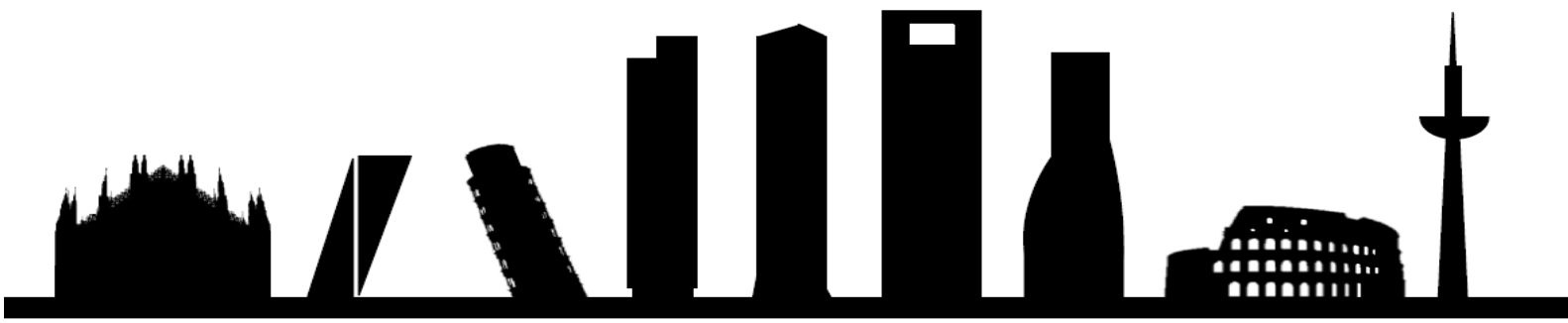
FIRST JOINT SPANISH-ITALIAN MEETING ON CANNABINOID RESEARCH

XIII REUNIÓN ANUAL SEIC

Madrid, November 29th- December 1st 2012



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SCIENTIFIC PROGRAME

Thursday, November 29th

11:30-13:00	Registration and poster setting-up
13:00-13:30	Opening Daniela Parolaro (IRES representative) Manuel Guzmán (SEIC President)
13:30-14:30	Opening lecture (Introduced by Walter Fratta) Rafael Maldonado, Pompeu i Fabra University, Barcelona, Spain "Involvement of the endocannabinoid system in addictive behaviour"
14:30-16:00	Lunch
16:00-18:15	Oral communications (Session I) "Cannabinoids and neuropsychiatry" (Chaired by Andrés Ozaita and Miriam Melis)
16:00-16:15	Introduction (by Miriam Melis)
16:15-16:30	CANNABIS AND PSYCHOSIS: AN UPDATE. L.A. Núñez-Domínguez
16:30-16:45	STUDY OF THE ENDOCANNABINOID SYSTEM IN POSTMORTEM BRAIN OF SCHIZOPHRENIC SUBJECTS. C. Muguruza, J.J. Meana, S.P. Alexander and L.F. Callado
16:45-17:00	INVOLVEMENT OF SEROTONIN 2A RECEPTORS IN THE REGULATION OF THC-INDUCED EFFECTS IN MICE. X. Viñals, R. Maldonado and P. Robledo
17:00-17:15	INVOLVEMENT OF PKC-GAMMA IN THE DELETERIOUS EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL ON MEMORY CONSOLIDATION. M. Gomis-González, A. Busquets-Garcia, E. Puighermanal, R. Maldonado and A. Ozaita
17:15-17:30	INTERLEUKIN-1 BETA RELEASED BY ACTIVATED MICROGLIAL CELLS MEDIATES THE CEREBELLAR FUNCTIONAL DEFICITS ASSOCIATED TO REPEATED CANNABIS EXPOSURE. L. Cutando, A. Busquets, E. Puighermanal, M. Gomis-González, J.M. Delgado-García, A. Gruart, R. Maldonado and A. Ozaita
17:30-17:45	ADOLESCENT DELTA-9-TETRAHYDROCANNABINOL (THC) RESULTS IN ABNORMAL GLUTAMATE AND GABA NEUROTRANSMISSION WITHIN THE ADULT PREFRONTAL CORTEX. E. Zamberletti, S. Beggiato, C. Nazzaro, P. Prini, M. Tramarin, L. Ferraro, R. Tonini, S. Tanganelli, T. Rubino and D. Parolaro
17:45-18:00	CHRONIC (-)CANNABIDIOL IMPROVES DEPRESSIVE-LIKE BEHAVIORS IN OBX MICE LIKELY VIA 5-HT1A AND CB1 RECEPTOR MODULATION. R. Linge, A. Pazos and A. Díaz
18:00-18:15	MATERNAL DEPRIVATION INDUCES SEX- AND REGION- DEPENDENT CHANGES IN THE GENETIC EXPRESSION OF THE BRAIN CANNABINOID SYSTEM IN

	ADOLESCENT RATS. V. Echeverry-Alzate, J.A. López-Moreno, S. Peñasco, E.M. Marco, A.B. López-Rodríguez, E. Giné and M.P. Viveros
18:15-19:15	Coffee break and poster session
19:15-20:30	Oral communications (Session I continued) "Cannabinoids and neuropsychiatry"
19:15-19:30	ENDOCANNABINOID-MEDIATED PLASTICITY AT INHIBITORY SYNAPSES ONTO MIDBRAIN DOPAMINE NEURONS AS A POSSIBLE MARKER OF VULNERABILITY TO ADDICTION. M. Melis and M. Pistis
19:30-19:45	THE HYPOCRETIN RECEPTOR-1: A NEW TARGET TO MODULATE THE REINFORCING PROPERTIES OF CANNABINOIDS. A. Flores, R. Maldonado and F. Berrendero
19:45-20:00	MORPHINE SELF-ADMINISTRATION AND EXTINCTION DIFFERENTIALLY MODULATE CB2 AND μ-OPIOID RECEPTORS ON LYMPHOCYTES AND MONOCYTES FROM LEWIS AND FISCHER344 RATS. M. Ucha, S.M. Coria, A. Higuera-Matas, D. Roura, R. Santos, A. Selvas, M. Miguéns, M.A. Assis and E. Ambrosio
20:00-20:15	THE HUMAN SINGLE NUCLEOTIDE POLYMORPHISM C385A IN THE FAAH ENZYME IS ASSOCIATED WITH ALCOHOL INTOXICATION. K.M. Bühler, E. Huertas, V. Echeverry-Alzate and J.A. López-Moreno
20:15-20:30	PHARMACOLOGICAL CHARACTERIZATION OF JWH-018, A CANNABINOID COMPOUND OF "SPICE" DRUGS. M.A. De Luca, P. Caboni, Z. Bimpisidis, V. Valentini, M. Melis, M. Marti and G. Di Chiara

Friday, November 30th

9:00-10:45	Oral communications (Session II) "Cannabinoids and neurobiology" (Chaired by Eva M. Marco and Tiziana Rubino)
9:00-9:15	Introduction (by Eva M. Marco)
9:15-9:30	SMELL MORE TO EAT MORE: CANNABINOIDS INCREASE FOOD INTAKE THROUGH INHIBITION OF ODOR HABITUATION. E. Soria-Gómez, L. Bellocchio, M. Bendahmane, S. Rühle, F. Remmers, L. Reguero, T. Desprez, A. Wadleigh, I. Matias, T. Wiesner, A. Cannich, D. Verrier, P. Vincent, H.C. Pape, G. Ferreira, F. Massa, B. Lutz, M. Guzman, P. Grandes, H. Gurden and G. Marsicano
9:30-9:45	O-7460, A NOVEL FLUOROPHOSPHONATE INHIBITOR OF THE BIOSYNTHESIS OF THE ENDOCANNABINOID 2-ARACHIDONOYLGLYCEROL WITH POTENTIAL ANTI-OBESITY EFFECTS. T. Bisogno, A. Mahadevan, R. Coccurello, J.W. Chang, M. Allarà, Y. Chen, A. Lichtman, B.F. Cravatt, A. Moles and V. Di Marzo
9:45-10:00	CHRONIC PHARMACOLOGICAL BLOCKADE OF CB1 RECEPTORS REDUCES BINGE-TYPE EATING BEHAVIOUR IN FEMALE RATS. M. Scherma, L. Fattore, V. Satta, F. Businco, B. Pigliacampo, S.R. Goldberg, C. Dessy, W. Fratta and P. Fadda
10:00-10:15	OBESITY-DEPENDENT CANNABINOID MODULATION OF NEUROGENESIS IN ADULT HIPPOCAMPUS. P. Rivera, M. Pérez-Martín, A. Pastor, M.J. Luque-Rojas, F.J. Pavón, A. Serrano, F.J. Bermúdez-Silva, R. de la Torre, F. Rodríguez de Fonseca and J. Suárez
10:15-10:30	LEPTIN-CONTROLLED OREXIN/ENDOCANNABINOID INTERACTIONS IN THE MOUSE PERIAQUEDUCTAL GREY: ROLE IN THE REGULATION OF THE

DESCENDING ANTINOCICEPTIVE PATHWAY. R. Imperatore, L. Cristino, L. Luongo, M.A. Di Grazia, S. Boccella, S. Petrosino, S. Maione and V. Di Marzo

10:30-10:45 MOTOR EFFECTS OF THE NON-PSYCHOTROPIC PHYTOCANNABINOID CANNABIDIOL THAT ARE MEDIATED BY 5-HT1A RECEPTORS. F. Espejo-Porras, C. Palomo, J. Fernández-Ruiz, R.G. Pertwee, R. Mechoulam and C. García

10:45-11:00 CANNABINOID RECEPTOR TYPE 1 (CB1R) ACTIVATION MEDIATES PRESYNAPTIC SILENCING BY EPAC-DEPENDENT MECHANISMS. J. Ramírez-Franco, D. Bartolomé-Martín, B. Alonso, M. Torres and J. Sánchez-Prieto

11:00-12:00 Coffee break and poster session

12:00-14:30 Oral communications (Session III)
"Cannabinoids and neurodegeneration"
(Chaired by Eva de Lago and Fabiana Piscitelli)

12:00-12:15 Introduction (by Fabiana Piscitelli)

12:15-12:30 DOSE-DEPENDENT THERAPEUTIC EFFECTS OF THE MAGL INHIBITOR JZL184 IN A CHRONIC MODEL OF MULTIPLE SCLEROSIS. A. Bernal-Chico, A. Pérez-Samartín, R. Rodríguez-Puertas, C. Matute and S. Mato

12:30-12:45 EVALUATION OF THE EFFECTS OF A SATIVEX-LIKE COMBINATION OF PHYTOCANNABINOIDS AS A DISEASE-MODIFYING THERAPY IN TWO EXPERIMENTAL MODELS OF MULTIPLE SCLEROSIS. A. Feliú, M. Moreno-Martet, M. Mecha, E. de Lago, F. Carrillo-Salinas, J. Fernández-Ruiz and C. Guaza

12:45-13:00 CHANGES IN ENDOCANNABINOID RECEPTORS AND ENZYMES AND BENEFICIAL EFFECTS OF A SATIVEX®-LIKE COMBINATION OF PHYTOCANNABINOIDS IN AN EXPERIMENTAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS. M. Moreno-Martet, J. Fernández-Ruiz, J.A. Ramos and E. de Lago

13:15-13:30 Δ^9-THC AND CANNABIDIOL ENRICHED BOTANICAL EXTRACTS REDUCE THE ALZHEIMER-LIKE PHENOTYPE IN APP/PS1 MICE. E. Aso, S. Juvés, R. Maldonado and I. Ferrer

13:30-13:45 THE ENDOCANNABINOID SYSTEM IS INVOLVED IN THE NEUROPROTECTIVE EFFECTS OF MINOCYCLINE AFTER TRAUMATIC BRAIN INJURY IN MICE. A.B. López-Rodríguez, E. Siopi, C. Marchand-Leroux, L. M. García-Segura, M. Jafarian-Tehrani and M.P. Viveros

13:45-14:00 TARGETED LIPIDOMICS PROFILING OF INJURED RAT BRAIN: N-OLEOYL-GLYCINE AND ITS POSSIBLE MECHANISM OF ACTION. F. Piscitelli, A. Lichtman, L. Sim-Selley, R. Hamm, T. Bisogno, V. Di Marzo and R. Mechoulam

14:00-14:15 FATTY ACID AMIDE HYDROLASE DELETION POTENTIATES IN VIVO GLIAL ACTIVITY IN RESPONSE TO ACUTE BRAIN INJURY. C. Vázquez, M. Moreno, L. Ruiz-Valdepeñas, E. Tomás, S. Ruiz de Martín, R. María Tolón and J. Romero

14:15-14:30 EFFECT OF THE ENDOCANNABINOID N-ARACHIDONOYL-DOPAMINE (NADA) IN THE HYPOXIA RESPONSE PATHWAY. R. Soler-Torronteras, M. Pérez, C. García-Limones, M.A. Calzado and E. Muñoz

14:30-16:00 Lunch

16:00-17:45 Oral communications (Session IV)
"Cannabinoids in the periphery"
(Chaired by Tiziana Bisogno and Inés Díaz-Laviada)

16:00-16:15	Introduction (by Inés Díaz-Laviada)
16:15-16:30	CHROMENOPYRAZOLEDIONES: CANNABINOID-QUINONE DERIVATIVES WITH ANTITUMOR ACTIVITY IN VITRO AND IN VIVO. P. Morales, D. Vara, M. Goméz-Cañas, J. Guzman, Y. Quiroz, C. Olea-Azar, P. Goya, J. Fernández-Ruiz, I. Díaz-Laviada and N. Jagerovic
16:30-16:45	ROLE OF CANNABINOIDS IN PROSTATE CANCER: FOCUS ON CBD PRO-APOPTOTIC MECHANISMS AND POTENTIAL CLINICAL APPLICATIONS. A. Ligresti, R. Verde, L. De Petrocellis, L. Cristino, A. Schiano Moriello, P. Orlando and V. Di Marzo
16:45-17:00	ROLE OF THE CB_2 CANNABINOID RECEPTOR IN ERBB2-DRIVEN BREAST CANCER PROGRESSION. E. Pérez-Gómez, C. Andradas, M.M. Caffarel, G. Moreno-Bueno, J.M. Flores, S. Blasco-Benito, M. Guzmán and C. Sánchez
17:00-17:15	THE ENDOCANNABINOID SYSTEM CONTROLS SKELETAL MUSCLE CELL PROLIFERATION AND DIFFERENTIATION VIA CB1 RECEPTOR ACTIVATION. F.A. Iannotti, C. Silvestri, A. Martella, F. Piscitelli and V. Di Marzo
17:15-17:30	THE NON-PSYCHOTROPIC CANNABINOID CANNABICHROMENE INHIBITS NITRIC OXIDE PRODUCTION IN MACROPHAGES AND AMELIORATES EXPERIMENTAL INFLAMMATORY BOWEL DISEASE. B. Romano, I. Fasolino, F. Borrelli, R. Capasso, F. Piscitelli, P. Orlando, V. Di Marzo and A.A. Izzo
17:30-17:45	THCV EFFECTIVELY DECREASE LIPID LEVELS IN VARIOUS BIOLOGICAL SYSTEMS AND HAS INSULIN SENSITIZING EFFECTS IN HEPATOCYTES. C. Silvestri, A. Martella and V. Di Marzo
17:45-19:00	Coffee and poster session
19:00-20:30	SEIC business meeting (only for SEIC members)
21:00	Dinner (Provided by the organization)

Saturday, December 1st

9:00-10:00	Presentation of the best publications by young researchers in 2012 (Introduced by Cristina Sánchez and Alessia Ligresti)
10:00-11:45	Round table: "Recent advances in the development of cannabinoid-based medicines and their clinical possibilities" (Chaired by Vincenzo Di Marzo and Javier Fernández-Ruiz) • Geoffrey Guy or Stephen Wright, GW Pharmaceuticals • Eduardo Muñoz, VivaCell Biotechnology • Justo García de Yébenes, Hospital Ramón y Cajal • Juan Sepúlveda, Hospital 12 de Octubre • José Martínez-Orgado, Hospital Puerta de Hierro
11:45-12:15	Pause
12:15-13:15	Closing lecture (Introduced by Julián Romero) Mauro Maccarrone, Campus Bio-Medico University of Rome, Italy "Regulation of endocannabinoid signalling by the membrane environment: from receptors to metabolic enzymes"
13:15	Closing – Finger Food

Poster Session

INFLAMMATION IN ALZHEIMER DISEASE: EFFECTS OF CANNABINOIDS
D. Aguirre-Rueda and S.L. Valles

P.2
CANNABINOID COMPONENTS ARE DIFFERENTIALLY DISTRIBUTED DURING DEVELOPMENT
I. Buceta, A. Urtasun, P. Grandes, and I. Elezgarai

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OVEREXPRESSION OF CANNABINOID RECEPTOR CB2 mRNA IMPAIRS CRANIAL NERVE DEVELOPMENT
L. Callén, G. Marras, V. Casadó, A. Cortés, J. Mallol, C. Lluís, E.I. Canela, M. Ori, R. Franco, I. Nardi and P.J. McCormick

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ULTRASTRUCTURAL LOCALIZATION OF THE CB1 RECEPTOR IN THE MUSCLE MITOCHONDRIA
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PALMITOYLETHANOLAMIDE COUNTERACTS IRRITABLE BOWEL SYNDROME (IBS)-LIKE ACCELERATED UPPER GASTROINTESTINAL TRANSIT IN MICE
R. Capasso, E. Pagano, A. Sabatino, I. Fasolino and A.A. Izzo

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CANNABIGEROL QUINONE EXERTS THERAPEUTIC EFFECTS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS
F. Carrillo-Salinas, M. Mecha, A. Feliú, L. Mestre, I. Cantarero, C. Navarrete, E. Muñoz and C. Guaza

P.7
PRENATAL CORTICOSTERONE ADMINISTRATION AND STIMULATION OF THE ENDOCANNABINOID SYSTEM DURING ADOLESCENCE MODULATE EMOTIONAL RESPONSES AND CB1 RECEPTORS IN MICE
C. Ceci, E.M. Marco, S. Macrì, V. Mela, M. López-Gallardo, M.-P. Viveros and G. Laviola

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CANNABIDIOL ADMINISTRATION TO NEWBORN RATS AFTER HYPOXIA-ISCHEMIA ENHANCES NUROPROLIFERATION AND MYELINIZATION
M. Ceprián, M.R. Pazos, F. Penna, N. Mohammed, M. Santos and J. Martínez-Orgado

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CB_1 CANNABINOID RECEPTORS LOCATED ON GLUTAMATERGIC TERMINALS CONFER NEUROPROTECTION IN HUNTINGTON'S DISEASE
A. Chiarlone, C. Blázquez, E. Resel, L. Bellocchio, J.J. Ferrero, E. Soria-Gómez, O. Sagredo, C. Benito, J.M. Flores, M. Sendtner, J. Romero, J. Sánchez-Prieto, B. Lutz, J. Fernández-Ruiz, G. Marsicano, I. Galve-Roperh and M. Guzmán

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ID-1 INHIBITION IS CORRELATED TO CBD ANTI-PROLIFERATIVE EFFECT IN U87-MG CELLS
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EPIGENETIC MECHANISMS IN ALZHEIMER DISEASE: ROLE OF FATTY ACID AMIDE HIDROLASE IN LATE-ONSET AD SUBJECTS AND IN A RARE CASE OF MONOZYGOTIC TWINS DISCORDANT FOR AD
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THE CB1 CANNABINOID RECEPTOR DRIVES CORTICOSPINAL MOTOR NEURON SPECIFICATION THROUGH THE CTIP2/SATB2 TRANSCRIPTIONAL REGULATION AXIS
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CB2 AGONISTS HALT AXONAL DEGENERATION IN A MOUSE MODEL OF THE NEUROMETABOLIC DISEASE ADRENOLEUKODYSTROPHY
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ACUTE CANNABIGEROL ADMINISTRATION RECOVERS PHENCYCLIDINE-INDUCED COGNITIVE DEFICITS AND NEGATIVE-LIKE SYMPTOMS IN RATS
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2-ARACHIDONOYLGLYCEROL MODULATES EARLY STAGES OF ENDOTHELIAL/LEUKOCYTE INTERACTIONS BY UP-REGULATING SELECTINS
V. Gasperi, M.V. Catani D. Evangelista, I. Savini, L. Avigliano and M. Maccarrone

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DISTRIBUTION OF PHOSPHOLIPIDS IN THE BRAIN OF CB1 KNOCKOUT MICE BY IMAGING MASS SPECTROMETRY
E. González de San Román, I. Manuel, S. Mato, A. Veloso, R. Fernández, M.T. Giralt, C. Matute, J.A. Fernández and R. Rodríguez-Puertas

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CHRONIC CANNABIDIOL IMPROVES THE SCHIZOPHRENIA-LIKE SIGNS INDUCED BY REPEATED TREATMENT WITH MK-801 IN MICE
F.V. Gomes, E. Del-Bel, M.P. Viveros and F. Guimarães

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ENVIRONMENTAL STIMULATION DURING DEVELOPMENT MODULATES INDIVIDUAL BEHAVIOURAL AND NEUROCHEMICAL RESPONSES TO CANNABINOID AGONISTS IN MICE
S. Macrì, C. Ceci, L. Altabella, R. Canesea and G. Laviola

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EMOTIONAL REGULATION IN INFANT MALE RATS IS MEDIATED BY PLASMA CORTICOSTERONE LEVELS AND BRAIN ENDOCANNABINOID LIGANDS
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V. Micale, A.L. Terzian, F. Drago, A. Sulcova and C.T. Wotjak

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CANNABIDIOL ADMINISTERED AFTER HYPOXIA-ISCHEMIA TO NEWBORN PIGS IMPROVES NEUROBEHAVIORAL PERFORMANCE
M.R. Pazos, M. Vroomen, H. Lafuente, L. Barata, N. Mohammed, M. Ceprián, M. Santos, F.J. Alvarez and J. Martínez-Orgado

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OLEOYLETHANOLAMINE DOES NOT AFFECT BINGE-TYPE EATING BEHAVIOUR IN FEMALE RATS
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ADOLESCENT THC EXPOSURE AND VULNERABILITY TO DRUG ABUSE
M. Scherma , C. Dessì, L. Fattore, W. Fratta and P. Fadda

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CHARACTERIZATION OF THE ENDOCANNABINOID SYSTEM IN GLIOMA STEM-LIKE CELLS AND ITS ROLE AS POTENTIAL TARGET FOR GLIOMA TREATMENT
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PHYSICAL ACTIVITY UP-REGULATES LYMPHOCYTE FATTY ACID AMIDE HYDROLASE ACTIVITY BY INCREASING CIRCULATING IL-6 LEVELS
M. Tantimonaco, R. Ceci, A. Rossi, S. Sabatini, G. Duranti, A. Parisi, V. Gasperi and M. Maccarrone

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THE ENDOCANNABINOID/ENDOVANILLOID SYSTEM: A NEW POTENTIAL TARGET FOR OSTEOPOROSIS
F. Rossi, G. Bellini, L. Luongo, M. Torella, S. Mancusi, L. De Petrocellis, D. Siniscalco, S. Perrotta, B. Nobili, V. di Marzo and S. Maione

ORAL 1.1

CANNABIS AND PSYCHOSIS: AN UPDATE

LA. Núñez Domínguez

Centro Médico, Pamplona, Navarra, España

From 2002 until now, some research has been carried out trying to discover new data about the relationship between cannabis use and psychosis, new aspects of this relationship. In this presentation I show wich aspects (sociodemographics, age of beginning, genetics,...) may contribute to explain this relationships

ORAL 1.2

STUDY OF THE ENDOCANNABINOID SYSTEM IN *POSTMORTEM* BRAIN OF SCHIZOPHRENIC SUBJECTS

C. Muguruza[1], J.J. Meana[1,2], S.P. Alexander[3] and L.F. Callado[1,2]

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There is a growing body of evidence suggesting that alterations in the endocannabinoid (EC) system may be involved in the pathophysiology of schizophrenia. In this sense, previous studies have reported altered EC levels in cerebrospinal fluid and blood of schizophrenic patients. Additionally, previous findings from our research group demonstrate an opposite change in the levels of the two most common ECs, 2-arachidonoylglycerol (2-AG) and arachidonoylethanolamine (anandamide, AEA) in the prefrontal cortex of subjects with schizophrenia compared to matched controls.

The aim of the present study was to further investigate the status of the different EC system components in the *postmortem* prefrontal cortex of schizophrenic subjects.

For that purpose, brain samples were collected from 20 patients with diagnosis of schizophrenia (DSM-IV), and 20 controls matched by age, gender and *postmortem* delay. In these samples, mRNA expression levels of the major brain cannabinoid receptor (CB1 receptor) and the two main EC degrading enzymes (FAAH and MAGL) were measured by qRT-PCR assays. Moreover, functional assays were carried out in order to determine the CB1 receptor dependent G-protein activation and the enzyme activity of FAAH and MAGL.

The qRT-PCR analysis of mRNA expression of CB1, FAAH and MAGL in the prefrontal cortex revealed no statistically significant differences between schizophrenic and control subjects for any of the genes (Student´s t-test $p=0.10$, $p=0.19$ and $p=0.85$ respectively).

The co-analysis of the concentration-dependent stimulation curves of the [^{35}S]GTPγS binding produced by WIN 55,212-2 (10^{-12}-10^{-3} M), revealed no statistically significant differences between schizophrenic and control subjects either in terms of potency (EC_{50}) ($F[1,405]=0.23$, $p=0.63$) or maximal effect (E_{max}) ($F[1,405]=0.03$, $p=0.87$).

The FAAH activity for increasing concentrations of the substrate AEA was fitted to a Michaelis-Menten hyperbola. The maximum enzyme velocity (V_{max}) and the concentration of the substrate at half of the V_{max} (K_m) values were not significantly different in the prefrontal cortex of schizophrenic and control subjects (curves co-analysis: $F[2,113]=2.79$; $p=0.07$). No differences between schizophrenia and control group were found in the MAGL activity assessed with a single concentration of the substrate 2-OG (Student´s t-test $p=0.99$).

These results demonstrate that the expression and function of the CB1 receptor and the degrading enzymes FAAH and MAGL are not altered in the prefrontal cortex of schizophrenic subjects, and point towards other mechanisms underlying the alterations of EC levels observed in schizophrenia.

ORAL 1.3

INVOLVEMENT OF SEROTONIN 2A RECEPTORS IN THE REGULATION OF THC-INDUCED EFFECTS IN MICE

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There is evidence establishing a link between serotonin and endocannabinoid signalling. Thus, it has been shown that the activation of 5HT2AR stimulates the release of endocannabinoids, possibly mediating many of the actions of 5HT2AR. However, little is known about the implication of 5HT2AR in the effects induced by cannabinoids. In this study we have used 5HT2AR knockout (KO) and wild-type (WT) mice to evaluate locomotor activity, hypothermia and analgesia following acute administration of delta-9-tetrahydrocannabinol (THC), the main psychoactive compound of the cannabis sativa plant. In addition, anxiety-like behaviour was evaluated using anxiolytic and anxiogenic doses of THC in the elevated plus maze. Moreover, amnesic-like effects in the object recognition task, WIN 55212 reinforcing properties in the self-administration paradigm and cannabinoid dependence have been evaluated as well. 5HT2AR KO mice presented a reduction in spontaneous activity after THC administration whereas WT mice did not show this hypolocomotor effect. Also, an anxiolytic dose of THC (0.3 mg/kg), which induced significant effects in WT animals, did not produce a significant reduction of anxiety in 5HT2AR KO animals in the elevated plus maze. Moreover, 5HT2AR KO mice showed a lower global withdrawal score in comparison with WT animals. On the other hand, no differences between genotypes were obtained in analgesia either in the hot plate test or in the tail immersion paradigm. Therefore, our results indicate that 5HT2AR are involved in the regulation of cannabinoid-induced responses in mice, suggesting the presence of bilateral interactions between 5HT2AR and the endocannabinoid system.

ORAL 1.4

INVOLVEMENT OF PKC-GAMMA IN THE DELETERIOUS EFFECTS OF DELTA9-TETRAHYDROCANNABINOL ON MEMORY CONSOLIDATION

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Delta9-tetrahydrocannabinol (THC), the main psychoactive component in Cannabis sativa plant, modulates several intracellular signaling pathways in the brain, some of them related to its memory impairing effects. Using biochemical, pharmacological, genetic and behavioral approaches on mice, we found that acute administration of THC (3 mg/kg) promotes the phosphorylation of hippocampal protein kinase C (PKC) isoforms in their catalytic domain through a N-methyl-D-aspartate receptor (NMDAR)-dependent manner. Moreover, THC also modulates the phosphorylation of neurogranin, a calmodulin regulating protein abundantly expressed in brain regions involved in cognitive function, and the phosphorylation of the myristoylated alanine-rich C-kinase substrate (MARCKS), involved in the regulation of cell shape and dendritic spine maintenance. Interestingly, both proteins together with neuromodulin, which phosphorylation was not modulated by THC, are preferential targets from the PKC-gamma isoform. Considering the results we evaluated the relevance of PKC-gamma signaling in the cognitive effects of THC in mice lacking this PKC isoform (PKC-gamma KO mice). For this purpose, we used four memory tasks: object recognition, context recognition, cued fear conditioning and active avoidance. In all these paradygms reduced amnesic-like effects of THC were revealed in PKC-gamma KO mice compared to wild-type controls. In contrast, other pharmacological effects of THC, such as hypothermia and analgesia were not modified in the PKC-gamma KO mice. Finally, the cognitive performance was evaluated after a sub-chronic treatment of THC. In the object-recognition test, PKC-gamma KO mice were only sensitive to the amnesic-like effects of THC after 4 days of treatment while the control mice were sensitive to the amnesic-like effects of THC from the first day of THC exposure. Our results show a role of PKC-gamma signaling in the cognitive deficits produced by THC. Thus, the modulation of calcium/calmodulin signaling by neurogranin phosphorylation, and the regulation of structural plasticity through MARCKS phosphorylation may underlie the PKC-gamma role in the cognitive deficits produced by THC.

ORAL 1.5

INTERLEUKIN-1 BETA RELEASED BY ACTIVATED MICROGLIAL CELLS MEDIATES THE CEREBELLAR FUNCTIONAL DEFICITS ASSOCIATED TO REPEATED CANNABIS EXPOSURE.

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Chronic cannabis consumption has been associated to cerebellar dysfunction in humans. We have mimicked such a deficit in mice through the repeated exposure to delta9-tetrahydrocannabinol (THC). Under our experimental conditions chronic THC administration modifies microglia morphology towards a reactive phenotype mainly in the molecular layer of the cerebellum, increasing the expression of several neuroinflammatory markers, such as interleukin 1β (IL-1β). This neuroinflammatory phenotype was markedly increased five days after spontaneous THC withdrawal and correlated with deficits in motor coordination and conditioned cerebellar learning. A key role of CB1 cannabinoid receptor (CB1R) down-regulation in the molecular layer of the cerebellum as result of chronic THC exposure was observed since the neuroinflammatory phenotype was readily detectable in the cerebellum of CB1R constitutive knockout mice (CB1–/–) and in mice lacking CB1R in the cerebellar parallel fibers (CB1α6–) but not in the hippocampus, a brain area with a high density of CB1R.

Quantitative real time PCR analysis revealed a clear enhancement of IL-1β mRNA levels in the cerebellum of CB1–/– and CB1α6– mice, resembling that found in THC-withdrawn mice. Interestingly, IL-1β is an important pro-inflammatory cytokine mainly produced in the brain by microglial cells and directly regulates neuronal function though IL-1β receptors (IL-1R). Thus, the direct action of IL-1β on IL-1R present in Purkinje cells has been associated to an increase on cellular excitability that could directly affect cerebellar output. In this regard, we demonstrated that acute pharmacological blockade of IL-1β receptors drastically reduced the cerebellar deficits observed in THC-withdrawn, CB1–/– and CB1α6– mice. These results show the crucial role of cerebellar microglia in the performance of cerebellar-dependent functions, and reveal microglial reactivity as a key element in the cerebellar deficits associated to repeated cannabis exposure.

ORAL 1.6

ADOLESCENT DELTA-9-TETRAHYDROCANNABINOL (THC) RESULTS IN ABNORMAL GLUTAMATE AND GABA NEUROTRANSMISSION WITHIN THE ADULT PREFRONTAL CORTEX

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Exposure to delta-9-tetrahydrocannabinol (THC) during adolescence results in long-term disturbances of cognitive performances and emotional reactivity in adult female rats. Interestingly, no behavioral abnormalities are observed when THC is administered in adulthood, suggesting a specific vulnerability of the adolescent brain to the long-term adverse effects of cannabinoids.
Since maturation of the prefrontal cortex (PFC) is one of the most important processes during adolescence, THC may predominantly affect the maturation of specific neurocircuits within this brain region, leading to abnormal behavioral responses in adulthood.
Therefore, in the present study, we investigated the impact of adolescent THC exposure on the maturation of glutamate and GABA systems in the PFC.
In control animals, a significant decrease of N2B subunits from mid to late adolescence and a significant increase of N2A subunits from late adolescence to adulthood are present in the PFC. Moreover, changes in the scaffolding protein PSD95 are also observed in control animals together with an increase in the amount of GluA2-containing AMPA receptors.
In contrast, altered rearrangement of NMDA and AMPA receptor subunits occurs after adolescent THC exposure. Indeed, the amount of GluN2B-containing NMDA receptors and GluA1-containing AMPA receptors is significantly higher in adult THC-treated rats compared to controls. Moreover, adolescent THC impairs the physiological fluctuation of PSD95 levels and leads to reduced basal glutamate release in the PFC.
In the developmental period from mid adolescence till adulthood, maturation processes also occurs at GABA synapse. Indeed, the levels of the enzyme responsible for GABA synthesis, GAD67, constantly increase during this developmental period in the PFC of control animals. Once again, adolescent THC treatment disrupts the physiological development of the GABAergic system. In fact, GABA synthesis and release are reduced in adulthood after adolescent THC treatment compared with controls.
At structural and functional levels, synaptic connectivity, investigated by means of Golgi-cox staining, is altered in the PFC of THC-treated animals compared with controls and, moreover, adolescent THC exposure leads to a significant reduction of endocannabinoid-mediated LTD in adulthood.
These data demonstrate that adolescent THC exposure disrupts physiological synapse maturation and circuit refinement in the PFC, eventually leading to functional impairments and abnormal behavioral reactivity.

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ORAL 1.7

CHRONIC (-) CANNABIDIOL IMPROVES DEPRESSIVE-LIKE BEHAVIORS IN OBX MICE LIKELY VIA 5-HT_{1A} AND CB_1 RECEPTOR MODULATION

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The major non-psychoactive constituent of marihuana, (-)Cannabidiol (CBD), has been recently proposed as a potential therapy for mood disorders. Its anxiolytic effect after acute administration has been certainly demonstrated. However, the consequences of chronic treatment, particularly in an animal depressive-like model, remain unknown.

In this study we have tested the effect of chronic treatment with CBD in the bulbectomy (OBX) mice model of comorbid depression and anxiety, both in behavioral paradigms and in the functionality of CB_1 and 5-HT_{1A} receptor in different brain areas.

Materials and methods: Sham operated and OBX mice received CBD or vehicle for two weeks. Aversive open field and sucrose intake test were conducted for behavioral characterization, and G-protein coupling to CB_1 and 5-HT_{1A} receptors was analyzed by [^{35}S]GTPγS binding.

Results: After chronic CBD administration, a decrease in peripheral hyperactivity of OBX mice was observed in the open field, consistent with the reversal of reduced sucrose intake exhibited by OBX anhedonic mice. We found interesting changes in the functionality of 5-HT_{1A} and CB_1 receptors in limbic areas, such as amygdale, hippocampus and entorhinal cortex where CBD counteracted the enhanced 5-HT_{1A} functionality prompted by OBX, or in hypothalamus where the increased CB_1 functionality after OBX was reduced similarly.

Conclusions: Taken together, these results indicate that CBD improves the disrupted emotional state of OBX mice, acting like an antidepressant drug, and that the modulation of 5-HT_{1A} and CB_1 receptors functionality in limbic areas could underlie these effects.

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ORAL 1.8

MATERNAL DEPRIVATION INDUCES SEX- AND REGION- DEPENDENT CHANGES IN THE GENETIC EXPRESSION OF THE BRAIN CANNABINOID SYSTEM IN ADOLESCENT RATS

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We have previously found that maternal deprivation (MD, 24h at postnatal day 9) in rats induce diverse behavioural and neuroimmunoendocrine alterations as well as disturbances in the development of diverse brain areas that affect neurons, glia and synaptic plasticity (Marco et al Neuropharmacol 2012 in press; Llorente et al J Neuroendocrinol. 2011, 23:329-44; Llorente et al Neuroscience. 2012, 201:12-9; Viveros et al Psychoneuroendocrinology. 2009, Suppl 1:S217-26). Notably, by western blotting and immunohistochemical analyses, we have revealed alterations in the developing cannabinoid system, i.e. in CB1 and CB2 receptors, endocannabinoid levels and enzymes involved in their synthesis and degradation (Marco et al Neuropharmacol 2012; Suarez et al Hippocampus. 2009, 19:623-32 & Brain Res. 2010, 1349:162-73). In the present study we have addressed the effect of MD on the gene expression of the main components of the brain cannabinoid system in adolescent Wistar rats of both sexes. We analysed *Cnr1, Faah, Mgll, Dagl_a, Dagl_b, Nape, Pparg, Cox-2, Tprv1, Cb2_a, Cb2_b,* and *Gpr55* in the prefrontal cortex, ventral and dorsal striatum, hippocampus and amygdala. *Cnr1* and *Mgll* were the most abundantly expressed genes in all the five regions studied, whereas *Pparg, Gpr55* and *Cb2_a, Cb2_b* genes were the lowest expressed. MD induced different genetic expression patterns depending on the brain structure and the sex of the animals. In males, the main effect of MD was an increase in the genetic expression of all the genes studied in the prefrontal cortex whereas in females this increase was observed in the hippocampus. The data indicate a region dependent vulnerability of the cannabinoid system to the effects of early life stress in male and female rats.

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ORAL 1.9

ENDOCANNABINOID-MEDIATED PLASTICITY AT INHIBITORY SYNAPSES ONTO MIDBRAIN DOPAMINE NEURONS AS A POSSIBLE MARKER OF VULNERABILITY TO ADDICTION

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Addiction is a psychiatric disorder, whose aetiology involves interaction of inherited predispositions and environmental factors. Both clinical and preclinical findings indicate that there are important genetic and sex-dependent variations in vulnerability to drug addiction, and that such differences may be mediated by the same biological mechanisms.
Addictive drugs share the properties of being self-administered by laboratory animals, and of activating the brain reward circuitry, which stems from the ventral tegmental area (VTA) where dopamine (DA) cells are located. Endocannabinoids serve as retrograde signaling molecules at many synapses in the brain, including the VTA, and regulate reward seeking by modulating DA signaling. We took advantage of significant sex differences in cannabinoid self-administration displayed by Lister Hooded (LH) female and male rats, and of one of the few pairs of lines of rats selectively bred for their voluntary alcohol preference or aversion, that is Sardinian alcohol-preferring (sP) or nonpreferring (sNP) rat line. We have found that depolarization-induced suppression of inhibition (DSI), a form of endocannabinoid-mediated short term synaptic plasticity, is differently expressed by two discrete sets of inhibitory synapses arising from rostral and caudal afferents onto VTA DA neurons. This phenomenon is selectively mediated by the endocannabinoid 2-arachidonoylglycerol (2-AG), which activates presynaptic type 1-cannabinoid (CB1) receptors. However, the two discrete DSI do not seem to depend upon differences in CB1 number and/or function, but rather on the rate 2-AG is degraded. Thus, 2-AG by differently depressing inhibitory synapses arising from either rostral or caudal afferents might indirectly alter DA neuron functional state, and enhance the responsiveness of the reward pathway to phasic DA.
Given that both LH female rats and sP rats are vulnerable phenotypes, and that they share this endocannabinoid-mediated form of short term plasticity, our results suggest that differences in equipment of the endocannabinoid system machinery might control specific sources of vulnerability.

ORAL 1.10

THE HYPOCRETIN RECEPTOR-1: A NEW TARGET TO MODULATE THE REINFORCING PROPERTIES OF CANNABINOIDS

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Emerging evidence suggests that hypocretins (orexins), classically related to the regulation of feeding, circadian cycle and arousal, are also involved in addictive-like behaviour. In this study, we evaluated the role of these hypothalamic neuropeptides in the reinforcing properties of the synthetic cannabinoid WIN 55,212-2 by using intravenous self-administration in mice. The acute administration of the hypocretin receptor-1 (HcrtR-1) antagonist SB334867 (10 mg/kg) after the acquisition of WIN 55,212-2 self-administration (12.5 μg/kg/infusion) blocked this behavioural response. Moreover, mice pre-treated with SB334867, as well as HcrtR-1 knockout mice, achieved a lower breaking point in the progressive ratio schedule, suggesting also an involvement of this receptor in the motivation to self-administer WIN 55,212-2. In contrast, the acute injection of the hypocretin receptor-2 (HcrtR-2) antagonist TCS OX2 29 (10 mg/kg) did not affect either WIN 55,212-2 self-administration or motivation for the drug. Additionally, pre-treatment with SB334867 before each session during the acquisition period of WIN 55,212-2 self-administration reduced the percentage of mice achieving the acquisition criteria, although no significant differences were found in active nose-poking. In agreement, HcrtR-1 knockout mice also showed a decrease in the percentage of acquisition of the operant behaviour and a reduction of active nose-poking. In contrast, chronic pre-administration of TCS OX2 29 had no effect in any of these parameters. According to the behavioural data, *in vivo* microdialysis studies revealed that the enhancement in dopamine extracellular levels in the nucleus accumbens induced by THC (0.3 mg/kg) was blocked in HcrtR-1 knockout mice. Taken together, these results suggest that the HcrtR-1 is involved in the reinforcing properties of cannabinoids, possibly through modulation of cannabinoid-induced dopaminergic transmission, revealing a possible new target for the treatment of cannabinoid dependence.

ORAL 1.11

MORPHINE SELF-ADMINISTRATION AND EXTINCTION DIFFERENTIALLY MODULATE CB_2 AND μ-OPIOID RECEPTORS ON LYMPHOCYTES AND MONOCYTES FROM LEWIS AND FISCHER344 RATS

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Although opiate addiction treatment has improved considerably over the past few decades the risk of relapse still remains too high. In addition, there seems to be a high degree of individual variation in this phenomenon. It is well known that morphine exposure is able to alter the endogenous opioid and cannabinoid systems at the central nervous system level. Interestingly, morphine also modulates the immune response by means of its actions on opioid receptors and related intracellular signaling proteins that are expressed in lymphocytes. For this reason, in this study we decided to analyze the expression of μ opioid and CB_2 receptors on peripheral immune subpopulations by flow cytometry after morphine self-administration (1 mg/kg/injection) and extinction training (7 or 15 days) in Lewis and Fischer344 rats. These strains differ in their vulnerability to the rewarding effects of several drugs and have been typically used to model genetic factors affecting the proneness to drug addiction.

Under basal conditions, Fischer344 rats showed higher levels of T-cells than Lewis rats, on the contrary they displayed less B cells. Also Fischer344 rats had more monocytes expressing μ receptor than Lewis rats. After 7 days of extinction, we found increased levels of μ expressing T-cells in both strains. When the extinction was prolonged to 15 days, both strains showed increased levels of CB_2 expressing splenic immune cells, and μ expressing B-cells. We also found an elevated proportion of CB_2-expressing monocytes but only in Lewis rats.

These results suggest that morphine self-administration and extinction differently modulate the expression of certain elements of the immune cells according to the individual genetic background. Furthermore, it should be possible that some effects reported here after extinction could be implicated in mechanisms underlying relapse to morphine seeking. This later approach could contribute to develop novel therapeutic strategies for opiate addiction treatment.

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ORAL 1.12

THE HUMAN SINGLE NUCLEOTIDE POLYMORPHISM C385A IN THE FAAH ENZYME IS ASSOCIATED WITH ALCOHOL INTOXICATION

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In the *FAAH* gene there is a single nucleotide polymorphism (SNP) that causes the codon CCA (proline residue) to change to ACA (threonine residue) in the position 129 of the FAAH protein (P129T or C385A or rs324420). This non-synonymous SNP is also found in mouse, rat, pig and cow suggesting that it is highly conserved in mammals. Interestingly, it has been repeatedly associated with several neuropsychiatric disorders and among them drug addiction. Here, we aimed to investigate whether the C385A variant was associated with alcohol, nicotine and cannabis consumption. For this purpose we used 180 university students. DNA samples were collected from saliva and genotyped by direct sequencing and TaqMan assays. Genotypes were grouped as AX (AA and AC alleles) and CC due to that A is the rare allele. Additionally, the participants were asked to rate multiple food, drug and neutral pictures on a pleasant-unpleasant analogue scale. We found a very significant association between heavy alcohol consumption during the weekends and CC carrier individuals. It was not found any association between nicotine and cannabis with the C385A variant. In order to perform better predictions, we performed a multiple regression model where several explanatory variables were included (e.g., nicotine consumption and pictures rating). We found that including three variables more along with the C385A variant it would be possible to predict above of the 30% of the variance associated with heavy alcohol consumption among individuals. These results suggest that the FAAH enzyme would be implicated in the early stages of alcohol addiction and it may be used as a biomarker, even before the individuals reach a diagnosis of alcoholism. Furthermore, we provide conclusive evidence that the use of genotype and behavioural information provides a better fit model than a simpler model in the prediction of heavy alcohol consumption.

ORAL 1.13

PHARMACOLOGICAL CHARACTERIZATION OF JWH-018, A CANNABINOID COMPOUND OF "SPICE" DRUGS

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'Spice' is a smokable herbal mixture marketed under many brand names as herbal alternatives to Cannabis. Spice users report experiences similar to those produced by Cannabis and regular users may develop addiction and experience withdrawal and symptoms. Spice contains dried, shredded plant material and chemical additives that could be responsible for its psychoactive cannabis-like effects. JWH-018 (1-pentyl-3-(1-naphthoyl) indole), a potent CB1 and CB2 synthetic agonist has been analytically identified in different specimens of Spice drugs. Here, we investigated the rewarding properties of JWH-018 by *in vivo* microdialysis, intravenous self-administration behavior (SA) and *ex vivo* electrophysiological studies in separate groups of male Sprague-Dawley rats. In microdialysis studies, we observed that JWH-018, at the dose of 0.25 mg/kg ip, preferentially stimulates extracellular dopamine (DA) release in the nucleus accumbens (NAc) shell with respect to core and prefrontal cortex and that this effect was blocked by CB1 receptor antagonists rimonabant and AM 251 (1 mg/kg ip 30 min before JWH-018). In SA studies, rats implanted with a jugular catheter were trained to self-administer JWH-018 (10 and 20 µg/kg/inf iv) in single daily 1h session for 11 weeks, under an initial Fixed Ratio (FR) 1 schedule, than increased to FR3. Active nose-poking significantly increased over inactive ones from the 21st SA session (20 µg/kg/inf iv, FR3, acquisition phase) and the reinforcing effect was blocked by rimonabant (1 mg/kg ip, 30 min before SA session). However, when vehicle was substituted for JWH-018 (37th SA session, extinction phase), a very low reduction of SA behavior was observed. But, the replacement of vehicle with JWH-018, significantly increased the rate responding and the number of injections (reacquisition phase). Whole cell patch clamp recordings from DA neurons of the ventral tegmental area revealed that JWH-018 is able to decrease GABAA-mediated postsynaptic currents in a dose-dependent fashion. This effect is mimicked by the CB1 agonist WIN55,212-2, and fully blocked by rimonabant. These results suggest that the JWH-018 shares with other drugs of abuse the property of stimulating DA transmission and of being self-administered by rats. Furthermore, in order to assess a more comprehensive pharmacological characterization of the effects of JWH-018 in the control of some physiological parameters, behavioral studies in CD-1 mice have been performed. We observed that JWH-018 (0.1-3 mg/kg ip) reproduces even in mouse model the typical "tetrad effects" that characterize the acute symptoms following the assumption of cannabinoids, such as hypothermia, the increase of the pain threshold, the marked catalepsy and hypokinesia of the animal. All effects were blocked by AM251 (3 mg/kg ip).

ORAL 2.1

SMELL MORE TO EAT MORE: CANNABINOIDS INCREASE FOOD INTAKE THROUGH INHIBITION OF ODOR HABITUATION

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By favoring the retrieval and selection of food sources, olfaction is a key determinant of feeding behavior. Activation of type-1 cannabinoid receptors (CB_1) on cortical glutamatergic neurons promotes fasting-induced food intake, but the exact locus of this control is unknown. Here, we show that CB_1 receptors regulate feeding behavior and odor habituation in fasted animals by controlling glutamatergic inputs from olfactory cortical areas to the olfactory bulb. This circuit is responsible for the orexigenic action of both endo- and exo-cannabinoids, such as THC. Therefore, we propose that the control of olfactory processes is a key determinant of the promotion of stimulated food-intake by the endocannabinoid system.

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ORAL 2.2

O-7460, A NOVEL FLUOROPHOSPHONATE INHIBITOR OF THE BIOSYNTHESIS OF THE ENDOCANNABINOID 2-ARACHIDONOYLGLYCEROL WITH POTENTIAL ANTI-OBESITY EFFECTS

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The development of potent and selective inhibitors of the biosynthesis of the endocannabinoid 2-AG via diacylglycerol lipases (DAGL) α and β is just starting to be considered as a novel and promising source of pharmaceuticals for the treatment of disorders that might benefit from a reduction of endocannabinoid tone, such as hyperphagia in obese subjects. Here we describe the synthesis and pharmacological characterization *in vitro* and *in vivo* of 1-((fluoro(methyl)phosphoryl)oxy)-3-isopropoxypropan-2-yl oleate (O-7460).

The new compound, O-7460 exhibited high potency (IC_{50}=690 nM) against the human recombinant DAGLα, and selectivity (IC_{50}>10 μM) towards COS-7 cell and human monoacylglycerol lipase (MAGL), and rat brain fatty acid amide hydrolase (FAAH). O-7460 did not exhibit measurable affinity for human recombinant CB_1 or CB_2 cannabinoid receptors (K_i>10 μM). Moreover, O-7460 inhibited DAGLα in a seemingly irreversible way since its inhibitory effect persisted after repeated washing of the DAGLα membrane preparations. In mouse neuroblastoma N18TG2 cells stimulated with ionomycin, O-7460 (10 μM) reduced de novo biosynthesized 2-AG levels.

When administered to mice, O-7460 dose-dependently (0-12 mg/kg, i.p.) inhibited the intake of a high fat diet over a 14 h observation period. We also showed, for the first time, that acute DAGLα inhibition by O-7460 also caused a reduction of body weight, since mice treated with the highest dose of the compound, unlike mice treated with vehicle, not only did not gain weight over the observation period, but even experienced a small but statistically significant reduction in body weight. Importantly, these effects of O-7460 were accompanied by its reversal of HFD-induced elevation of both hypothalamic and hepatic 2-AG levels.

In conclusion, O-7460 might be considered a useful pharmacological tool to investigate further the role played by 2-AG both *in vitro* and *in vivo* under physiological as well as pathological conditions, and provides proof-of-concept to the idea that DAGL inhibitors might be developed into new anti-obesity drugs.

ORAL 2.3

CHRONIC PHARMACOLOGICAL BLOCKADE OF CB1 RECEPTORS REDUCES BINGE-TYPE EATING BEHAVIOUR IN FEMALE RATS

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The endocannabinoid system has been shown to control food intake in both animals and humans, modulating either rewarding or quantitative aspects of the eating behaviour, and those actions are mediated by the CB1 receptors. Binge eating disorder (BED) is defined as an intermittent and uncontrollable consuming of an unusual amount of food during short periods of time and typically involves highly palatable foods rich in calories and fat composition. Plasma levels of endocannabinoid anandamide (AEA) resulted significantly enhanced in overweight/obese patients with BED suggesting that alterations in the endocannabinoid signalling could be involved in the pathophysiology of BED. In this study we investigated whether the pharmacological manipulation of the endocannabinoid transmission may be effective in modulating the aberrant eating behaviour present in a validated rat model of BED. Binge-type eating was induced in female rats by providing limited access to an optional source of fat dietary (margarine). Rats were divided into three groups, all with ad libitum access to chow and water: Control (C), with no access to margarine; Low-restriction (LR), with 2h margarine access 7 days/week; High-restriction (HR), with 2h margarine access 3 days/week. As compared to LR group, HR group displayed higher consumption of margarine accompanied by an increasing in body weight. The cannabinoid CB1/CB2 receptor agonist Δ^9-tetrahydrocannabinol (THC) significantly increased margarine intake selectively in LR rats, while the fatty acid amide hydrolase inhibitor URB597 showed no effect. The CB1 receptor inverse agonist/antagonist rimonabant dose-dependently reduced margarine intake in HR rats. Notably, in HR rats, chronic treatment with a low dose of rimonabant induced a selective long-lasting effect on margarine intake that did not develop tolerance, and produced a significant and persistence reduction of body weight. Thus, chronic pharmacological blockade of CB1 receptors reduces binge eating behaviour in female rats and may prove effective in treating BED, with an associated significant reduction in body weight.

ORAL 2.4

OBESITY-DEPENDENT CANNABINOID MODULATION OF NEUROGENESIS IN ADULT HIPPOCAMPUS

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Obesity can induce alterations in brain physiology affecting neurogenesis and produce changes in the endocannabinoid signaling (ECS) influencing the regulation of energy balance. However, it is unclear whether cannabinoid signaling may modulate neurogenesis in obese animals. Here we analyzed the impact of obesity defined by three different approaches: standard diet (STD, normal chow), high-carbohydrate diet (HCHD, 70% carbohydrate) and high-fat diet (HFD, 60% fat), and the response to a subchronic treatment with the CB1 receptor inverse agonist AM251 (3 mg/kg) on the cell proliferation of a relevant neurogenic region, the subgranular zone of the dentate gyrus (SGZ), and the immunohistochemical expression of the enzymes that produce (DAGL□ and NAPE-PLD) and degrade (MAGL and FAAH) endocannabinoids in the hippocampus.
We found evidence of an interaction between the diet-induced obesity and CB1 signaling in the regulation of hippocampal cell proliferation. Our results showed that AM251 reduced caloric intake and body weight in obese rats. We described for the first time that AM251 can modulate the hippocampal cell proliferation in HFD-obese rats only. Thus, we observed an increase in the number of 5-bromo-2-deoxyuridine-labelled (BrdU+) cells in the SGZ. These BrdU+ cells expressed the neuron-specific □III-tubulin. These results suggest that obesity may impact cell proliferation in the brain, and provide support for a role of CB1 signaling regulation of neurogenesis in response to obesity. Results also indicated that AM251 induced a modulation of the expression of ECS-related proteins in the hippocampus of animals exposed to hypercaloric diets. These effects were differentially restricted to either 2-AG or anandamide signaling pathways, in a diet-dependent manner. AM251-treated rats fed HCHD showed a drop of the DAGL□/MAGL ratio, whereas AM251-treated rats fed HFD showed a decrease of the NAPE-PLD/FAAH ratio. These results are consistent with the reduced levels of hippocampal endocannabinoids found after food restriction. Regarding the CB1 expression, AM251 induced specific changes focused in the CA1 stratum pyramidale of HFD-fed rats. These results indicated that cannabinoid antagonist AM251 modulates ECS-related proteins in the rat hippocampus in a diet-specific manner. Overall, these findings suggest that the hippocampal neurogenesis and endocannabinoid signaling participate in the physiological adaptations to different caloric diets.

ORAL 2.5

LEPTIN-CONTROLLED OREXIN/ENDOCANNABINOID INTERACTIONS IN THE MOUSE PERIAQUEDUCTAL GREY: ROLE IN THE REGULATION OF THE DESCENDING ANTINOCICEPTIVE PATHWAY

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In the ventrolateral periaqueductal gray (vlPAG), activation of excitatory output neurons projecting monosynaptically to OFF cells in the rostral ventromedial medulla (RVM) causes antinociceptive responses via OFF cells stimulation and ON cell inhibition (Behbehani et al., *Pain* 1990). We demonstrated that this descending nociceptive pathway is under the control of cannabinoid receptor type-1 (CB1) (Maione et al*., J Pharmacol. Exp. Ther.* 2006). On the other hand, 2-AG deeply affects nociception via CB1 stimulation, and its concentration is higher in the PAG and RVM of wt mice during neuropathic pain (Petrosino et al. *Neuropharmacology, 2007).* Orexins are hypothalamic peptides known to modulate arousal, feeding, reward and antinociception via orexin receptors (OX-R). In obese *ob/ob* leptin knock-out mice, OX-A expression increases in the fibers projecting to vlPAG and 2-AG levels increases in the lateral hypothalamus (Cristino et al., submitted). Recently, Ho and collaborators demonstrated that orexin-A (OX-A), by activating OX-AR (OX-A receptor) in the vlPAG of rats, stimulates the synthesis of 2-AG and retrograde inhibition of the tonically active GABAergic circuit (disinhibition) thus inducing disinhibition of the descending nociceptive pathway (Ho et al., *J Neurosci.,* 2011). Interestingly, during neuropathic pain the levels of leptin increase substantially, and *ob/ob* mice do not develop mechanical allodynia (Maeda et al., *PNAS,* 2009). On this basis we hypothesized the existence of a leptin-controlled orexin/endocannabinoid interaction in the modulation of the pain network leading to nociception. We have investigated this hypothesis using a combination of electrophysiological (*in vivo* recording), immunohistochemical (OX-A, OX-AR and CB1 single and multiple localization), ultra-structural (CB1/OX-A immunogold labeling on symmetric or asymmetric synapses), biochemical (determination of endocannabinoid levels in the PAG) and behavioral (nociception in the "plantar test" and in spontaneous and tail-flick-related activities of RVM neurons) approaches in wt and *ob/ob* mice. RESULTS: 1. OFF (anti-nociceptive) and ON (pro-nociceptive) cells are more and less active, respectively, in *ob/ob* compared to wt. 2. A significant increase of number and intensity of OX-A fibers was observed in the PAG of *ob/ob* mice and was accompanied by a two-fold increase of pre-prorexin mRNA expression in the LH compared to wt. 3. OX-AR/DAGLalpha expression colocalized in a limited subset of PAG neurons. 4. CB1 receptors were expressed at symmetric (inhibitory) synapses to OX-AR-expressing neurons. 5. In vivo leptin administration or OX-AR inhibition by SB-334867 (an OX-AR selective antagonist) induced a depression of pro-nociceptive (ON) cells in the RVM of *ob/ob* mice without affecting wt mice. CONCLUSIONS: We provide evidence supporting that the heterosynaptic endocannabinoid spread in the vlPAG after OX-AR activation is under the negative control of leptin. The leptin deficiency-related increase of OX-A signalling in the PAG is accompained by incresed activation of OX-AR which are GqPCRs and by increased levels of 2AG and could initiate the GqPCR-PLC-DAGL-2AG retrograde inhibition onto tonic GABAergic transmission in the vlPAG, thus leading to potentiation of antinociception.

ORAL 2.6

MOTOR EFFECTS OF THE NON-PSYCHOTROPIC PHYTOCANNABINOID CANNABIDIOL THAT ARE MEDIATED BY 5-HT_{1A} RECEPTORS

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The broad presence of CB_1 receptors in the basal ganglia, mainly in GABA- or glutamate-containing neurons (Fernández-Ruiz, BJP 156: 1029-1040, 2009), as well as the presence of $TRPV_1$ receptors in dopaminergic neurons (de Lago et al., Brain Res. 1007: 152-159, 2004) and the identification of CB_2 receptors in some neuronal subpopulations within the basal ganglia (Lanciego et al., J. Psychopharmacol. 25: 97-104, 2011), explain the powerful motor effects exerted by those cannabinoids able to activate/block these receptors. By contrast, cannabidiol (CBD), a phytocannabinoid with a broad therapeutic profile, is generally presented as an example of a cannabinoid compound with no motor effects due to its poor affinity for the CB_1 and the CB_2 receptor (Mechoulam et al., Chem. Biodivers. 4: 1678-1692, 2007), and despite its activity at the $TRPV_1$ receptor (Bisogno et al., BJP 134: 845-852, 2001). However, recent evidence suggests that CBD may interact with the serotonin 5-HT_{1A} receptor to produce some of its beneficial effects (Fernández-Ruiz et al., BJCP, in press, 2012). This may enable CBD to directly influence motor activity through the well-demonstrated role of serotoninergic transmission in the basal ganglia. We have investigated this issue in rats using three different pharmacological and neurochemical approaches. First, we compared the motor effects of various i.p. doses of CBD with the selective 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT; i.p.). Second, we investigated whether the motor effects of CBD are sensitive to 5-HT_{1A} receptor blockade but not to CB_1 receptor antagonism. Finally, we looked at possible synergies of CBD with 8-OH-DPAT. Our results demonstrated that: (i) only high doses of CBD (>10 mg/kg) altered motor behavior using a computer-aided actimeter; (ii) these alterations were restricted to vertical activity (rearing) with only modest changes in other parameters; (iii) similar effects were produced by 8-OH-DPAT (1 mg/kg), although this agonist affected exclusively vertical activity, with no effects on other motor parameters, and it showed always more potency than CBD; (iv) the effects of 8-OH-DPAT (1 mg/kg) and CBD (20 mg/kg) on vertical activity were reversed by the 5-HT_{1A} receptor antagonist WAY-100,635 (0.5 mg/kg; i.p.); (v) the effects of CBD (20 mg/kg) on vertical activity were not reversed by the CB_1 receptor blocker rimonabant (0.1 mg/kg; i.p.); and (vi) the effect of 8-OH-DPAT on vertical activity was associated with an increase in serotonin content in the basal ganglia, a neurochemical change not produced by CBD (20 mg/kg). We are presently investigating whether CBD is able to enhance motor effects of a subeffective dose of 8-OH-DPAT (0.1 mg/kg). Collectively, these results suggest that CBD may influence motor activity, in particular vertical activity, and that this effect is dependent on its ability to target the 5-HT_{1A} receptor, possibly as an allosteric modulator, a mode of action that it has been proposed might account for its anti-emetic effect (Rock et al., BJP 165: 2620-2634, 2012).

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ORAL 2.7

CANNABINOID RECEPTOR TYPE 1 (CB1R) ACTIVATION MEDIATES PRESYNAPTIC SILENCING BY EPAC-DEPENDENT MECHANISMS

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Presynaptically silent synapses are mature synapses in terms of release machinery, synaptic vesicles (SV) content and calcium influx in response to stimulation, but fail to release neurotransmitter in response to a strong depolarization. While the physiological role of presynaptic silencing starts to be elucidated, the molecular mechanisms underlying synaptic silencing remain poorly understood. Here we show, by combining two different approaches to monitorize the SV cycle (FM1-43 and VGluT1::phluorin), that a long activation period of the cannabinoid receptor type one (CB1R) with HU-210 (5μM, 10 minutes) increased the number of silent synapses from 1% to 30% approximately in 7DIV cultured cerebellar granule neurons. This effect required a prolonged receptor activation, since we were not able to induce silencing with shorter incubation periods. The same effect was observed at different extracellular calcium concentrations (ranging from 0.25 to 5 mM $CaCl_2$), suggesting that this phenomenon does not rely on VDCC (Voltage Dependent Calcium Channels) inhibition. Furthermore, the adenylyl cyclase activator forskolin (50μM, 10 minutes) fully prevented the induction of silent synapses. The coincubation of forskolin with the selective PKA inhibitor H89 (10μM, 30 minutes) still prevented the induction of silencing and the treatment with the selective PKA activator 6Bnz-cAMP (200μM, 5 minutes) was not sufficient to counteract CB1R-induced silencing, thus indicating that synaptic numbing does not arise from a decrease in PKA activity. In addition, the selective activator of the cAMP- regulated guanine nucleotide exchange factor (EPAC) 8-pCpt2'-O-Me-cAMP (50μM, 10 minutes) also prevented the appearance of presynaptically silent boutons, indicating that an EPAC-dependent mechanism, rather than a PKA-dependent one, is involved in switching synapses between different states. Post-hoc immunocytochemistry revealed that the susceptibility to CB1R-mediated silencing of a given synapse was determined by the quotient of RIM1α and CB1R in individual boutons. Finally we corroborated these findings with ultrastructural observations that showed a withdrawal of the SVs closer (less than 10nm) to the Active Zones (AZ) when cells were faced to HU-210 treatment, this effect was also observed in cortical synaptosomes but was absent in synaptosomes coming from *cnr1*$^{-/-}$ mice. It is concluded that the cannabinoid-induced presynaptic silencing involves the retrieval of synaptic vesicles from the plasma membrane through a cAMP-EPAC-dependent mechanism, rendering synapses ineffective in terms of synaptic vesicles and neurotransmitter release.

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ORAL 3.1

DOSE-DEPENDENT THERAPEUTIC EFFECTS OF THE MAGL INHIBITOR JZL184 IN A CHRONIC MODEL OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic disease of the human central nervous system that is characterized by focal lesions with inflammation, infiltration of immune cells, demyelination and axonal damage. Experimental autoimmune encephalomyelitis (EAE) is the most widely used animal model to study MS, because it reproduces the autoimmune and neuroinflammatory components of the disease. Activation of cannabinoid CB_1/CB_2 receptors is considered a potential therapeutic strategy for the treatment of MS, based on the evidence that exogenous cannabinoid agonist exert neuroprotective and immunosuppressive effects in experimental models of the disease. Nevertheless, the therapeutic use of synthetic agonists acting on brain cannabinoid receptors is somehow limited by the possible adverse responses related to memory and learning impairment. An alternative approach that could avoid this limitation consists of enhancing the concentration of the endocannabinoids anandamide and/or 2-arachidonoylglycerol (2-AG) by increasing their synthesis or decreasing their degradation. In addition, recent evidence indicates that inhibition of monoacylglycerol lipase (MAGL), the enzyme responsible for 2-AG hydrolysis, may provide neuroprotection during neuroinflammation independently of CB_1/CB_2 receptors activation, by uncoupling 2-AG degradation from proinflammatory prostaglandin signaling. The aim of this study was to analyze the effects of JZL184, a selective MAGL inhibitor, in the EAE model of MS. Chronic EAE was induced in C57BL/6 mice by immunization with myelin oligodendrocyte glycoprotein in Freund's adjuvant supplemented with *Mycobacterium tuberculosis*. Mice were treated daily with JZL184 (8 mg/kg and 32 mg/kg) or vehicle from the peak of the motor symptoms (~12 days post-immunization, dpi) to the end of the experiment (40 dpi). Comparison of the motor score curves indicated that both doses of JZL184 ameliorated the deficits observed in vehicle-treated mice during the disease course. Nevertheless, the benefitial effect of the 32 mg/kg dose at the motor level was no longer evident in mice scored at 40 dpi. Consistent with this result, chronic treatment with 8 mg/kg JZL184 reduced the conduction latency of the corticospinal tract measured at the end of the experiment, as well as the number and size of inflammatory lesions in spinal cord, whereas chronic administration of the high dose of the MAGL inhibitor had no effect on these parameters. The possible changes in the coupling ability of brain cannabinoid receptors to $G_{i/o}$ proteins in mice chronically treated with both doses of JZL184 is currently being examined by means of [^{35}S]GTPγS autoradiography. These findings suggest that chronic administration of inhibitors of 2-AG degradation may be a promising therapeutic strategy for the treatment of MS.

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ORAL 3.2

EVALUATION OF THE EFFECTS OF A SATIVEX-LIKE COMBINATION OF PHYTOCANNABINOIDS AS A DISEASE-MODIFYING THERAPY IN TWO EXPERIMENTAL MODELS OF MULTIPLE SCLEROSIS

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Sativex®, a phytocannabinoid-based medicine constituted by an equimolecular combination of Δ^9-tetrahydrocannabinol (Δ^9-THC)- and cannabidiol (CBD)-enriched botanical extracts, has been approved for the treatment of spasticity and pain associated to multiple sclerosis (MS) in various countries. In this study, we investigated the therapeutic potential of a Sativex-like combination of phytocannabinoids as a disease-modifying therapy in two experimental models of MS: Theiler's virus induced demyelinating disease (TMEV-IDD) and experimental autoimmune encephalomyelitis (EAE). In the TMEV-IDD model, Sativex-like combination of phytocannabinoids (10 mg/kg), Δ^9-THC-enriched botanical extract (5 mg/kg) or CBD-enriched botanical extract (5 mg/kg) were administered (i.p.) for 10 consecutive days to susceptible mice (SJL/J) once established symtomatology. Each independent treatment was effective in improving the motor disturbances associated with the TMEV-IDD and slowed the disease progression. The most significant findings with Sativex® treatment in spinal cord histological analysis include a reduction of cellular infiltrates, decreased microglial reactivity and less axonal damage in comparison to TMEV-infected mice that received vehicle. Myelin proteins and demyelination were also affected by Sativex®. We are investigating the participation of cannabinoid receptors in these effects by using specific antagonists for CB_1, CB_2 and PPARγ receptors. Similar positive effects (marked attenuation of neurological deficits) were found in the second experimental MS model, EAE mice, but, in this case, we used 20 mg/kg of the Sativex-like combination of phytocannabinoids. Interestingly, the treatment with Δ^9-THC-enriched botanical extract alone (20 mg/kg) resulted equally effective when compared with the Sativex-like combination of phytocannabinoids, but the treatment with CBD-enriched botanical extract (20 mg/kg) produced only a modest recovery. Collectively, these data support the idea that the beneficial effects of Sativex® for specific symptoms in MS might be extended to the control of disease progression, presumably based on the anti-inflammatory and neuroprotective properties of the two phytocannabinoids that constitute this medicine. The issue deserves further investigation at the clinical level.

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ORAL 3.3

CHANGES IN ENDOCANNABINOID RECEPTORS AND ENZYMES AND BENEFICIAL EFFECTS OF A SATIVEX®-LIKE COMBINATION OF PHYTOCANNABINOIDS IN AN EXPERIMENTAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Different cannabinoid compounds, i.e. Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabinol, selective CB_2 receptor agonists or fatty acid amide hydrolase (FAAH) inhibitors, afforded neuroprotection in the experimental model of amyotrophic lateral sclerosis (ALS) generated by overexpression of a mutated form of superoxide dismutase-1 (SOD-1). By contrast, these mice have been poorly studied to determine the alterations caused by the disease in those elements of the endocannabinoid system targeted by the above treatments. In the present study, we addressed two objectives: (i) to analyze the changes in endocannabinoid receptors and enzymes, using RT-PCR analysis, in the spinal cord of SOD-1 transgenic mice at an advanced phase in the disease progression (17-18 weeks old); and (ii) to evaluate the cannabis-based medicine Sativex®, which is a combination of botanical extracts enriched in both Δ^9-THC and cannabidiol (CBD), as a disease-modifying therapy in this experimental ALS model, based on the potentiality of this combination to act through different mechanisms frequently activated by cannabinoid compounds. Our biochemical studies proved a significant increase of CB_2 receptors and NAPE enzyme in SOD-1 transgenic males and only CB_2 receptors in SOD-1 transgenic females. Moreover, trends toward an increase were also found for MAGL and DAGL enzymes in both genders, but not in CB_1 receptors and FAAH enzyme. Pharmacological experiments consisted of a daily administration of Sativex®-like combination of Δ^9-THC- and CBD-enriched botanical extracts, at a dose of 40 mg/kg (equivalent to 20 mg/kg for each phytocannabinoid), starting when both wild-type and SOD-1 transgenic mice were 10 weeks old (the first symptoms in these animals typically appear at this age). Our results demonstrated that the treatment of SOD-1 transgenic mice with the Sativex®-like combination of Δ^9-THC- and CBD-enriched botanical extracts: (i) partially attenuated the weight loss typical of these animals, but this positive effect was only found in males not in females; (ii) delayed the progression of neurological deficits, in particular in females; and (iii) slightly increased animal survival, an effect observed in both genders. In summary, our results provide support to the possibility that Sativex® may serve as a novel disease-modifying therapy in ALS, a disorder with a poor therapeutic outcome at present with only one medicine already approved, Rilutek®, but with a modest efficacy on disease progression. Anyway, more preclinical studies in additional models of ALS, i.e. TDP-43 transgenic mice, will be necessary before to go to the clinical evaluation of Sativex® in ALS patients.

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ORAL 3.5

Δ^9-THC AND CANNABIDIOL ENRICHED BOTANICAL EXTRACTS REDUCE THE ALZHEIMER-LIKE PHENOTYPE IN APP/PS1 MICE

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Several findings indicate that cannabinoids provide neuroprotection against different sources of neuronal damage, including the Aβ peptide. The present study was aimed to evaluate two *Cannabis sativa* extracts mainly containing delta-9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) in an animal model of Alzheimer's disease (AD).

We administered a non-psychoactive dose of the Δ^9-THC botanical extract (0.75 mg/kg, i.p.), the CBD botanical extract (0.75 mg/kg, i.p.) or the combination of both, once daily during 5 weeks to wild-type and APP/PS1 mice aging 6 months (early symptomatic phase). Both Δ^9-THC and CBD extracts improved the memory, but not the learning in APP/PS1 mice, whereas the combination of both compounds induced a general cognitive improvement, as revealed the two-object recognition and the active avoidance tests. In spite of the positive effect in APP/PS1 mice, the chronic administration of Δ^9-THC induced memory impairment in wild-type mice, warning about the effect of Δ^9-THC on healthy subjects. Interestingly, the combination of both extracts decreased the soluble $A\beta_{1\text{-}42}$ contents, which is the most toxic form of the Aβ peptide, suggesting a cannabinoid effect on the Aβ processing. Moreover, the combination of Δ^9-THC and CBD reduced astrocytic and microglial reaction to Aβ deposition, as well as some other inflammatory parameters, confirming previous evidence about the antiinflammatory properties of cannabinoids compounds. Additional molecular pathways related to the cannabinoid extracts effects were uncovered by a gene expression array.

In conclusion, the mechanisms underlying the cognitive improvement induced by the combination of Δ^9-THC and CBD extracts in APP/PS1 mice are multiple and include the modulation of Aβ metabolism and neuroinflammation, among others. Thus, medicines based on cannabinoids could offer a multi-faceted approach against AD.

ORAL 3.6

THE ENDOCANNABINOID SYSTEM IS INVOLVED IN THE NEUROPROTECTIVE EFFECTS OF MINOCYCLINE AFTER TRAUMATIC BRAIN INJURY IN MICE

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Traumatic brain injury (TBI) and its consequences represent one of the leading cause of death in young individuals. This type of lesion triggers an increase in extracellular calcium that activates many cascades and mediates glial activation and the release of potential harmful molecules, such as cytokines and reactive oxygen species. It also causes local and diffuse brain edema, axonal injury and functional impairment. Since glial activation plays a key role in the development of this secondary damage, it seems likely that controlling this step could be beneficial and could lead to neuroprotective effects. Recent studies show that minocycline, a highly lipophilic semi-synthetic derivative of the antibiotic tetracycline, suppresses microglial activation, reduces the lesion volume and decreases TBI-induced locomotor hyperactivity from 48h up to 3 months**.** The endocannabinoid system (ECS) plays an important role mediating compensatory and reparative mechanisms under pathological situations like brain traumatisms. Thus, the ECS controls inflammation by acting on glial and endothelial cells, involving some mechanisms that are shared with minocycline neuroprotective pathways (modification of calcium currents, p38-MAPK activation, caspase-3 expression, NF-kβ pathways or nitric oxide production). On the basis of these premises, we hypothesized that the ECS could be involved in the neuroprotective effects of minocycline. To address this hypothesis, we used a TBI model in mice in combination with selective CB1 and CB2 receptor antagonists (AM251 and AM630, respectively) and analyzed the possible involvement of cannabinoid receptors in the effects of minocycline. The results obtained provided the first evidence for the involvement of endocannabinoid system in the neuroprotective action of minocycline on brain edema, neurological impairment, diffuse axonal injury, astrogliosis and microglial activation since these effects were blocked by CB1 and CB2 receptor antagonists in a mouse model of traumatic brain injury.

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ORAL 3.7

TARGETED LIPIDOMICS PROFILING OF INJURED RAT BRAIN: *N*-OLEOYL-GLYCINE AND ITS POSSIBLE MECHANISM OF ACTION

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Traumatic brain injury (TBI) is the leading cause of death in the young age group and the most commonly identified cause of epilepsy in adult populations older than 35 years. At present, there are no effective drugs to treat brain injury (see Mechoulam and Shohami, *Mol. Neurobiol*. 2007 for review). The role of the endocannabinoid system in neuroprotection is well established. Several groups reported enhanced levels of anandamide (AEA) after acute injury and in response to TBI there is local and transient accumulation of 2-arachidonoylglycerol (2-AG) at the site of injury, peaking at 4 h and sustained up to at least 24 h (see Shohami et al., *Br. J. Pharmacol.* 2011 for review). Furthermore, very recently, Naqvi and co-workers (Naqvi, Rudrauf et al. 2007) found that cigarette smokers presenting with TBI, with damage at the level of the insula, experience a cessation of smoking. Given the reinforcing role of endocannabinoids and CB_1 receptors in nicotine self-administration, it is possible that TBI is accompanied by reduced endocannabinoid levels in the insula. Moreover, a study from Cohen-Yeshurun and co-workers (*J. Cereb. Blood Flow Metab.*, 2011) reported the role of N-arachidonoyl-L-serine (AASer) as a new neuroprotective lipid mediator after TBI, thus raising the opportunity to investigate also the levels of these compounds and other endocannabinoid-like molecules in brain areas involved in TBI and their possible mechanism of action. The aim of this study was, therefore, to investigate further the alterations of endocannabinoid levels in a model of rat TBI and to discover new endocannabinoid molecules possibly involved in this process through the use of very sensitive and specific "targeted lipidomics" methods involving high resolution LC-ESI-IT-ToF (Liquid Chromatography-ElectroSpray Ionization-Ion Trap-Time of Flight). Rats underwent TBI using the lateral fluid percussion model (LFP). The prefrontal cortex, hippocampus and insular cortex, both ipsi- and contra-lateral to the injury were dissected, and lipids extracted. TBI led to decreased 2-AG and/or AEA levels, in both ipsi- and contra-lateral brain areas. On the other hand, we observed that the levels of two *N*-acylethanolamines with neuroprotective and anti-inflammatory properties, *N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA), tended to increase only in ipsilateral areas. Finally, we identified *N*-oleoylglycine (OlGly) in the prefrontal cortex and hippocampus of the injured hemisphere. The intriguing possibility that the reduced levels of endocannabinoids following TBI might be also accompanied by decreased nicotine self-administration, and that *N*-oleoylglycine, OEA and PEA might instead play a role in neuroprotection is currently being investigated. Moreover, the hypothesis that OlGly, like *N*-arachidonoylglycine (Kohno et al., *Biochem. Biophys. Res. Commun.*, 2006), might be a natural ligand for the orphan receptor GPR18 and, therefore, exert neuroprotective effects by acting at this target is currently under investigation.

ORAL 3.8

FATTY ACID AMIDE HYDROLASE DELETION POTENTIATES IN VIVO GLIAL ACTIVITY IN RESPONSE TO ACUTE BRAIN INJURY

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Glial cells are involved in the inflammatory responses of the brain against different kinds of insults. Astrocytes and microglia are the main types of cells that result activated after an alteration of the brain parenchyma homeostasis. We have recently reported that the enhancement of the endocannabinoid tone through the genetic deletion of the fatty acid amide hydrolase (FAAH) induces profound phenotypic changes in glial cells in vitro[1]. In the present experiments we have used an in vivo model of acute insult to the brain in order to explore the effects of FAAH gene deletion on glial responses. To that end, we generated a new transgenic mouse ($Cx3cr1^{eGFP/+}/FAAH^{-/-}$) that exhibits green fluorescence in microglial cells while lacking the enzyme FAAH and compared them with their corresponding controls ($Cx3cr1^{eGFP/+}/FAAH^{+/+}$). To study glial activity in vivo, we used an intravital multiphoton microscopy system[2,3]. By opening a small cranial window (3mm diameter), we could observe cellular responses against acute laser injuries (15microns diameter) induced in the brain parenchyma. Our data indicate that the absence of FAAH exacerbated the microglial response by increasing their ability to direct cell processes towards the sites of injury. Furthermore, this effect was mediated by cannabinoid CB_1 receptors as was prevented by the preincubation with SR141716. In addition, astrocytic hemichannels exhibited a differential pattern of activation in mice lacking FAAH, which could partially account for the observed effect in microglial cells. These results confirm the relevant impact that FAAH gene deletion has on glial function.

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ORAL 3.9

EFFECT OF THE ENDOCANNABINOID N-ARACHIDONOYL-DOPAMINE (NADA) IN THE HYPOXIA RESPONSE PATHWAY

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Hypoxia is the condition in which the organism is deprived of adequate oxygen supply. This deprivation causes damage in tissues and organs and is related with cardiorespiratory disorders, cancer and neurodegenerative diseases. Neurodegeneration refers to the processes whereby damaged neuronal cells deteriorate or degenerate and eventually die, and represent one of the world's major unsolved health problems. There is increasing evidence that endocannabinoids can exert anti-inflammatory and neuroprotective effects through receptor (CBs and TRPV1)-dependent and -independent mechanisms.
The objective of this study was to determine the ability of the major endocannabinoids to regulate the hypoxia response pathway. Our results show that some endocannabinoids such as N-Arachidonoyl-Dopamine (NADA) and N-Oleoyl-Dopamine (OLDA) but not Anandamide, induce the stabilization and activation of the hypoxia-inducible factor 1 alpha (HIF-1α) through CB1- and TRPV-1-independent mechanism. Recent findings demonstrated that the induction of this transcription factor exert neuroprotective effects through the induction of intrinsic adaptive mechanisms in neuronal as well as in non- neuronal cells. We show that NADA regulates the expression of HIF-1α through E3 ubiquitin ligase SIAH2 (seven in absentia homolog 2). NADA induces SIAH2 activity, resulting in the stabilization and accumulation of HIF-1α. We also found that NADA induces PHD3 degradation and stimulates HIF-1α-mediated transcription. Accordingly, NADA treatment increases endothelial cell tube formation in a model of angiogenesis, strongly corroborating the finding that this endocannabinoid has the ability to simulate hypoxic physiological conditions. Moreover, kinase pathway analysis, network construction and identifying upstream regulators revealed that DNA-PK, GSK3β, SYK, p38MAPK and AKT1 kinases are involved is the signalling pathway activated by NADA in neuronal cells. Altogether our results demonstrated a novel role for the endocannabinoid NADA in the control of the hypoxia response pathway. Since the endogenous levels of NADA could be increased pharmacologically our results open new research avenues for the management of neuroinflammatory and neurodegenerative diseases.

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ORAL 4.1

CHROMENOPYRAZOLEDIONES: CANNABINOID-QUINONE DERIVATIVES WITH ANTITUMOR ACTIVITY IN VITRO AND IN VIVO

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A large diversity of biological signaling-pathways are implicated in the pathogenesis of cancer. Appropriate treatment of neoplasms often depends on pharmaceutical intervention at multiple pathways, with a combination of different drugs. In this context, targeting different anticancer modes of action in a single molecule is a challenge. Our interest in cannabinoid ligands and antiproliferative agents lies in developing molecules which structure includes cannabinoid and quinone features. Antineoplastic effects of quinones have been widely reported. [1] On the other hand, increasing evidence showed that cannabinoids can modulate tumor growth, apoptosis and angiogenesis in various types of cancer. Quinones, related to chromenopyrazole[2] previously reported by us, have been designed and synthesized as anticancer agents using the multi biological target concept that involves quinone cytotoxicity and cannabinoid antitumor properties. Our binding assays indicate that one of our chromenopyrazoledione[s3] has affinity for the cannabinoid receptors. Moreover, electrochemical studies show that it could form stable radicals able to generate oxidative stress. Their antitumor activity was evaluated in vitro by human cancer cell cytotoxicity assays. In vivo human hepatocellular carcinoma and prostate cancer proliferation studies in mice are reported for one of them.

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ORAL 4.2

ROLE OF CANNABINOIDS IN PROSTATE CANCER: FOCUS ON CBD PRO-APOPTOTIC MECHANISMS AND POTENTIAL CLINICAL APPLICATIONS

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TRPM8, a Ca^{2+}-permeable channel belonging to the transient receptor potential family, is an androgen-regulated protein, which is thought to play an important role in the pathophysiology of prostate epithelial cells. This receptor is also known to be a target of cannabinoids, thereforewe investigated the effect of eleven non-THC cannabinoids with functional antagonism to this channel, which were tested on AR-positive (LNCaP and 22RV1) and -negative (DU-145 and PC-3) PCCs. All the analyses performed selected CBD as the most efficacious compound on cell growth inhibition and on apoptosis induction. In particular, the pro-apoptotic effect of CBD was accompanied by a remarkable transcriptional up-regulation of several pro-apoptotic markers (i.e. PUMA and CHOP) and a considerable elevation of and intracellular Ca^{2+} suggesting the involvement of an apoptotic intrinsic pathway mediated by mitochondrial and/or ER stress. The possible mechanism(s) through which this CBD-mediated apoptotic process might occur has been also investigated with respect to possible cellular targets related to estrogens. In particular, we observed in LNCaP cells a CBD-induced up-regulation of GPR30 expression, which, by enhancing $[Ca^{2+}]_i$ mobilization and down-regulating AR expression, might contribute to the pro-apoptotic actions of the cannabinoid. Moreover, we also have preliminary results suggesting that CBD dose-dependently stimulates LNCaP cell autophagy. Finally, CBD pro-apoptotic efficacy was also confirmed on androgen-insensitive neuroendocrine-like (NED) cells obtained from LNCaP cells after prolonged serum deprivation- and dbcAMP/IBMX-induction, according to the available literature. Thus we decided to differently differentiate NED cells (by cultivating them in culture conditions leading to a possibly more "human-like" model of androgen resistance) in order to investigate CBD potential therapeutic action on the transition to this highly metastatic form of prostate carcinoma.

ORAL 4.3

ROLE OF THE CB_2 CANNABINOID RECEPTOR IN ERBB2-DRIVEN BREAST CANCER PROGRESSION

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A large body of evidence has demonstrated that plant-derived, endogenously produced and synthetic cannabinoids exert antitumoral actions in different models of cancer, including cell cultures, xenografted animals and genetically engineered mice. They inhibit cancer cell proliferation, adhesion, migration and induce cell death by apoptosis. However, little is known about the role of the endocannabinoid system in tumor physio-pathology. In particular, although strong evidence point to the CB_2 cannabinoid receptor as target for anti-cancer therapy, there is no information about its role in tumor generation and progression.
To shed light on this issue, we have generated animals with two genetic modifications, specifically, ErbB2 overexpression directed to the mammary epithelium, which triggers the spontaneous generation of breast tumors, and genetic ablation of the CB_2 cannabinoid receptor.
First of all, we have observed that human Her2-positive tumors express higher levels of CB_2 receptor than the Her2-negative tumors. In the animal model, we observed that the absence of CB_2 receptors produced a striking delay in tumor appearance, reduced the number of tumors generated per animal, slowed down their growth and diminished the percentage of animals with lung metastasis. We have also observed that animals lacking CB2 receptors present different levels of the endogenously produced cannabinoid anandamide than the corresponding wild type animals. Preliminary results suggest that the differences in anandamide levels between genotypes may control the general homeostasis of the mammary gland, including its oncogenic transformation and tumor progression. Together, these results suggest that CB_2 receptors, by modulating the endogenous endocannabinoid tone, play a pivotal role in ErbB2-driven breast tumor generation and progression.

ORAL 4.4

THE ENDOCANNABINOID SYSTEM CONTROLS SKELETAL MUSCLE CELL PROLIFERATION AND DIFFERENTIATION VIA CB_1 RECEPTOR ACTIVATION

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Skeletal muscle differentiation is strictly regulated by coordinated changes in the expression and function of several classes of genes determining the transition from proliferating myoblasts to non-proliferating multinucleated myotubes. In this present study, using murine C_2C_{12} cells as an experimental paradigm, we aimed at investigating the expression profile and the functional role played by the endocannabinoid system (ECS) during myoblast proliferation and differentiation. We report that while the endogenous levels of anandamide (AEA) was not significantly modified upon C_2C_{12} myoblast differentiation, 2-arachidonoyl-glycerol (2-AG) levels dramatically declined. In support of this evidence, by means of Q-PCR, we performed a wide mRNA expression analysis for the entire set of genes related to ECS metabolism and function such as those encoding for the receptors (CB_1, CB_2, $TRPV_1$), the anabolic (NAPEPLD, $ABDH_4$, GDE_1, $PTPN_{22}$, $DAGL_{\alpha/\beta}$) or catabolic (FAAH, MAGL, $ABDH_{6/12}$) enzymes. Through this analysis, we suggest that the changes in AEA and 2-AG levels during the myotube formation might be due to the differential transcriptional activity of some of the aforementioned genes.
Exposure of C_2C_{12} myoblast to AEA (3 μM) and 2-AG (1-3 μM), as well as to URB567 (1 μM) and JZL184 (1 μM), two selective inhibitors of FAAH and MAGL, respectively, caused the reduced expression of skeletal muscle genes normally up-regulated during myotube formation such as Myogenin (Myog) and TroponinT-1 (TnT-1) as revealed by Q-PCR analysis. On the other hand, AEA (1μM) and 2-AG (1μM) caused a marked increase in myoblast proliferation as measured by ^{3}H-thymidine incorporation. Interestingly, the expression of both CB_1 and CB_2 receptors increased within 3 days of C_2C_{12} differentiation, with CB_1 showing the highest degree of up-regulation within the first 24 h of exposure to differentiation media (DM), as revealed by Q-PCR and western blot analysis. The use ACEA (1-3 μM), or the antagonists SR141716 (1-3 μM) and AM251 (1-3 μM), all considered pharmacological tools selective for the CB1 receptor, as well of the selective CB_2 agonist, JWH-133, or antagonist, AM-630, allowed us to conclude that the effect of the ECS on myoblast proliferation and/or differentiation is largely dependent on CB_1 receptor activation. The inhibitory effect of ACEA (1-3 μM) as well as 2-AG (1-3 μM) on myotube formation was also determined by immunocytochemical evaluation of Myosin Heavy Chain (MyHC) expression and of the fusion index at 3 days of myoblast exposure to differentiation media (MD), in the presence of above tools. Finally, the use in myoblasts of siRNA selective for CB_1 receptors, confirmed the data obtained using CB_1-selective ligands.
Collectively, this evidence suggests a possible regulatory mechanism afforded by the ECS during skeletal muscle cell proliferation and differentiation, and highlights a novel role for CB_1 receptors during myogenesis.

ORAL 4.5

THE NON-PSYCHOTROPIC CANNABINOID CANNABICHROMENE INHIBITS NITRIC OXIDE PRODUCTION IN MACROPHAGES AND AMELIORATES EXPERIMENTAL INFLAMMATORY BOWEL DISEASE

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Cannabis preparations have been traditionally employed to treat inflammatory diseases, including inflammatory bowel disease [1]. Cannabichromene is a non-psychotropic phytocannabinoid able to activate TRPA1 channels and to inhibit endocannabinoid inactivation, both of which are involved in inflammatory processes [2,3]. We examined the effects of this phytocannabinoid on murine peritoneal macrophages activated by lipopolysaccharide (LPS) and its efficacy in a murine model of colitis. LPS caused a significant production of nitrites, associated to up-regulation of anandamide, iNOS, COX-2 and CB_1 receptors, down-regulation of cannabinoid CB_2 receptors and complete suppression of transient receptor potential ankyrin 1-type (TRPA1) channels mRNA expression. Cannabichromene significantly reduced LPS-stimulated nitrite levels, an effect associated with reduction of IL-10 and interferon-γ levels. The effect of cannabichromene on nitrite production was mimicked by selective cannabinoid receptor agonists as well as by carvacrol and cinnamaldehyde (two TRPA1 agonists)**.** The effect of cannabichromene on nitrite production was enhanced by AM251 and rimonabant (two cannabinoid CB_1 receptor antagonists) and unaltered by SR144528 (a cannabinoid CB_2 receptor antagonist). LPS-induced anandamide, iNOS, COX-2, cannabinoid receptor and TRPA1 changes were not significantly modified by cannabichromene, which, however, increased oleoylethanolamide levels. *In vivo*, cannabichromene ameliorated dinitrobenzenesulphonic-induced colitis, as revealed by reduction of colon weight/colon length *ratio* and myeloperoxidase activity. It is concluded that cannabichromene exerts anti-inflammatory actions in activated macrophages - with tonic cannabinoid CB_1 signalling being negatively coupled to this effect - and ameliorates experimental inflammatory bowel disease

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ORAL 4.6

THCV EFFECTIVELY DECREASE LIPID LEVELS IN VARIOUS BIOLOGICAL SYSTEMS AND HAS INSULIN SENSITIZING EFFECTS IN HEPATOCYTES

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The endocannabinoid system (ECS) has quickly emerged as a key player in the regulation of energy homeostasis and its overactivity especially, though not exclusively, within the liver is associated with the development of insulin-insensitivity and metabolic syndrome. The accumulation of triglycerides in the liver is linked to obesity, dyslipidemia, type 2 diabetes and metabolic syndrome in general, thus strategies to decrease hepatic triglyceride levels hold significant therapeutic potential. We have assayed the potential of THCV (which can behave as both a CB1 antagonist at low concentrations and CB1/CB2 agonists at higher concentrations) to decrease lipid levels in hepatocytes and in a fish model of lipid mobilization as well as testing ability of THCV to regulate glucose homeostasis/insulin sensitivity in murine models of obesity. We have found that, in hepatocytes treated with oleic acid to up-regulate triglyceride accumulation, to mimic hepatosteatosis, THCV rapidly decreased lipid stores in a concentration dependent manner, possibly through increased lipolysis. The dependence of THCV on cannabinoid receptor CB1 for this effect appears to be minimal based on pharmacological and knockdown studies. Utilization of zebrafish embyos to analyse the ability to affect mobilization of yolk fat stores in the absence of food intake revealed that THCV significantly increased the rate of yolk absorption in a time- and dose-dependent manner. In mice, while THCV did not modulate food intake or weight gain, it reduced glucose intolerance in *ob*/*ob* mice while it increased insulin sensitivity in diet-induced obese mice. In vitro models of hepatic insulin resistance showed that THCV, in a manner more efficacious than a CB1 antagonist, dose-dependently sensitized cells to insulin-dependent activation of AKT, a key modulator of glucose homeostasis. These data indicate that THCV has significant positive, and partly CB1 receptor-independent, effects on lipid metabolism and may hold therapeutic potential for the treatment of the metabolic syndrome.

POSTER 1

INFLAMMATION IN ALZHEIMER DISEASE: EFFECTS OF CANNABINOIDS

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The hallmark in Alzheimer's disease (AD) is both accumulation of beta-amyloid (Aβ) plates and the presence of TAU protein inside neurons. Furthermore, glial cell activation, occurs after plates appear in brain damaged producing astrogliosis and microglia activation. In recent years, many studies show the potential positive effects of cannabinoids in various pathologies. Our group has shown inflammation in astrocytes in primary culture comparing Aβ with control cells. Here we determined the action of cannabinoids on inflammation induced by Aβ in astrocytes in culture. Protein expression levels were detected by ELISA and Western- blot techniques in astrocytes in primary culture treated with Aβ and/or cannabinoids. Using Aβ (10 μM) during 24 h, an increase of pro-inflammatory mediators (TNF-α and IL-1β), compared with control astrocytes was detected. Treatment with Win 55, 212-2 (10 μM) produced increase of anti-inflammatory mediators (PPAR-γ) and decrease of pro-inflammatory mediators, such as TNF-α and IL-1β, protecting cells to the toxic effect of Aβ.

POSTER 2

CANNABINOID COMPONENTS ARE DIFFERENTIALLY DISTRIBUTED DURING DEVELOPMENT

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The cerebellar cortex is subjected to morphological changes during postnatal development. Recent works point out the role of endocannabinoids in those processes such as axonal growth and guidance (Oudin et al., 2011).

CB_1 is expressed in parallel and climbing fibre synaptic terminals (Kawamura et al., 2006). As to the 2-AG biosynthetic enzyme, DAGL-α is expressed in Purkinje cell dendritic spine necks (Yoshida et al., 2006). Due to the migration and the synaptic establishment processes suffered by cerebellar cortex during the first three postnatal weeks, we wondered whether components of the endocannabinoid system may show transitory localizations in different cerebellar compartments during early postnatal development compared to adult.

We performed immunohistochemical confocal microscopy and preembedding immuno-electron microscopy assays to determine the precise localization of CB_1 and DAGL-α at postnatal day 5 (P5), P12, P21 and adult.

Our results indicate that CB_1 is present in parallel fibre axons while they are suffering their migration and axon elongation processes. They get to synaptic terminal position when parallel fibres make synapses with the Purkinje cell spines. At P12, DAGL-α is mainly expressed at Purkinje cell somatic proximal spines and dendrites and less in distalmost dendrites poorly developed, corroborating the results of Yoshida et al. (2006). Furthermore, we demonstrate a granule cell axon localization of DAGL-α mostly at P5 when these fibres are elongating and where CB_1 receptor is also present.

These results, suggest that DAGL-α dependent eCB signalling through CB_1 could play an important role at maturation processes that take place in the cerebellum during the postnatal development.

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POSTER 3

OVEREXPRESSION OF CANNABINOID RECEPTOR CB_2 MRNA IMPAIRS CRANIAL NERVE DEVELOPMENT.

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Although upregulation of CB_2 receptors have been involved in several brain injuries, mainly associate with neuroinflammation, little is known about its putative role during brain development. Taking adventage of *Xenopus laevis* embryo model as a neural development model, we studied the role of CB_2 receptors in this process by means of gain of function *in vivo* studies. Embryos overexpressing CB_2 receptors showed an impairment of and strong impairment of maxillomandibullar (VII), glossopharyngeal (IX) and vagal (X) cranial nerve development by a process that implies differential signaling, activation of the Akt pathway whilst inhibiting the ERK 1/2 MAPK pathway and inducing an up regulation of genes involved in placodal development. Those resultsindicate that CB_2 receptors can act breaking the timing switch in cell fate and preventing neural differentiation.

POSTER 4

ULTRASTRUCTURAL LOCALIZATION OF THE CB1 RECEPTOR IN THE MUSCLE MITOCHONDRIA

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CB_1 receptor is the main cannabinoid receptor expressed in the brain, but it is also present in peripheral organs where it participates in energy metabolism. Our recent data have shown that CB_1 receptors are localized in mouse neuronal mitochondrial membranes where they regulate cellular respiration and energy production (Benard et al., 2012). However, nothing is known concerning the presence of CB_1 receptors on peripheral mitochondria.

The goal of this investigation was to determine whether CB_1 cannabinoid receptors are localized in striate muscle mitochondria, an organ where the endocannabinoid system is present (Crespillo et al., 2011). Highly specific CB_1 antibodies were used in combination with a pre-embedding silver-intensified immunogold method for high resolution electron microscopy. Gastrocnemius muscles were removed from transcardially perfusion-fixed wild-type (CB_1-WT) mice and mutant littermate mice constitutively lacking the CB_1 receptor gene (CB_1-KO).

CB_1 immunoparticles were distributed on mitochondrial membranes of CB_1-WT but not of CB_1-KO mutants. The statistical analysis revealed that about 26% of mitochondria were immunopositive in CB_1-WT, whereas, these values dropped to 0.37% in CB_1-KO mice. The CB_1 immunolabelling density was significantly different in WT and KO animals (0.231 versus 0.003 particles/ μm, respectively). Interestingly, CB_1-KO muscles had significantly fewer mitochondria than WT (0.36 versus 1.06 mitochondria/ μm^2). Mitochondria of CB_1-KO mice displayed also a trend to be smaller than CB_1-WT (mitochondrial area: 0.091 and 0.068 μm^2 in WT and KO, respectively; p=0.7935).

In conclusion, these anatomical data show that CB_1 receptors, like in the brain, are also located in muscle mitochondrial membranes, where they might exert an intracellular regulation of metabolism through control of mitochondrial functions.

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POSTER 5

PALMITOYLETHANOLAMIDE COUNTERACTS IRRITABLE BOWEL SYNDROME (IBS)-LIKE ACCELERATED UPPER GASTROINTESTINAL TRANSIT IN MICE

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Irritable bowel syndrome (IBS) is one of the most common gastrointestinal ailments among those seeking health care for gastrointestinal disorders [1]. Palmitoylethanolamide (PEA) is an endogenous fatty acid amide chemically related to the endocannabinoids anandamide which was originally identified in peanut oil and egg yolk [2]. In some countries, PEA-containing preparations are sold as food supplements to relieve some of the symptoms associated to irritable bowel syndrome (IBS). Here, we have evaluated the effect of PEA in a model of functional bowel disorder that mimics aspects of post-inflammatory-IBS in humans [3]. **Methods** A model of enhanced upper gastrointestinal transit induced by intracolonic oil of mustard (OM) in mice was used [3]. Gastrointestinal transit was measured by the charcoal method. In vitro, PEA was evaluated in the mouse ileum on acetylcholine- and EFS-induced contractions as well as on spontaneous contractility. **Results** OM administration developed a severe colitis that peaked at day 3 and was absent by day 14. Twenty-eight days after its administration, OM increased upper gastrointestinal transit. PEA (1-10 mg/kg, IP) reduced upper gastrointestinal transit both in control and OM-treated mice, being it more active in delaying motility in OM-treated mice than in control mice. Palmitic acid (1-10 mg/kg, IP) also reduced GI motility. In vitro, PEA had small inhibitory effects on spontaneous and stimulated intestinal contractility. **Conclusions** PEA preferentially inhibits motility in a IBS-like model of accelerated gastrointestinal transit. Such results might provide a pharmacological basis able to explain the empirical use of PEA-containing preparations by IBS patients.

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POSTER 6

CANNABIGEROL QUINONE EXERTS THERAPEUTIC EFFECTS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Phytocannabinoids (pCBs) without psychotropic effects are considered of special interest as novel therapeutic agents in CNS diseases. These pCBs include cannabidiol (CBD), cannabigerol (CBG), Δ9tetrahydrocannabivarin (Δ9THCV) and cannabidivarin (CBDV). We have developed a series of new cannabinoid quinones, among them the CBG quinone (VCE-003) that shows PPARγ and CB2 receptors agonism. In addition we have found that VCE-003 activates the Nrf2/ARE pathway in neuronal cell lines. In the present study, we investigated the therapeutic potential of VCE-003 in the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS) by immunization con MOG $_{33-55}$. VCE-003 (5mg/Kg ip, daily) was administered to susceptible C57/BL6 mice at the onset of symptomatology. Clinical score and weights of mice were daily recorded until the day of sacrifice (28 days post-immunization). VCE-003 treatment delayed the onset of disease and ameliorated the symptomatology. Histological analysis of spinal cord of EAE mice treated with VCE-003 showed decreased microglia reactivity and reduced cellular infiltrates, in particular $CD4^+$ T lymphocytes. Double labeling with Neurofilament and the myelin protein, RIP indicated that VCE-003 diminished the axonal damage. Demyelination was evaluated by Luxol fast blue labeling. Changes in the expression of several cytokines, adhesion molecules and Nrf2-dependent genes were determined by qRT-PCR. The implication of PPARγ and CB2 receptors in the beneficial effects of VCE-003 in the EAE model of MS is being investigated by using specific receptors antagonists. Taken together our results support the potential of VCE-003 for the treatment of MS and other chronic inflammatory diseases.

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POSTER 7

PRENATAL CORTICOSTERONE ADMINISTRATION AND STIMULATION OF THE ENDOCANNABINOID SYSTEM DURING ADOLESCENCE MODULATE EMOTIONAL RESPONSES AND CB1 RECEPTORS IN MICE.

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Experimental evidence indicates that the central endocannabinoid (eCB) system sustains the activity of the hypothalamus-pituitary-adrenal (HPA) axis in mediating individual emotional responses. An alteration in their maturational trajectories, during highly plastic developmental stages (e.g. prenatal life and adolescence), may persistently adjust individual behavioural phenotype. Here we attempted to investigate, in outbred CD1 mice, whether exposure to antenatal stressors may influence short- and long-term emotional and molecular consequences of an enhancement of the eCB system during adolescence. To mimic prenatal stress, pregnant mice were supplemented with corticosterone (prenatal corticosterone group, PNC) in their drinking water (33.3 mg/l); their adolescent offspring (postnatal days 29–38) received daily injections of fatty acid amide hydrolase URB597 (0.4 mg/kg, i.p.) or its corresponding vehicle (Tween-80, 5%, and 0.9% saline, 95%). We observed that prenatal corticosterone administration resulted, in adolescence, in a reduction in body weight and general locomotion, while in adulthood, in increased anxiety-related behaviour and reduced expression of CB1 receptors in hippocampus, striatum and cerebellum. Additionally, long-term repeated URB597 exposure during adolescence reduced locomotor activity, increased anxiety and depressive-like (reduced motivation in a progressive ratio schedule) behaviours. CB1 receptors were up-regulated in striatum and hippocampus and down-regulated in the cerebellum. Finally, we observed a complex interaction in the long-term effects of HPA and eCB system developmental modulation. Specifically, we observed that PNC individuals exposed to URB597: (1) failed to show further reductions in locomotion (2) exhibited increased anxiety-like behaviour and (3) showed a differential regulation of CB1 receptors compared to control mice exposed to URB597. Specifically, compared to their respective controls (PNC vehicle), PNC-URB597 mice showed reduced CB1 receptors in prefrontal cortex, and no variation in hippocampus, striatum and cerebellum. Present results provide support to the hypothesis that changes in the developmental trajectories of the HPA axis and of the eCB system may persistently affect individual emotional responses and reactivity to cannabinoid compounds later in life.

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POSTER 8

CANNABIDIOL ADMINISTRATION TO NEWBORN RATS AFTER HYPOXIA-ISCHEMIA ENHANCES NUROPROLIFERATION AND MYELINIZATION.

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Background: We have previously observed that administration of cannabidiol (CBD) to newborn rats after a hypoxic-ischemic (HI) insult led to the increase of proliferative cells in brain. In the present work we aimed to characterize the target cells of this effect.

Methods: unilateral HI brain damage was induced in newborn Wister rats (7-10 day-old: P7-P10) by exposure to hypoxia (10% FiO2) for 120 min after left carotid artery electrocoagulation under anaesthesia. Ten minutes after the end of HI pups were treated s.c. with vehicle (HV, n=9) or with the CBD 1 mg/kg single dose (HC, n=9). Other pups remained as controls (SHM, n= 11). One or seven days after HI rats were sacrificed, transcardially perfused with paraformaldehide (PFH) 4% and their brains removed and cut off into coronal slices for immunohistochemical study on the subventricular zone (SVZ) and hippocampus (HPC). KI67 was used to detect proliferating cells. GFAP, IBA-1 and Olig-2 were used as astrocyte, microglial and immature oligodendrocyte specific markers, respectively. Myelin basic protein (MBP) fluorescence measurement was used to quantify the effect on myelinization

Results: in HV the density of KI67+ cells in the ipsilateral HPC was reduced 1 day postHI, increasing slightly in the following 7 days (from 72±6% to 107±7% contralateral hemisphere); such un increase was not observed in the ipsilateral SVZ (from 112±2 to 111±5% from 1 to 7 days postHI). CBD augmented the density of KI67+ in HPC and the magnitude of the increase in the following 7 days (from 106±2 to 144±1%, all $p<0.05$ vs. HV). The latter increase was also observed in SVZ in CBD treated animals (from 106±5 to 118±1%). Double staining revealed that KI67+ cells were mostly microglial cells and immature oligodendrocytes. MBP analysis showed that CBD led to a 10-fold increase of MBP fluorescence in ipsilateral hemisphere as compared to HV.

Conclusions: CBD has a neuroproliferative effect in immature brain after HI. This effect includes immature oligodendrocytes, leading to a positive effect on myelinization.

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POSTER 9

CB_1 CANNABINOID RECEPTORS LOCATED ON GLUTAMATERGIC TERMINALS CONFER NEUROPROTECTION IN HUNTINGTON'S DISEASE

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Endocannabinoids prevent excessive synaptic activity by engaging CB_1 cannabinoid receptors, the same receptors targeted by Δ^9-tetrahydrocannabinol, the major active component of marijuana. CB_1 receptors are the most abundant G protein-coupled receptors of the brain, and are very highly expressed in GABAergic terminals of the forebrain, in which they inhibit GABA release. Smaller amounts of CB_1 receptors reside on terminals of principal neurons, in which they blunt glutamatergic transmission. Despite the widely reported neuroprotective activity of CB_1 receptors, the precise relevance of those two pools in neurodegenerative processes is still unknown. Here, we show that CB_1 receptors are severely down-regulated in striatal GABAergic neurons but remain unaffected in corticostriatal glutamatergic neurons both in a transgenic mouse model of Huntington´s disease (HD), R6/2 mice, and in post-mortem specimens of HD patients. Administration of the *N*-methyl-D-aspartate receptor antagonist MK-801 prevented Huntington's disease-like neurodegeneration in R6/2 mice, while the $GABA_A$ receptor antagonist picrotoxin was ineffective. Pharmacological delivery of MK-801, but not of picrotoxin, compensated the deleterious effects produced by CB_1 receptor genetic elimination in R6/2 mice. Moreover, induction of striatal excitotoxicity in conditional mutant mice lacking CB_1 receptors in GABAergic and/or glutamatergic neurons of the forebrain provided direct evidence for the prominent neuroprotective activity of CB_1 receptors located on glutamatergic neurons. Taken together, these findings support that maintenance of CB_1 receptors in glutamatergic terminals blunts neurodegeneration in Huntington's disease, and suggest that this specific receptor pool may constitute a therapeutic target to achieve neuroprotection in Huntington's disease patients.

POSTER 10

ID-1 INHIBITION IS CORRELATED TO CBD ANTI-PROLIFERATIVE EFFECT IN U87-MG CELLS

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Several studies have demonstrated that cannabidiol (CBD) possesses anti-proliferative, anti-apoptotic effects and inhibits cancer cell migration and invasion. CBD effect depends on multiple cellular targets that control tumorigenesis through the modulation of different intracellular signaling pathways, with regard to the cancer type considered. Strong evidence now suggests that the Id family of helix-loop-helix proteins control cellular processes related to tumor progression. In addition, up-regulation of Id-1 has been found in many types of human cancer and its expression levels are also associated with advanced tumor stage. Moreover recent papers demonstrated that CBD inhibits human breast cancer cell proliferation and invasion through differential modulation of the extracellular signal-regulated kinase (ERK) and reactive oxygen species (ROS) pathways, and that both pathways lead to down-regulation of Id-1 expression. CBD anti-proliferative effect on glioma cells is known to involve ROS production. More recently, we also demonstrated down-regulation of both ERK and Akt pathways as consequence of CBD treatment. Thus, based on these data we decided to investigate the role of Id-1 protein in CBD antitumor effect driven by ROS, ERK and Akt pathways in glioma cells.

We first evaluated Id-1 expression in U87-MG human glioma cells. As demonstrated by Western Blot analysis, Id-1 level is high in glioma cells in comparison with the very low expression observed in astrocytes colture. CBD treatment causes a down-regulation of this protein level at concentrations causing inhibition of glioma cell proliferation (MTT test).

Subsequently, we investigated the possible correlation between ROS production, Id-1 levels and CBD anti-proliferative effect. As previously reported, in U87-MG cells the ROS scavenger □-tocopherol (TOC) reversed CBD anti-proliferative effect (MTT test). Moreover, our data demonstrate its ability to counteract the CBD-induced Id-1 inhibition (Western Blot).

Finally we tried to correlate the CBD-induced inhibition of ERK and Akt phosphorilation with the Id-1 inhibition observed. We found that both ERK inhibitor, U0126, and PI3K inhibitor, LY294002, could significantly down-regulate Id-1 expression (Western Blot), compared to untreated control, corresponding to reduced U87-MG proliferation (MTT test).

These data confirm that CBD inhibition of U87-MG cell proliferation depends on the modulation of different intracellular pathways.

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POSTER 11

EPIGENETIC MECHANISMS IN ALZHEIMER DISEASE: ROLE OF FATTY ACID AMIDE HYDROLASE IN LATE-ONSET AD SUBJECTS AND IN A RARE CASE OF MONOZYGOTIC TWINS DISCORDANT FOR AD

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Alzheimer disease (AD) is the most frequent form of dementia in the elderly, affecting more than 25 million people worldwide. Since epigenetic differences could contribute to phenotypic differences, we investigated epigenetic mechanisms occurring in Late Onset (after age 65) AD (LOAD) subjects (and age-matched controls (CT)) and in a couple of monozygotic (MZ) twins discordant for AD, which provides a unique opportunity to evaluate the role of environmental factors in the etiology of the disease. Current treatments for AD provide only palliative approaches [1] and in the search of new targets for its therapy, the endocannabinoid system (ECS) recently emerged as a promising candidate due to its role in neuroinflammatory and neurodegenerative diseases [2]. We have studied possible gene transcription changes and the epigenetic regulation of ECS components occurring in AD using peripheral blood mononuclear cells (PBMCs), accessible cells with potential for biomarker discovery in neuroinflammatory disorders [2]. We found a selective increase in fatty acid amide hydrolase (*faah*) gene expression in LOAD subjects when compared to CT as well as in the AD twin versus the healthy with no changes in the mRNA levels of any other gene of ECS elements. Consistently, we also observed a reduction in DNA methylation at *faah* gene promoter in LOAD subjects and even more pronounced in the AD twin. When data were stratified for LOAD subjects according to functional and cognitive tests, a significantly lower level of DNA methylation at *faah* gene was observed in those with lower levels of MMSE (this with severe AD) compared to the rest of the samples. Present findings suggest the involvement of FAAH in the pathogenesis of AD, highlight the importance of epigenetic mechanisms in enzyme regulation and unravel epigenetic differences potentially helpful in the understanding of environmental factors and phenotypic differences in MZ twins. Moreover, our date point to FAAH as a new potential biomarker for AD in easily accessible peripheral cells.

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POSTER 12

THE CB_1 CANNABINOID RECEPTOR DRIVES CORTICOSPINAL MOTOR NEURON SPECIFICATION THROUGH THE CTIP2/SATB2 TRANSCRIPTIONAL REGULATION AXIS

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The generation and specification of pyramidal neuron subpopulations during development relies on a complex network of transcription factors. The CB_1 cannabinoid receptor is the major molecular target of endocannabinoids and marijuana active compounds. This receptor has been shown to influence neural progenitor proliferation and axonal growth, but its involvement in neuronal specification and the functional impact in the adulthood caused by altering its signaling during brain development are unknown. Here we show that the CB_1 receptor, by preventing Satb2-mediated repression, increased Ctip2 promoter activity and $Ctip2^+$ neuron generation. The neurogenic fate-determination unbalance found in complete $CB_1^{-/-}$ mice or in glutamatergic neuron-conditional Nex-$CB_1^{-/-}$ mice induced overt alterations in corticospinal motor neuron generation and subcerebral connectivity, thereby resulting in an impairment of skilled motor function in adult mice. Likewise, genetic deletion of CB_1 in Thy1-eYFP-H mice elicited corticospinal-tract development alterations. Altogether, these data demonstrate that the CB_1 receptor, by coupling endocannabinoid signals from the neurogenic niche to the intrinsic proneurogenic Ctip2/Satb2 axis, is required for the generation of deep-layer cortical neurons, thus dictating appropriate subcerebral projection neuron specification and corticospinal motor function in the adulthood.

POSTER 13

ANTI-INFLAMMATORY AND ANTINOCICEPTIVE EFFICACY OF PALMITOYLETHANOLAMIDE IN A RAT MODEL OF OSTEOARTHRITIS

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Osteoarthritis (OA), the most common form of arthritis, is characterized by extensive remodelling of subchondral bone and permanent destruction of articular cartilage leading to joint pain. The most commonly used drugs are non-steroidal anti-inflammatory drugs (NSAIDs). However, their prolonged use induces serious side effects. For this reason the identification of alternative drugs is crucial for the OA pathology. For this purpose, animal models are useful to effectively mimic the human pathology, such as the model obtained by a single intra-articular injection of sodium mono-iodoacetate (MIA) (2mg/25µl) in the intra-patellar ligament of the knee of male Wistar rats. Local injection of MIA, an inhibitor of glycolysis, disrupts chondrocytes metabolism and produces cartilage degeneration. The aim of this study was to investigate, in such an animal model, the anti-inflammatory and antinociceptive efficacy of palmitoylethanolamide (PEA), an endogenous lipid analogous of the endocannabinoid anandamide, which displays, when exogenously administered, anti-inflammatory and antinociceptive efficacy, as demonstrated by our research group to in mouse model of neuropathy associated with chronic constriction injury of the sciatic nerve (Costa et al., Pain, 2008). As expected, MIA-treated rats developed knee swelling, mechanical allodynia, thermal hyperalgesia, motor impairment (calculated as sciatic functional index) and a significant cartilage erosion, compared with non-OA rats. The oral administration of PEA 50 mg/kg once a day for 15 days reduced such symptoms and slowed the degradation of cartilage. PEA efficacy was superimposable and in some cases greater than that evoked by 10 mg/kg nimesulide, one of the most employed NSAIDs for OA treatment, so suggesting a therapeutic use of PEA in clinic. Furthermore, PEA repeated treatment didn't evoke adverse effects while the repeated treatment with nimesulide, led to the development of duodenal ulcers, one of the major adverse effects limiting NSAIDs use in humans. Since OA patients showed elevated levels of pro-inflammatory and pro-algogen mediators such as TNF-α and NGF in their synovial fluid, and since PEA is able to inhibit *in vitro* mast cell degranulation so reducing TNF-α and NGF release, experiments were performed to determinate the levels of such markers, in order to postulate PEA mechanism of action. As expected, NGF and TNF-α levels were increased in the synovial fluid of OA rats. A significant reduction in NGF levels was detected in the synovial fluid of animals treated with PEA 50 mg/kg, while the same PEA treatment did not affect TNF-α increase. Further experiments will be performed in order to measure this mediator at an early stage of the disease, characterized by a greater inflammatory component. Basing on these data, an hypothesis of the mechanism of action of PEA can be formulated. Locally, PEA could interact with cannabinoid-like receptors expressed by mast cells by inhibiting their degranulation and the release of TNF-α and NGF, so protecting from the cartilage damage. Concomitantly, at spinal cord level, PEA could interact with cannabinoid-like receptors expressed by microglial cells thus inhibiting the release of cytokines and the recruitment of inflammatory cells. In view of these results we can propose that the efficacy of PEA in OA should be further characterize since it could represent a viable alternative for the osteoarthritis treatment than the classical NSAIDs.

POSTER 14

CB2 AGONISTS HALT AXONAL DEGENERATION IN A MOUSE MODEL OF THE NEUROMETABOLIC DISEASE ADRENOLEUKODYSTROPHY

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X-linked adrenoleukodystrophy (X-ALD) is an inherited disorder characterized by axonopathy and demyelination in the central nervous system and adrenal insufficiency. Main X-ALD phenotypes are: (i) an adult adrenomyeloneuropathy (AMN) with axonopathy in spinal cords, (ii) cerebral AMN with brain demyelination (cAMN) and (iii) a childhood variant, cALD, characterized by severe cerebral demyelination. Loss of function of the ABCD1 peroxisomal fatty acid transporter and subsequent accumulation of very long-chain fatty acids (VLCFA) are the common culprits to all forms of X-ALD. The mouse model for X-ALD exhibits a late-onset neurological phenotype with locomotor disability and axonal degeneration in spinal cords. Axonal degeneration is a main contributor to disability in progressive neurodegerative diseases in which oxidative stress is often associated as pathogenic factor. Recently, we have identified oxidative damage, inflammation and energetic failure as an early event in life, and the excess of VLCFA as a generator of radical oxygen species (ROS) and oxidative damage to proteins in X-ALD. The present study shows that chronic administration of the selective cannabinoid receptor type 2 (CB2) agonist (JWH133), at symptomatic stage in a mouse model of X-ALD, halts microgliosis and axonal degeneration.

POSTER 15

ACUTE CANNABIGEROL ADMINISTRATION RECOVERS PHENCYCLIDINE-INDUCED COGNITIVE DEFICITS AND NEGATIVE-LIKE SYMPTOMS IN RATS

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Animal and human models have suggested that cannabinoids might possess antipsychotic properties. To date, only the antipsychotic properties of cannabidiol (CBD), a non-psychoactive cannabinoid found in the plant Cannabis sativa, have been deeply investigated. Based on their wide range of pharmacological actions with potential therapeutic interest, it is conceivable that, besides CBD, other phytocannabinoids may possess antipsychotic potentials. In particular, cannabigerol (CBG), another phytocannabinoid, has been recently reported to interact in vitro and in vivo with 5-HT1A receptors, whose modulation seems to represent a promising target for treating some of the negative, cognitive and affective symptoms of schizophrenia.

In contrast to CBD, no studies investigated the potential antipsychotic effect of CBG in vivo.

Therefore, in this study we evaluated the effects of CBG in a pharmacological model of schizophrenia based on the sub-chronic administration of the non competitive NMDA receptor antagonist, phencyclidine (PCP), which has been recognized as a model to reproduce some negative symptoms and cognitive deficits in rats.

To this aim, starting from PND 60, animals were treated with PCP 5 mg/kg once a day for 7 days followed by 7 days of withdrawal. At PND 75, CBG was administered 30 minutes prior the test session and animals were tested in the novel object recognition test, social interaction test and forced swim test.

As expected, in the classic variant of the novel object recognition test, sub-chronic PCP induced a significant reduction of the discrimination index, indicating the presence of a cognitive impairment. PCP-induced cognitive deficit was even worsened in the spatial version on the test. CBG administration did not alter the recognition memory in control animals but, interestingly, in both variants of the test, pre-treatment with CBG prevented the development of the cognitive deficit in PCP-treated animals.

In the social interaction test, sub-chronic PCP treatment reduced the time spent in active social behaviours and induced aggressiveness. CBG per se did not alter social behaviours nor aggressiveness but, in PCP-treated rats restored social behaviours and counteracted PCP-induced increase in aggressive events.

Finally, in the forced swim test, PCP per se significantly increased the time spent in immobility, simultaneously reducing the time spent in climbing and swimming activities. CBG treatment per se did not alter these parameters but, interestingly, its administration in PCP-treated rats completely counteracted the increase in the immobility time.

The present results demonstrate, for the first time, that the non-psychotropic phytocannabinoid, CBG, may possibly exert antipsychotic properties. In particular, CBG administration is effective in preventing the cognitive deficits and negative symptoms induced by a well validated pharmacological model of psychotic syndrome in rats.

Acknoledgements*: CBG was kindly provided by GW Pharmaceuticals.*

POSTER 16

2-ARACHIDONOYLGLYCEROL MODULATES EARLY STAGES OF ENDOTHELIAL/LEUKOCYTE INTERACTIONS BY UP-REGULATING SELECTINS

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At sites of inflammation, damaging stimuli promote adhesiveness and migration of leukocytes on vascular endothelial cells, through a multi-step cascade that involves: i) capture and rolling of leukocytes on endothelium, ii) activation, arrest and firm adhesion and, finally, iii) para-and trans-cellular migration. To date, accumulated evidence indicates that endocannabinoids (eCBs) and their congeners play a role as immunomodulators, nonetheless their role is still under debate. Moreover, since literature data mainly relate to single cell types or analysis of late stages of the inflammatory process, we characterized the effect of eCBs in early events of endothelium (ECV304)/leukocyte (Jurkat) interactions. RT-PCR, Western blot, ELISA and confocal microscopy analysis showed that 2-AG was able to initiate and complete the leukocyte adhesion cascade. A short exposure of ECV304 to 2-AG primed them towards a pro-inflammatory state, through exposure of specific selectins. Commitment to inflammation was permanent, since activated ECV304 released tumour necrosis factor-α (TNF-α) for up to 24 hours, despite the removal of 2-AG after 1 hour of incubation. TNF-α-containing medium, derived from 2-AG-treated ECV304, promoted leukocyte recruitment: conditioned media and co-culture studies showed that Jurkat cells enhanced the expression of L-selectin and P-selectin-glycoprotein-ligand-1, and increased adhesion and trans-migration. 2-AG indirectly promotes leukocyte recruitment into inflamed sites by acting on endothelial cells, thus representing a potential therapeutic target for treatment of inflammatory diseases.

POSTER 17

DISTRIBUTION OF PHOSPHOLIPIDS IN THE BRAIN OF CB1 KNOCKOUT MICE BY IMAGING MASS SPECTROMETRY

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Lipids, and phospholipids (PL) in particular, are the major constituents of cell membranes. The PL in brain cells are not only essential for the formation of the connecting network (myelin, axons, dendrites, spines, synapses), but they are also precursors of lipid species that participate in signalling processes and neurotransmission. Some of these "neurolipids", such as the endocannabinoids, activate G protein coupled receptors (GPCR). The CB1 cannabinoid receptor is the main subtype involved in CNS synapses. Therefore, the localization of the different species of phospholipids in CB1 knockout mice would contribute to the understanding of the metabolic involvement of this class of lipids with the endocannabinoid signalling.

The analysis of lipids in biological samples is a difficult task, due to the differences in detection and abundance of the different classes of lipids. As a result of recent improvements, remarkable images which reveal the distribution of complex lipids in tissues are now generated by matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS).

MALDI IMS has been used to detect different lipid species in the CNS of CB1 KO mice. Some of the lipids that have been analysed are phosphatidylcholine (PC) PC(36:4), PC(38:4), PC(38:6), PC(40:6), which may be precursors of anandamide (AEA) or 2-arachidonoylglycerol (2-AG) and phosphatidylinositol (PI) PI(18:1/20:4), PI(18:0/20:4), PI(18:0/22:6) and phosphatidylethanolamine (PE) PE(16:0p/20:4), PE(16:0p/22:6), PE(18:0/20:4), PE(18:0/22:6), PE(18:0/22:4) species, as possible precursors of 2-AG. The preliminary results have not shown there to be an adaptive modulation in two month-old CB1 ko mice. The reported levels of AEA and 2-AG could account for these results.

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POSTER 18

CHRONIC CANNABIDIOL IMPROVES THE SCHIZOPHRENIA-LIKE SIGNS INDUCED BY REPEATED TREATMENT WITH MK-801 IN MICE

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It has been suggested that cannabidiol (CBD), a major non-psychotomimetic constituent of Cannabis sativa, may have antipsychotic-like effects. However, the antipsychotic properties of repeated treatment with CBD has not been investigated. Thus, we evaluate if the repeated treatment with CBD would attenuate the behavioral changes induced by chronic administration of MK-801, a NMDA receptor antagonist. Methods: Male C57BL/6J mice (6 weeks of age when experiment began) received daily ip injection of MK-801 (0.1, 0.5 or 1 mg/kg) for 14, 21 or 28 days. Twenty-four h after the last injection, the animals were submitted to prepulse inhibition test (PPI; pulse: 105dB/20ms; prepulse: 75, 80 and 85dB/10ms, noise: 65 dB). After that, we investigated if repeated treatment with CBD (15, 30 and 60 mg/kg) would attenuate the impairments induced by chronic treatment (28 days) with MK-801 (1 mg/kg) in PPI. This experiment was similar to the first one except that the CBD treatment started on the 6th day after the start of treatment with MK-801 and continued until the end of treatment. In a third experiment, we investigated if CBD would attenuate the MK-801-induced behavioral changes in social interaction and object recognition test. Results: We observed impairment in PPI (prepulse: 75 and 80dB) only after the treatment with MK-801 at a dose of 1 mg/kg for 28 days. In addition, CBD (30 and 60 mg/kg) was able to attenuate the behavioral changes in the PPI induced by repeated treatment with MK-801. Repeated treatment with MK-801 also impaired the social interaction and object recognition, an effect attenuated by CBD. Moreover, CBD by itself did not change the PPI, social interaction or object recognition behaviour/responses. Conclusion: These results indicate that the repeated treatment with CBD was able to reverse the psychotomimetic-like effects observed after chronic administration of NMDA receptor antagonists, a model that seems to represent the changes observed in patients with schizophrenia. The data support the view that CBD may have antipsychotic properties.

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POSTER 19

ENVIRONMENTAL STIMULATION DURING DEVELOPMENT MODULATES INDIVIDUAL BEHAVIOURAL AND NEUROCHEMICAL RESPONSES TO CANNABINOID AGONISTS IN MICE.

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Clinical and experimental studies indicate that adverse environmental conditions may favour the onset of emotional disturbances and increase the vulnerability towards the effects of the consumption of psychoactive drugs. Conversely, stimulating environmental conditions may exert a protective or compensatory role. Neurobiological systems such as the hypothalamic-pituitary-adrenal (HPA) and the endocannabinoid system (ECS) interact to modulate the expression of emotions. Here we analysed how exposure to stimulating environments during development (childhood and adolescence) modulates individual response to the administration of high doses (0.3 mg/kg) of an ECS agonist (JWH-018) during adolescence. We exposed newborn CD1 mice to a moderate dose of corticosterone (33.3 mg/l) supplemented in the maternal drinking water; an independent group of adolescent mice were housed in environmental enrichment (EE) conditions (physical and social stimuli were provided). Exposure to JWH-018 during adolescence is associated, in the short-term, with hypomotility, analgesia and reduced body temperature; in the long-term, adolescent exposure to JWH-018 is associated with anhedonia, anxiety, increased rearing, and major alterations in brain concentrations of the following metabolites (measured through ^{1}H imaging-guided magnetic resonance spectroscopy): glutamate, glutamine, phospho-choline plus glicero-phospho-choline, creatine plus phospho-creatine, inositol, N-acetyl-aspartate. Beside exerting independent behavioural and neurochemical effects, exposure to stimulating environments contrasts some of the consequences of JWH-018 administration on behaviour (hypolocomotion during adolescence and increased anxiety in adulthood) and brain metabolic profiles. These data support the hypothesis that moderate neonatal stress, environmental enrichment and pharmacological stimulation of the ECS contribute to the expression of emotions throughout the entire course of development.

POSTER 20

EMOTIONAL REGULATION IN INFANT MALE RATS IS MEDIATED BY PLASMA CORTICOSTERONE LEVELS AND BRAIN ENDOCANNABINOID LIGANDS

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Accumulating evidence demonstrates that, while being orchestrated by hypothalamus-pituitary axis corticosteroids, the individual response to stress is also sustained by the endocannabinoid (eCB) system. Whilst the participation of the eCB system in stress response and emotional homeostasis in adult animals has been extensively studied, this role has been scarcely considered in developing animals. Herein, we aimed to investigate the participation of the eCB system in acute stress responsiveness in neonate male rats. Male rat pups of 12 days of age were faced with a social stressor, repeated isolation from mother and siblings. The stressful nature of such a stimulus was confirmed by the evaluation of emotional and endocrine responses. A reduction in locomotion and an increase in the duration of isolation-induced ultrasonic vocalizations were observed as a consequence of the isolation challenge together with a significant increase in circulating corticosterone levels. Notably, a specific decrease in anandamide content was observed within the hippocampus, whereas no changes were observed for 2-arachidonoyl glycerol levels, or within the striatum. Therefore, the eCB system seems to play a pivotal role in the immediate stress response to a social challenge in the neonate rat. The influence of prenatal exposure to stressful episodes - by the repeated restraint of dams during gestation - was also evaluated and no changes in the above described responses were observed. Present data support that the HPA axis and the eCB system participate in stress reactivity from early life stages. Consequently, maintaining their developmental trajectories within homeostatic levels during sensitive age windows seems to be crucial to the appropriate establishment of adult emotional and stress reactivity.

POSTER 21

GENETIC DISSECTION OF THE ROLE OF CANNABINOID CB1 RECEPTORS IN THE SCHIZOPHRENIA-LIKE PHENOTYPE OF MICE

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Cannabis and endocannabinoids (ECs) seem to play a pivotal role in the genesis of schizophrenia (SCZ). The cannabinoid CB1 receptor (CB1R) are mainly distributed in brain regions implicated in emotional responses such as amygdala, prefrontal cortex and hippocampus. Within these structures, they are located pre-synaptically on GABAergic and glutamatergic neurons and their activation leads to inhibitory effect on neurotransmitter release. Although several evidences suggest that alteration of CB1Rs could be involved in the pathophysiology of SCZ, it is unclear which of these neuronal subpopulations containing CB1 receptors could be direct involved. Thus, the aim of the present study was to dissect the role of CB1R in the schizophrenia-like phenotype of mice. For this purpose conventional (ubiquitous deletion of CB1R: $CB1^{-/-}$) or conditional (specific deletion of CB1R on cortical glutamatergic neurons: "$GluCB1^{-/-}$" or in dopamine D1 receptor expressing neurons "$D1CB1^{-/-}$") knockout mice were submitted to a battery of behavioral tests to assess the locomotor activity in the open field test (as index of positive symptoms) the social behavior in the social interaction and social investigation tests (as index of negative symptoms) and the attention deficit in the prepulse inhibition of startle responses (as index of sensorimotor gating deficit). Conventional ($CB1^{-/-}$) or conditional CB1 mutant mice ($GluCB1^{-/-}$ or $D1CB1^{-/-}$) did not show any difference both in the locomotor activity and in the acoustic startle response of the prepulse inhibition test as compared to wild type animals, suggesting that total or partial deletion of CB1 receptors did not induce neither positive- or attention deficits-like symptoms. Since a moderate social activity deficit has been found in mutant animals, these results suggest that deletion of CB1R in specific neuronal subpopulation could be associated to some features of schizophrenia-like phenotype.

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POSTER 22

EXPOSURE OF ADOLESCENT MICE TO THC SHAPES IMMUNE RESPONSE IN ADULTHOOD

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Cannabis use has characteristics of very early onset, and it is known that more than 25% of teens have used this drug during their life. It has been recently shown that THC can affect immune system function. We investigated whether the use of cannabis in adolescence would cause effects on the immune system that can persist in adulthood.

We measured T-cells and macrophage cytokine production. We used Balb C /J male mice youngs and adults, divided into 3 experimental groups:young and adult mice treated with THC or vehicle for 10 days, and whose immune parameters were evaluated at the end of treatment adult mice treated in adolescent age and housed for 47 days, when the immunological assessments were performed.

The THC effects on the immune system were investigated using the following experimental protocol: 5 mg/kg for 3 days, 10 mg/kg for 3 days and finally 15 mg/kg for 4 days. These doses were chosen as, accordingly to the literature, plasma THC concentration reached in mouse plasma is comparable to that measured in human heavy smokers. In order to study acquired immunity, young and adult mice were immunized with KLH to induce an antigen-specific reaction and Th1/Th2 balance was assessed by measuring the production of IFNγ, and IL4.In order to assess macrophage function we used peritoneal macrophages stimulated in vitro with LPS; IL1β and TNFα were assessed as proinflammatory cytokines, while IL10 was evaluated as antinflammatory cytokine. All cytokines were measured by specific ELISA.

At the end of the 10 day treatment in both young and adult mice, a significant reduction in the production of IFNγ was observed, while IL4 was significantly increased. When we measured immune response after immunization of adult mice that had been treated in adolescence, we observed that the production of IFNγ was still lower and that also the IL4 level was decreased compared with vehicle mice. Regarding the macrophage function, the THC treatment induced in both young and adult mice a significant increase of IL10 while TNF and IL1β are decreased. Particularly interesting were the results obtained in mice treated in adolescent age with THC respect to vehicle ; in fact, we observed an opposite effect since IL1β and TNFα levels were significantly increased while IL10 production was lower, indicating a switch towards a proinflammatory phenotype of the macrophage. These effects on IL10 take place at transcriptional level since we observed similar result by measuring IL10 mRNA with RT-PCR.

These results indicate that the immune system is profoundly altered by treatments with THC, with the presence of a deregulated response. We can conclude that THC has significant effects on the immune response that last long after its administration.

POSTER 23

CANNABIDIOL ADMINISTERED AFTER HYPOXIA-ISCHEMIA TO NEWBORN PIGS IMPROVES NEUROBEHAVIORAL PERFORMANCE

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Background: Cannabidiol (CBD) activation of $5HT_{1A}$ receptors is related with some of its behavioural effects (vg. antianxiety) and is involved in CBD neuroprotection after a hypoxic-ischemic (HI) insult. The aim of the present work was to assess whether the neuroprotective effect of CBD in big mammals as newborn pigs after HI was associated with motor and behavioural beneficial effects.

Methods: 1 day-old piglets underwent a HI insult by interrupting carotid blood flow and decreasing inspired oxygen fraction to 10% for 20 min. Then, piglets were allowed to spontaneously recover and then kept in a warmed cage for 72 h. Thirty min after HI piglets received vehicle (ethanol:solutol:saline 2:1:17; HV, n=8) or CBD 1 mg/kg (HC, n=9). During the HI insult and then every 24 h cerebral activity was assessed by amplitude-integrated EEG (aEEG). A neurobehavioral score was carried out every 24 h, including tone, coordination and movement assessment, as well as specific behavioural items as aggressiveness/avoidance, eating behaviour and playfulness. At the end of the experiment the piglets were euthanized and their brains removed, stored in formaldehide 4% and then processed for histological (Nissl) study in cortex and hippocampus. Non HI piglets served as controls (SHM, n=4).

Results: CBD treatment reduced HI brain damage 72 h postHI, restoring brain activity (aEEG amplitude: 86±3%, 70±2% and 90±2% basal amplitude for SHM, HV and HC, $p<0.05$) and reducing neuronal loss in cortex (normal neurons 1413±42, 994±88 and 1293±62 per mm^2 for SHM, HV and HC, $p<0.05$) and hippocampus (normal neurons 133±8, 103±14 and 131±10 for SHM, HV and HC, $p<0.05$). CBD treatment improved motor performance (tone: 4±0, 2.7±0.3 and 3.8±0.1 points; barrow: 3±0, 2.4±0.2 and 2.9±0.1 points; walking: 4±0, 2.8±0.3 and 3.6±0.2 points, for SHM, HV and HC, all $p<0.05$). CBD restored eating behaviour (4.6±0.3, 3.4±0.5 and 4.7±0.2 points, for SHM, HV and HC, $p<0.05$), leading to faster recovery of suckling (60±4 vs 31±0.6 h postHI for HV and HC, $p<0.05$) and greater milk volume intake (460±40, 177±30 and 335±25 mL, for SHM, HV and HC, $p<0.05$). CBD abolished HI-induced aggressiveness (0/4, 5/7 and 0/9 for SHM, HV and HC, $p<0.05$) and restored playful behaviour (4/4, 3/7 and 9/9 for SHM, HV and HC, $p<0.05$).

Conclusions: CBD administration after Hi to newborn pigs reduces brain damage, leading to significant recovery of motor and behavioural performance. CBD effects on piglet behaviour suggest some anti-anxiogenic effect.

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POSTER 24

HIGH RESOLUTION ANATOMICAL LOCALIZATION OF THE TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 (TRPV1) IN THE MOUSE DENTATE GYRUS

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Recent evidence has demonstrated that the endocannabinoid anandamide, in addition to cannabinoid receptors, also activates the transient receptor potential vanilloid type-1 (TRPV1), a nonselective cation channel. In the CNS, TRPV1 activation by anandamide triggers a non-cannabinoid dependent long-term depression (LTD) upon medial perforant path stimulation in the hippocampal dentate molecular layer (Chávez et al., 2010). Despite these findings, nothing is known about the subcellular distribution of TRPV1 in this dentate layer. The aim of this study was to investigate this question by means of TRPV1 antibodies combined with a highly sensitive pre-embedding immunogold method for high resolution electron microscopy.

TRPV1 immunoparticles were mostly localized in postsynaptic compartments, particularly in dentate granule cell dendritic spines (44.83%±7.50%) and shafts (29.02%±4.05%) distributed throughout the outer 2/3 of the molecular layer. In addition, only about 10% of the synaptic terminals were TRPV1-immunolabeled in this target region of the glutamatergic perforant path. In contrast, TRPV1 in the inner 1/3 of the molecular layer, the main target of the excitatory mossy cell axon terminals, was poorly expressed (positive spines: 12.83%±5.52%; dendrites: 17.27%±3.50%; terminals: 4.33%±1.89%). Importantly, TRPV1 immunolabeling was not observed in the TRPV1-KO hippocampal dentate molecular layer (spines: 4.03%±2.25%; dendrites: 4.99%±2.33%; terminals: 5.45%±1.75%), meaning that the antibody used was highly specific. TRPV1-KO mice were confirmed by genotyping techniques.

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POSTER 25

DEVELOPMENT AND CHARACTERIZATION OF SELECTIVE CB_2 RECEPTOR LIGANDS BASED ON MODIFICATIONS OF SR144528 STRUCTURE

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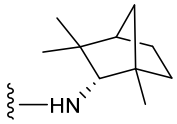
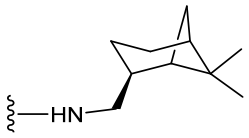
Comparing the distribution of the two classic cannabinoid receptors in the body, it appears well-demonstrated that the CB_2 receptor has a more restricted distribution than the CB_1 receptor. In the CNS, the CB_1 receptor is abundant in neuronal cells in concordance with its key role in regulation of synaptic processes, whereas the CB_2 receptor is located preferentially in glial elements, but particularly when they become activated by different types of insults. Ligands that selectively target and activate the CB_2 receptor may be used for their anti-inflammatory, anti-tumoral and neuroprotective properties in various neurological disorders, with the advantage of being devoid of psychoactive effects associated with the activation of CB_1 receptors. Selective CB_2 receptor ligands have been developed so far including agonists (i.e. HU-308, JWH-133, AM1421) or antagonists (i.e. SR144528, AM630). In this study, we have modified the chemical structure of SR144528 to generate novel analogs that retain selectivity for the CB_2 receptor and that may be used to either activate or block this receptor. Among a total number of 14 compounds designed, synthesized and characterized for their binding to CB_1 or CB_2 receptors, we obtained two compounds (**1a** and **1d**) with affinity in the nanomolar range for the CB_2 receptor and negligible or poor binding at the CB_1 receptor:

Compounds	Structure (-R)	Ki for CB_1 (nM)	Ki for CB_2 (nM)
1a		1470	5.7
1d		>5000	72.2

We have also studied the functional activity of these ligands as agonists, antagonists or inverse agonists, using cultured BV-2 cells, which release significant amounts of prostaglandin E2 (PGE2) when exposed to LPS. This effect is attenuated by CB_2 receptor agonists or enhanced by CB_2 receptor antagonists/inverse agonists (Oh et al., Neurosci. Lett. 474: 148-153, 2010). In our hands, compound **1a** showed characteristics of CB_2 receptor agonist as it reduced PGE2 levels and this effect was reversed by SR144528, whereas compound **1d** appears to work more as a CB_2 receptor antagonist/inverse agonist. We plan to develop *in vivo* studies with these compounds to determine their ability to regulate glial-mediated influences on neuronal survival in experimental models of neurodegenerative disorders.

*Both authors contributed equally to this work

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POSTER 26

CONTINUOUS ACCESS TO A HIGHLY PALATABLE FOOD FROM INFANCY ALTERS THE RESPONSE TO AN ACUTE DOSE OF AM251 AND FEEDING BEHAVIOUR

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The weaning period is a transitional stage from breastfeeding to adult feeding where rapid growth and development occurs. Manipulating dietary conditions during this stage can increase the risk for developing diseases like obesity in adult life. The endocannabinoid system has an important role in energy balance, appetite, satiety and it is associated with the modulation of the reinforcing and motivational properties of food. In the last few years, several endocannabinoid receptor antagonists have been discovered and studied for drug development against diseases like obesity. One of them is AM251, an inverse agonist of CB1 receptors. It has been shown that this compound can reduce food intake, but it is especially effective in reducing intake of palatable food. Here we studied two groups of male wistar rats. One of them was weaned on a standard chow diet ad libitum, and the other one was weaned on a free choice diet, where rats were given two types of meal ad libitum: standard chow and highly palatable food (composed of a mixture of chocolates). Food intake and weight gain were measured during the infant, adolescent and adult periods. We also studied the development of inflexible intake of food behaviour and food preference by a test for compulsive feeding. At 13-14 postnatal weeks (adulthood), both groups, previously deprived of food, were administered a dose of AM251 (3mg/kg). Then, food intake for both groups and both types of meal was measured. We found that the group weaned on a free choice diet gained more weight than the control group, developed an increasing inflexibility to standard chow consumption when palatable food was limited and their preference for palatable food gradually increased across different stages of life. In contrast, the group weaned on standard chow exhibited higher preference for palatable food when it was available but didn't show inflexible feeding behaviour. In the AM251 test, we also found that administration of this drug decreased total food intake in both groups, but the group weaned on a standard chow had significant less preference for palatable food. However, the group weaned on a free choice diet with inflexible feeding behaviour had a significant reduction of standard chow intake but not less preference for palatable food. These results suggest that continuous access to palatable food from infancy could alter the motivational and reinforcing networks involved in food intake, the CB1 receptors distribution and, therefore, the response to CB1 antagonist drugs and feeding behaviour.

POSTER 27

PRECISE SUBCELLULAR LOCALIZATION OF THE SYNTHESIZING AND DEGRADING ENZYMES OF ENDOCANNABINOIDS IN THE MOUSE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS

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It is well established that the endocannabinoid system and the hypothalamus, including the ventromedial nucleus (VMH), are associated with homeostatic and behavioral functions, such as regulation of food intake and energy balance. In a previous study, we showed that CB_1 receptors in the VMH are mainly localized in inhibitory GABAergic and also in cortical and subcortical excitatory glutamatergic synaptic terminals. However, the distribution of the enzymes responsible for the synthesis and degradation of the main endocannabinoids (2-AG and anandamide) in the VMH is not known. Therefore, the aim of this study was to investigate in the VMH the precise ultrastructural distribution of the enzymes DAGL-α and MAGL (for the synthesis and degradation of 2-AG respectively) and NAPE-PLD and FAAH (for the synthesis and degradation of anandamide, respectively). For this purpose, a high resolution pre-embedding silver-intensified immunogold method for electron microscopy was used.
As a summary, DAGL-α was distributed in postsynaptic membranes of dendritic elements, as well as in dendritic spine heads and necks. MAGL appeared in both postsynaptic dendrites and presynaptic terminals. The distribution pattern of NAPE-PLD was also in axon terminals and dendrites, while FAAH was preferentially distributed in postsynaptic dendritic profiles.
Current extensive statistical analysis will define the contribution of the endocannabinoid-related enzymes to the presynaptic and postsynaptic compartments of synapses demonstrated to localize CB_1 in the VMH. This anatomical knowledge shown here will contribute understanding the complex regulation of appetite by the endocannabinoid system.

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POSTER 28

OLEOYLETHANOLAMINE DOES NOT AFFECT BINGE-TYPE EATING BEHAVIOUR IN FEMALE RATS

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In recent years, converging evidence led to hypothesise a link between defects in the endocannabinoid system and eating disorders, including binge-eating disorder (BED). For example, elevated plasma levels of anandamide (AEA) were found in women with BED, which was supposed to drive the binge episodes and reinforce the rewarding effects of palatable foods, promoting the cycle of binge eating. AEA belongs to the family of the N-acylethanolamines which also includes the fatty acid amides oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), which are ligands for alpha-type peroxisome proliferator-activated nuclear receptors (PPARα). In contrast to AEA, OEA has anorectic properties: pharmacological studies showed that OEA decreases food intake and body weight gain through a cannabinoid receptor-independent mechanism. We have recently shown that the CB1 receptor inverse agonist/antagonist rimonabant reduced the aberrant eating behaviour present in a validated rat model of BED. This follow-up study was undertaken to investigate whether OEA and PEA were effective under the same experimental conditions. Binge eating behaviour was induced in animals by giving them a sporadic (3 days/week) and limited (2h) access to a high fat diet (margarine) in addition to a continuous access to chow and water (HR group). In these animals, the intake of margarine becomes significantly greater than in animals with limited daily access to margarine (LR group), and remains stable over prolonged periods of time. Drug treatments commenced only once binge eating behaviour was firmly established (Induction phase: 3-4 weeks). Treatments were the following: (i) vehicle, OEA 2.5 and 5 mg kg^{-1}; (ii) vehicle, PEA 1 and 2 mg kg^{-1}. Drug injections were given intraperitoneally (i.p.) 24h, 4h and 10min prior to the margarine access period. Results showed that, in contrast to rimonabant, subchronic treatment with OEA and PEA did not affect binge-type eating behaviour induced by a limited access to a high fat diet, suggesting that these compounds are likely not useful in the treatment of BED.

POSTER 29

ADOLESCENT THC EXPOSURE AND VULNERABILITY TO DRUG ABUSE

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Emerging evidence suggest that the use of cannabis during adolescence might lead to neurobiological changes that can affect adult brain function and behaviour. Given the role of the endocannabinoid system in this critical phase of life and in the expression of drug reward-related behaviors, the aim of this study was to investigate whether cannabis exposure during adolescence might increase reinforcing effects of abused drugs, such as nicotine, heroin and cannabinoids, in adulthood. For this purpose behavioural and neurochemical studies have been conducted. Male adolescent rats (45 postnatal day, PND) were treated intraperitoneally with increasing doses of Δ^9-tetrahydrocannabinol (THC, 2.5, 5 and 10 mg/kg) twice/day for 11 consecutive days. Once animals reached the adulthood (75 PND), we studied the effects of THC exposure on acquisition of nicotine (30 μg/kg/infusion), heroin (30 μg/kg/infusion) and WIN55,212-2 (12.5 μg/kg/infusion) intravenous self-administration behaviour using a continuous fixed-ratio (FR-1)reinforcement schedule. Faster acquisition and higher rate of drug intake was considered as index of vulnerability to drug abuse. In a different set of animals that underwent the same drug treatment, dopamine release in the shell of the nucleus accumbens was measured both in basal condition and after a drug challenge in order to evaluate possible modifications at the level of the mesolimbic dopaminergic system, a brain circuit crucially modulating reward and drug dependence. Behavioural data from nicotine self-administration showed no significant difference between the two groups of animals, although THC group showed lower, but not significant, responding with respect to the control group. On the other hand, THC exposure increased responding for both heroin and WIN55,212-2 as compared to control groups. Interestingly, neurochemical data showed a significant difference between the two groups of treatment with respect to drug challenge, with a significant lower release of dopamine after nicotine (0.4 mg/kg i.p.) and WIN55,212-2 (0.3 mg/kg i.v.) challenge, and a significant increase after heroin (0.06 mg/kg i.v.) injection in THC treated animals with respect to control group. Altogether, these results provide support to the hypothesis that, although with different mechanisms, early cannabis exposure may increase the vulnerability to heroin and cannabis, but not nicotine, abuse in adulthood.

POSTER 30

CHARACTERIZATION OF THE ENDOCANNABINOID SYSTEM IN GLIOMA STEM-LIKE CELLS AND ITS ROLE AS POTENTIAL TARGET FOR GLIOMA TREATMENT

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In the past few years, increasing interest has been addressed to the development of new and more efficacious cancer therapies. Nonetheless little progress has been achieved in the treatment of malignant gliomas, particularly in regard to the high-grade gliomas, such as glioblastoma multiforme (GBM). The resistance of malignant gliomas to therapy is attributed not only to their rapid growth and invasion, but also to the presence within the heterogeneous tumor mass of a small population of cells displaying stem cell properties and characterized by the capability of self-renewal, multipotent differentiation, initiation of tumor tissues and resistance to therapy. These cells are named as glioma stem-like cells (GSLCs) and it is now believed that a more efficient way to improve GBM treatment resides in the development of therapies that may selectively eliminate GSLCs. Over the past years, cannabinoids have been shown to exert anti-proliferative, and pro-apoptotic effects in gliomas, both *in vitro* and *in vivo*. Moreover, other mechanisms contributing to cannabinoid antiglioma effects are currently emerging, as their ability to interfere with tumour neovascularisation, cancer cell migration, adhesion and invasion . Based on these evidences, our aim was to characterize the endocannabinoid system in GSLCs and to evaluate the effect of cannabinoid treatment on their viability.

To this purpose we first considered three different lineages of GSLCs obtained from patients with diagnosis of primary glioblastoma - 0627, R8 and R53 – and evaluated the expression in these cells of the principal constituents of the endocannabinoid system: cannabinoid receptors 1 and 2 (CB1 and CB2) and the two enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Western Blot analysis showed that none of the three lineages expressed significant levels of CB1 receptor nor of FAAH, whereas all the three lineages expressed CB2 receptor. As far as MAGL is concerned, R8 and R53 merely expressed low levels of the enzyme, while it was not detectable in 0627 lineage.

Based on these data, we then evaluated the effect on these latter cells of two major phytocannabinoids - the CB1-selective, psychoactive compound Δ^9-tetrahydrocannabinol (Δ^9-THC) and the non-psychotropic cannabidiol (CBD) – and of the CB2 synthetic agonist, JWH-133, after different times of exposure. All of these compounds were able to reduce neurosphere viability, though with different extent, already after 24 hours-treatment, as assessed by MTT assay. The role of CB2 receptor in mediating the observed effect was also evaluated exposing the cells to the CB2 antagonist SR144528.

Our preliminary data promote further studies to better elucidate the mechanisms of action of these compounds and to evaluate the effect of newly synthesized CB2 agonists, in view of their potential employment as effective tools in glioma therapy.

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POSTER 31

PHYSICAL ACTIVITY UP-REGULATES LYMPHOCYTE FATTY ACID AMIDE HYDROLASE ACTIVITY BY INCREASING CIRCULATING IL-6 LEVELS

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Both endocannabinoids (eCB) and interleukin (IL)-6 levels change during physical activity, thus suggesting their involvement in modulation of exercise-related biological processes, including metabolism homeostasis, insulin sensitivity and inflammation. In order to investigate whether a regular physical activity might affect the activity of fatty acid amide hydrolase (FAAH), five active subjects, performing aerobic exercise (swimming, running, cycling) for 8.1 ± 3.4 hours/week, and five sedentary subjects were enrolled for this study. We observed that the active group showed basal plasmatic IL-6 levels and lymphocyte FAAH activity significantly higher than those measured in the sedentary group. Increased IL-6 levels and FAAH activity seemed to be associated, as i*n vitro* treatment of lymphocytes (isolated from sedentary individuals) with 10 ng/ml IL-6 for 48 h significantly increased FAAH activity and expression. Transient transfection experiments showed that IL-6 induced the expression of a reporter gene under the control of a region of human *faah* promoter, containing a CRE-like element. Consistently, a mutation in the CRE-like element abolished the IL-6 up-regulation, thereby indicating that the cytokine regulate FAAH activity at transcriptional level and that the CRE-like element is necessary for IL-6-mediated induction of FAAH promoter.
In conclusion, we suggest that increased IL-6, associated to regular physical activity, leads to enhancement of FAAH activity, thus modulating eCB tone in blood.

POSTER 32

PPARγ REGULATES THE ANTITUMORAL PROPERTIES OF CANNABINOIDS IN HEPATOCELLULAR CARCINOMA CELLS

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Hpatocellular carcinoma (HCC) is one of the most challenging liver carcinomas to treat and currently, no curative treatments are available for late-stage metastatic or recurrent HCC. In this study, we show that the cannabinoids Δ^9-Tetrahydrocannabinol (THC) and JWH-015 exhibit antitumoral activity against HCC cells through PPARγ. Both cannabinoids increased the activity and intracellular level of PPARγ mRNA and protein in a time-dependent fashion. THC and JWH-015dose-dependently enhanced neutral lipid accumulation in HepG2 and HUH-7 cells. Such effect was abolished by the PPARγ inhibitor GW9662. Moreover, genetic ablation with siRNA as well as pharmacological inhibition of PPARγ decreased the cannabinoid-induced cell death and apoptosis. Likewise, GW9662 totally blocked the antitumoral action of cannabinoids in xenograft induced HCC tumors in mice.

We have previously described that THC and JWH-015 induce autophagy in HCC cells through AMPK activation. Here, we examined the between PPARγ and these signaling pathways. Down-regulation of PPARγ expression with siRNA did not blocked cannabinoid-induced AMPK activation suggesting that PPARγ and AMPK are two independent pathways. However, PPARγ knocking down with siRNA induced accumulation of the autophagy markers LC3-II and p62 suggesting that PPARγ is necessary for the autophagy flux.

Taken together, we demonstrate that the antiproliferative action of the cannabinoids THC and JWH-015 on HCC cells in vitro and in vivo are modulated by up-regulation of PPARγ-dependent pathways.

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POSTER 33

THE ENDOCANNABINOID/ENDOVANILLOID SYSTEM: A NEW POTENTIAL TARGET FOR OSTEOPOROSIS

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Bone is a highly metabolically active tissue and its formation and resorption is at the base of bone remodelling, which continues throughout life. It is generally recognized that removal of bone is the task of osteoclasts (OCs), while its neo-formation relies on osteoblasts (Datta et al., 2008). The critical importance of a balanced bone remodelling is demonstrated by human diseases, i.e. osteoporosis, in which a net increase in bone resorption is responsible of skeleton weakening and fracture risk (Teitelbaum et al., 2000).

Human osteoclasts express functional TRPV1 channels, CB1/CB2 cannabinoid receptors and endocannabinoid/endovanilloid synthetic/catabolic enzymes. Pharmacologic manipulation of this system can modulate osteoclast activity (Rossi et al., 2010).

In this study, through multidisciplinary approaches, we demonstrate that enzymes and receptors of the endocannabinoid/endovanilloid system are differently expressed in osteoclasts from menopausal women without or with osteoporosis. Overall, in osteoclasts from osteoporotic patients, TRPV1 channels are up-regulated and, if persistently stimulated with resiniferatoxin (RTX), become clustered to the plasma membrane whilst inducing a massive over-expression of CB2 receptors, the counterpart receptor system for bone mineralization and remodelling via osteoclast inhibition.

Accordingly, treatment with the CB2 agonist JWH-133 [5 μM] is able to induce a reduction of TRAP levels in menopausal healthy and osteoporotic OCs. Moreover, TRPV1 silencing is able to significantly reduce TRAP expression in osteoporotic OCs, whereas the non-targeting siRNA control is ineffective, and treatment with the vanilloid antagonist I-RTX [2.5 μM] modulates TRAP expression only in cells treated with the non-targeting siRNA control.

By providing new evidence for a critical functional cross-talk between CB2 and TRPV1 receptors in osteoporosis, we speculate that TRPV1 desensitization, or its enhanced trafficking, together with TRPV1 agonist-induced CB2 receptor overexpression, might be critical to minimize calcium entry in osteoclasts, which could be in turn responsible of cell over-activation and higher bone resorption.

In this direction, drug cocktails or hybrid molecules, designed to simultaneously stimulate CB2 receptors and activate/desensitize or antagonize TRPV1 channels (may prove useful in pathological conditions where an unbalanced osteoblast/osteoclast activity is observed. Our data pave the way to the use of TRPV1 agonist together with CB2 agonists in osteoporosis.

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