSIS. M. Moreno-Martet, M. Timmers, J. Fernández-Ruiz, E. de Lago, W. Robberecht

16:15-16:30 O-2.9
REGULATION OF AMYLOID-INDUCED NEUROINFLAMMATION BY ENDOCANNABINOIDS. C. Vázquez, R.M. Tolón, M. Caraza, E. Calleja, A. Gutiérrez, J. Romero

16:30-16:45 O-2.10
THE ANTI-INFLAMMATORY EFFECT OF A CANNABIGEROL DERIVATIVE IN EAE INVOLVES MULTIPLE CELLULAR TARGETS. F. Carrillo-Salinas, C. Navarrete, M. Mecha, A. Feliú, J.A. Collado, I. Cantarero, E. Muñoz, C. Guaza

16:45-17:00 O-2.11
CB1 CANNABINOID RECEPTOR-DEPENDENT ACTIVATION OF MTORC1/PAX6 SIGNALING DRIVES TBR2 EXPRESSION AND BASAL PROGENITOR EXPANSION IN THE DEVELOPING MOUSE CORTEX. J. Díaz-Alonso, T. Aguado, A. de Salas-Quiroga, Z. Ortega, I. de Prada, M.Á. Pérez-Jiménez, E. Aronica, M. Guzmán, I. Galve-Roperh

17:00-18:30 Café y sesión de pósters

18:30 Asamblea de la SEIC

21:30 Cena del congreso (Hotel Silken Ramblas)

Sábado, 30 de noviembre

9:00-10:15 Premios a las Mejores Publicaciones 2013
(presentados por Andrés Ozaita y Emilio Fernández Espejo)
Mejor Publicación Predoctoral
Mejor Publicación Posdoctoral

10:15-12:00 Sesión de comunicaciones orales
"Sistema endocannabinoide: otros efectos"
(moderadores: Juan Suárez y Ekaitz Agirregoitia)

10:15-10:30 Presentación (Juan Suárez)

10:30-10:45 O-3.1
NOCICEPTIVE, EMOTIONAL AND COGNITIVE BEHAVIOURS IN A MOUSE MODEL OF OSTEOARTHRITIS PAIN: INVOLVEMENT OF CB1 AND CB2 CANNABINOID RECEPTORS. C. La Porta, S. A. Bura, F. Navarrete, J. Manzanares, R. Maldonado

10:45-11:00 O-3.2
THE ROLE OF GLUTAMATERGIC CB1 CANNABINOID RECEPTORS IN THE REGULATION OF OBESITY. A. Aparisi-Rey, I. Ruiz de Azúa, S. Guggenhuber, B. Lutz

11:00-11:15 O-3.3
EFFECT OF CANNABINOID DURING OOCYTE IN VITRO MATURATION ON IN VITRO FERTILIZATION AND EMBRYO DEVELOPMENT IN MOUSE. E. Agirregoitia, A.P. López-Cardona, N. Agirregoitia, A. Gutiérrez-Adán

11:15-11:30 O-3.4
ROLE OF THE MIDKINE / ANAPLASIC LYMPHOMA KINASE AXIS IN THE RESISTANCE TO CANNABINOID ANTITUMORAL ACTION IN GLIOMA STEM CELLS. I. López-Valero, D. Dávila, M. Lorente, S. Torres, J. González, M. Guzmán, G. Velasco

11:30-11:45 O-3.5
DUAL ROLE OF THE CB2 CANNABINOID RECEPTOR IN HER2-POSITIVE BREAST CANCER. E. Pérez-Gómez, S. Blasco-Benito, C. Andradas, M.M. Caffarel, E. Moreno, E. García-Taboada, R. Hernando-Llorente, E.I. Canela, P.J. McCormick, M. Guzmán, C. Sánchez

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THE ORPHAN RECEPTOR GPR55 CONFERS PRO-METASTATIC ADVANTAGES ON BREAST CANCER CELLS IN VITRO AND IN VIVO. C. Andradas, E. Pérez-Gómez, S. Blasco-Benito, P. Dillenburg-Pilla, D. Megías, M. Quintanilla, JS. Gutkind, M. Guzmán, C. Sánchez

12:00-12:30 Café

12:30 Entrega de premios y Clausura

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REGULATION OF APOPTOTIC PATHWAYS BY AM630, AN ANTAGONIST/INVERSE AGONIST AT THE CANNABINOID CB2 RECEPTOR, IN MOUSE BRAIN. M. Álvaro-Bartolomé, G. Salort, J.A. García-Sevilla

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THE ACYLETHANOLAMIDES OEA AND PEA SHOW ANTI-INFLAMMATORY PROPERTIES IN LPS-INDUCED NEUROINFLAMMATION. A. Said, M. Antón, F. Alén, J.R. Caso, J. Pavón, J.C. Leza, F. Rodríguez de Fonseca, L. Orio, B. García-Bueno

P.3.
ANTIINFLAMMATORY EFFECTS OF OLEOYLTHETHANOLAMIDE IN A MODEL OF BINGE ALCOHOL DRINKING. M. Antón, F. Alén, R. Gómez de Heras, J. Pavón, J.C. Leza, F. Rodríguez de Fonseca, B. García-Bueno, L. Orio

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THE TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 IS REVEALED IN DENTATE INHIBITORY SYNAPSES BY HIGH RESOLUTION IMMUNOELECTRON MICROSCOPY. M.J. Canduela, J.L. Mendizabal-Zubiaga, A. Ramos, L. Reguero, N. Puente, P. Grandes

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NEGATIVE EMOTIONAL ALTERATIONS DURING SPONTANEOUS THC WITHDRAWAL ARE NOT MODULATED BY HYPOCRETIN RECEPTOR-1. A. Flores, R. Maldonado, F. Berrendero

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CORTICAL DEVELOPMENT ALTERATIONS INDUCED BY EMBRYONIC Δ9-TETRAHYDROCANNABINOL ADMINISTRATION ALTER SKILLED MOTOR ACTIVITY AND SEIZURE SUSCEPTIBILITY. J. Díaz-Alonso, A. de Salas-Quiroga, D. Vega, D. García-Rincón, Z. Ortega, B. Lutz, M. Guzmán, I. Galve-Roperh

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P.13.
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P.17.
DEAZA-ANALOGUES OF SR144528: DESIGN, SYNTHESIS, PHARMACOLOGICAL EVALUATION AND DOCKING STUDIES OF SELECTIVE CB2 RECEPTOR LIGANDS. G. Ragusa, M. Gómez-Cañas, P. Morales Lázaro, J. Fernández-Ruiz, N. Jagerovic, D.P. Hurst, P.H. Reggio, M. García Arencibia, G. Murineddu

P.18.
MATERNAL COMSUMPTION OF HIGHLY PALATABLE FOOD DURING PERINATAL PERIOD ALTERS ADIPOSITY, ANXIETY-RELATED BEHAVIOUR AND THE RESPONSE TO AM251 IN OFFSPRING. M.T. Ramírez-López, Vázquez, F. Alén, M. Antón, R.N. Blanco, M. Gutiérrez-Morales, D. Ouro, L. Orio, F. Rodríguez de Fonseca, R. Gómez de Heras

P.19.
THE CB1 CANNABINOID RECEPTOR IS PREDOMINANTLY LOCALIZED AT ASYMMETRIC EXCITATORY SYNAPSES IN THE GRANULE CELL LAYER OF THE MOUSE MAIN OLFACTORY BULB. L. Reguero, N. Puente, I. Elezgarai, E. Soria, G. Marsicano, P. Grandes

ORAL 1.1.

BASOLATERAL/BASOMEDIAL AMYGDALA CB1 EXPRESSION IN A MODEL OF DRUG SEEKING INCUBATION

D. Roura-Martínez[1], R. Santos-Toscano[1], M. Miguéns[2], A. Higuera-Matas[1], E. Ambrosio[1]

[1]Departamento de Psicobiología, Facultad de Psicología, UNED, 28040, Madrid,
[2]Departamento de Psicología Básica I, Facultad de Psicología, UNED, 28040, Madrid.

It was suggested in 1986 that cue-induced drug craving in cocaine addicts progressively increases over the first several weeks of abstinence and remains high for extended periods of time, a phenomenon called *incubation of craving.* During the past decade, investigators have identified an analogous incubation phenomenon in rodents, in which time-dependent increases in cue-induced drug seeking are observed after withdrawal from intravenous drug self-administration. Most of the data available regarding this phenomenon come from cocaine self-administration experiments while information regarding incubation of opiate drugs seeking is scarce. In our experiments we study the incubation of cocaine and heroin craving in parallel and have recently observed a dramatic increase in the adrenal glands weight in animals after one day of both cocaine and heroin withdrawal. Interestingly, this effect disappeared after protracted abstinence. The amygdaloid complex is a target of the stress hormones secreted by these glands. In addition, the pathway linking this limbic region with the nucleus accumbens has been implicated in the incubation phenomenon. Moreover, endocannabinoids in the basolateral amygdala mediate stress-induced modulation of memory, an important component of the incubation effect. Taking all these pieces of evidence in consideration, we decided to analyze the gene expression of the CB_1 receptor in the basolateral/basomedial amygdala of rats that had self-administered cocaine, heroin or their saline controls, after 1 or 30 days of forced abstinence. According to our preliminary results, no changes in CB_1 gene expression were observed neither after acute nor protracted withdrawal. Our next goal will be to measure the gene expression of the enzymes involved in the synthesis and degradation of anandamide and 2-arachidonoylglycerol to gain a more detailed understanding of the role of the endocannabinoid system in the incubation of drug seeking.

This work was supported by grants from the Spanish Ministry of Health, Social Services and Equality (grant PND-2012I057), the Carlos III Health Institute (grant RD12/0028/0020) and Comunidad de Madrid (grant S2010/BMD-2308; CANNAB Consortium).

ORAL 1.2.

BLOCKADE OF CANNABINOID CB1 AND CB2 RECEPTORS PREVENTS COCAINE-INDUCED REDUCTION OF CELL PROLIFERATION AND COCAINE-INDUCED INCREASE OF GLIOSIS IN ADULT MALE RATS

J. Suárez[1] , P. Rivera[1], S. Arrabal[1], A. Vargas[1], F.J. Pavón[1], A. Serrano[1], E. Castilla-Ortega[1], P. Galeano[1], E. Blanco-Calvo[2], F. Rodríguez de Fonseca[1]

[1]Laboratorio de Investigación (UGC Salud Mental), Instituto de Investigación Biomédica (IBIMA), Complejo Hospitalario de Málaga (Hospital Carlos Haya), Avda. Carlos Haya 82, Pabellón de Gobierno, 29010, Málaga, Spain. [2]Departament de Pedagogia i Psicologia. Facultat de Ciències de l'Educació. Universitat de Lleida. Avda. de l'Estudi General 4, 25001, Lleida, Spain.

Changes in the activity of the cannabinoid receptors CB1 and CB2 influence on neurogenesis, inflammation and cell death in the brain. However, it is still unknown how the impact of drug addiction on these cellular processes is modulated by the endocannabinoid system. In the present study, we evaluated how the pharmacological blockade of CB1 (Rimonabant, 3 mg/kg) and CB2 (AM630, 3 mg/kg) affects cell proliferation (BrdU), found in the subventricular zone (SVZ) of the lateral ventricles and the dentate subgranular zone (SGZ), as well as apoptosis (cleaved caspase-3) and gliosis (GFAP and Iba-1) in the striatum and hippocampus, during acute or sub-chronic (5 days) cocaine administration (20 mg/kg). Results showed that acute cocaine decreased the number of $BrdU^+$ cells in SVZ and SGZ. In contrast, sub-chronic cocaine reduced the number of $BrdU^+$ cells in SVZ only. Both acute and sub-chronic cocaine increased the number of cleaved caspase-3^+, $GFAP^+$ and $Iba1^+$ cells in the hippocampus. Repeated AM630 or Rimonabant administration increased the reduced number of $BrdU^+$ cells observed in SVZ and SGZ, and reduced the increased number of cleaved caspase-3^+ and $GFAP^+$ cells observed in the striatum and hippocampus of sub-chronic cocaine-treated rats. Iba^+ cells were specifically decreased in the striatum after the co-administration of repeated cocaine with AM630 or Rimonabant. These results indicate that changes on neurogenic, apoptotic and gliosis processes, which were produced as a consequence of sub-chronic cocaine administration, were normalized by the pharmacological blockade of CB1 and CB2. This study stimulates to further research whether the pharmacological cannabinoid blockade could be a therapeutic strategy in the treatment of psychostimulant addiction.

This work was supported by grants from RD12/0028/0009, PNSD2010/143 and SAS111224.

O.1.3.

INVOLVEMENT OF CANNABINOID RECEPTORS IN THE COGNITIVE DEFICTS ASSOCIATED WITH NICOTINE WITHDRAWAL

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Abstinence from smoking produces a range of withdrawal symptoms including impaired attention and memory. Some studies suggest that relapse to tobacco use after a period of abstinence may occur to ameliorate these cognitive deficits. Moreover, poor cognitive performance during nicotine abstinence has been shown to predict more rapid smoking resumption. The objective of this study was to investigate the possible neurobiological mechanisms underlying this nicotine effect. Nicotine was administered by using Alzet osmotic minipumps (25 mg/kg/day/14days) and withdrawal was precipitated by the administration of the nicotinic receptor antagonist, mecamylamine (2 mg/kg). A deficit in memory consolidation was observed when animals performed the object recognition task 24 hours after the precipitation of withdrawal, and this cognitive deficit was still present at least during 4 days. Interestingly, memory impairment was abolished by the administration of the CB_1 antagonist rimonabant (1 mg/kg), and the CB_2 antagonist, SR144528 (1 mg/kg). In agreement, this effect was also blocked in CB_1 and CB_2 receptor knockout mice. The metabotropic glutamate receptor 5 (mGluR5) is known to be involved in the biosynthesis of endocannabinoids. We observed that administration of the mGluR5 antagonist, MTEP (1 mg/kg), prevented the memory impairment associated with nicotine abstinence. Futhermore, mTOR pathway and protein synthesis were found to be involved in this effect. Changes in glutamate receptor surface expression in the hippocampus are related to plasticity and cognition. However, no effect of nicotine withdrawal on AMPA or NMDA receptor surface expression, measured by biotinylation, was found 60 minutes after the precipitation of nicotine abstinence. These results suggest that the activation of the endocannabinoid system could be, at least in part, responsible for the cognitive deficits observed during nicotine abstinence.

O.1.4.

CEREBELLAR CYCLOOXYGENASE-2 (COX-2) PLAYS A CRUCIAL ROLE IN THE CEREBELLAR FUNCTIONAL DEFICITS ASSOCIATED TO REPEATED CANNABIS EXPOSURE

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Heavy cannabis exposure has been associated to cerebellar dysfunction in humans. Possible involvement of some neuroinflammatory molecules such as interleukin 1β (IL-1β) or Cyclooxygenase-2 (COX-2) in the alteration of the cerebellar functions has been previously demonstrated. Moreover, it has been described that neuronal COX-2 is expressed in the plasma membrane of cerebellar Purkinje cells and its expression is up-regulated following brain insults, via glutamatergic and inflammatory mechanisms. We have previously reported that the repeated exposure to delta9-tetrahydrocannabinol (THC) in mice mimics the residual cerebellar functional deficits observed in heavy cannabis consumers. Under these experimental conditions, quantitative real time PCR analysis revealed a clear enhancement of COX-2 mRNA expression level, as well as significant modifications in the expression of prostaglandin receptors genes (Ptger1, Ptger2, Ptger3 and Ptger4). Moreover, we determined that these changes are readily detectable in the cerebellum of cannabinoid receptor type 1 (CB1) constitutive knockout mice (CB1 KO), suggesting that CB1 receptor down-regulation in the cerebellar molecular layer regulates the COX-2 expression. These results correlated with the motor coordination deficits and the enhancement in the Purkinje cells excitability observed in CB1KO mice. This excitability was evaluated by measuring the expression of the activity-regulated cytoskeleton-associated protein (Arc/Arg3.1). Interestingly, the pharmacological blockade of COX-2 after a sub-chronic administration of NS-398 (10mg/kg; 4 days; once per day), significantly reduced the motor coordination deficits as well as the Arc/Arg3.1 increased expression observed in the CB1 KO mice. These results suggest the important role of COX-2 in the performance of cerebellar-dependent functions, and reveal this enzyme as a crucial element in the cerebellar deficits associated to repeated cannabis exposure.

O.1.5.

COGNITIVE IMPAIRMENT INDUCED BY DELTA-9-TETRAHYDROCANNABINOL OCCURS THROUGH HETEROMERS BETWEEN CANNABINOID CB1 AND SEROTONIN 2A RECEPTORS

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One of the major consequences of cannabis use is memory impairment. Behavioural experiments using WT and 5-HT2AR KO animals revealed that these receptors are necessary for the amnesic- and anxiolytic-like effects of delta-9-tetrahydrocannabinol (THC) but not for the analgesic, hypothermic and hypolocomotor effects of THC. In trying to understand the molecular basis of these differences we found that type 1 cannabinoid receptors (CB1R) and serotonin 2A receptors (5-HT2AR) form heteromers in cells and brain tissue using both energy transfer techniques and the Proximity Ligation Assay. CB1R-5HT2AR heteromers were detected in approximately 60-70% of cortical, hippocampal and striatal neurons of WT mice. Interestingly, we showed for the first time that the formation of the heteromer leads to a shift in G-protein coupling for 5-HT2AR from Gq to Gi proteins. In a cell model, we found that cAMP signalling was greatly reduced via the heteromer upon co-activation of both receptors as compared to the single receptor activation, while ERK 1/2 signalling was not affected. This molecular dissociation could possibly account for the differential effects observed on specific behavioural responses. Cross-talk and cross-antagonism via the heteromer were observed in cells and brain tissue from WT but not KO mice lacking 5-HT2AR. Furthermore, the cross-antagonism has also been confirmed in behavioural experiments using C57BL/6J mice as both the amnesic- and anxiolytic-like effects induced by THC were blocked by the administration of the 5-HT2AR antagonist, MDL 100,907. These data reveal a novel molecular mechanism for the functional interaction between CB1R and 5-HT2AR mediating cognitive impairment.

O.1.6.

MEDIAL BUT NOT LATERAL ORBITOFRONTAL CB_1 GENE EXPRESSION PREDICTS IMPULSIVE CHOICE IN RATS

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Impulsivity is the tendency to act prematurely without foresight. It is a multifaceted construct that can be divided in impulsive choice and impulsive action. Impulsive choice can be studied using delay discounting paradigms whereby subjects choose between an immediate but small reward and a delayed but larger reward. Using this behavioural approach, THC has been found to reduce impulsive choice in rats. Behavioral and neurobiological evidence suggests that the different varieties of impulsivity depend on distinct cortico-striatal substrates. The orbitofrontal cortex (OFC) is implicated in a variety of adaptive decision-making processes and human and animal studies suggest that there is a functional dissociation between medial and lateral OFC (mOFC and lOFC, respectively) subregions when performing certain choice procedures. Indeed, mOFC-lesioned rats show increased, whereas lOFC-lesioned rats show decreased preference for the larger-delayed reward in a delay discounting experiment. In this study we wanted to examine if this double dissociation would be present at the level of CB_1 receptors. We submitted Wistar rats to a delay discounting procedure and used non-linear regression to analyze their discounting curves. Cluster analysis was performed to separate rats in two groups according to their performance. We found that medial but not lateral orbitofrontal CB_1 gene expression predicted the impulsive choice of rats. Moreover, a significant difference was observed in mOFC CB_1 gene expression levels between high and low impulsive animals when separated by cluster analysis. These results suggest that manipulations of the endocannabinoid system might be a potential therapeutic approach to treat impulse control disorders.

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O. 1.7.

ANANDAMIDE AND 2-AG MODIFY THE EXPRESSION OF CONDITIONED FEAR IN OPPOSITE DIRECTIONS

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We systematically investigated the role of the endocannabinoid system in the expression of auditory-cued fear memories in mice. Administration of the CB1 antagonist/inverse agonist SR141716 (3 mg/kg) caused an increase in conditioned freezing upon repeated tone presentation. Blockade of CB2 receptors by AM630 (3 mg/kg), in contrast, had opposite effects during the first tone presentation, with no effects of the TRPV1 antagonist SB366791 (1 and 3 mg/kg). Administration of the CB2 agonist JWH133 (3 mg/kg) failed to affect the freezing response, whereas the CB1 agonist CP55,940 (50 μg/kg) augmented this behavior. The endocannabinoid uptake inhibitor AM404 (3 mg/kg), but not VDM11 (3 mg/kg), significantly reduced the freezing response. Its co-administration with SR141716 or SB366791 confirmed an involvement of both CB1 and TRPV1. Inhibition of AEA degradation by the FAAH inhibitor URB597 (1mg/kg) had similar consequences as AM404. Inhibition of 2-AG degradation by the MAGL inhibitor JZL184 (4 and 8 mg/kg), in contrast, caused a pronounced increase in freezing, similar to CP55,940. This provides first evidence for opposite consequences of 2-AG vs. AEA in the same behavioral dimension. As revealed in conditional CB1-deficient mutants, fear-promoting effects of 2-AG depended on CB1 signaling in GABAergic neurons. Our findings suggest that increased AEA levels mediate acute fear relief via CB1 on glutamatergic neurons, while increased 2-AG levels promote the expression of conditioned fear via CB1 on GABAergic neurons. This dichotomy in endocannabinoid action has to be considered for the exploitation of the endocannabinoid system as a treatment of psychiatric disorders associated with exaggerated fear responses.

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O.1.8.

ENDOCANNABINOIDS, MATERNAL CARE AND SLEEP

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Endocannabinoids (eCBs) are sleep inducers, as several studies have shown. Maternal care (MC) is a crucial early stimulation for healthy development, as it has been proven in studies in which rats have been MC deprived. Several changes occur in the behavior, and in the brain once pups have been subjected to MC deprivation. Among other observations is the reduction in sleep expression. Likewise, MC deprivation seems to facilitate substance use disorder (SUA). In this study our goal was to reproduce the sleep loss observed after MC deprivation in rats. To obtain this information rats were MC deprived (MCD) from post natal day (PND)2 to PND16. Control pups remained under MC. Then they were allowed to grow until they reach adulthood (PND90). They were permanently implanted with a set of electrodes for sleep recordings. Rats were habituated to the recording conditions, then recorded for 24h after the systemic administration of vehicle (grpMC, n=10; grpMCD, n=10) or oleamide (grpMC, n=10; grpMCD, n=10). In two additional grps (MC and MCD), we evaluated the expression of Cannabinoid Receptor 1 (CB1) in the frontal cortex (FC), in the hippocampus (HP) and in the nucleus accumbens (NAc). Likewise, we evaluated the microanatomical characteristics of the neurons in the FC and NAc in MC and MCD rats. Results indicated that MCD rats exhibit less sleep as compared to MC rats. In addition, oleamide (an eCBs), restores sleep in MCD rats. CB1 receptor was found reduced in the FC and in the HP; while was augmented in the NAc. Dendrite arborization was reduced while spines augmented in the FC and NAc of MCD. These results indicate that MCD induces changes in the sleep-waking cycle associated to changes in the expression of CB1 receptor. Likewise, dendrites in FC and NAc were affected. We conclude that MC is important to maintain the expression of CB1 receptor and the correct development of dendrites. We also conclude that changes in the CB1 affect the normal expression of sleep.

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O.1.9.

A PERIPHERAL ENDOCANNABINOID MECHANISM FOR STRESS-INDUCED AMNESIA

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Memory consolidation is a labile process under the direct impact of emotional experiences, but little is known concerning the underlying mechanisms. The endocannabinoid system plays an important role in the modulation of both emotions and memory, although the integration of these functions in the memory consolidation processes has not been addressed. This study reveals that type-1 cannabinoid (CB1) receptors mediate stress-induced amnesia. Using a model for declarative memory in mice, the novel object-recognition test, we found that stress and the arousal state of the animal determines the consolidation outcome in this non-emotional memory. Such a memory trace was obliterated under different acute stress conditions or corticosterone administration. These amnesic-like effects of stress and corticosterone were not observed after pharmacological or genetic blockade of CB1 receptors. Using several cell type-specific conditional CB1 receptor knockout mouse lines, we found that CB1 receptors present in noradrenergic dopamine β-hydroxylase-expressing neurons are necessary for stress-induced amnesia, whereas CB1 receptors in other brain neuronal populations were not involved in this response. Interestingly, the peripherally acting CB1 receptor antagonist AM6545 or the removal of the adrenal glands prevented the amnesic-like effect of stress. Moreover, peripheral antagonism of beta-adrenergic receptor-mediated signaling prevented the protective effect of CB1 receptor inhibition on memory.

In conclusion, peripheral noradrenergic transmission determines the consolidation of non-emotional memories and this function is under the direct control of peripheral CB1 receptors. The elucidation of this mechanism opens novel therapeutic approaches for the treatment of memory- and stress-related disorders through peripherally acting drugs for CB1 cannabinoid receptors.

O.1.10.

EVALUATION OF THE ENDOCANNABINOID SYSTEM IN *POSTMORTEM* BRAIN OF SUBJECTS WITH MAJOR DEPRESSION

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The potential role of the endocannabinoid system (ECS) in several mental disorders, including Major Depression (MD), has been widely discussed (Ashton and Moore, 2011). Thus, recent studies have reported altered serum concentrations of the main endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), in depressed women relative to matched controls (Hill et al., 2009). Additionally, alterations in the expression and functionality of the CB1 receptor have been also described in *postmortem* brain of patients with major depression (Hungund et al., 2004; Koethe et al., 2007; Choi et al., 2012). However, the status of endocannabinoids and other elements comprising the ECS on the brain of patients with MD remain to be elucidated.

The aim of the present study was to evaluate different ECS components in the *postmortem* prefrontal cortex (PFC) —Brodmann's area 9— of subjects with an *antemortem* diagnosis of MD (DSM-IV) (n=20) and control subjects matched by age, gender and *postmortem* delay (n=20). Additionally, in order to investigate the potential effect of antidepressant (AD) treatment, subjects with MD were divided in two groups —AD-free (n=7) and AD-treated (n=13)— according to the absence or presence of AD drugs in the toxicological screening.

In the PFC samples: 1) levels of 2-AG, AEA, DHEA, LEA, PEA and OEA were measured by quantitative liquid chromatography coupled to mass spectrometric detection; 2) mRNA expression levels of CB1 receptor and the two main endocannabinoid degrading enzymes (FAAH and MAGL) were measured by qRT-PCR assays and 3) [^{35}S]GTPγS binding assays and enzyme hydrolysis functional assays were carried out in order to determine the CB1 receptor dependent G-protein activation and the enzyme activity of FAAH and MAGL respectively.

No statistically significant differences were found between MD subjects and controls in the PFC levels of any of the endocannabinoids studied, even when discriminating between AD-free and AD-treated subjects. Likewise, no differences were observed in the mRNA levels of the degrading enzymes FAAH and MAGL between MD and control subjects regardless of the AD treatment. However, a statistically significant increase in the FAAH maximum enzyme velocity (V_{max}) was found in the PFC of AD-treated MD subjects compared to controls (1.36±0.09 vs. 1.01±0.09 nmol/min/mg protein; $p<0.01$), but not in those who were AD-free (1.04±0.14 vs. 0.97±0.06 nmol/min/mg protein; $p=0.66$). No significant differences were found neither in CB1 receptor mRNA levels nor in the CB1 receptor dependent stimulation of [^{35}S]GTPγS binding between MD subjects —AD-free or AD-treated— and controls.

These results showed that AD treatment is able to modulate the FAAH activity in the PFC of subjects with MD. However, the clinical relevance of this modulation exerted by currently available AD drugs remains to be investigated.

O.1.11.

SYNTHETIC CANNABINOIDS

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Synthetic cannabinoids are a new form of cannabinoids that presents an inportant increase of use in the last years. They han been shown as a “secure” form of cannabis use, altough some recent reports have demostrated diferent harmfull efects into general populations. I make a review of recent reports and discused the results

O.2.1.

ADDITIVE NEUROPROTECTIVE EFFECT OF CANNABIDIOL AND HYPOTHERMIA IN HYPOXIC-ISCHEMIC PIGLETS

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Background: The proportion of newborns beneficed from hypothermia (HT) after brain hypoxia-ischemia (HI) is limited. Thus, looking for synergistic therapies is warranted. Cannabidiol (CBD) has shown neuroprotective effects in animal models of newborn HI brain damage.
Objective: To test the possible additive neuroprotective effects of CBD and HT.
Design/Methods: Sedated and ventilated piglets (1-2 day-old) underwent HI brain damage (hypoxia -FiO2 10%- + bilateral carotid artery compression for 30 min). Then, normothermic (NT) piglets were maintained at 37-38 C using a warmed air blanket; HT piglets were cooled by a cool water mattress to 33-34 ºC. Thirty min after HI piglets received i.v. vehicle (VEH) or CBD 1 mg/kg. Six hours after HI brains were obtained for histological studies quantifying the number of neurons (Nissl) and astrocytes (GFAP) in parietal cortex and for proton magnetic resonance spectroscopy (H-MRS) to quantify Lac/NAA, Glu/NAA and GSH/Cr ratios. Blood samples were obtained for ELISA studies to quantify the IL1-Beta global levels. Similarly studied animals without HI insult served as controls (SHM).

Results:

Parameter	SHM+NT	VEH+NT	CBD+NT	SHM+HT	VEH+HT	CBD+HT
Histology						
Necrotic neurons (%)	4.7(1.3)	*25.5(4.8)*	***11.8(3.2)***	1(0.1)	*13.6(1.3)*	**3.7(0.9)**
Astrocytes (n)	33(3.2)	31.1(2.2)	***39.4(3.8)***	33.4(1.3)	*23.8(0.6)*	**34.3(1.5)**
Astrocyte size (pixel)	365(67)	392(57)	***459(33)***	391(20)	375(21)	***523(16)***
H-MRS						
Lac/NAA	2.4(0.2)	*6.6(2.3)*	**2.9(0.3)**	1.4(0.1)	*2.3(0.1)*	**1.6(0.1)**
Glu/NAA	0.51(.02)	*0.61(0.04)*	**0.49(0.03)**	0.42(0.02)	*0.45(0.02)*	**0.39(0.01)**
GSH/Cr	0.17(0.01)	*0.11(0.01)*	**0.17(0.01)**	0.16(0.01)	*0.14(0.01)*	**0.16(0.01)**
ELISA						
IL1-Beta (pg/mL)	226.4(98.3)	*1590.4(361)*	***689.6(172.3)***	157.1(1.2)	*237(49.9)*	**125.6(8.4)**

Mean(SEM). Lac: lactate. NAA: n-acylaspartate. Glu: glutamate. GSH: reduced glutathione.
(*Italic*) P<0.05 vs SHM; (**Bold**) p<0.05 vs VEH

Neuroprotection was CBD+HT>VEH+HT=CBD+NT>VEH+NT in terms of reduction of neuronal death and Lac/NAA ratio increase. CBD prevented HI-induced reduction in number of astrocytes and enhanced astrocyte activity. Excitotoxicity modulation was CBD+HT>VEH+HT=CBD+NT> VEH+NT. Antioxidant effect was CBD+HT>CBD+NT>VEH+HT>VEH+NT. CBD has significant anti-inflammatory effects.
Conclusions: CBD is at least as efficient as neuroprotectant as HT, both therapies showing additive neuroprotective effects.

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O.2.2.

ROLE OF 5HT1A RECEPTORS ON THE NEUROPROTECTIVE AND NEUROBEHAVIORAL EFFECTS OF CANNABIDIOL IN HYPOXIC-ISCHEMIC NEWBORN PIGS

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Background: Blockade of 5HT1A receptors (5HT1AR) antagonizes the neuroprotective effects of cannabidiol (CBD) as shown in the first 6 hours after a hypoxic-ischemic (HI) insult in newborn pigs (Pazos et al, Neuropharmacology 2013). The aim of the present work was to study whether or not 5HT1A blockade interferes with CBD neuroprotective and neurobehavioral effects in a longer period-72 h.

Methods: 1 day-old piglets were studied for 72 h after a HI insult (carotid clamp and FiO2 10% for 20 min). Thirty min after HI piglets received vehicle (HV, n=8) or CBD 1 mg/kg single dose or in 3 repeated doses u.i.d (respectively, HC1, n=5; and HC3, n=6). Non HI piglets served as controls (SHM, n=4). Other animals were similarly treated but receiving the 5HT1AR antagonist WAY100630 1 mg/kg/12 h for 3 days, 15 min before the corresponding VEH or CBD dose (respectively, HVW, n=4; HC1W, n=4; HC3W, n=4; and SHMW, n=3). Every 24 h brain activity was assessed by amplitude-integrated EEG (aEEG) and a neurobehavioral score was carried out including eating behaviour assessment. Object-related and social playfulness activity was assessed by video recording, and anxiety was quantified by the restless time during holding for aEEG recording.

Results: CBD treatment restored brain activity (aEEG) and neurobehavioral performance 72 h postHI (Table). In addition, CBD induced an anxiolytic effect and restored playfulness. There were no differences between HI piglets receiving single dose or three doses of CBD. CBD neuroprotective effects as shown by brain activity and motor performance was blunted by 5HT1AR antagonism in animals receiving CBD single dose but not in those receiving CBD for three days. CBD effects on anxiety and playfulness responses, however, were reversed by 5HT1AR antagonism in all CBD-treated animals no matter the dosage schedule. The 5HT1AR antagonist did not worse HI effects on vehicle-treated animals and had no effects on sham animals than mild hyperactivity and hyperphagia.

Item	SHM	HV	HC1	HC3	SHMW	HVW	HC1W	HCW3
aEEG (μV)	18(2)	*14(1)*	20(3)	19(2)	18(1)	*15(2)*	***12(3)***	18(2)
NBS (pts)	35.5(0.5	*29(2)*	35(1)	34.6(1)	34.6(1)	*28.6(1)*	***32.6(1)***	34.5(1)
Eating bhv (pts)	4.6(0.3)	*3.5(0.4)*	4.8(0.1)	4.8(0.2)	*3.8(0.2)*	4.3(0.2)	***3.1(0.3)***	4.4(0.1)
Restless (min)	3.5(1)	*6.7(1)*	*2.2(1)*	*2.4(1)*	3.6(1)	*6.6(1)*	***5.2(1)***	***6.3(1)***
Object play (%)	14(3)	*17(8)*	22(6)	24(8)	*42(13)*	15(3)	***10(2)***	***11(3)***
Social play (%)	52(6)	23(8)	40(6)	37(8)	*27(8)*	*7(2)*	***16(5)***	***15(6)***

Items measured 72 h postHI. *Italic:* $p<0.05$ vs. SHM. **Bold:** $p<0.05$ vs HC

Conclusions: 5HT1AR activation is involved in CBD neuroprotection in the first 24 hours postHI. Later on CBD is able to induce neuroprotection by 5HT1AR-independent mechanisms. 5HT1AR activation mediates anxiolytic and some behavioural effects of CBD.

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O.2.3.

CANNABIDIOL PROMOTES OLIGODENDROCYTE SURVIVAL AFTER HYPOXIA-ISCHEMIA IN NEWBORN RATS

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Background: Hypoxic-ischemic (HI) insults enhance the proliferation of oligodendrocyte (OL) precursors (preOL) in immature brain. Survival of those proliferating preOL, however, is very poor because those cells are particularly sensitive to oxidative stress and inflammaiton. This leads eventually to hypomyelinization, which plays a key role in the genesis of cerebral palsy. We described that cannabidiol (CBD) increases the number of proliferative cells in newborn rat brain after a hypoxic-ischemic (HI) insult. In the present work we aimed to determine how CBD treatment affects OL survival.
Methods: Unilateral HI brain damage was induced in newborn Wister rats (7-10 day-old: P7-P10) by exposure to hypoxia (10% FiO2) for 112 min after left carotid artery electrocoagulation under anaesthesia. Ten minutes after the end of HI pups received s.c. vehicle (HV, n=18) or CBD 1 mg/kg single dose (HC, n=24). Other pups remained as controls (SHM, n= 16). Seven or 30 days after HI rats were sacrificed, transcardially perfused with paraformaldehide (PFH) 4% and their brains cut off into coronal slices for immunohistochemical study on the subventricular zone (SVZ) in P14 rats: KI67 was used to detect proliferating cells, IBA-1 to detect microglial cells and Olig-2 and SOX-10 to detect undetermined and determined preOL, respectively. In P37 Myelin basic protein (MBP) fluorescence in the external capsule was used to quantify the presence of mature OL.
Results: 7 days postHI there were no differences between groups in the number of KI67+ cells in SVZ (0.53±0.03, 0.55±0.05 and 0.49±0.04 cells/area for SHM, HV and HC respect). CBD prevented the HI-induced decrease of undetermined preOL cells (0.49±0.03, 0.44±0.01 and 0.50±0.02 Olig2+ cells/area for SHM, HV and HC respect, $p<0.05$); such an effect did not reach statistical significance regarding determined preOL (0.54±0.0.3, 0.49±0.08 and 0.62±0.07 Sox10+ cells/area for SHM, HV and HC respect, $p=0.06$). 30 days postHI, HI led to a significant decrease of MBP signal in vehicle treated rats that was prevented by CBD (0.49±0.06, 0.32±0.07 and 0.52±0.08 MBP i.u./pixel for SHM, HV and HC respect, $p<0.05$). This effect of CBD on OL is associated with a decrease of oxidative stress (GSH/Cr ratio by H^+-MRS) and inflammation (TNFα by westernblot) by CBD in brain 7 days after HI, as reported.
Conclusions: CBD administration preserves OL maturation after a HI insult, preventing HI-induced hypomyelinization. This is likely related at least in part to the anti-inflammatory as well as the antioxidant effect of CBD

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O.2.4.

OLEOYLETHANOLAMIDE AND PALMITOYLETHANOLAMIDE EXERT NEUROPROTECTION OF CULTURE CORTICAL NEURONS SUBJECTED TO HYPOXIA

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Perinatal hypoxia-ischemia encefalopathy is defined as a neurological syndrome where the newborn suffers from acute ischemia and hypoxia during the perinatal period. It is quite important to develop new therapeutic approaches. The acylethanolamides oleoylethanolamide (OEA) and palmithoylethanolamide (PEA) are lipid compounds with neuroprotective properties in animal models of neurodegenerative diseases. However their role as neuroprotective drugs in brain damage derived from acute hypoxia remains to be fully determined. These acylethanolamides act through PPARα receptors mostly, although vanilloid TRPV receptors are also involved, and they are potentially therapeutic tools for acute neurodegeneration secondary to hypoxia/ischemia.

We have performed in vitro studies with culture cortical neurons from mouse pups (P2) in order to evaluate the protective efficacy of acylethanolamides, as well as the cannabinoid anandamide and acyl-sulfamides with activity at the PPARα receptor for comparison. Culture medium was made hypoxic by 20-min bubbling with 100% nitrogen. Neurons were obtained from parieto-temporal cortex. The LDH assay was employed for analyzing cell death. The findings revealed that both OEA and PEA exert neuroprotective effects given either before or after hypoxia. Effective doses were 40 and 20 μM OEA, and 40, 20 and 10 μM PEA. These responses were not modified when using neurons obtained from PPARα KO mice, or pretreatment with antagonists of TRPV1 (SB452533) and TRPV4 receptors (RN1734), suggesting that all of these receptors are not involved in the neuroprotective effects. Anandamide was found to be cytotoxic if given after hypoxia. Acyl-sulfamides were devoid of protective effects except for stearylpropylsulfamide at the highest dose (40μM).

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O.2.5.

SEXUAL DIMORPHISMS IN TIME-RELATED SEQUELAE AND RECOVERY COURSE AFTER TRAUMATIC BRAIN INJURY IN MICE. A FOCUS ON CB1 AND CB2 CANNABINOID RECEPTORS

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Traumatic brain injury (TBI) constitutes the primary cause of death in young individuals. This type of lesion triggers intracellular signalling cascades that activate glial cells leading to the secondary damage of TBI. This includes neurotoxicity, neuroinflammation, blood-brain barrier disruption, oxidative stress, brain oedema, axonal injury and functional impairment. We have recently provided the first evidence for the involvement of CB1 and CB2 cannabinoid receptors in the neuroprotective action of minocycline in a TBI murine model (male mice). Moreover, our results suggested that CB2 receptor has a relevant role modulating beta-amyloid peptide (β-APP) accumulation since a CB2R antagonist, when administered alone, enhanced the effect of TBI on diffuse axonal damage (Lopez-Rodriguez et al. Cerebral Cortex, 2013 in press). There are evidences indicating that there are sex differences in secondary damage after brain trauma and that hormonal fluctuations during the reproductive cycle play an important role in controlling the outcome after TBI (Roof and Hall. Journal of Neurotruma, 2000; 17(5): 637-88). The aim of this study was to determine the possible existence of sexual dimorphisms in time-related sequelae and recovery course, in relation to lesion severity, behavioural alterations and molecular changes, including CB1 and CB2 receptors, after TBI. Our results show that TBI induced, in both sexes, a neurological impairment 24h and 48h after lesion that recovered 2 weeks after lesion. However, when we measured oedema formation in the contralateral hemisphere, we observed that TBI induced an increase in the percentage of water content in males at 24h and 48h after TBI that disappeared 2 weeks after lesion whereas in females, the levels of oedema did not differ from the control at any time after lesion. The expression of CB1 mRNA decreased at 24h and 48h after TBI in males, whereas not changes were found for this parameter in females. CB2 mRNA expression levels increased progressively in males whereas in females there was a peak at 24h after lesion that recovered two weeks after trauma. Ongoing western blot analyses of the cannabinoid receptors and behavioural testing will allow us to discuss the functional significance of these sexual dimorphisms.

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O.2.6.

A RESTRICTED POPULATION OF CB_1 CANNABINOID RECEPTORS WITH NEUROPROTECTIVE ACTIVITY

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The CB_1 cannabinoid receptor, the main molecular target of endocannabinoids and cannabis active components, is the most abundant GPCR in the mammalian brain. The CB_1 receptor is particularly expressed on GABAergic terminals of the forebrain, while smaller amounts reside on terminals of glutamatergic neurons. Despite the widely-reported neuroprotective activity of the CB_1 receptor in animal models, the precise pathophysiological relevance of those two CB_1 receptor populations in neurodegenerative processes is unknown. Here, we first induced excitotoxic damage in the mouse brain (a) by administering quinolinic acid to conditional mutant animals lacking CB_1 receptors in either GABAergic or glutamatergic neurons, and (b) by manipulating corticostriatal glutamatergic terminals with a *designer receptors exclusively activated by designer drugs* pharmacogenetic approach. We next examined the alterations that occur in the R6/2 mouse, a well-established model of Huntington's disease, (a) upon fully knocking-out CB_1 receptors, and (b) upon deleting CB_1 receptors selectively in corticostriatal glutamatergic terminals or striatal GABAergic neurons. Altogether, the data show that the restricted pool of CB_1 receptors located on glutamatergic terminals plays an indispensable role in the neuroprotective activity of the endocannabinoid system, thereby suggesting that these precise CB_1 receptor molecules constitute the therapeutic target that might achieve neuroprotection in patients.

O.2.7.

NEUROPROTECTION WITH CANNABIGEROL IN HUNTINGTON'S DISEASE: STUDIES IN 3-NITROPROPIONATE-LESIONED OR R6/2 TRANSGENIC MICE

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The phytocannabinoids Δ^9-tetrahydrocannabinol and cannabidiol, used alone or in combination, have shown to be neuroprotective in experimental models of Huntington's disease (HD). These effects were provided by their capability to activate CB_1 and/or CB_2 receptors, but also through mechanisms independent of the cannabinoid receptors (e.g. activation of nuclear receptors of PPAR family). In the present study, we have investigated the non-psychotropic phytocannabinoid cannabigerol (CBG), which is devoid of any activity at the cannabinoid receptors, but it is antioxidant, can activate PPAR-γ receptors and serve as an antagonist of serotonin $5HT_{1A}$ receptors. In our hands, the compound was extremely active as a neuroprotectant compound in mice lesioned with the mitochondrial toxin 3-nitropropionate (3NP), an experimental model of HD useful to investigate compounds with antioxidant properties. In these animals, the administration of CBG (10 mg/kg, i.p., 4 days) improved the motor deficits typical of 3NP-lesioned mice, as a result of its positive effects in the preservation of striatal neurons against 3NP toxicity. CBG also attenuated the appearance of astrogliosis (labelled with GFAP), microgliosis (labelled with Iba-1) and the up-regulation of pro-inflammatory markers (e.g. COX-2, iNOS, TNF-α, IL-6) induced by 3NP, and improved the levels of antioxidant defenses (e.g. catalase, glutathione, superoxide dismutase-1) that were significantly reduced by 3NP insult. We also investigated the neuroprotective properties of CBG in R6/2 mice, a transgenic murine model of HD which produces a very aggressive pathological phenotype that recapitulates most of the cytotoxic mechanisms that operate in the human pathology. In these mice, the administration of CBG (10 mg/kg, i.p., 6 weeks) also produced certain beneficial effects which originated a partial recovery in the deteriorated rotarod performance typical of R6/2 mice. Using HD array analysis, we were able to identify a series of genes that have been frequently linked to this disease given their role in the regulation of gene transcription (e.g. symplekin, Sin3a, Rcor1, histone deacetylase 2, and huntingtin-associated protein 1), GABA transmission (e.g. δ subunit of the GABA-A receptor), and calcium homeostasis (e.g. hippocalcin). We found the expression of these genes altered in R6/2 mice and partially normalized by CBG. We also observed an improvement in the levels of some biochemical markers that are typically affected in these mice (e.g. BDNF, IGF-1, PPAR-γ), as well as a reduction in the accumulation of aggregates of mutant huntingtin (EM48 immunostaining) in the striatal parenchyma by the treatment with CBG. In conclusion, CBG appears to have a promising neuroprotective profile for the treatment of HD, in particular against the mitochondrial dysfunction and oxidative injury caused by 3NP. It was also active in R6/2 mice, but it is possible that it needs to be combined with another phytocannabinoid in these mice to enhance its therapeutic effects.

O.2.8.

POTENTIAL OF CANNABINOID CB_1 AND CB_2 RECEPTORS IN A ZEBRAFISH MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a chronic neurodegenerative disease, mainly affecting young adults and devoid of a successful treatment aimed at altering the disease progression and offering neuroprotection. For experimental purposes, human ALS has been replicated in different species including the zebrafish (*Danio rerio*), which has recently emerged as a powerful tool to study neurodegenerative diseases because of their rapid development, easiness of growth and gene expression manipulation. Previous work has demonstrated that zebrafish has in its genome all the key elements (e.g. endocannabinoid receptors and enzymes) of the endocannabinoid signaling system. The pharmacological manipulation of this system may serve to investigate novel neuroprotective therapies in this experimental model, given the beneficial effects derived from targeting CB_1 and/or CB_2 receptors to decrease excitotoxicity, microglial activation, neuroinflammation and oxidative stress. In the present study we have used a morpholino antisense oligo silencing strategy to study the role of CB_1 and CB_2 receptors in a zebrafish model of ALS. Our preliminary results show that the CB_2 receptor could play a role in the axonal elongation during the zebrafish development and that the silencing of the CB_1 receptor could play a beneficial effect in the axonopathy caused by the injection of mutated $hSOD^{G93A}$ mRNA in zebrafish embryos. When confirmed, these data may provide support to the idea of a cannabinoid-based therapy to delay disease progression in ALS.

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O.2.9.

REGULATION OF AMYLOID-INDUCED NEUROINFLAMMATION BY ENDOCANNABINOIDS

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It has been proposed that the inhibition of the activity of the fatty acid amide hydrolase (FAAH) may provide some benefits in certain brain diseases and, more specificaly, may provide neuroprotection in areas where intense neuroinflammation takes place. The blockade of this enzyme leads to an increase in the levels of anandamide and other acylethanolamines that may trigger CB1-mediated as well as CB1–independent effects. Previous reports, however, suggest that the inactivation of FAAH may also provoke undesired effects. We have employed an in vivo model of chronic brain damage to explore this question. We have used a murine model of chronic neuroinflammation induced by the amyloid peptide (5xFAD), in which high levels of beta-amyloid are produced in the brain. This model includes the formation of amyloid plaques as well as amyloid deposition in blood vessels walls, and leads to cognitive impairment at 5-6 months of age. The genetic inactivation of FAAH in these mice led to an increase of cytokine production. Paradoxically, these mice showed an improved memory acquistion as well as a decrease in plaque deposition. Thus, we conclude that the inhibition of FAAH may promote a pro-inflammatory environment in the mouse brain but leading to beneficial effects in terms of decreased brain damage and improved cognitive function.

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O.2.10.

THE ANTI-INFLAMMATORY EFFECT OF A CANNABIGEROL DERIVATIVE IN EAE INVOLVES MULTIPLE CELLULAR TARGETS

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Cannabinoids have been proposed as promising therapeutic agents in multiple sclerosis (MS) by their capability to alleviate specific MS symptoms, like spasticity, and by their immunomodulatory and neuroprotective properties. Our previous results showed that a derivative of cannabigerol, the cannabigerol quinone VCE-003 ameliorates neurological deficits and severity of MOG-induced EAE in mice by activation of CB2 and PPAR-γ receptors. We also observed a reduction of cell infiltrates, mainly $CD4^+$cells, and inhibition of Th-1 and Th-17 responses in the spinal cord of EAE mice treated with VCE-003. Here, we have addressed whether peripheral immunosuppressive mechanisms of VCE-003 can be involved in its actions on EAE. The possibility that brain endothelial cells are targets of VCE-003 was also investigated. The main results at peripheral level are: i) VCE-003 inhibits T cells proliferation and cellular cycle progression without apoptosis in human primary T cells; ii) VCE-003 reduces Th1 cytokines, chemokines, IL-17 and other proinflammatory cytokines secretion in activated primary T cells; iii) VCE-003 is inhibiting the transcriptional activity of IL-2, TNF-α and IL-17 in Jurkat cells and, iv) VCE-003 reduces the polarization of macrophages to a pro-inflammatory M1 profile. In addition, VCE-003 acts in brain endothelial cells and diminishes in a dose response way the production of VCAM-1 suggesting the existence of a new obstacle for cell trafficking into the CNS. Based on its immunomodulatory and pleiotropic effects, VCE-003 represents a potential therapeutic agent for the treatment of human immune diseases with both inflammatory and autoimmune components.

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O.2.11.

CB_1 CANNABINOID RECEPTOR-DEPENDENT ACTIVATION OF MTORC1/PAX6 SIGNALING DRIVES TBR2 EXPRESSION AND BASAL PROGENITOR EXPANSION IN THE DEVELOPING MOUSE CORTEX

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The CB_1 cannabinoid receptor regulates cortical progenitor proliferation during embryonic development, but the molecular mechanism of this action remains unknown. Here we report that CB_1-deficient mouse embryos show premature cell cycle exit, decreased number of Pax6- and Tbr2-positive cells, and reduced mammalian target of rapamycin complex 1 (mTORC1) activation in the ventricular and subventricular zones. Pharmacological stimulation of CB_1 receptors in cortical slices and progenitor-cell cultures activated the mTORC1 pathway and increased the number of Pax6- and Tbr2-expressing cells. Likewise, acute CB_1 receptor knockdown *in utero* reduced mTORC1 activation and Tbr2-positive cell generation. Luciferase reporter assays and ChIP analyses revealed that the CB_1 receptor drives Tbr2 expression downstream of Pax6 induction in an mTORC1-dependent manner. Characterization of CB_1 receptor expression in human samples from type IIb focal cortical dysplasia, a developmental cortical malformation associated to overactivation of the mTORC1 pathway, revealed an enrichment of CB_1 receptors in phospho-S6-positive undifferentiated cortical cells. Altogether, our results demonstrate that the CB_1 receptor exerts a crucial role in tuning dorsal telencephalic progenitor proliferation by sustaining the transcriptional activity of the Pax6/Tbr2 axis via the mTORC1 pathway, and suggest that CB_1 receptor/mTORC1-mediated expression of cortical progenitor identity determinants may contribute to human developmental cortical alterations.

O.3.1.

NOCICEPTIVE, EMOTIONAL AND COGNITIVE BEHAVIOURS IN A MOUSE MODEL OF OSTEOARTHRITIS PAIN: INVOLVEMENT OF CB1 AND CB2 CANNABINOID RECEPTORS

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Osteoarthritis is a degenerative joint disease of which chronic pain is the main symptom and the first reason of complaint in patients. Osteoarthritis pain is often associated with emotional and cognitive alterations that impair the quality of life of patients. Thus, an appropriate treatment able to improve not only pain manifestations, but also the emotional and cognitive symptoms is essential for an effective management of osteoarthritis. The purpose of this study was to investigate the role of the cannabinoid receptor 1 (CB1R) and 2 (CB2R) in the nociceptive, affective and cognitive alterations associated with osteoarthritis pain. The intra-articular injection of monosodium iodoacetate (MIA) was used to induce osteoarthritis pain in wild-type and knockout mice for CB1R (CB1KO) and CB2R (CB2KO). We first analysed the affective and cognitive consequences of this chronic pain exposure by evaluating the anxiety-like behaviour (elevated plus-maze test) and memory functions (object recognition test) at different time points after the intra-articular injection of MIA. Then, we also evaluated the ability of the selective CB1R agonist, ACEA (1 and 5 mg/Kg, i.p.), and the selective CB2R agonist, JWH133 (1 and 5 mg/Kg, i.p.), to improve the nociceptive manifestations (von Frey model) as well as the emotional (elevated plus-maze test) and cognitive deficits (object recognition test) observed in this mouse model of osteoarthritis. Mice that received the intra-articular injection of MIA showed an alteration of the anxiety-like behavior, as revealed by a decrease in the percentage of entries and time spent in the open arm of the elevated plus-maze. Interestingly, these alterations induced by MIA appeared more pronounced in CB1KO when compared with wild-type mice. The presence of osteoarthritis pain also produced memory impairment, since mice injected with MIA showed a decrease of the discrimination index in the object recognition task. No significant differences were revealed between wild-type and knockout mice for CB1R or CB2R in the memory deficits produced by MIA. The systemic administration of ACEA or JWH133 produced an improvement of the nociceptive responses, and the emotional and cognitive alterations observed in the MIA model of chronic joint pain.

These results revealed that CB1R, but not CB2R, is mainly involved in the control of the affective alterations associated with this osteoarthritis pain model. However, neither CB1R nor CB2R seem to play a major role in the memory deficits observed in this osteoarthritis model. Interestingly, both CB1R and CB2R agonists ameliorated the nociceptive manifestations as well as the affective and cognitive alterations associated with this chronic pain state, suggesting a potential interest of these compounds for osteoarthritis treatment.

O.3.2.

THE ROLE OF GLUTAMATERGIC CB1 CANNABINOID RECEPTORS IN THE REGULATION OF OBESITY

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Obesity is becoming one of the most important public health problems of the 21st century representing a link to a number of severe associated diseases. Dieting and physical exercise have been proven to be insufficient in the treatment of this condition which is characterized in humans by an abnormal increase of the percentage of body fat (above 25% in women and 30% in men). Consequently, new therapeutic approaches are required to counteract the increasing prevalence in adults and children. The influence of cannabinoids on food intake and energy balance is getting the attention of the scientific community during the last years.

Here we present a study aiming at deciphering the role of a specific population of CB1 cannabinoid receptors expressed in Glutamatergic neurons (Glu-CB1R) in the regulation of diet-induced obesity. By treating a conditional Knock-out mouse line where the CB1R gene has been deleted specifically in forebrain Glutamatergic neurons (Glu-CB1R-KO) with a super High-Fat Diet (60% Fat; sHFD) we have observed that the absence of Glu-CB1R in Glu-CB1R-KO mice leads to a resistance to develop obesity as compared to their WT littermates. This resistance is only seen when mice are fed with sHFD. The obesity-resistant phenotype is explained by a reduced food intake that was further demonstrated using a pair-feeding and fasting/refeeding paradigms. Additionally, Glu-CB1R-KO mice showed a better performance in glucose and insulin tolerance test as compared to their WT littermates, corroborating the metabolic benefit of the lack of Glu-CB1R.

Interestingly, the olfactory capacity of Glu-CB1R-KO mice seems to be impaired, indicating a potential involvement of the Anterior Olfactory Nucleus (AON) in the phenotype. Accordinglly, we wanted to investigate whether this resistance can be reverted after the onset of obesity. For this purpose we performed an experiment were Glu-CB1R-KO and -WT mice were intracranially injected in the AON with 2 different Adeno-Associated Viruses containing either a floxed stop cassette only (AAV-empty) or the same cassette followed by the CB1R gene (AAV-CB1R). Interestingly, the re-expression of CB1R only in Glutamatergic neurons of the AON in Glu-CB1R-KO mice was sufficient to block the obesity-resistant phenotype of these mice 12 weeks after the onset of the treatment with sHFD. In summary, our results strongly demonstrate that the population of Glu-CB1R in the AON is a very attractive target, paving the way for a new treatment of obesity. To our knowledge, this is the first study that correlates cannabinoid influence on olfaction and obesity, opening a new clinical perspective.

O.3.3.

EFFECT OF CANNABINOID DURING OOCYTE IN VITRO MATURATION ON IN VITRO FERTILIZATION AND EMBRYO DEVELOPMENT IN MOUSE

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The number of couples with sterility problems attending fertility programs is arising, and the success in fecundation or subsequent development is not totally accomplished due to the fact that many biochemical and physiological aspects of the reproduction are still unknown. There are some evidences about the cellular communication exerted by the endocannabinoids during the development events but, nothing is known about the role exerted by this system in the resumption of oocyte meiosis, a key process in achieving potentially fertilizable cells. Our study aims to analyze the involvement of cannabinoids in in vitro maduration (IVM) of mice oocytes to verify if the presence of cannabinoids in the culture media improve in vitro fertilization (IVF) rates and the subsequent in vitro development into blastocyst stage.

Immunofluorescence and PCR experiments showed the presence of CB1 and CB2 receptors in oocytes during the different stages of both in vitro and in vivo oocyte maturation. Pharmacological activation of CB1 receptor during IVM using different doses of HU-210 synthetic cannabinoid demonstrated that cannabinoid is involved in a more efficient IVF and in a better in vitro development of the blastocyst prevenient from IVM oocytes. Moreover, the immunofluorescence study of the phosporylation of the kinases PKB/Akt, MAPK and CDC2, involved in different signalling pathways of meiosis, after cannabinoid treatment of oocytes at 30 min and 17 hours of IVM showed that, at least, the PKB/Akt pathway was modulated during IVM.

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

O.3.4.

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

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Introduction: Cancer stem cell derived from glioma, named here glioma stem cells (GSCs), have been proposed to be responsible - at least in part – for the high invasiveness and resistance to standard chemo / radio- therapy exhibited by glioblastoma multiforme (GBM), the most frequent and aggressive class of malignant primary brain tumour.
Identification of the signalling mechanisms (including changes in the levels of growth factors or stimulation of their receptors) that regulate GSCs proliferation and survival is therefore crucial for the development of more efficacious therapies to fight GBM.
Although cannabinoid administration has been shown to inhibit tumor growth of glioma xenografts in mice, our group has found that increased expression of the growth factor Midkine (Mdk) and subsequent activation of Anaplasic Lymphoma Kinase (ALK) are critically involved in glioma resistance to cannabinoid antitumoral action in glioma cell lines. Previously, Mdk has also been involved in resistance to anti-cancer therapies and in stem cell regulation, so targeting the Mdk/ALK axis in GSCs could be a good therapeutic strategy to enhance the efficacy of antitumoral action of cannabinoids.
Objectives: The main objective of this work is therefore to analyse the role of the Mdk/ALK axis in the resistance to cannabinoid antitumoral action, as well as to develop novel therapeutic strategies based on combination of cannabinoids with inhibitors of the Mdk/ALK via or with temozolomide (TMZ), the benchmark agent used for the treatment of gliomas.
Results: (i) Levels of MDK were much higher in cultures of GSCs than in those of their corresponding differentiated counterparts. (ii) MDK genetic inhibition (using siRNA) or neutralization (using specific anti-MDK antibodies) sensitizes GSCs to cannabinoid antitumoral action. (iii) Combined administration of cannabinoids with TMZ or inhibitors of Mdk/ALK axis exerts a strong antiproliferative action in cultures of GSCs.
Conclusion: These findings support that the MDK / ALK axis plays an important role in the regulation of the proliferation and self-renewal of GSCs as well as the resistance of these cells to cannabinoid action. Moreover, combination of cannabinoids with TMZ or inhibitors targeting this axis could be a potential therapeutic strategy to eliminate the GSC population in GBM patients.

O.3.5.

DUAL ROLE OF THE CB_2 CANNABINOID RECEPTOR IN HER2-POSITIVE BREAST CANCER

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A large body of evidence has demonstrated that plant-derived, endogenously-produced and synthetic cannabinoids exert anti-tumoral actions in different models of cancer, including cell cultures, xenografted animals and genetically-engineered mice. However, little is known about the biological role of the endocannabinoid system in tumor physio-pathology.
In this study, we focused on the role of the CB_2 cannabinoid receptor in tumor generation and progression and on its role as a target for cannabinoid-based anti-tumoral therapies.
On the one hand, we observed that CB_2 confers pro-oncogenic properties on breast cancer cells by activating Her2 signaling, specifically the non-receptor tyrosine kinase SRC. On the other hand, we show that pharmacological activation of CB_2 produces anti-tumoral actions, both *in vivo* and *in vitro*, in human Her2-positive breast cancer cells. Our preliminary data indicate that exogenous cannabinoids disrupt the pro-oncogenic signaling induced by the CB_2/Her2/SRC axis, resulting in the opposite effect (anti-tumoral actions).
Taken together, our results demonstrate that CB_2 plays a dual role in Her2-positive breast cancer and start to shed light on the molecular mechanism underlying the well-known biphasic effects of cannabinoids on cell growth/survival. In more general terms, our results support the unprecedented notion that the CB_2 cannabinoid receptor is a pivotal regulator of Her2 oncogenic signaling in breast cancer.

O.3.6.

THE ORPHAN RECEPTOR GPR55 CONFERS PRO-METASTATIC ADVANTAGES ON BREAST CANCER CELLS *IN VITRO* AND *IN VIVO*

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Emerging evidence point to an important role of the orphan G protein-coupled receptor GPR55 in tumor generation and growth. Thus, we have previously demonstrated that this receptor promotes cancer cell proliferation *in vitro* and *in vivo*, and confers oncogenic advantages to tumor cells. Metastasis is the last and most lethal step during tumor progression, where cancer cells acquire the ability to leave the primary tumor and colonize distal niches. In this work we aimed at studying whether GPR55 participates in the control of the metastatic process. Our results show that it drives breast cancer cell migration and invasion via G_q heteromeric G proteins *in vitro*. This effect was accompanied by the activation of matrix metalloproteinases and the upregulation of metastatis-inducing genes such as MMP1, CXCL1 and ANGPTL4. Furthermore, GPR55 promotes breast cancer tumor growth and lung colonization *in vivo*. Together, our data support the involvement of GPR55 on cancer metastasis, and suggest that pharmacological blockade of this receptor could be a new strategy to manage metastatic breast cancer.

P.1.

REGULATION OF APOPTOTIC PATHWAYS BY AM630, AN ANTAGONIST/INVERSE AGONIST AT THE CANNABINOID CB_2 RECEPTOR, IN MOUSE BRAIN

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CB_1 receptors have been shown to display constitutive activity; e.g. a tonic activation of Fas/FADD (Fas-associated death domain) receptor complex suggesting that endocannabinoids could induce pro-apoptotic actions in brain. Moreover, the CB_1 receptor agonist WIN55212-2 acutely reduced FADD and rimonabant (SR141716A), inactive by itself (neutral antagonist), antagonized this effect in mouse brain. CB_2 receptors also display constitutive activity in vitro, and the selective ligand AM630 has been classified as a CB_2 receptor protean ligand; i.e. the compound can behave as an agonist, neutral antagonist or inverse agonist. The aim of this work was to investigate the in vivo pharmacological nature of AM630 modulating pro-apoptotic FADD and other apoptotic factors in mouse brain.

Groups of male CD1 Swiss mice were acutely treated with drug-vehicle (n=14), AM630 (1 and 10 mg/kg, i.p., 1.5 h, n=3-5), and JWH133 (a CB_2 receptor agonist, 1 and 3 mg/kg, i.p., 1 h, n=6-6). For comparison, other mice were acutely treated with the CB_1 receptor antagonists rimonabant (3 mg/kg, i.p., 2 h, n=5) and AM281 (10 mg/kg, i.p., 1.5 h, n=8). The animals were killed by decapitation at the indicated times. Dimeric FADD, oligomeric p-Ser191 FADD (associated with non-apoptotic actions), phosphorylated (p) and total (t) JNK (c-Jun NH_2-terminal protein kinase), cytochrome c, AIF and truncated-AIF (t-AIF, released from mitochondria), nuclear PARP-1 (polyADP-ribose-polymerase) and its main fragment were quantified in the cerebral cortex by Western immunoblot analyses with specific antibodies, and the content of beta-actin was used as a loading (negative) control.

The CB_2 ligand AM630, but not the CB_2 agonist JWH133, markedly increase the content of FADD (72% and 172%, $p<0.001$) and p-Ser191 FADD (61% and 178%, $p<0.001$) in mouse brain cortex, without altering the ratio p-FADD/FADD when compared with that in controls. In contrast, the CB_1 receptor antagonists rimonabant and AM281 did not significantly alter the content of brain FADD. Notably, JWH133 decrease (20%-40%, $p<0.05$) and AM630 increase (115%-186%, $p<001$) the activation of pro-apoptotic JNK (ratio of p-JNK to t-JNK). Moreover, AM630 (dose 10 mg/kg), but not JWH133, also increased the content of mitochondrial pro-apoptotic cytochrome c (24%, $p<0.01$) and t-AIF/AIF ratio (30%, $p<0.05$). In line with these findings, the CB_2 ligand AM630 (10 mg/kg) increased the cleavage of PARP-1 (95%, $p<0.01$), a marker of apoptosis. In contrast, the CB_1 receptor antagonist AM281 did not alter the content of these pro-apoptotic factors in brain.

The CB_2 agonist JWH133 downregulated pro-apoptotic JNK whereas the CB_2 ligand AM630 upregulated the activity of this kinase. Therefore, AM630 appears to behave as an inverse agonist at the CB_2 receptor. AM630 also upregulated other pro-apoptotic factors such as FADD or cytochrome c. In addition, the cleavage and release of AIF (t-AIF form, increased) from mitochondria results in PARP-1 fragmentation and DNA damage (a caspase-independent mechanism) in mouse brain cortex, indicating that this drug is not a neutral antagonist at the CB_2 receptor. In contrast, AM281, like rimonabant, appears to behave as a neutral antagonist at the CB_1 receptor regulating these pro-apoptotic factors. The findings also suggest that the CB_2 inverse agonist AM630 could induce abnormal brain cell death in vivo.

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P.2.

THE ACYLETHANOLAMIDES OEA AND PEA SHOW ANTI-INFLAMMATORY PROPERTIES IN LPS-INDUCED NEUROINFLAMMATION

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The acylethanolamides are lipid mediators that include endogenous ligands of the peroxisome proliferator-activated nuclear receptor alpha (PPARα) such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA). OEA and PEA regulate satiety and pain processes but little is known about their effect as modulators of brain inflammation. Lipopolysaccharide challenge (LPS, 0.5 µg/kg, i.p.) was used as model of neuroinflammation, and the anti-inflammatory properties of OEA and PEA (10 mg/kg, i.p.) were assessed in frontal cortex of LPS-injected male adult Wistar rats. LPS administration induced the release of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), the expression of nuclear factor kappaB (NFκB) and its inhibitory cytosolic subunit IκBα, and other inflammatory related mediators such as the inducible isoforms of nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) in frontal cortex 3h after LPS administration. Pretreatment with OEA and PEA prevent the increase in TNF-α but has no effect in IL-6. OEA and PEA pre-treated rats also showed reductions in LPS-induced NFκB and IκBα increases in nuclear and cytosolic extracts, respectively, and the expression of iNOS. Additionally, OEA and PEA prevent the increase in lipid peroxidation induced by LPS (measured by malondialdehyde accumulation). Pretreatment of LPS-injected rats with the PPARα agonist WY14643 reproduced the anti-inflammatory/neuroprotective profile of OEA and PEA, suggesting a role for these acylethanolamides as modulators of pathologies with neuroinflammatory component.

P.3.

ANTIINFLAMMATORY EFFECTS OF OLEOYLTHETHANOLAMIDE IN A MODEL OF BINGE ALCOHOL DRINKING

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Alcohol abuse induces neuroinflammation and damage to the brain. Alcohol is frequently consumed in a specific pattern of binge drinking which contributes to develop an alcohol use disorder. The purpose of this study is to investigate the effects of alcohol binge drinking in brain and peripheral markers of inflammation and to evaluate the anti-inflammatory properties of oleoylethanolamide (OEA), an endogenous member of the acylethanolamide´s family that plays also a role in satiety and pain processes. Male Wistar rats were exposed to a binge pattern of alcohol by intragastric administration of 3 g/kg of alcohol 3 times per day during 4 consecutive days. OEA (10 mg/kg, i.p.) was administered i.p. previous each alcohol gavage administration. We measured blood alcohol levels during the experiment, and brain tissue samples were taken at different time points after the end of alcohol binge protocol. Our results show that this model of alcohol binge drinking induces the expression of several inflammatory parameters in blood and frontal cortex. We characterized the temporal profile of expression of cytokines such as interleukin-1beta (IL-1β) and tumoral necrosis factor alpha (TNF-α) in blood and cortex, the expression of nuclear factor kappa B (NFκB), and the inflammatory and oxido-nitrosative enzimes COX-2 and iNOS, as well as the level of lipid peroxidation in frontal cortex 1h, 6h and 24h after the last alcohol administration. We observed that repeated injections of OEA decreases the rise in plasma corticosterone levels induced by binge alcohol without modify the levels of blood alcohol. Pretreatment with OEA also reduces other plasmatic parameters altered by alcohol, such the release of TNF-α, and the expression of the subunit p65 of NFκB in nuclear extracts of cortex, and is able to prevent the upregulation of toll-like receptors (TLR)-4 mRNA induced by alcohol exposure. Our results suggest that OEA may interfere with the inflammatory cascade of mediators induced by alcohol showing potential beneficial effects to prevent alcohol neuroinflammation.

P.4.

THE TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 IS REVEALED IN DENTATE INHIBITORY SYNAPSES BY HIGH RESOLUTION IMMUNOELECTRON MICROSCOPY

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The transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel that acts primarily as pain sensor in the periphery but also modulates neurotransmitter release and synaptic plasticity in the brain. TRPV1 function must lay on its anatomical distribution in the peripheral and central nervous system regions involved in the physiological roles of the channel. However, the anatomical localization of TRPV1 is well established in the periphery, but in the brain it is a matter of debate. We have recently shown that TRPV1 is highly concentrated in postsynaptic dendritic spines to asymmetric perforant path synapses in the outer 2/3 of the ML, being poorly expressed at the excitatory hilar mossy cell synapses in the inner 1/3 of this layer. However, the TRPV1 distribution at inhibitory synapses in the dentate molecular layer is still an open question.

To investigate this, we have used TRPV1 antibodies combined with a highly sensitive pre-embedding immunogold method for high resolution electron microscopy. TRPV1 immunoparticles were observed in dentate granule cell dendrites receiving symmetric inhibitory synapses.

The silver-intensified gold particles were mostly confined to postsynaptic membranes and distributed at a relative short distance from the inhibitory synaptic contacts. Importantly, the TRPV1 pattern distribution at inhibitory synapses disappeared in the molecular layer of TRPV1-knockout mice.

These findings give additional knowledge on the fine TRPV1 localization in the rodent hippocampus by means of high resolution electron microscopy.

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P.4.

ASTROGLIOSIS AND CHONDROITIN SULPHATE PROTEOGLYCANS ACCUMULATION IN THEILER' S VIRUS MODEL: EFFECT OF SATIVEX

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The accumulation of extracellular matrix (ECM) and glial scar formation are considered important factors for the failure of regeneration in CNS injury and multiple sclerosis (MS). Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) is a suitable model to evaluate alterations of ECM associated to demyelination/remyelination process. Previous results of our group showed that Sativex ® treatment ameliorates symptomatology and axonal damage in TMEV-IDD. The aim of the present study was to address whether Sativex® treatment affects astrogliosis and the expression of chondroitin sulphate proteoglycans (CSPGs) in TMEV-IDD at the chronic phases of the disease. As astrocytes are the main source of CSPGs we also analyze the regulation of their production by Sativex® using in vitro approaches.

Our results show the presence of strong astrogliosis (GFAP and Vimentin staining) associated with a deposition of CSPGs (CS56) in the spinal cord of TMEV-infected mice at 80 dpi which is decreased following Sativex® treatment. Using purified primary astrocyte cultures we observe that a combination of TGFβ1 and bFGF induce the production and delivery of brevican and neurocan. Stimulated-astrocytes treated with Sativex® show reduced synthesis and release of CSPGs at doses of 0.5 and 1 μM, suggesting that astrocytes can be a new target of Sativex® actions.

As CSPGs are known to be involved in remyelination failure in human MS and in murine demyelinating models, our results might be relevant for reparative mechanisms mediated by Sativex®.

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P.6.

NEGATIVE EMOTIONAL ALTERATIONS DURING SPONTANEOUS THC WITHDRAWAL ARE NOT MODULATED BY HYPOCRETIN RECEPTOR-1

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Recent data suggest that abstinence from marijuana smoking produces a consistent withdrawal pattern in humans, including symptoms such as anxiety, apathy, decreased appetite and sleep disorders, which may be relevant for the maintenance of cannabis addiction. Limited animal research has been carried out to model these negative affective alterations during spontaneous cannabinoid withdrawal. On the other hand, hypocretinergic system has been associated with the regulation of emotional behaviour. Thus, hypocretins promote arousal and anxiety-like states and modulate depressive-like responses. The objective of this study was to investigate the potential negative affective effects of spontaneous cannabinoid withdrawal in mice and their possible modulation by hypocretins. Male hypocretin receptor-1 (Hcrtr-1) knockout mice and their wild type littermates were injected with Δ9-tetrahydrocannabinol (THC) (20 mg/kg, i.p.) or vehicle twice daily for 7 days. Spontaneous emotional withdrawal symptoms were evaluated 1 and 3 weeks after the last drug injection. Anxiety-like responses were determined by the elevated plus-maze and the black and white box paradigms. Depressive-like behaviour was assessed by the tail suspension and the forced swim tests. Preliminary data indicate that THC produces an anxiogenic-like response after one week of abstinence when compared with vehicle. This effect was similar in both wild type and Hcrtr-1 knockout mice. In contrast, no depressive-like states were revealed at this time point for none of the groups. Interestingly, the anxiogenic-like response was maintained after three weeks of abstinence. In addition, at this time point THC-abstinent mice also showed a depressive-like phenotype. This effect was not modified in the absence of the Hcrtr-1. These results identify the presence of emotional alterations following 1 and 3 weeks of protracted abstinence from chronic THC exposure, which do not seem to be modulated by Hcrtr-1.

P.7.

CORTICAL DEVELOPMENT ALTERATIONS INDUCED BY EMBRYONIC Δ^9-TETRAHYDROCANNABINOL ADMINISTRATION ALTER SKILLED MOTOR ACTIVITY AND SEIZURE SUSCEPTIBILITY

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Developmental cannabinoid exposure has been shown to induce long-lasting behavioral alterations in adult mice. However, the impact of embryonic administration of plant-derived Δ^9-tetrahydrocannabinol (THC) and its neurobiological mechanism of action remain obscure. Here we investigated the developmental and functional consequences of embryonic THC exposure (i.p. administration to pregnant female mice from gestational day 12.5 to 16.5, 3mg/Kg) in wild-type (WT) and CB_1-deficient littermates. Analysis of motor activity revealed that embryonic THC exposure impaired the skilled motor function (assessed by the skilled reaching- and staircase pellet reaching-tests), but did not affect unskilled activity and general motor activity. Immunofluorescence characterization of cortical excitatory populations revealed that embryonic cannabinoid administration induced deep-layer projection neuron alterations. In addition, the use of transgenic Thy1-YFP mice, that label layer V pyramidal neurons, confirmed the existence of THC-induced alterations in subcortical projection neurons, likely responsible of the deficits observed in skilled motor activity. Ongoing analysis after vital retrograde tracing of corticospinal motor neurons labelled in the cervical spinal cord of THC- and vehicle-treated WT and $CB_1^{-/-}$ mice will define the contribution of the CB_1 cannabinoid receptor in THC-induced alterations. In addition, the latency to the GABA antagonist pentylenetetrazol-induced seizures was decreased in THC-exposed WT but not $CB_1^{-/-}$ mice. Subsequent analysis of the inhibitory neuronal lineage revealed a shift in the distribution of GABAergic interneurons of THC-treated animals. Finally, gene expression analysis in the cortex and hippocampus of THC-administered mice pointed to altered differentiation of the excitatory and inhibitory neuronal populations and indicated a pro-epileptic transcriptional signature. These findings demonstrate that prenatal cannabinoid exposure exerts functional alterations in the adult brain, owing to the role of CB_1 cannabinoid receptors in the differentiation of subcortical long-range projection neurons and the appropriate coordination of excitatory and inhibitory neuronal specification.

P.8.

ROLE OF CANNABINOID CB1 AND CB2 RECEPTORS IN BRAIN DAMAGE FOLLOWING HYPOXIA-ISCHEMIA IN ADULT MICE

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A great deal of research is now being pursued in order to develop new therapies for brain injury and the recovery of brain function. The endogenous cannabinoid system seems to play an important role in the neuropathology associated with ischemic brain damage. Thus, the aim of this study was to clarify the involvement of cannabinoid CB1 and CB2 receptors (R) in brain damage produced by hypoxia-ischemia (HI) in adult mice. Mice lacking the CB1R and CB2R and their respective WT controls were anesthetized and their left common carotid artery was permanently ligated. Following recovery, mice were placed in a hypoxia chamber at 10% oxygen for 60 min with a constant temperature of 38ºC ± 0.5. Behavioural tests including the rotarod, the object recognition, the open field, and the Irwin tests were conducted 24h, 72h and 7 days after HI. Immediately after testing, animals were sacrificed and their brains removed and stored for future analysis of the lesions and immunohistochemical assessment. The results showed loss of neurons in primary motor, somatosensory, insular and piriform cortices, dorsal striatum, ventral and dorsal hippocampus of lesioned mice with respect to sham operated animals. On the other hand, areas such as the cingulate and secondary motor cortices, and the ventral striatum were spared. Astrocyte reactivity in lesioned animals was observed only in the hippocampus. The extent of the lesion was not significantly different between genotypes, although higher mortality was observed in CB2R than in CB1R KO mice. In contrast, deletion of CB1R induced more neurological deficits than CB2R deletion. These data suggest that CB1R and CB2R differentially modulate brain injury induced by HI in adult mice.

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P.9.

A COMBINED PRECLINICAL THERAPY OF CANNABINOIDS AND TEMOZOLOMIDE AGAINST GLIOMA

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Introduction: Cannabinoids, the active components of marijuana and their derivatives, are currently investigated due to their potential therapeutic application for the management of cancer. Specifically, Δ^9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD) - the two major ingredients of marijuana – have been shown to inhibit tumor growth in a number of animal models of cancer, including glioma. The antitumoral effect of THC relies, at least in part, on the stimulation of autophagy-mediated apoptosis in tumor cells.
Objectives: Optimizing cannabinoid-based anticancer therapies in preclinical models of glioma
Methods: Tumor xenografts were induced in nude mice by subcutaneous injection of 5 x 10^6 U87 cells. Orthotopic mouse model of glioma was generated injecting 3 x 10^5 U87 cells into the striatum of nude mice. Animals were treated using different routes of administration.
Results: (i) intraperitoneal or oral administration of THC or THC + CBD reduces the growth of glioma xenografts with similar efficacy than the local administration of these agents. (ii) administration of THC, CBD or THC+CBD-loaded microparticles reduced tumour growth with the same efficacy than a daily local administration of the equivalent cannabinoids in solution. (iii) local or oral delivery of THC or THC+CBD in combination with temozolomide produced a very strong synergic reduction in tumour growth in subcutaneous and intracranial glioma xenografts.
Conclusion: The combined treatment of cannabinoids and temozolomide using different vias of administration produces a strong anticancer activity in animal models of glioma.

P.10.

OPERANT MODEL OF FOOD ADDICTION IN MICE

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

P.11.

CB_1 AND GPR55 RECEPTORS FORM FUNCTIONAL HETEROMERS IN STRIATUM

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The endocannabinoid system has emerged as a major player in brain neuromodulation, participating in the control of neurotransmitter release and regulating processes such as motor activity, memory and learning and motivational responses. Accordingly, it is known that the cannabinoid CB_1 and CB_2 receptors form functional heteromers in brain that are considered as therapeutic targets for neurodegenerative diseases. GPR55 receptor was considered a member of the cannabinoid receptor family, but it is not activated by cannabinoids and its endogenous ligand is L-α-lysophosphatidylinositol. GPR55 receptor activation may explain physiological effects that are non-CB_1/CB_2 mediated. This receptor is expressed in the CNS, although its exact function remains unclear. Based on the similar signalling, localization and distribution displayed by CB_1 and GPR55 receptors, the aim of this work is the study whether GPR55 form heteromers with CB_1.For this purpose, CB_1-GPR55 receptor heterodimerization was studied in mammalian cells cotransfected with the cDNA for human GPR55 and CB_1 by Bioluminescence Resonance Energy Transfer, Reporter gene and ERK phosphorylation assays. Furthermore, the presence of CB_1-GPR55 heteromer in the brain was determined in rat striatum slices by measuring MAPK signalling. Our results demonstrated that CB_1 receptor can form heteromers with GPR55 receptor in transfected mammalian cells. Within CB_1-GPR55 receptor heteromer, CB_1 receptor antagonist, SR141716, can block the effect of GPR55 receptor agonist, CID1792197, in MAPK activation. This cross-antagonism phenomenon was used as a "biochemical fingerprint" of CB_1-GPR55 receptor heteromers, allowing their identification in the rat striatum. Taken together, the data suggest the occurrence of CB_1-GPR55 receptor heteromers in the striatum and more give insight into the mechanism by which CB_1 receptor can negatively modulate GPR55 receptor function, opening new avenues for drug discovery and therapeutic targets for neurodegenerative diseases.

P.12.

ALTERATIONS IN THE ENDOCANNABINOID MACHINERY IN ALTERNATIVE MICROGLIA *IN VITRO*

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The regulation of inflammation in the central nervous system (CNS) is necessary to prevent the irreversible cellular damage that occurs in neurodegenerative diseases like Multiple Sclerosis. Under pathological conditions, much is known about how microglial cells play a pivotal role becoming rapidly activated, changing both phenotype and morphology, and displaying several functions like antigen presentation, phagocytosis and cytokine production. On the other hand, little is known about how microglial cells serve a protective role in the CNS, acquiring an alternative state (also referred to as M2-type microglia) that is associated with tissue repair, anti-inflammatory cytokine production and extracellular matrix reconstruction. Compared to classically activated microglia, NOS2, IL-12, IL-1β, IL-6 and TNFα induction are suppressed in alternative activated microglia when stimulated with IL-4 and IL-13, whereas repair genes like Arginase1 and Ym-1 are activated. However, the participation of the endocannabinoid machinery in the acquisition of the M2-type microglia phenotype has not been addressed yet.
In this study, we have investigated the possible alterations of the endocannabinoid system in the alternative activated primary microglia *in vitro*, stimulated with the anti-inflammatory cytokines IL4 and IL13. M2-type microglia cells showed an increase in the Arginase-1 and IGF-1 levels, as it has been previously described in M2-type macrophages. Using RT-PCR, we analyzed the mRNA levels of the classical cannabinoid receptors CB1 and CB2, and of the enzymes of synthesis (NAPE and DAGL) and degradation (FAAH and MAGL) of AEA and 2-AG, respectively. M2-type microglia express a higher expression of CB2 and of the synthesis enzyme of AEA (NAPE); moreover, this alternative activated cells express lesser expression of both degradation enzymes (FAAH and MAGL), suggesting a change in the endocannabinoid system towards an increase in the production and signaling of classical cannabinoids like AEA and 2-AG .
Our results highlight the importance of the endocannabinoid system in the alternative state of microglia, and raise new questions about its role in the repair functions in the damaged CNS.

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P.13.

CB2 RECEPTOR LIGANDS: BINDING, IN SILICO DRUG-LIKE PROPERTIES, AND HUMAN SERUM ALBUMIN INTERACTIONS

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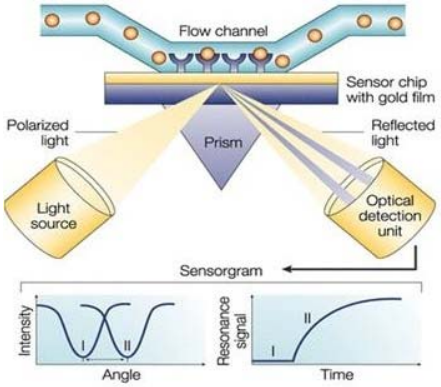
Based on our previous studies,[1] a new library of chromenopyrazoles was designed, synthesized, characterized and tested at the cannabinoid receptors. These compounds exhibit a selective CB_2 ligand profile. Due to the high percentage of drug development failures associated to insufficient pharmacokinetic properties, characterization of drug candidate compounds in this field gets increasingly important. Thus, it is necessary to obtain data at early stages of the discovery process. After administration, a drug is first carried through the blood stream by absorption, permeation and transport processes. Then, the distribution is crucial for the drug to reach a specific target in organ or tissue. The active drug is eliminated by metabolic processes and excreted. ADME (administration, distribution, metabolism, excretion) properties of the new synthesized CB2 ligands have been calculated in silico using QikProp from Maestro Software.
An important factor in this ADME process is the interaction with soluble protein in blood. In fact, level of plasma protein binding in blood contributes to drug activity, efficacy and possible side effects. Since human serum albumin (HSA) is the most abundant protein in blood, the binding strength of a selection of our new CB2 ligands has been tested using surface plasmon resonance.



P.14.

OEA DISRUPTS ANHEDONIA AND HYPOTHALAMIC FEVER MARKERS IN LPS-INJECTED RATS

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Oleoylethanolamide (OEA) is an endogenous lipid mediator of the acylethanolamide´s family which has shown anti-inflammatory activity in a model of lipopolysaccharide (LPS)-induced neuroinflammation (this meeting). In this study we tested the efficacy of OEA to ameliorate different behavioral parameters reflecting LPS-induced sickness behavior and study the neural correlates of fever in rats treated with LPS and OEA. Adult male control Wistar rats and rats receiving a LPS challenge (0.5 µg/kg, i.p.) were pretreated with OEA (10 mg/kg, i.p.) and tested for anhedonia in a saccharine preference test. Rectal temperature was also recorded several hours after LPS injection. Additionally, hypothalamic samples were collected from parallel experimental groups in order to study the effect of OEA in neural markers of fever. Our results show that LPS-treated animals decrease the total fluid intake compared with vehicles immediately after LPS administration and up to 30 h, and gradually decreases the preference for saccharine, being the effect significant from 8 h up to 30 h after LPS. Pretreatment with OEA in LPS-injected rats also reduced the total fluid intake compared with vehicles but restores the preference for saccharine to the level of controls at any time studied. Pretreatment with OEA in control rats did not modify the total fluid intake nor the preference for saccharine. LPS injection induces a biphasic curve in rectal temperature, showing the animals hypothermia 1 and 3 h after the injection and hyperthermia 24 h after the administration. Those changes in rectal temperature correlate with a hypothalamic mRNA upregulation of the pro-inflammatory cytokine IL-1β, the pyretic enzyme cyclooxygenase-2 and the microsomal prostaglandin E synthase-1 (mPGE), which regulates PGE2 production, 3h after LPS-injection. Interestingly, OEA inhibits the LPS-induced upregulation of those fever markers in the hypothalamus showing potential anti-pyretic effects. More intriguingly, OEA tends to increase COX-2 expression in control rats showing different roles in pathological or normal conditions.

P.15.

EVALUATION OF PLASMA N-ACYL-ETHANOLAMINES IN COCAINE ADDICTION: IMPACT OF PSYCHIATRIC COMORBIDITY

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Introduction: Cocaine is associated with serious health problems including psychiatric comorbidity. There is a need for the identification of biomarkers for the correct stratification of cocaine addicts. Several studies have evaluated circulating endocannabinoid-related lipids as biomarkers of inflammatory, metabolic and mental disorders. However, little is known in substance use disorders. This study characterizes both N-acyl-ethanolamines (NAEs) and 2-acyl-glycerols in cocaine addicts diagnosed with cocaine use disorders (CUD).
Methodology and patients: CUD-subjects seeking outpatient treatment (N=88), and age-/gender-/body mass-matched healthy control volunteers (N=46) were recruited for this study. CUD-subjects were also examined for the presence of psychopathological comorbidity using the semi-structured interview PRISM according to the DSM-IV-TR. Plasma acyl derivatives were quantified by LC-MS/MS and predictive models were generated through multivariate analyses.
Results: The results indicate that plasma acyl derivatives are altered in cocaine addiction. While NAEs were found to be increased, 2-acyl-glycerols were decreased in CUD-subjects *versus* controls. A predictive model based on these lipids as explanatory variables was developed to distinguish CUD-subjects from controls providing high discriminatory power. However, these alterations were not influenced by the severity of cocaine addiction. Also, we have demonstrated that some acyl derivatives are specifically influenced by the comorbidity of other psychiatric disorders. As an example, the plasma level of the monounsaturated NAE N-palmitoleoyl-ethanolamine was found to be elevated in CUD-subjects diagnosed with mood and anxiety disorders (51% and 34%, respectively) compared with non-comorbid CUD-subjects.
In summary, our findings support the monitoring of plasma endocannabinoids and acyl congeners as predictors of CUD and comorbid psychiatric disorders. Their use as biomarkers might allow a better stratification of cocaine addicts for therapeutic purposes.

This work was supported by grants from Instituto de Salud Carlos III, Red de Trastornos Adictivos UE-FEDER 2006 (RD06/0001/0000 and RD06/0001/1009) and 2012 (RD12/0028/0001 and RD12/0028/0009); Plan Nacional sobre Drogas 049/2009; Consejería de Economía, Innovación y Ciencia, Junta de Andalucía UE-FEDER (CTS-433); and Consejería de Salud, Junta de Andalucía (SAS 111224).

P.16.

CANNABIS SOCIAL CLUBS AS A NEW SOURCE OF OBSERVATIONAL DATA

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The creation of so-called "cannabis social clubs" in Spain is a social phenomenon that has developed exponentially in recent years. The existence of this form of association allows users continuously access to a source of quality cannabis without having to resort to the black market. On the other hand, many patients found in cannabis a source of relief for their conditions, and often turn to these associations with the idea of getting a product that meets appropriate quality of production and avoid having to resort to illegal sources.
In the present study we analyze observational data obtained from the work done by a family physician to these kind of patients in different Spanish associations. In all cases the patients received a questionnaire where they provided data such as route of administration, daily amount used, degree of improvement of symptoms and eventual adverse side effects resulting from their self-medication with cannabis, among others.
Methods: A questionnaire was applied to patients at the first consultation with the doctor. In this questionnaire, the questions were related to the onset of cannabis use, route of administration, the condition for which cannabis is used for medicinal purposes, knowledge of such consumption by other health professionals, level of experience with cannabis prior to onset of the disease and the need for dose modification over treatment.
Results: As remarkable data we can point that patients use cannabis inhaled in cigarettes (49,99%), oral (33,33%) or sublingual (16,66%). Most of them use less than 1g per day (55,55%), have informed their doctors about their cannabis consumption (48,98%), have not had to modify the dose in the last three months (71,42%) and use cannabis for diseases involving pain (36,25%) or cancer-related symptoms (12,5%).
Conclusions: While the patients have no access to other sources of cannabinoids, the use of cannabis in natural way remains a valid option for patients who improve their symptomatology using cannabis in its original form.

P.17.

DEAZA-ANALOGUES OF SR144528: DESIGN, SYNTHESIS, PHARMACOLOGICAL EVALUATION AND DOCKING STUDIES OF SELECTIVE CB_2 RECEPTOR LIGANDS

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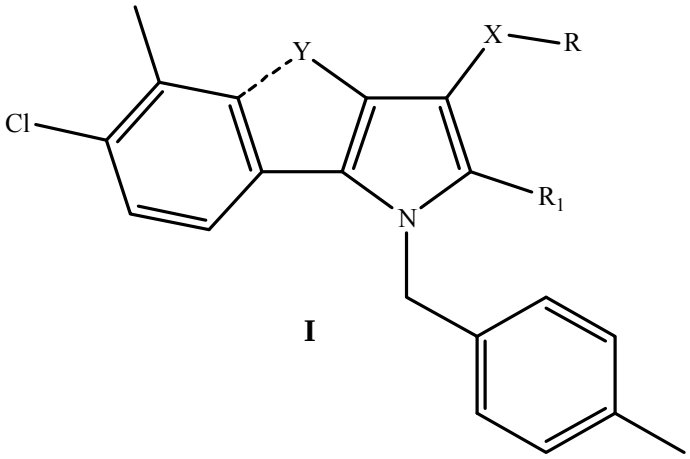
Increasingly experimental evidences show that targeting the CB2 receptors has the potential to become therapeutically beneficial for the treatment of many diseases, in particular in the CNS. To explore this potential, it is necessary to develop compounds with high selectivity for the CB2 receptor, including not only compounds able to activate this receptor, which may be used as therapeutic agents, but also those with antagonist/inverse agonist activity which may be used as experimental tools. In this context, our continuing efforts in the research of novel cannabinoid receptor ligands led us to design, synthesize and evaluate new deaza-analogues (general formula **I)** of the selective CB2 receptor antagonist SR144528.



I

In this study, we postulated that the modification of the pyrazole structure of SR144528 could generate some analogues with selectivity for the CB2 receptor and which can be used to modulate the activity of this receptor. We designed and synthesized a total of 20 compounds, which were evaluated for their binding to CB1 or CB2 receptors. Two of these compounds, amides **6** and **10** showed high affinity and selectivity for the CB2 receptor, as SR144528. In an *in vitro* assay to determine their activity at the CB2 receptors, both derivatives behaved as antagonists/inverse agonists of this receptor. Lastly, molecular modeling studies have been carried out using a model of the CB2 receptor in its inactivated state (CB2R) in order to identify key residues involved in the interactions between ligands and CB2 receptor. In conclusion, we have synthesized some SR144528 derivatives that retain its ability to block the receptor and can be used to identify CB2 receptor-mediated effects, or even to be evaluated in experimental models of cerebral malaria, an encephalopathy derived from Plasmodium infection in which the blockade of CB2 receptors has been found to have therapeutic value.

P.18.

MATERNAL COMSUMPTION OF HIGHLY PALATABLE FOOD DURING PERINATAL PERIOD ALTERS ADIPOSITY, ANXIETY-RELATED BEHAVIOUR AND THE RESPONSE TO AM251 IN OFFSPRING

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Over the last few years, several studies have shown the importance of early life nutrition for the development of chronic diseases like obesity in adulthood. Obesity has been associated with an overactivation of endocannabinoid system. This system is involved in energy balance, appetite, satiety, motivation and it also contributes to the modulation of stress-related behaviours. In this way, blockade of CB1 receptor by drugs, like AM251, can reduce food intake, particularly of palatable food, but can increase stress-related behaviours too. Here we studied male offspring from two groups of female wistar rats. They consumed two types of diet during pre-mating, pregnancy and lactation period. Dams assigned to the control group(C) were given standard chow ad libitum, and dams assigned to the palatable group (P) were given simultaneously standard chow and a mixture of chocolate ad libitum. The offspring were divided in other two groups at weaning and offered the same diets: control group and palatable group. Food intake and weight gain were measured during the infant, adolescent and adult periods. We also studied food preference, anxiety-related behaviour and tested the response to an acute dose of AM251 (3mg/kg) by measuring food intake of both types of meal for all rats after its administration. We found that the offspring from P dams weighed less than controls at birth, and the group weaned on palatable food (PP) remained weighed less than the group from control dams weaned on palatable food(CP), until 19 postnatal week. However, offspring from P dams tended to be more hyperphagic and had significantly higher percentage of body fat than offspring from C dams. The group from control dams weaned on standard chow(CC) exhibited the highest preference for palatable food when it was available. In contrast, the group from palatable dams weaned on palatable food tended to have higher chocolate preference across time. Furthermore, offspring from palatable dams spent significantly more time than the control group in close arms in elevated plus maze. In the AM251 test, we also found that administration of this drug significantly decreased standard chow intake in offspring from palatable dams but not chocolate intake. In contrast, AM251 significantly reduced chocolate intake in offspring from control dams but not standard chow intake. These results suggest that exposure to highly palatable food during perinatal period could program adiposity and the motivational and reinforcing networks involved in food intake, including the CB1 receptors distribution. Therefore, the response to CB1 antagonist drugs and anxiety-related behaviour could be altered by a perinatal diet.

P.19.

THE CB_1 CANNABINOID RECEPTOR IS PREDOMINANTLY LOCALIZED AT ASYMMETRIC EXCITATORY SYNAPSES IN THE GRANULE CELL LAYER OF THE MOUSE MAIN OLFACTORY BULB

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The endocannabinoid system is a key player in the central regulation of energy balance. Indeed, CB_1 receptors are distributed through brain regions regulating food intake. At the same time, it is well established that CB_1 activation modulates excitatory and inhibitory synaptic transmission in the brain. However, the contribution of CB_1 to the molecular architecture of the excitatory and inhibitory synaptic terminals in the granule cell layer of the main olfactory bulb (MOB) is not known.

The aim of this study was to investigate the subcellular distribution of CB_1 in the granule cell layer of the mouse MOB. Immunocytochemical techniques for light and high resolution electron microscopy were applied to olfactory bulbs of *CB_1*-WT, *CB_1*-KO and conditional mutant mice bearing a selective deletion of CB_1 in cortical glutamatergic (Glu-*CB_1*-KO) or GABAergic neurons (GABA-*CB_1*-KO).

In the light microscope, strong CB_1 immunostaining was localized in the MOB granule cell layer of *CB_1*-WT and GABA-*CB_1*-KO, but drastically decreased in Glu-*CB_1*-KO. Furthermore, the CB_1 immunostaining pattern completely disappeared in *CB_1*-KO. At the electron microscopic level, ~30% of synaptic terminals in the granule cell layer were CB_1 immunopositive, while only 10% of them contained CB_1 immunoparticles in Glu-*CB_1*-KO. The CB_1 immunolabeling virtually disappeared in the *CB_1*-KO granule cell layer. The CB_1 distribution pattern was further analyzed in asymmetric excitatory synaptic terminals. Hence, almost 40% of asymmetric terminals unequivocally identified by ultrastructural features were CB_1 immunopositive, decreasing to 3% in Glu-*CB_1*-KO and virtually disappearing in *CB_1*-KO.

The anatomical data shown here indicate that CB_1 in the granule cell layer of the mouse MOB, in contrast to other brain regions, is predominantly localized at asymmetric excitatory synaptic terminals where it may play an important role in the control of food intake through olfactory processes.

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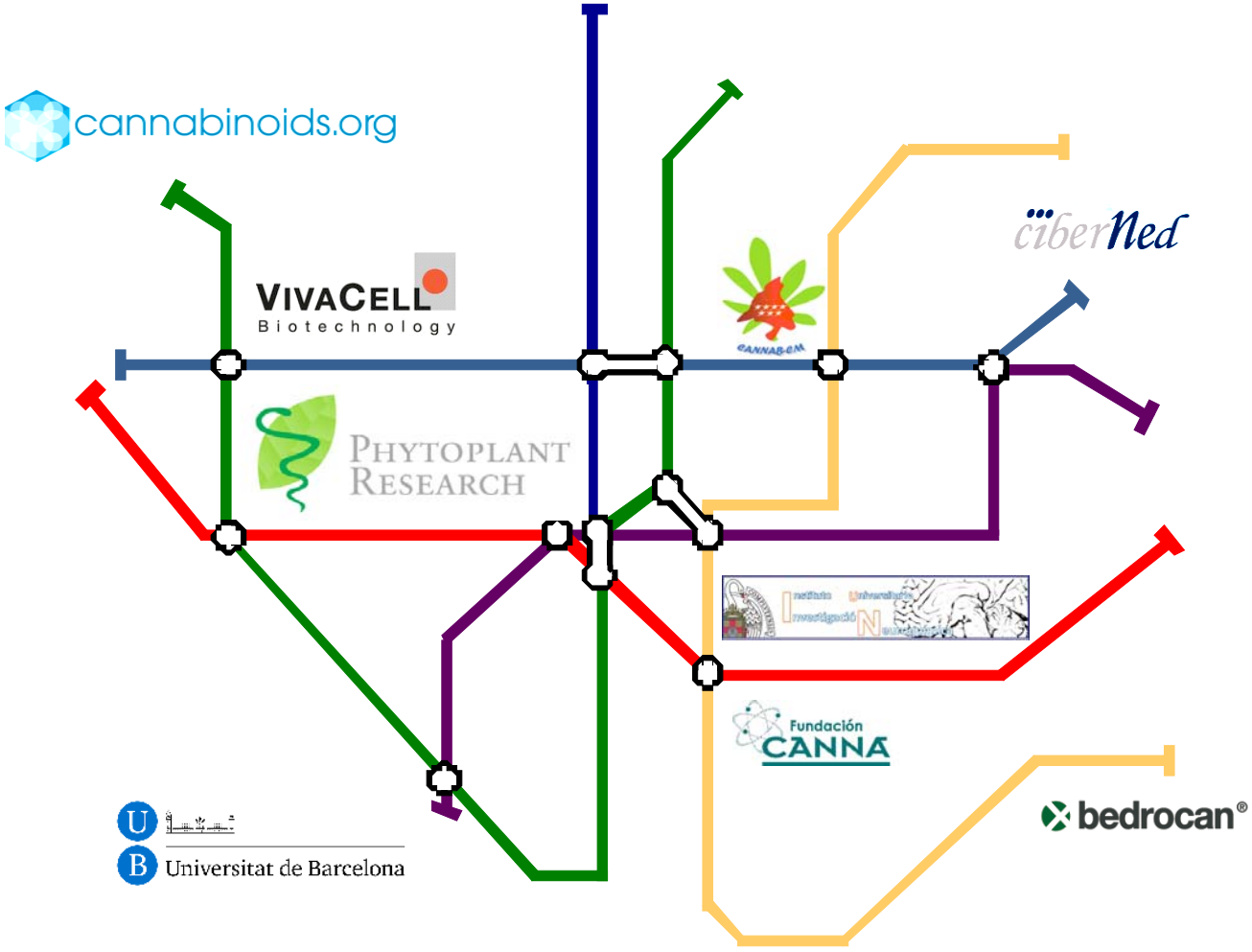

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