25ª REUNIÓN ANUAL

Sociedad Española de Investigación sobre Cannabinoides

seic

MADRID

27 – 29 de Noviembre de 2025

COMITÉ ORGANIZADOR

Alejandro Higuera (Universidad Nacional de Educación a Distancia)

Nadine Jagerovic (Instituto de Química Médica-CSIC, Madrid)

Onintza Sagredo (Universidad Complutense de Madrid)

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Sociedad Española de Investigación sobre Cannabinoides

Departamento de Bioquímica y Biología Molecular

Facultad de Medicina

Universidad Complutense de Madrid

28040 Madrid (España)

PATROCINADORES ESTRATÉGICOS

OTROS PATROCINADORES

PROGRAMA CIENTÍFICO

Jueves, 27 de noviembre

11:00-11:45 **Registro, entrega de documentación**
(Facultad de Educación, UNED. C/ Juan del Rosal, 14 28040, Madrid)

11:45-12:00 **Inauguración**

- Cristina Sánchez (Presidenta de la SEIC)
- Javier Sanz Gozalo (Vicerrector de investigación, transferencia del conocimiento y divulgación científica de la UNED)
- Alejandro Higuera (Comité organizador local, UNED)

12:00-13:00 **Conferencia inaugural:**

"Endocannabinoids orchestrate lipid networks to curb inflammation"

Mauro Maccarrone

Catedrático de Bioquímica
Departamento de Ciencias Biotecnológicas y Clínicas Aplicadas Universidad de L'Aquila, L'Aquila, Italia

Presentado por Javier Fernández

13:00-14:00 **1ª Sesión de comunicaciones orales:**
Química Médica
Moderada por Eddy Sotelo

13:00-13:15 **O1.1**
STRUCTURE-BASED OPTIMIZATION OF N-ADAMANTYL-ANTHRANILAMIDE DERIVATIVES AS SELECTIVE CANNABINOID CB2 RECEPTOR LIGANDS
<u>Fanizzi A</u>, Graziano G, Alberga D, Delre P, Brea J, Ligresti A, Riganti C, Abate C, Loza M, Sotelo E, Mangiatordi G, Contino M, Stefanachi A, Leonetti F

13:15-13:30 **O1.2**
CHANGING THE PARADIGM IN CB2 SCREENING WITH FLUORESCENT HIGH-CONTENT ASSAYS
<u>Majellaro M</u>, Gioé Gallo C, Ortigueira S, Camino T, González L, Loza MI, Brea J, Sotelo E

13:30-13:45 **O1.3**
CANNABINOID-LOADED NANOSYSTEMS TARGETED TO VCAM-1 AS A POTENTIAL ANTI-THEROSCLEROTIC THERAPY
<u>Martín-Navarro L</u>, Herrera-González MD, Claro-Cala CM, Álvarez-Fuentes J, Martín-Banderas L

13:45-14:00 **O1.4**
CONFORMATIONAL RESTRICTION OF DESIGNER DRUGS REVEALS SUBTYPE-SELECTIVE AND BIASED CB2 AGONISTS WITH NEUROPROTECTIVE EFFECTS
<u>Ortigueira S</u>, Gioé-Gallo C, Prieto-Díaz R, Contino M, Stefanachi A, Loza M, Brea J, Navarro G, Sotelo E

14:00-15:00 **Comida**

15:00-16:00 **2ª Sesión de comunicaciones orales: Funciones cognitivas**

Moderada por Edgar Soria

15:00-15:15 **O2.1**

ASTROCYTIC AND NEURONAL ENDOCANNABINOIDS IN THE OLFACTORY BULB COOPERATE WITH NORADRENERGIC SIGNALING TO DETERMINE THE SOCIAL AND COGNITIVE CONSEQUENCES OF SOCIAL TRANSMISSION OF STRESS.

Gómez-Sotres P, Eraso-Pichot A, Kazyken G, Gisquet D, Cannich A, Bains JS, Marsicano G

15:15-15:30 **O2.2**

HIPPOCAMPAL ENDOCANNABINOIDS MODULATE INCIDENTAL ASSOCIATIONS ACROSS SENSORY MODALITIES

Barrera-Conde M, Fundazuri UB, Gisquet D, Rampini E, Gómez-Sotres P, Ferreira G, Marsicano G

15:30-15:45 **O2.3**

ASTROCYTIC RELEASE OF 2-AG MODULATES NEURONAL ACTIVITY AND BEHAVIOR

Fernández-Rodrigo A, Gómez-Sotres P, Eraso-Pichot A, Kazyken G, Cannich A, Gisquet D, Marsicano G

15:45-16:00 **O2.4**

INDUCTION OF HEPATIC STEATOSIS BY SUCROSE-RICH DIET WAS ASSOCIATED WITH LIVER ENDOCANNABINOID DYSREGULATION, AND CAUSED COGNITIVE IMPAIRMENT AND NEUROINFLAMMATION: BENEFITS OF A CANNABIS OIL-BASED THERAPY

Degrave V, Rodríguez Cueto C, Ferreira MR, D'Alessandro ME, Fernández-Ruiz J, Oliva ME

16:00-17:00 **Café y 1ª sesión de pósteres** (de P1 a P12)

17:00-18:00 **Hot Topic:**

"The VICT3R project: applying virtual control groups to advance on efficient neuroscience research"

Olga Valverde

Catedrática del Departamento de Medicina y Ciencias de la Vida Universidad Pompeu Fabra, Barcelona

Presentado por Alejandro Higuera

18:00-19:00 **3ª Sesión de comunicaciones orales: Circuitos motivacionales y metabolismo energético**

Moderada por María Ceprián

18:00-18:15 **O3.1**

CANNABINERGIC REGULATION OF HABENULAR CIRCUITS

Sangroniz-Beltrán L, Rodriguez-Cedrés C, López N, Ceprián M, Egaña-Huguet J, Fedorov D, Santas-Martin JA, Bonilla del Río I, Pablo Reyes, Ducourneau E, Ramos-Miguel A, Piriz J, Grandes P, Marsicano G, Ferreira G, Mato S, Soria-Gómez E

18:15-18:30	**O3.2** EFFECTS OF Δ9-TETRAHYDROCANNABINOL ON THE REWARDING PROPERTIES OF DIETS ENRICHED IN SATURATED FATTY ACIDS Roca M, Salinas-Blasco T, Ibias J, Ambrosio E, Sanz-Martos AB, Del Olmo N
18:30-18:45	**O3.3** CB1 CANNABINOID RECEPTOR SIGNALING REGULATION OF CORTICAL AND BEHAVIORAL ALTERATIONS INDUCED BY SATB2-DEFICIENCY Gavaldà-Vives T, Simón-Sánchez S, Apostolova G, Guzmán M, Aguado T, Galve-Roperh I
18:45-19:00	**O3.4** COMBINED PERIPHERAL CB1R BLOCKADE AND INCRETIN THERAPY ACHIEVE ROBUST WEIGHT LOSS AND METABOLIC BENEFITS IN HUMANIZED CB1R MICE Ponce-Díaz FJ, Ruiz-Pino F, Romero de la Rosa B, Lastres-Cubillo I, García-Martín A, Tena-Sempere M, Muñoz E

Viernes, 28 de noviembre

9:30-10:30	**4ª Sesión de comunicaciones orales:** **Drogas y psiquiatría** Moderada por Koldo Callado
9:30-9:45	**O4.1** MODELING CANNABIS-INDUCED PSYCHOTIC-LIKE STATES IN MICE Manzanares-Sierra I, Pinho J, Ramon-Duaso C, Castany S, Busquets-García A
9:45-10:00	**O4.2** mTOR/S6 SIGNALING DYSREGULATION AS A CONVERGENT PATHWAY LINKING CANNABIS EXPOSURE TO INCREASED SCHIZOPHRENIA RISK Unzueta-Larrinaga P, Olabarrieta E, Diez-Alarcia R, Callado LF, Urigüen L
10:00-10:15	**O4.3** ADOLESCENT BINGE DRINKING INDUCES LONG-TERM REORGANIZATION OF HIPPOCAMPAL ENDOCANNABINOID SIGNALING AND BEHAVIOR IN ADULT FEMALE MICE Lekunberri L, Serrano M, Saumell-Esnaola M, Ocerin G, García-Del Caño G, Mimenza A, Aretxabala X, Bonilla del Río I, Rico-Barrio I, Grandes P, Gerrikagoitia I, Puente N
10:15-10:30	**O4.4** POLYDRUG-INDUCED NEUROADAPTATIONS: ENDOCANNABINOID SYSTEM INVOLVEMENT IN THE INCUBATION OF COCAINE AND ETHANOL SEEKING Garrido-Matilla L, Vera-Fernández C, Spano E, Puig-Martínez N, Marcos A, Ambrosio E
10:30-11:30	**Café y 2ª sesión de pósteres** (de P13 a P25)

11:30-12:45	**5ª Sesión de comunicaciones orales:** **Enfermedades neurodegenerativas** Moderada por Estefanía Moreno
11:30-11:45	**O5.1** HETEROMERIC INTERACTION BETWEEN CB_1R AND OX_2R IN ALZHEIMER: A PROMISING THERAPEUTIC TARGET TO ENHANCE NEURONAL NEUROPROTECTIVE RESPONSES Capó A, Rebassa JB, Sotiroff R, Badia P, Tardà M, Closa P, Raïch I, Lillo J, Reyes-Resina I, Navarro G
11:45-12:00	**O5.2** THE ENDOCANNABINOID SYSTEM AS A THERAPEUTIC TARGET IN ALZHEIMER'S DISEASE: EFFECTS OF CHRONIC FAAH INHIBITION IN THE 5XFAD MODEL Martín Pérez L, López Escobar A, Bravo Pérez-Plá I, Álvarez Gallardo L, Rodríguez I, Ruiz de Martín Esteban S, Martínez Relimpio AM, Romero J, Grande MT
12:00-12:15	**O5.3** ASTROGLIAL LIPID METABOLISM: A LINK BETWEEN CANNABINOID DRUGS AND ALZHEIMER DISEASE? Ramon-Duaso C, Pinho J, Mato-Blanco X, Berenguer-Molins P, Perera-Bel J, Busquets-Garcia A
12:15-12:30	**O5.4** RESTORATION OF CB1 RECEPTOR FUNCTION IN HIPPOCAMPAL GABAERGIC NEURONS RESCUES MEMORY DEFICITS IN HUNTINGTON´S DISEASE MODELS Di Franco N, Bengoetxea de Tena I, Sánchez-Ruiz A, Pereda-Velarde A, Enfedaque F, Gónzalez-Arias C, Miquel Rio LM, Bortolozzi A, Rodríguez-Puertas R, Costas-Insua C, Molina-Porcel L, Vázquez-Oliver A, Ozaita A, Guzmán M, Perea G, Ginés S
12:30-12:45	**O5.5** THE INHIBITION OF MONOACYLGLYCEROL LIPASE AFFORDS PROTECTION IN A MOUSE MODEL OF PARKINSON'S DISEASE Torres X 1, Santamaría A, Ramírez-Carreto R, Chavarría A
12:45-13:45	**Mesa redonda:** **"25 años de SEIC: ¿Quiénes somos? ¿De dónde venimos? ¿Hacia dónde vamos?"** **Javier Fernández, Manuel Guzmán, Julián Romero, Pedro Grandes, Carmen Guaza** Moderada por Ester Aso
13:45-14:45	**Comida**

14:45-16:00 **6ª Sesión de comunicaciones orales:**
Enfermedades neurológicas y neuroinflamación.
Moderada por Carmen Rodríguez

14:45-15:00 **O6.1**
THERAPEUTIC POTENTIAL OF CB_2 RECEPTOR ACTIVATION OR SEROTONERGIC MODULATION IN A MOUSE MODEL OF DRAVET SYNDROME
Guimaré JA, Satta V, García-Guirado G, Sierra A, Hernández-Fisac I, Grether U, Fernández-Ruiz J, Sagredo O

15:00-15:15 **O6.2**
NEUROINFLAMMATION DISRUPTS ENDOCANNABINOID-MEDIATED CONTROL OF ASTROCYTIC LACTATE SUPPLY
Baraibar AM, Colomer T, Bernal-Chico A, Sánchez-Martín E, Carmona N, Nasu Y, Bonilla-Del Río I, Puente N, Grandes P, Fernández-Moncada I, Marsicano G, Mato S

15:15-15:30 **O6.3**
NEUROPROTECTIVE AND NEUROIMMUNOMODULATORY POTENTIAL OF MAGL INHIBITION IN A TDP-43-BASED ALS MURINE MODEL
Martín-Baquero R, Rodríguez-Cueto C, García-Carracedo L, Martín ED, Grether W, Fernández-Ruiz J, de Lago E

15:30-15:45 **O6.4**
MULTIMODAL IMMUNOREGULATORY MECHANISMS OF CANNABIDIOL IN A MITOCHONDRIAL EPILEPSY MODEL
Van der Walt G, Kouchaeknejad A, Yu J, Quintana A, Gertsch J, Puighermanal E

15:45-16:00 **O6.5**
CANNABINOID TYPE-1 RECEPTOR GENETIC HEMIDELETION REMODELS SYNAPTIC TRANSCRIPTOME IN FRAGILE X SYNDROME MOUSE MODEL
de los Reyes-Ramírez L, Petre-Munteanu A, Bergadà-Martínez A, Ozaita A

16:00-17:00 **Café, 3ª sesión de pósteres** (de P26 a P38)

17:00-17:15 **Homenaje a Paco Molina**

17:15-18:15 **Asamblea General Ordinaria de socios/as de la SEIC**

18:15-18:30 **Asamblea General Extraordinaria de socios/as de la SEIC**

21:00 **Cena Congreso en el Restaurante Café del Rio**
(Av. de Portugal, 1, Moncloa - Aravaca, 28011 Madrid)

10:00-11:15	**7ª Sesión de comunicaciones orales: Envejecimiento, y cáncer** Moderada por María Salazar
10:00-10:15	**O7.1** CNR1 RS80 6371 POLYMORPHISM, COGNITIVE FUNCTION, EMPATHY AND CORTISOL LEVELS IN OLDER ADULTS <u>Baliyan S</u>, Castillejo L, Grec J, Díaz-Silveira C, Galiana A, Donaire C, Díaz-Mardomingo MC, García-Herranz S, Venero C
10:15-10:30	**O7.2** EXPLORING COGNITIVE ALLEVIATION THROUGH THE MODULATION OF THE ENDOCANNABINOID SYSTEM DURING PHYSIOLOGICAL AGING IN MICE <u>Ciaran-Alfano L</u>, Galera-López L, de los Reyes-Ramírez L, Ozaita A
10:30-10:45	**O7.3** CANNABINOIDS ADMINISTRATION ENHANCES THE ANTICANCER ACTIVITY OF BEVACIZUMAB IN PRECLINICAL MODELS OF GLIOMA <u>Tovar-Ambel E</u>, Sanz-Gálvez A, Mugica-Urruzola E, Lorente M, Sepúlveda-Sánchez JM, Bhogal P, Velasco G
10:45-11:00	**O7.4** CANNABINOID ADMINISTRATION AS A THERAPEUTIC STRATEGY TO IMPROVE GLIOBLASTOMA RESPONSE TO RADIOTHERAPY <u>Sanz-Gálvez A,</u> Salvador-Tormo N, Tovar-Ambel E, Múgica-Urruzola E, Bragado B, Gutiérrez-Uzquiza Á, Lorente M, Velasco G
11:00-11:15	**O7.5** THERAPEUTIC POTENTIAL OF HER2-CB2R HETEROMERS IN HER2 POSITIVE BREAST CANCER. Seijo-Vila M, <u>Balsinde S</u>A, Blasco-Benito S, Tundidor I, Rubert-Hernández M, Pérez-Gómez E, Sánchez C
11:15-11:30	**Premio a la mejor publicación predoctoral 2025** LONG-TERM CONSEQUENCES OF ADOLESCENT EXPOSURE TO THE SYNTHETIC CANNABINOID AB-FUBINACA IN MALE AND FEMALE MICE <u>Izquierdo-Luengo C</u>, Ponce-Renilla M, Ten-Blanco M, Arnanz MA, Tolón RM, Pereda-Pérez I, Berrendero F Presentado por Leyre Urigüen
11:30-11:45	**Premio a la mejor publicación postdoctoral 2025** ACTIVATION OF CENTRAL CANNABINOID TYPE 2 RECEPTORS, BUT NOT ON PERIPHERAL IMMUNE CELLS, IS REQUIRED FOR ENDOCANNABINOID-MEDIATED NEUROPROTECTION IN PARKINSON'S DISEASE <u>Ayerra L</u>, Abellanas MA, Vidaurre C, Basurco L, Tavira A, Luquin E, Clavero P, Mengual E, Collantes M, Peñuelas I, Ruiz de Martin-Esteban S, Grether U, Hillard CJ, Romero J, Hervás-Stubbs S, Aymerich MS Presentado por Andrés Ozaita
11:45-12:30	**Café**
12:30-13:00	**Entrega de premios y clausura**

PÓSTERES

PRIMERA SESIÓN DE PÓSTERES

P1. THE INTERPLAY BETWEEN CB_2 AND NMDA RECEPTORS IN PARKINSON'S DISEASE
Reyes-Resina I, Lillo J, Raïch I, Rebassa JB, Capó T, Badia P, Navarro G

P2. CANNABINOID CB_1 RECEPTOR ACTIVATION MITIGATES N-METHYL-D-ASPARTATE RECEPTOR (NMDA)-MEDIATED NEUROTOXICITY
Navarro G, Raïch I, Rebassa JB, Lillo J, Pérez-Olives C, Capó A, Cubeles E, Cordomí A, Saura CA, Sotelo E, Reyes-Resina I, Franco R

P3. NEUROGLIAL CB_1 RECEPTORS CONTROL NAVIGATION STRATEGIES
Egaña-Huguet J, Sangroniz-Beltrán L, Baraibar AM, Reyes-Velásquez P, Landgraf N, Torres-Maldonado P, Rodríguez-Cedrés C, Julio-Kalajzic F, Piriz J, Ramos A, Grandes P, Marsicano G, Mato S, Ceprián M, Soria-Gómez E

P4. CANNABIDIOL AS A MULTIFACETED THERAPEUTIC AGENT: MITIGATING ALZHEIMER'S DISEASE PATHOLOGY AND ENHANCING COGNITIVE FUNCTION
Lillo J, Raïch I, Rebassa JB, Griñán-Ferré C, Bellver-Sanchis A, Reyes-Resina I, Franco R, Pallàs M, Navarro G

P5. METABOLISM, PHARMACOKINETICS, AND BIOAVAILABILITY OF CANNABIGEROL IN HORSES FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION WITH MICELLAR AND OIL FORMULATIONS.
Serrano-Rodríguez JM, Miraz R, Ledesma Escobar C, Ferreiro-Vera C, Priego Capote F, Muñoz-Juzado A, Sánchez de Medina V, Sánchez de Medina A

P6. HETEROMERIZATION OF OLFACTORY AND CANNABINOID RECEPTORS: IMPLICATIONS FOR NEUROPROTECTIVE GPCR SIGNALING
Garrigós C, Capó T, Badia P, Serrano-Marín J, Rebassa JB, Lillo J, Franco R

P7. DIFFERENTIATION STATE-DEPENDENT METABOLIC PROFILES OF OLIGODENDROCYTES: MODULATION BY CANNABINOID CB1 RECEPTORS
Bernal-Chico A, Sánchez-Martin E, Baraibar AM, Uribe A, Colomer T, Ruiz A, Fernández-Moncada I, Battefeld A, Pagano Zottola AC, Bellocchio L, Marsicano G, Mato S

P8. OX_1R ACTIVATION NEGATIVELY REGULATES CB_1R EFFECTS IN ALZHEIMER'S DISEASE
Rebassa JB, Raïch I, Capó A, Lillo, Irene Reyes-Resina I, Navarro G

P9. THE CANNABIS RESEARCH COMMUNITY AND PALESTINE GENOCIDE
Galve-Roperh I, Aguado T, Callado LF, Campos A, Caudevilla F, Fernández-Ruiz J, Ginés S, Gomes F, Guzmán M, de Lago E, Mato S, Morán J, Sagredo O, Sampaio L, Soria E, Urigüen L

P10. SEX-DEPENDENT MODULATION OF EMOTIONAL AND COGNITIVE PROCESSES BY PREFRONTAL CB_1 RECEPTORS
Ceprián M, Egaña-Huguet J, Godoy L, Sangroniz-Beltran L, Aranguren-Alberdi A, Aguirrebengoa O, Reyes-Velasques P, Torres-Maldonado P, Ramos-Miguel A, Mato S, Soria-Gómez E

P.11 THE THERAPEUTIC EFFECT OF THE SYNTHETIC CANNABINOID URB447 IN INFECTION-SENSITIZED NEONATAL RATS MAY BE MEDIATED BY A DECREASE IN NEUROINFLAMMATION
Pereda I, Alart JA, Chillida M, Beldarrain G, Cantalapiedra P, Abarrategi A, Herrero de la Parte B, Álvarez A, Alonso-Alconada D

P.12 DEVELOPMENT OF GRAB SENSORS FOR UNRAVELING THE MOLECULAR DYNAMICS OF THE GLIAL PURINERGIC SYSTEM AND THE ECS IN FAAH KNOCKOUT CONTEXT
López Escobar A, Sánchez Martínez C, Martín Pérez L, Bravo Pérez-Plá I, Álvarez Gallardo L, Rodríguez I, Ruiz de Martín Esteban S, Grande MT, Cravatt BF, Romero Paredes J, Martínez Relimpio AM

SEGUNDA SESIÓN DE PÓSTERES

P.13 EFFECTS OF ARACHIDONIC AND DOCOSAHEXAENOIC PUFA ENRICHMENT IN THE CELL MEMBRANE ON CB1 RECEPTOR FUNCTION
Martínez-Gardeazabal J, Cœugnet A, Bengoetxea de Tena I, Pereira-Castelo G, Moreno-Rodríguez M, Bellocchio L, Marsicano G, Villete S, Rascol E, Jobin ML, Manuel I, Alves ID, Rodríguez-Puertas R

P.14 FATTY ACID AMIDE HYDROLASE REGULATES THE MICROGLIAL RESPONSE TO ACUTE CORTICAL INJURY IN AN ALZHEIMER'S DISEASE MOUSE MODEL
Álvarez Gallardo L, Bravo Pérez-Plá I, Martín Pérez L, López-Escobar A, Ruiz de Martín Esteban S, Martínez-Relimpio AM, Grande MT, Mostany R, Cravatt BF, Romero J

P.15 EXPLORING CANNABINOID RECEPTOR EXPRESSION IN THE OLFACTORY NEUROEPITHELIUM OF INDIVIDUALS WITH MAJOR PSYCHIATRIC DISORDERS
Menéndez-Pérez C, Villar-Conde S, Cachan-Vega C, Fernández-Guisasola P, Menéndez-Coto N, Potes-Ochoa Y, Coto-Montes A, Franco R, Martínez-Pinilla E

P.16 SEX-DEPENDENT EFFECTS OF CANNABIDIOL ON NEUROTRANSMITTER RECEPTORS AND PKA/MTOR SIGNALING IN OFFPRING´S HIPPOCAMPUS AFTER PERINATAL ETHANOL EXPOSURE
de Ceglia M, Rodríguez-Pozo M, Rubio-Rodríguez P, García-Martos P, Preining P, Gavito A, Rodríguez de Fonseca F, Suárez J, Rivera P

P.17 CELL TYPE-SPECIFIC ROLE OF CB1 RECEPTORS IN THE ANTERIOR INSULAR CORTEX: IMPLICATIONS FOR EMOTIONAL AND COGNITIVE REGULATION
Mut-Arbona P, Horta G, Bellocchio L, Marsicanno G, Schmeißer M, Ruiz de Azúa I, Lutz B, Maldonado R, Martín-García E

P.18 RANDOMIZED CONTROLLED TRIAL OF HEMP-DERIVED CANNABIDIOL WITH AND WITHOUT LOW-DOSE THC FOR THE TREATMENT OF CHRONIC PAIN
Hutchison K, Mueller R, Bolts O, Guha A

P.19 EFFECTS OF A Δ^9-TETRAHYDROCANNABINOL AND CANNABIDIOL COMBINATION ON THE DYSREGULATED DOPAMINERGIC SYSTEM IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE
Subirana P, Bonaventura J, Aso E

P.20 PKC-GAMMA DELETION DISRUPTS CB1R SIGNALLING MECHANISMS RELEVANT FOR COGNITIVE PERFORMANCE
Galera-López L, Gomis-González M, Fontanet-Bosque C, Bengoetxea de Tena I, Moreno E, Martinez-Gardeazabal J, Romero-Pérez R, Pastor A, Busquets-Garcia A, de la Torre R, Maldonado R, Casadó V, Rodríguez-Puertas R, Ozaita A

P.21 CAN CANNABINOID ADMINISTRATION REDUCE THE IMMUNOSUPPRESSIVE MICROENVIRONMENT IN GLIOBLASTOMA?
Mugica-Urruzola E, Tovar Ambel E, Sanz Gálvez A, Monreal S, Sepúlveda JM, Salvador N, Lorente M, Velasco G

P.22 EXPLORING PYRIMIDINE-2-ONE SCAFFOLDS AS INNOVATIVE POSITIVE ALLOSTERIC MODULATORS OF THE CB_2 RECEPTOR
Andújar-Arias A, Azuaje J, García-Mera X, Brea J, Loza MI, Navarro G, Sotelo E

P.23 CANNABIDIOL MODULATES THE INFLAMMATORY CARGO OF EXTRACELLULAR VESICLES TO CONFER NEUROPROTECTION IN A MURINE MODEL OF NEUROLOGICAL EFFECTS OF LONG-COVID
Lemes Marques B, Cassaro Lírio PH, Martins RB, Arruda E, Reis Almeida FB, Campos AC

P.24 INTRANASAL ADMINISTRATION OF FLUORESCENT CANNABIDIOL-LOADED NANOPROBE EXHIBITS ANTIDEPRESSANT-LIKE AND NEUROPROTECTIVE EFFECTS IN NEUROINFLAMMATORY AND ALZHEIMER'S DISEASE MOUSE MODELS
Lirio PHC, Rudolph D, Marques BL, Benati RB, Vicente MA, Ramos F, Alves F, Feldmann C, Gaspari PDM, Alberici LC, Campos AC

P.25 UNRAVELLING WOMEN'S FERTILITY: ENHANCING THE PERFORMANCE OF UHPLC-HESI-MS/MS FOR PROFILING ENDOCANNABINOIDS IN FOLLICULAR FLUID
González-Infante E, Salvoch M, Grande I, Musatadi M, Ayala-Cabrera J, Diaz M, Lainz L, Villate O, Matorras R, Olabarrieta E, Agirregoitia N, Usobiaga A, Zuloaga O

P.25 BIS ENDOCANNABINOIDS SIGNALING REGULATES SHARP-WAVE RIPPLES FUNCTION ACROSS LEARNING
Martinez-Felix A, Abad-Perez P, del Castillo-Berges I, Ramon-Duaso C, Busquets-Garcia A, Martin-Fernandez M,.Valero M.

TERCERA SESIÓN DE PÓSTERES

P.26 COMPUTATIONAL EXPLORATION OF GPR12 MODULATORS: A STRUCTURE-BASED APPROACH
Fidalgo-Casares L, Montoliu E, Porzio S, Jagerovic N, Gómez-Cañas M, Morales P

P.27 THC IMPAIRS MEMORY CONSOLIDATION THROUGH GLUTAMATERGIC DYSREGULATION IN THE CA1 REGION
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ENDOCANNABINOIDS ORCHESTRATE LIPID NETWORKS TO CURB INFLAMMATION

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Resolution of inflammation is the cellular and molecular process that acts as a fail-safe on immune events to avoid uncontrolled acute inflammation that would lead to chronic phlogosis and impairment of tissue function. This pivotal homeostatic process is determined by the production and activity of more than 40 bioactive lipids, altogether termed specialized pro-resolving mediators (SPMs). The latter compounds act in concert to: i) hinder the influx of immune cells at the inflamed site, ii) induce phagocytic clearance of apoptotic cells (i.e., efferocytosis), and iii) oppose to the pro-inflammatory activationof macrophages and lymphocytes. Here, I show that also endocannabinoids (eCBs) – i.e., the endogenous ligands to cannabinoid receptors 1 and (CB1 and CB2) – act as bona fide SPMs in a CB2-dependent manner, inducing efferocytosis and driving the biosynthesis of selected SPMs in primary human M1-like macrophages. In addition, I report that the synergyof eCBs with different bioactive lipids to curb inflammation extends also to sphingosine-1-phosphate (S1P) signaling, through multiple mechanisms: i) receptor heterodimerization and coregulation, ii) mutual metabolic modulation, and iii) integrated regulation of downstream effectors. Overall, the dynamic interplay between eCBs, SPMs andS1P offers a compelling example of thecomplexity of interactions among lipid signals, emphasizing the importance of integrated approaches in the study of bioactive lipid biochemistry.

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THE VICT3R PROJECT: APPLYING VIRTUAL CONTROL GROUPS TO ADVANCE ON EFFICIENT NEUROSCIENCE RESEARCH

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The VICT3R project (Developing and implementing VIrtual Control groups To reducE animal use in toxicology Research) (www.vict3r.eu), funded by the European Innovative Health Initiative, aims to create a comprehensive database of historical control data to replace concurrent control groups with Virtual Control Groups (VCGs). VICT3R's goal is to enhance ethical standards and align with the 3Rs principles (Replacement, Reduction, and Refinement) while maintaining or even improving research consistency and reliability.

Primarily focused on pharmaceutical repeated-dose toxicity (RDT) studies, the project addresses key challenges, such as data standardisation and curation, implementation of AI approaches, and comparative validation, that are, however, equally relevant to other study types and research areas. The resulting database is also designed to support the analysis of study design variability, protocol optimization, and retrospective testing. Consequently, the methodologies and computational tools developed hold significant potential well beyond RDT studies.

To explore this broader applicability, we examined the ALURES database (the EU Commission's Animal Use Reporting System) and found that basic research - predominantly conducted in academic institutions - represents the most animal-intensive area, with rodents, particularly mice, in nervous system-related studies accounting for the largest proportion of animal use. Although attractive, academic research faces distinct challenges that complicate VCG application, including high variability in study designs and test protocols, informational gaps, and the lack of adequate - standardized, interoperable, sustainable - databases in a wide range of research areas. To begin narrowing down these challenges, we applied Large Language Model (LLM) text-mining analysis to the ALURES Non-Technical Summaries (NTS). This analysis revealed that pharmacological and psychopharmacological studies, pain and brain injury models, and other psychiatric and neurodegenerative models, were particularly prominent. The recurrent use of these paradigms suggests that they are especially suitable for VCG development; however, the associated tests, apparatus, and methods, particularly behavioural assessments, also require extensive standardization and careful metadata recruitment.

To achieve this, we are training a similar LLM to extract key information from published studies and build a comprehensive database with animal-level resolution and VCG generation capability. Curation of such a potentially vast database must be performed by the research community in open platform. Remarkably, an ongoing survey on VCGs and data reuse across more than 60 academic institutions has demonstrated a strong academic commitment to shared databases, standardization and data reuse critical steps for enhancing research efficiency and reproducibility.

Therefore, we invite the cannabinoid research community, given its close and collaborative relevance to these areas, to join this effort and help foster data reuse to accelerate the reduction of animal use and establish new research standards and tools.

LONG-TERM CONSEQUENCES OF ADOLESCENT EXPOSURE TO THE SYNTHETIC CANNABINOID AB-FUBINACA IN MALE AND FEMALE MICE

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Adolescence represents a critical window of vulnerability to psychoactive substances, including synthetic cannabinoids, whose use has been linked to an increased risk of psychiatric disorders later in life. Among them, AB-FUBINACA, an indazole carboxamide compound commonly detected in Spice/K2 products, stands out for its high potency at cannabinoid receptors. In this study, we explored the long-term consequences of adolescent AB-FUBINACA exposure in male and female mice. AB-FUBINACA treatment revealed several sex-dependent behavioral alterations. In this sense, the administration of this synthetic cannabinoid in female, but not male, mice induced psychotic-like symptoms which were associated with changes in dendritic arborization and density of mature dendritic spines in pyramidal neurons of the prefrontal cortex, as well as with an up-regulation of differentially expressed genes in this brain area. Together, our findings suggest that AB-FUBINACA exposure during adolescence may trigger persistent neurobiological disturbances, particularly in females, shedding light on the long-term risks associated with synthetic cannabinoid use during this developmental stage.

Keywords: Synthetic cannabinoids, Adolescence, Psychosis.

ACTIVATION OF CENTRAL CANNABINOID TYPE 2 RECEPTORS, BUT NOT ON PERIPHERAL IMMUNE CELLS, IS REQUIRED FOR ENDOCANNABINOID-MEDIATED NEUROPROTECTION IN PARKINSON'S DISEASE

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Neuroinflammation is a key feature of Parkinson's disease (PD). The cannabinoid receptor type 2 (CB2R) is expressed by cells of the innate and adaptive immune systems. Inhibition of monoacylglycerol lipase (MAGL) with JZL184 increases the levels of the endocannabinoid 2-arachidonoylglycerol (2-AG), which is neuroprotective for dopaminergic neurons. The aim of this study was to determine whether the neuroprotective effect of MAGL inhibition is mediated by CB2R activation on specific immune cell populations. Experimental parkinsonism was induced by chronic administration of MPTP and probenecid. A specific increase in CD4+ T cell infiltration was detected in the midbrain of parkinsonian mice and was reduced by administration of JZL184. JZL184 had no effect in CB2R KO mice, suggesting that CB2R is required for neuroprotection. In the brain, CB2R expression was restricted to myeloid cells and lymphocytes, and increased in microglia under parkinsonian conditions. Administration of a central CB2R agonist, JWH133, exerted a beneficial effect similar to that of JZL184, whereas the peripheral agonist RO304 lacked neuroprotective activity. These results were confirmed using chimeric mice. In silico analysis, showed that transcripts related to 2-AG biosynthesis are downregulated in the midbrain microglia from PD patients. Our results show that activation of CB2R in the brain prevents nigrostriatal degeneration, CD4+ T cell infiltration and TNFα production in the midbrain of parkinsonian mice. The reduced 2-AG signaling in microglia from PD patients suggests that activation of microglial CB2R may be an interesting strategy for the treatment of PD.

Keywords: CD4(+) T cells; Cannabinoid receptor type 2; Endocannabinoid system; Microglia; Neuroprotection; Parkinson’s disease.

STRUCTURE-BASED OPTIMIZATION OF *N*-ADAMANTYL-ANTHRANILAMIDE DERIVATIVES AS SELECTIVE CANNABINOID CB2 RECEPTOR LIGANDS

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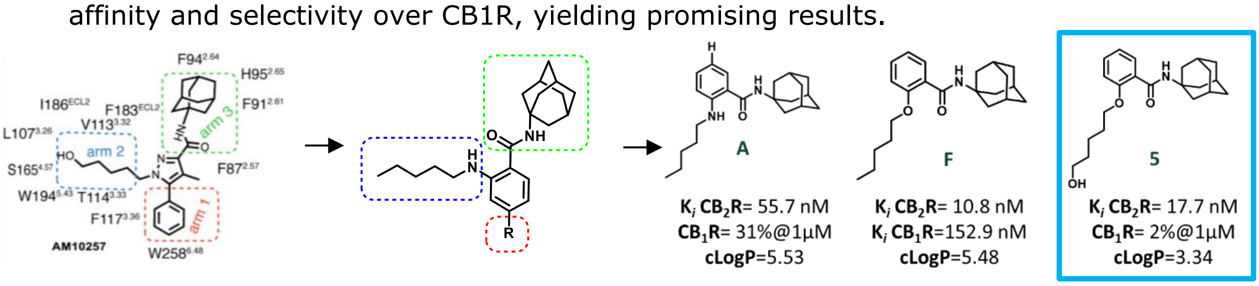
The endocannabinoid system (ECS) is a conserved lipid signaling network that regulates physiological homeostasis. It includes two main G-protein-coupled receptors: CB1R, primarily expressed in the central nervous system (CNS), and CB2R, mainly found in the immune system. Unlike CB1R, CB2R suppresses inflammation without inducing psychoactive effects, making it a promising target for inflammatory and neurodegenerative diseases. However, its structural similarity to CB1R limits the development of selective ligands. Pharmaceutical research aims to develop optimized CB2R-selective ligands with improved drug-like properties for therapeutic applications. In 2023, we synthesized, evaluated, and published *N*-adamantyl-anthranil amide derivatives as CB2R-selective ligands[1]. Our design was based on the proposed binding mode of the potent CB2R antagonist AM10257[2], known as the "three-arm pose." Although our derivatives demonstrated promising receptor affinity and selectivity, their lipophilicity values did not satisfy pharmaceutical requirements. To address this limitation, we designed compounds with improved pharmacokinetic properties by introducing polar substituents into the alkyl chain of arm 2[3]. These synthesized compounds were then tested for CB2R affinity and selectivity over CB1R, yielding promising results.



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Keywords:
Endocannabinoid system (ECS), CB2 receptor, Agonist, Anticancer

CHANGING THE PARADIGM IN CB2 SCREENING WITH FLUORESCENT HIGH-CONTENT ASSAYS

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Cannabinoid receptors (CB_1R and CB_2R) are G protein-coupled receptors (GPCRs) involved in key physiological and pathological processes, including pain modulation, inflammation, immune response, and neurodegeneration.[1] Previously, we synthesized and pharmacologically characterized the largest and most diverse library of synthetic cannabinoid receptor agonists (SCRAs) to date, comprising 64 enantiopure ligands.[2] This work allowed the identification of novel structure–activity (SAR) and structure–selectivity relationships (SSR) critical for the rational design of selective CB_2 modulators. Neurotoxicity profiling of these derivatives, generally higher than Δ^9-THC and CBD, provided additional insights for the development of safer CB_2-targeted compounds. This work is one of the examples of radioligand binding assays being used as the gold-standard methodology; however, their reliance on radioactivity underscores the need for more sustainable, versatile, and robust approaches, such as fluorescent-based assays. Fluorescent ligands offer remarkable advantages, including applicability in fluorescence microscopy, high-content screening (HCS), FRET, HTRF, and BRET, in both pre-transfected and living cells. Nevertheless, widespread adoption has been limited by the complexity of probe development and the scarcity of optimized ligands.[3] At Celtarys Research, we applied our proprietary fluorescent ligand technology to the CB_2 receptor, resulting in CELT-331, a red-emitting, CB_2-selective probe. This probe enables direct visualization of CB_2 expression via fluorescence microscopy and the development of a high-content screening (HCS) assay to study the binding affinity of various CB2 modulators. Initially, a set of fluorescent compounds was screened to validate the assay. Subsequently, a series of CB2 agonists (SCRAs) was evaluated, and the obtained data were compared with results from traditional radioligand binding experiments. The excellent correlation between the two experimental setups demonstrated the robustness and reliability of the developed HCS assay for CB2 modulator screening. This work laid the foundation for a new screening platform launched by Celtarys and supports the future development of ready-to-use kits, enabling any laboratory to perform the assay in-house using affordable and widely available equipment, along with robust, sustainable, and user-friendly experimental protocols

CANNABINOID-LOADED NANOSYSTEMS TARGETED TO VCAM-1 AS A POTENTIAL ANTI-THEROSCLEROTIC THERAPY

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Introduction: The CB2 cannabinoid receptor, due to its anti-inflammatory properties, is a promising target for treating atherosclerosis. However, high lipophilicity and low bioavailability limit the clinical use of its agonists, such as JWH-133. Our objective was to develop selective nanosystems to enhance the delivery of CB2 agonists directly to atherogenic regions. We synthesized biocompatible polymeric nanoparticles (NPs) loaded with JWH-133 and functionalized their surface with a VCAM-1 binding peptide (VBP) for specific targeting to the atheroma plaque.

Methods: NPs were produced via nanoprecipitation using PEGylated PLGAs. Encapsulation and drug loading were characterized by HPLC, and size and morphology by DLS and electron microscopy. VBP fun ctionalization efficiency was confirmed via maleimide chemistry and microBCA. We performed *in vitro* studies on toxicity (MTT), targeted cellular uptake (in TNFα-stimulated HUVECs), and anti-atherogenic effects (cytokine and gene expression). *In vivo* biodistribution and anti-atherogenic efficacy were evaluated in ApoE-/- mice.

Results: We obtained monodisperse NPs (170 nm, -26 mV) with remarkably high drug encapsulation (DL=15%) and successful VBP conjugation. The NPs showed no cytotoxicity, and VBP functionalization significantly enhanced accumulation in stimulated vascular cells, validating the targeting strategy. Molecular analysis revealed that the NPs reduced inflammation (*e.g.*, IL-6 and inflammatory genes such as *VCAM1* and *NFKB*). *In vivo* studies demonstrated significant accumulation in the heart (22%) and aorta (30%). Furthermore, 8 weeks of NPS administration improved the lipid and inflammatory profile and significantly decreased the development of atheroma plaques in the animal model.

Conclusion: These findings confirm the immense potential of these VBP-functionalized NPs as effective and selective vehicles for CB2 agonists to target and treat the atherosclerotic endothelium.

Keywords: PLGA-PEG NPs, CB2 agonist, atherosclerosis

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CONFORMATIONAL RESTRICTION OF DESIGNER DRUGS REVEALS SUBTYPE- SELECTIVE AND BIASED CB_2 AGONISTS WITH NEUROPROTECTIVE EFFECTS

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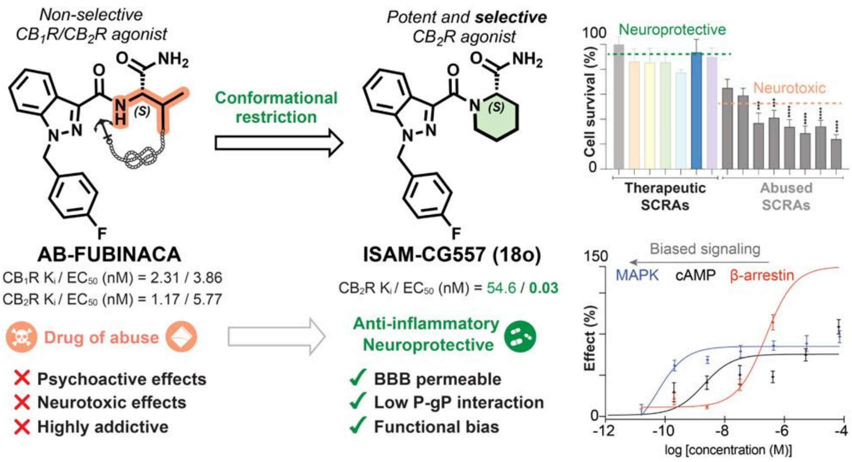
The cannabinoid receptor type 2 (CB_2R) has emerged as a promising target for neuroprotective therapies due to its key role in modulating immune responses and neuroinflammation. [1]In this study, we employed promiscuous (CB_1R/CB_2R) and highly potent designer ligands[2] as reference compounds to develop an extensive series of CB_2R agonists. The introduction of a central cyclic scaffold, following a conformational restriction design strategy, enabled the identification of derivatives displaying excellent CB_2R potency and affinity, enhanced subtype selectivity, and distinctive biased signalling profiles.

From these series, six representative compounds were evaluated and showed different bias toward β-arrestin, MAPK or G-protein signalling pathways. Additionally, these molecules displayed favourable pharmacokinetic properties, including high blood-brain barrier permeability, low P-glycoprotein interaction, and good microsomal stability, supporting their potential for central nervous system applications.

Overall, these findings highlight the efficacy of conformational restriction in transforming promiscuous and neurotoxicity of abused drugs into highly selective and biased CB_2R ligands with therapeutic potential in neurodegenerative disorders, while avoiding CB_1R-mediated psychoactive effects.



Keywords: CB_2 receptor, biased agonism, neuroprotection.

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ASTROCYTIC AND NEURONAL ENDOCANNABINOIDS IN THE OLFACTORY BULB COOPERATE WITH NORADRENERGIC SIGNALING TO DETERMINE THE SOCIAL AND COGNITIVE CONSEQUENCES OF SOCIAL TRANSMISSION OF STRESS.

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Mice can detect and react to the stress of other congeners, and this transmitted stress changes their social behavior and cognition. The smell of stress is processed by astrocytes in the olfactory bulb through a mitochondrial CB1-dependent mechanism impacting mitochondrial calcium dynamics. However, the underlying mechanism is still not fully characterized. Both neurons and astrocytes possess the enzymatic machinery to produce endocannabinoids, but the contribution of astrocytic endocannabinoids to olfactory function has yet not been studied. Moreover, accumulating evidence suggests that astrocytes are a key target of noradrenaline, another major neuromodulator.

Thus, we investigated whether noradrenaline and astrocytic endocannabinoids are involved in the processing of stress-related chemosignals from conspecifics and in the associated cognitive consequences. Using a combination of chemogenetic and optogenetic approaches, we found that the activity of noradrenergic projections from the locus coeruleus to the olfactory bulb is necessary for both olfactory exploration of a stressed partner and the amnesic effects of socially transmitted stress in novel object recognition, without impacting basal olfaction and memory.

Moreover, we used *Dagla*-flox mice to locally manipulate the DAGLA expression, - the gene encoding for the main enzyme responsible for the production of 2-AG -, in the olfactory bulb neurons and astrocytes, respectively. We found that astrocytic *Dagla* deletion abolished both anogenital exploration and the memory impairment associated with a stressed partner, whereas neuronal *Dagla* affected only the memory. These findings indicate distinct contributions of neuronal and astrocytic 2-AG production to the behavioral responses to social stress.

Altogether, our results reveal a complementary mechanism in which noradrenaline and endocannabinoids jointly modulate the olfactory processing of social stress signals, involving mitochondrial CB1 receptors in astrocytes.

HIPPOCAMPAL ENDOCANNABINOIDS MODULATE INCIDENTAL ASSOCIATIONS ACROSS SENSORY MODALITIES

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Decision-making is often influenced by stimuli that have not been directly associated with any reinforcer. For example, one might avoid a restaurant mentioned alongside another where they experienced food poisoning, despite having no direct negative experience at the first location. This indicates that an unreinforced unified mental representation of the stimuli (the two restaurants) was formed through processes known as incidental associations (IA). IAs are typically studied in rodents using sensory preconditioning paradigms, where animals are exposed to paired, low-salience stimuli (S1/S2). Subsequently, S1 is associated with a reinforcer, and specific mediated responses to S2 in the test phase indicate the formation of IAs between S1 and S2. Previous research has identified type-1 cannabinoid receptors (CB1R) located in the hippocampus as key players in IA formation in a sensory preconditioning paradigm involving odour and taste as sensory stimuli. Building on these findings, our project aims to determine whether IA formation engages common neural networks across paradigms underlying different sensory modalities. To investigate this, we used double transgenic mice expressing tamoxifen-inducible Cre-dependent TdTomato under the control of the cFos promoter (cFos-CreERT2-TdT). During preconditioning, light and sound were presented either in paired or unpaired configurations, while tamoxifen injections enabled specific tagging of cells activated by each condition. Mice were then subjected to a second preconditioning protocol involving paired odour and taste stimuli before being euthanized for c-Fos immunolabelling. Our data show significantly higher double-labelled neurons within the dorsal hippocampal CA3 and dentate gyrus in the light-sound paired group, suggesting that cells encoding general IAs may reside in these areas. Ongoing *in situ* hybridization will determine whether CB1R is expressed in these "IA cells". In parallel, we used the GRABeCB3.0 sensor to monitor *in vivo* endocannabinoid activity in the dorsal hippocampus by fiber photometry during both odour–taste and light–sound preconditioning protocols. These analyses indicate that preconditioning enhances hippocampal endocannabinoid activity in both preconditioning protocols, pointing to a shared CB1R-dependent mechanism. To causally test this hypothesis, CB1-flox mice were injected in the hippocampus with a virus expressing the tamoxifen-inducible Cre recombinase under the cFos promoter (AAV-cFos-CreERT2). Tamoxifen administration during the light–sound preconditioning will express the Cre in activated cells and induce CB1R deletion specifically in these cells. These mice will subsequently undergo the odour–taste protocol and be tested for mediated responses. If CB1R deletion in light–sound–activated cells abolishes mediated responding in the odour–taste task, this would provide strong causal evidence for the involvement of hippocampal endocannabinoid signalling in IA formation across sensory modalities. Overall, these findings could provide novel insight into how cannabinoids modulate the formation of IA and how their dysregulation might aberrantly promote associative processes, potentially contributing to psychotic-like states.

ASTROCYTIC RELEASE OF 2-AG MODULATES NEURONAL ACTIVITY AND BEHAVIOR

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Astrocytes are active regulators of brain function and they are able to modulate neuronal activity and behavior through the release of different gliotransmitters. Interestingly, the discovery of new gliotransmitters is advancing rapidly, opening new ways to understand how astrocytes sense the environmental cues and answer according to their needs, modulating the different neuronal circuits.

In previous work from our laboratory, we proposed that astrocytes are able to produce and release to neighboring neurons a lipid messenger, the endocannabinoid 2-arachidonylglycerol (2-AG), through an intracellular calcium-dependent mechanism. This astrocyte-derived eCB relies on the expression of the enzyme diacylglycerol lipase A (DAGLA). In the present work, we wanted to address which are the neuronal and behavioral consequences of removing these astroglial-derived endocannabinoids.

Using a DAGLA flox mouse model, we performed stereotaxic injections in the somatosensory cortex (SSC) of adult mice to specifically remove DAGLA from the astrocytes of this area (SSC-GFAP-DAGLA-KO). Electrophysiological activity was measured by performing patch-clamp recordings in the pyramidal neurons from layer V. Interestingly, we observed that the removal of DAGLA from astrocytes in the SSC produces an increase in the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) only in female mice while no differences were seen in spontaneous excitatory postsynaptic currents (sEPSCs). Interestingly, these females lacking DAGLA in astrocytes of the SSC showed a decreased performance in the rotarod test.

These results reveal a new mechanism of astrocyte-neuron communication, in which astrocytic production of 2-AG by Daglα may turn down the activity of the GABAergic neurons of the SSC to promote motor performance.

Our results confirm the role of astroglial-derived 2-AG in brain communication and establishes endocannabinoids as a possible signaling molecule by which astrocytes can control neuronal activity.

Keywords: astrocyte, Daglα, 2-AG.

INDUCTION OF HEPATIC STEATOSIS BY SUCROSE-RICH DIET WAS ASSOCIATED WITH LIVER ENDOCANNABINOID DYSREGULATION, AND CAUSED COGNITIVE IMPAIRMENT AND NEUROINFLAMMATION: BENEFITS OF A CANNABIS OIL-BASED THERAPY

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The global increase in Metabolic Syndrome (MetS) is mainly attributed to profound changes in dietary habits, particularly the excessive intake of saturated fats and refined sugars. The liver is one of the first organs affected, exhibiting steatosis, lipid imbalance, oxidative stress, and inflammation that contribute to the systemic alterations associated with MetS. MetS has also been linked to cognitive decline and an increased risk of neurodegenerative disorders. The endocannabinoid system (ECS) plays a crucial role in hepatic lipid metabolism and also in the neuroinflammatory response. While cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) show hepatoprotective and neuroprotective actions, the effects of full-spectrum cannabis oil remain unexplored.

Male Wistar rats were randomly divided and fed for 3 weeks with the following diets: reference diet (RD): standard commercial laboratory diet (GEPSA FEED), sucrose rich diet (SRD), and SRD+Cannabis Oil (SRD+CO): non-invasive oral administration of 1.5 mg/kg of cannabis oil (CBD:THC, 2:1 ratio). After 3 weeks, liver and medial prefrontal cortex (mPFC) tissues were collected. We evaluated in liver: triglyceride (TG) content, histological analysis, NAFLD activity score (NAS), activity of lipogenic and fatty acid oxidation enzymes, and the expression of endocannabinoid system (ECS)-related enzymes — NAPE-PLD, DAGL, FAAH, and MAGL — and CB1 receptor by quantitative PCR. In the mPFC, immunohistochemistry was performed to analyze Iba1 (microglia marker), S100β (astrocyte marker), and Ctip2 (marker of cortical layer V neurons). Cognitive performance was assessed through T-maze, and Novel Object Recognition (NOR) tests.

SRD-fed rats developed hepatic steatosis, with increased TG content as a consequence of enhanced fatty acid synthesis and reduced oxidation, as well as higher NAS score. They also showed upregulated DAGL expression and enhanced CB1 receptor levels in the liver indicating ECS overactivation. In parallel, SRD-fed rats exhibited impaired working memory and recognition performance in the NOR test accompanied by microglial activation and reduced neuronal markers in the mPFC. Cannabis oil administration exerted a hepatoprotective effect which resulted in a restoration of the endocannabinoid homeostasis with normalization in DAGL and CB1 expression. This was accompanied by behavioral improvements in cognitive performance and attenuation in glial activation and neuronal losses.

This work provides the first evidence that full-spectrum cannabis oil (CBD:THC 2:1) administration may work in metabolic disorders by preventing or mitigating hepatic impairment caused by sucrose overconsumption, as well as the resulting early signs of cognitive deterioration. To elucidate whether these cognitive benefits are direct (induced by an action on brain endocannabinoid elements), indirect (due to the hepatic improvement) or due to both will require further research.

Keywords: sucrose-rich diet; endocannabinoid system; neuroinflammation.

CANNABINERGIC REGULATION OF HABENULAR CIRCUITS

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The expression of aversive memories is critical for survival. In the brain, specialized circuits are in charge of regulating emotional states. Furthermore, malfunctioning of such brain regions could lead to the occurrence of mood disorders (e.g., anxiety and depression). The Medial Habenula (MHb) is a sub-ventricular structure coding stress-related stimuli, and its cellular diversity (e.g., tanycyte-like cells, mast cells, and different neuronal and astrocytic phenotypes) suggests a unique sensibility to metabolic states and immunological responses. Thus, we hypothesize that the MHb is a cellular hub linking peripheral and central information to modulate emotion. We previously showed that the global deletion of the cannabinoid receptor type-1 (CB1) from the astrocytes of the MHb impaired aversive responses. In this project, we aim to dissect the role of cannabinoid transmission in different MHb cell-types and to determine how stress- related stimuli, such as immunological or metabolic, act in the MHb to regulate emotional pathophysiology in male and female mice. To do this, we combine different methodological approaches, including behavioral genetics (e.g., specific CB1 deletion from astrocytes, MHb(GFAP)-*CB1*-KO), cellular structural analysis, peripheral quantification of immunological factors (e.g., cytokines), *in vitro* electrophysiology (e.g., patch clamp) and *in vivo* recording of MHb cellular activity (e.g., calcium imaging). Preliminary results show that MHb(GFAP)-*CB1*- KO females showed differences in innate emotional responses (e.g., light-dark box). In contrast, male KO mice displayed differences in the learned emotional behavior (e.g., fear conditioning). Interestingly, these phenotypes are also reflected at the peripheral (e.g., increased cytokine levels) and cellular levels (e.g., astrocyte morphology changes). Our data indicates that CB1 control of MHb-astrocytic activity is necessary for expressing innate and learned emotional responses in female and male mice, respectively.

Keywords: emotion, astrocytes, sex-differences.

EFFECTS OF Δ9-TETRAHYDROCANNABINOL ON THE REWARDING PROPERTIES OF DIETS ENRICHED IN SATURATED FATTY ACIDS

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Excessive consumption of high-fat diets has been implicated in the global rise of obesity. Although such diets are known to influence brain reward circuits, their potential addictive properties remain poorly understood. We previously demonstrated that a sugar-free diet enriched with saturated fatty acids (SOLF; Saturated OiL-enriched Food) exerts strong reinforcing effects in naïve male and female mice. As it is known that excessive consumption of palatable foods can engage neural mechanisms similar to those involved in drug addiction, and evidence shows that δ9-tetrahydrocannabinol (THC) can enhance food intake and affect reward circuits, in the present study we set to examine whether THC alters the motivational properties of SOLF. First, we replicated our previous self-administration protocol after chronic THC exposure and found that it reduced motivation to obtain SOLF, with effects persisting long-term in both sexes but observed only in females short-term. In addition, we have investigated the impact of acute THC treatment on food intake and preference between SOLF and a control diet in young female mice and analyzed gene expression in several regions critically involved in reward and motivation. Our results show that acute THC administration decreased SOLF intake and preference in females, accompanied by altered expression of Bdnf and dopamine D1 receptor in the nucleus accumbens (NAcc) and hippocampus, respectively. Finally, we studied synaptic transmission on the NAcc after SOLF intake in the presence and absence of THC to further explore the acute effect of diet and treatment in this area. Overall, our findings indicate that THC modulates feeding behavior and reward processes in a sex-dependent manner, attenuating the reinforcing properties of saturated fats and consequently reducing their consumption.

Keywords: high-fat diets, THC, reinforcement

CB_1 CANNABINOID RECEPTOR SIGNALING REGULATION OF CORTICAL AND BEHAVIORAL ALTERATIONS INDUCED BY SATB2-DEFICIENCY

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The endocannabinoid system (ECS) plays a key role in neurodevelopment. Prenatally, CB_1 cannabinoid receptors regulate multiple processes, including the migration and differentiation of cortical pyramidal neurons. Disruptions in the balance between the generation of superficial and deep-layer cortical pyramidal neurons are responsible for neuropsychiatric disorders, including the SATB2-associated syndrome (SAS). This neurodevelopmental disorder is characterized by intellectual disability and behavioral traits, including autistic features, often together with epileptic comorbidity. SAS is caused by mutations in the *SATB2* gene locus, which encodes a transcription factor essential for the specification of interhemispheric projection neurons that are predominant in the upper layers of the cortex. Since the ECS regulates the differentiation and maturation of pyramidal neurons and plays a role in modulating synaptic activity, we hypothesize that regulating cannabinoid signaling through CB_1 receptor antagonism during adolescence may attenuate some of the behavioral alterations involved in SAS etiopathology.

Previous research demonstrated that prenatal CB_1 receptor signaling manipulation partially rescues aberrant neuronal differentiation of *in vitro* SATB2-deficient cortical neurons. Now, we have generated a conditional knockout (cKO) mouse model that lacks SATB2 expression in forebrain pyramidal neurons from early stages of embryonic development. Behavioral characterization shows that adult SATB2 cKO mice exhibit hyperactivity, increased impulsivity, reduced anxiety and impaired novel social interaction. At the cellular level, SATB2 cKO mice exhibit a loss of upper-layer cortical neurons concomitant with an expansion of deep layer neurons. Furthermore, corpus callosum development is severely affected in these mice, indicating a shift in axonal projections to subcortical targets. The impact of CB_1 antagonist administration during adolescence (PND 22-36) in adult behavior traits and neuronal plasticity will be presented.

Keywords: cortical development, endocannabinoid system, SAS.

COMBINED PERIPHERAL CB1R BLOCKADE AND INCRETIN THERAPY ACHIEVE ROBUST WEIGHT LOSS AND METABOLIC BENEFITS IN HUMANIZED CB1R MICE

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The endocannabinoid system, through cannabinoid receptor-1 (CB1R), plays a central role in the regulation of appetite, adiposity, glucose metabolism, and inflammation. Although the CB1R inverse agonist rimonabant demonstrated robust anti-obesity efficacy, psychiatric adverse effects precluded its clinical use. Nimacimab, a peripherally restricted humanized IgG4 monoclonal antibody targeting a conformational epitope in CB1R extracellular loop 2, has been developed as a neutral antagonist with the potential to overcome these limitations.

To enable in vivo evaluation, we employed a humanized CB1R knock-in mouse, since Nimacimab does not bind rodent CB1R. Male diet-induced obese (DIO) mice and female post-weaning androgenized (PWA) mice expressing humanized CB1R were treated with Nimacimab, Tirzepatide, a dual GLP-1/GIP agonist, or their combination. Nimacimab reduced body weight, adiposity, and insulin resistance in both models, with significantly enhanced effects when combined with Tirzepatide in the DIO model. The combined therapy achieved approximately 35% weight loss in males and 15% in females, accompanied by reduced food intake, restored glucose tolerance, improved insulin sensitivity, and increased thermogenesis in brown and white adipose tissue. Plasma analysis showed normalization of leptin, adiponectin, and inflammatory cytokines, while histology demonstrated marked improvement in hepatic steatosis, inflammation, and fibrosis. Vascular function assessed by laser Doppler was restored, indicating improved peripheral perfusion. Proteomic profiling of liver tissue revealed distinct and complementary effects of each therapy: Nimacimab primarily corrected inflammatory and extracellular matrix pathways, Tirzepatide reversed mitochondrial and metabolic dysfunction, and the combination broadly normalized obesity-related molecular alterations, including a conserved 34-protein signature of obesity-associated liver disease across both models.

These findings demonstrate that peripheral CB1R blockade by nimacimab improves systemic, hepatic, and vascular dysfunction, and that combination with Tirzepatide yields synergistic metabolic benefits, supporting this therapeutic strategy for obesity and metabolic dysfunction–associated steatotic liver disease (MASLD).

Keywords: CB1R, Obesity, MASLD.

MODELING CANNABIS-INDUCED PSYCHOTIC-LIKE STATES IN MICE

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Cannabis is the most consumed illicit drug worldwide and its use has been linked with a major risk of psychotic symptoms, especially if used during adolescence. The neurobiological mechanisms underlying cannabis-induced positive psychotic-like states, such as impairments in reality perception, remain poorly understood, partly due to the lack of more precise animal behavioural models. Recently, complex socio-cognitive processes have been proposed to model positive psychotic-like behavioral alterations in rodents. In this context, our study proposes two novel behavioral paradigms to better understand cannabis-induced psychotic-like states. First, we are using a light-tone sensory preconditioning task in which neutral stimuli (light and tone) are associated in a preconditioning phase. During the conditioning stage one of the neutral stimuli (i.e. light) become reinforced, thus evoking conditioned responses to both neutral stimuli through mediated (ML) or direct learning (DL). Increasing the associations during the preconditioning phase, ML transitions into "reality testing" (RT), improving the discrimination between the real and the inferred stimuli, a trait commonly impaired in psychotic-like states. We first established a light-tone sensory preconditioning task in male mice, and also a RT protocol, in which an acute injection of delta-9-tetrahydrocannabinol (THC) (1mg/kg) prior the ML test, disrupted RT in a CB1-dependent manner, suggesting altered processing of mental sensory representations. On the other hand, we are currently investigating whether THC (1mg/kg) affects the mouse's perception of conspecifics with different internal states (e.g., under THC and/or ketamine treatments). Preliminary data suggests that THC1-treated mice fail to discriminate between control and high-dose THC-treated (10mg/kg) conspecifics. Based on these behavioral approaches, in the future, we aim to explore the molecular mechanisms underlying the THC-induced psychotic-like effects in order to identify novel therapeutic targets for treating cannabis-induced psychotic states.

Keywords: THC, sensory preconditioning, psychotic-like states, social discrimination

mTOR/S6 SIGNALING DYSREGULATION AS A CONVERGENT PATHWAY LINKING CANNABIS EXPOSURE TO INCREASED SCHIZOPHRENIA RISK

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Altered neurodevelopmental trajectories and exposure to cannabis during adolescence are key risk factors for schizophrenia; however, the molecular pathways that connect these factors to the fundamental features of the disorder remain poorly understood. Dysregulation of the mTOR/S6 signaling pathway, a key regulator of neuronal growth, synaptic plasticity, and protein synthesis, has been consistently implicated in schizophrenia pathophysiology. Emerging evidence suggests that cannabinoids, particularly Δ9-tetrahydrocannabinol (THC), modulate mTOR/S6 signaling, potentially exacerbating neurodevelopmental vulnerabilities and contributing to schizophrenia-relevant phenotypes. In this context, the objective of the study was to explore the mTOR/S6 pathway dysfunction and its potential link to schizophrenia by quantifying key signaling proteins in patient-derived olfactory neuroepithelial cells and in the cortex of a schizophrenia-like animal model, with a focus on the effects of adolescent THC exposure.

For this study, olfactory neuroepithelial cells were obtained via nasal exfoliation from patients with schizophrenia (n=10) and age- and sex-matched controls (n=10), cultured, and differentiated into neurons as previously described (Unzueta-Larrinaga et al., 2023). A “double-hit” mouse model of schizophrenia was generated (n=6 males, n=6 females per group) by prenatal maternal immune activation (Poly(I:C)) followed by adolescent-like period THC exposure from postnatal day 21 for 30 days. Levels of Akt, mTOR, S6, and their phosphorylated forms were measured in both neuronal cells and mouse cortical tissue using western blotting technique.

Analysis of the mTOR/S6 signaling pathway revealed dysregulations in both schizophrenia-like mouse model and human olfactory neuronal cells. In mice, total Akt and phospho(Thr450)-Akt levels were unaltered, whereas phospho(Ser2448)-mTOR and the phospho-mTOR/mTOR ratio were significantly reduced in both Poly(I:C)-Vehicle (-53.99% and -34.44%, respectively) and Poly(I:C)-THC groups (-64.40% and -46.97%, respectively). Phospho(Ser235/236)-S6 and the phospho-S6/S6 ratio were significantly decreased across Poly(I:C)-Vehicle (-33.20% and -37.34%) and Poly(I:C)-THC (-44.00% and -42.50%) groups, with a significant Poly(I:C) × THC interaction suggesting a synergistic effect. Consistently, in human-derived olfactory neuronal cells, total Akt and phospho(Thr450)-Akt were unaltered, while phospho(Ser2448)-mTOR and the phospho-mTOR/mTOR ratio were markedly reduced (-69.56% and -39.13%) in schizophrenia patients versus controls. Total S6 was significantly increased (+3709.22%), yet phospho(Ser235/236)-S6 and the phospho-S6/S6 ratio were strongly reduced (-93.65%).

In conclusion, patient-derived neuronal cells exhibited a profound dysregulation of the mTOR/S6 signaling axis, characterized by a reduced mTOR phosphorylation and an imbalance between total and activated S6. In mice, combined Poly(I:C) and THC treatment produced the most severe alterations, mirroring molecular abnormalities observed in patient-derived cells. These findings position dysregulated mTOR/S6 signaling as a convergent pathway linking cannabinoid exposure and neurodevelopmental insults to schizophrenia-increased risk.

Keywords: schizophrenia, THC, olfactory neuroepithelium.

ADOLESCENT BINGE DRINKING INDUCES LONG-TERM REORGANIZATION OF HIPPOCAMPAL ENDOCANNABINOID SIGNALING AND BEHAVIOR IN ADULT FEMALE MICE

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Adolescence is a critical period of brain maturation, highly vulnerable to alcohol exposure. Binge drinking (BD) during this stage can induce long-lasting neurobiological alterations; however, most studies have focused on males. Here, we examined the long-term effects of adolescent BD on hippocampal endocannabinoid (eCB) signaling, synaptic plasticity and behavior in adult female mice.

Female C57BL/6J mice underwent a “drinking-in-the-dark” (DID) protocol (postnatal day 32-56). After 2 weeks withdrawal, hippocampal slices containing the dentate gyrus were prepared for extracellular field recordings to assess excitatory synaptic transmission and eCB-mediated long-term depression (eCB-LTD) at medial perforant path-granule cell synapses. Behavioral assessments (open field, elevated plus maze, light/dark box and Barnes maze) assessed emotional and cognitive outcomes, and hippocampal synaptosomes were analyzed by Western blot.

In control females, medial perforant path stimulation induced robust eCB-LTD mediated by coordinated activation of CB1R and TRPV1. After adolescent ethanol exposure, eCB-LTD persisted but relied exclusively on TRPV1 signaling, indicating a loss of CB1R contribution. This form of synaptic plasticity still required mGluR1, NMDAR activation, anandamide and 2-arachidonoylglycerol synthesis, but also mGluR5, as well as extracellular and intracellular calcium influx. Basal expression of eCB-related proteins (CB1, PLCβ1, $G\alpha_o$, CRIP1a) was higher in females, while ethanol intake reduced $G\alpha_{i2}$, NAPE-PLD, and SNAP25, suggesting impaired eCB synthesis and synaptic integrity. Behaviorally, ethanol-exposed females showed altered emotional response and switched from serial to random strategy in the Barnes maze.

These findings reveal sex-specific adaptations in hippocampal eCB signaling. Despite preserved eCB-LTD through TRPV1-mediated compensation, adolescent BD disrupts CB1-related mechanisms and induces molecular and behavioral alterations, highlighting the complexity of endocannabinoid-mediated responses to early-life ethanol exposure.

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Keywords: alcohol, females, hippocampal neurobiology

POLYDRUG-INDUCED NEUROADAPTATIONS: ENDOCANNABINOID SYSTEM INVOLVEMENT IN THE INCUBATION OF COCAINE AND ETHANOL SEEKING

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Substance use disorders are chronic, relapsing conditions characterized by compulsive drug seeking, impaired control, and negative affective states during abstinence. During this period, craving for drug-associated cues progressively increases, a phenomenon known as incubation of craving. Drugs of abuse induce neural dysregulations involving overlapping neurochemical and neuroanatomical pathways, among which the endocannabinoid system (ECS) plays a key role. The ECS modulates synaptic transmission in the central nervous system by reducing neurotransmitter release at both excitatory and inhibitory synapses. Notably, CB1 receptors are abundant in motivation- and reward-related regions, including the ventral tegmental area, amygdala, hippocampus, and prefrontal cortex. It can be disrupted not only by exogenous cannabinoids but also by other drugs of abuse, such as cocaine and ethanol, leading to neuroadaptations that underlie addiction-related behaviours.

In this study, we evaluated the ECS in a rat model of incubation of craving following combined self-administration of cocaine+ethanol, given that most individuals who use cocaine also consume alcohol, and their co-use leads to more severe adverse consequences than either drug alone. Rats were trained in an extended-access self-administration paradigm, followed by a withdrawal period of either 2 or 30 days and a subsequent drug-seeking test. Thereafter, animals were sacrificed, and the hippocampus, prefrontal cortex, and striatum were dissected and preserved for further analyses. To assess ECS function, we quantified the gene expression of the CB1 receptor (*Cnr1*), its ligand-synthesizing enzymes (diacylglycerol lipase, *DAGL*; and N-acyl phosphatidylethanolamine phospholipase *D, NAPE-PLD*), and its ligand-degrading enzymes (monoacylglycerol lipase, *MAGL*; and fatty acid amide hydrolase, *FAAH*).

Our analyses revealed the incubation of cocaine+ethanol seeking in females but not in males. In the prefrontal cortex, females showed increased *Cnr1* expression in the cocaine+ethanol group compared with controls, along with decreased expression of *NAPE-PLD* and *MAGL* after 30 days of withdrawal compared to 2 days. In the hippocampus, changes were observed in both sexes: males displayed increased *Cnr1* expression after 2 days of withdrawal, while females showed this increase after both withdrawal periods, together with elevated *NAPE-PLD/FAAH* and *DAGL/MAGL* ratio compared to controls. These findings indicate greater ECS activity in the hippocampus of females that exhibited cocaine+ethanol seeking compared to males, who did not display this phenomenon. These preliminary results suggest that hippocampal activity of the endocannabinoid system may be involved in the incubation of cocaine+ethanol seeking, a process closely associated with relapse vulnerability.

Keywords: cocaine and ethanol polyconsumption; hypocamppus; endocannabinoid system.

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HETEROMERIC INTERACTION BETWEEN CB_1R R AND OX_2R IN ALZHEIMER: A PROMISING THERAPEUTIC TARGET TO ENHANCE NEURONAL NEUROPROTECTIVE RESPONSES

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Alzheimer's disease (AD) remains a significant health challenge, with an increasing prevalence globally. Recent research has aimed to deepen the understanding of the disease pathophysiology and to find potential therapeutic interventions. In this regard, G protein-coupled receptors (GPCRs) have emerged as novel potential therapeutic targets to palliate the progression of neurodegenerative diseases such as AD. Orexin and cannabinoid receptors are GPCRs capable of forming heteromeric complexes with a relevant role in the development of this disease. On the one hand, selective orexin 2 receptor (OX_2R) signalling modulation has been associated with the pathological progression of Alzheimer's. On the other hand, cannabinoid 1 receptor (CB_1R) overexpression takes place in a neuroinflammatory environment, favoring neuroprotective effects. Considering the high number of interactions between cannabinoid and orexin systems that have been described, regulation of this interplay emerges as a new focus of research. We here show a close interrelationship between those receptors in transfected cells and neuronal primary cultures where they are able to physically interact and affect the signaling of each other due to allosteric interaction within an CB_1-OX_2 receptor heteromer (CB1-OX2 Het). Interestingly, we observe a neuroprotective potentiation when coactivating the CB_1R and OX_2R simultaniously by reducing ROS production in primary cortex neuron cells treated with amyloid beta. In order to understand the mechanism through these receptors regulate each other, we first demostrated the heteromer formation in both heterologous cells and in primary cultures of neurons with BRET and PLA assays respectively. The most relevant finding, confirmed in both heterologous cells and primary neuron cultures, was that blocking OX_2R led to increased CB_1R-mediated signaling, and vice versa. This heteromer-specific feature suggests that specific antagonists of both receptors would potentiate the neuroprotective action of endocannabinoids and orexins with important implications for an AD therapy.

THE ENDOCANNABINOID SYSTEM AS A THERAPEUTIC TARGET IN ALZHEIMER'S DISEASE: EFFECTS OF CHRONIC FAAH INHIBITION IN THE 5XFAD MODEL

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The endocannabinoid system (ECS) is a complex network involved in maintaining neuronal homeostasis and regulating neuroinflammatory processes. Increasing evidence supports its potential as a therapeutic target for neurodegenerative disorders such as Alzheimer's disease (AD). The enzyme fatty acid amide hydrolase (FAAH), responsible for the degradation of the endocannabinoid anandamide, plays a crucial role in modulating neuroinflammation and glial activity.

In this study, we evaluated the effects of chronic pharmacological FAAH inhibition on glial responses and neuroinflammatory pathways in the 5xFAD mouse model of AD. Six-month-old male and female 5xFAD mice received the irreversible FAAH inhibitor PF-04457845 orally at a dose of 10 mg/kg for three months. Following treatment, behavioral tests assessing cognition, anxiety, and depressive-like behaviors were performed. In parallel, molecular and biochemical analyses were conducted to evaluate inflammatory mediators, glial activation, and markers of synaptic integrity.

Our findings revealed that FAAH inhibition attenuated neuroinflammatory responses, reducing astrocytic and microglial reactivity while promoting synaptic stability, particularly in females. The results indicate possible sex-related variations in the neuroinflammatory response following FAAH inhibition. Overall, the results support the notion that modulation of FAAH and the ECS may hold therapeutic potential in AD.

Keywords: Endocannabinoid system, FAAH inhibition, neuroinflammation

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ASTROGLIAL LIPID METABOLISM: A LINK BETWEEN CANNABINOID DRUGS AND ALZHEIMER DISEASE?

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Astrocytes play a fundamental role in Alzheimer's disease (AD), actively contributing to its behavioral deficits such as the cognitive alterations observed in this neurodegenerative disease. However, a better understanding of the mechanisms linking astroglial activity and AD could facilitate the development of targeted therapeutic approaches. Using the APP/PS1 mouse model of AD, we investigated sex-, genotype-, and cannabinoid-dependent effects across behavioral, molecular, and functional levels. We first performed a longitudinal behavioral characterization using EzTrack® and DeepLabCut™ to accurately quantify diverse behavioral responses including anxiety, cognition, social behavior or depressive-like responses. Notably, we observed significant sex-, genotype-, and cannabinoid-related differences across cognitive domains. Interestingly, whereas only male APP/PS1 mice displayed strong impairment in the Novel Object Recognition Task (NORT) that were prevented by chronic treatment with the CB1 agonist ACEA, female mice showed alterations in contextual fear conditioning that were not rescued by the cannabinoid treatment. To explore the contribution of astrocytes in the NORT, we selectively inhibited astrocytic activity in dorsal CA1 hippocampus during the consolidation phase in male WT and APP/PS1 mice. Strikingly, this intervention rescued the cognitive performance, suggesting a key role for astrocytic activity dynamics in recognition memory processes. Complementary, *in vivo* fiber photometry experiments were used to monitor astrocytic calcium dynamics in male and female mice while performing the NORT. Preliminary analyses revealed sex-, genotype-, and cannabinoid-dependent alterations in hippocampal astrocytic calcium activity, supporting astroglial involvement in AD-related memory impairments. At the molecular level, we conducted single-cell RNA sequencing on hippocampal tissue from 6-month-old male and female WT and APP/PS1 mice that had received cannabinoid or vehicle treatment during the presymptomatic period and underwent a NORT at 6 months of age. Interestingly, lipid metabolism–related genes were reduced in APP/PS1 males treated with vehicle and restored by ACEA treatment, indicating a specific cannabinoid modulation of these astrocytic metabolic pathways. Collectively, these findings reveal sex-specific behavioral and molecular alterations in AD, highlighting astrocytic lipid metabolism and calcium signaling as correlates of cognitive deficits observed in an AD mouse model, and demonstrate that modulating astrocyte activity during memory consolidation can restore recognition memory. Our study underlines astrocytes as promising targets for therapeutic intervention in Alzheimer's disease.

Keywords: Alzheimer's Disease, astrocytes, lipid metabolism, ACEA.

RESTORATION OF CB1 RECEPTOR FUNCTION IN HIPPOCAMPAL GABAERGIC NEURONS RESCUES MEMORY DEFICITS IN HUNTINGTON´S DISEASE MODELS

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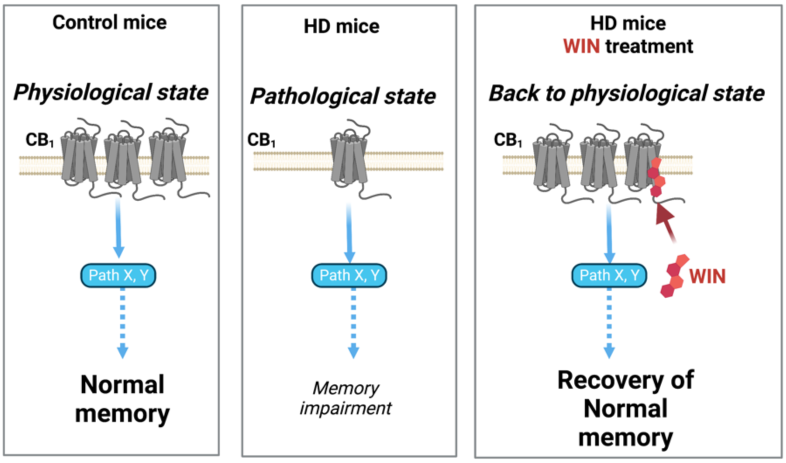
Background. Dysregulation of the endocannabinoid system (eCBS) and loss of CB1 receptors (CB1R) in the basal ganglia are well-established hallmarks of Huntington's disease (HD). Consequently, significant research has focused on targeting the eCBS to alleviate motor symptoms. However, beyond its recognized role in motor control, the eCBS is a complex signaling network essential for learning and memory. Despite the early onset of cognitive impairments in HD—often preceding motor dysfunction—the contribution of eCBS alterations to these deficits has remained largely unexplored.

Methods. We assessed hippocampal CB1R expression in human HD samples and two established HD mouse models (R6/1 and HdhQ7/111, including both sexes) using Western blotting, immunohistochemistry, and radioligand binding assays. To restore CB1R function, HD mice received systemic administration of the CB1R agonist WIN-55,212-2 or localized hippocampal infusion of viral vectors encoding CB1R. A multidisciplinary approach—encompassing behavioral, biochemical, electrophysiological, and morphological analyses—was employed to unravel the molecular mechanisms underlying CB1R-mediated modulation of HD-related cognitive deficits.

Results. Both human HD tissue and mouse models exhibited a selective reduction of CB1R—but not CB2R—in the hippocampus, accompanied by synaptic alterations and deficits in spatial, recognition, and working memory. In R6/1 mice, hippocampal depolarization-induced suppression of inhibition (DSI) was markedly impaired. Systemic WIN-55,212-2 treatment successfully rescued these synaptic and cognitive deficits. Notably, immunohistochemical analyses revealed that CB1R downregulation was restricted to hippocampal GABAergic interneurons. Targeted re-expression of CB1R in these interneurons using viral vectors was sufficient to restore hippocampus-dependent memory performance in HD mice.

Conclusion. These findings identify hippocampal CB1R dysfunction in GABAergic interneurons as a key contributor to the cognitive deficits observed in Huntington's disease. Restoring CB1R signaling in this neuronal population offers a promising therapeutic avenue to mitigate memory impairment in HD.

Perspectives. Alterations in CB1 receptor expression, including astrocytic CB1, have been observed in the hippocampus of HD mouse models. Targeted genetic restoration



of CB1 specifically in this region leads to significant improvement in long-term memory, while short-term memory remains unaffected. These preliminary findings highlight the therapeutic potential of modulating astrocytic CB1 signaling in HD and underscore the importance of regional and functional specificity when developing cannabinoid-based interventions.

THE INHIBITION OF MONOACYLGLYCEROL LIPASE AFFORDS PROTECTION IN A MOUSE MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disease worldwide. This disorder is associated with neuroinflammation and motor dysfunction secondary to loss of dopaminergic neurons in the nigrostriatal axis. Although current therapy consists mainly of levodopa, its long-term use evokes secondary effects. Consequently, finding new therapeutic alternatives is necessary. Among the most promising options is the inhibition of monoacylglycerol lipase (MAGL) through the administration of JZL184, a compound promoting the accumulation of 2-arachidonoylglycerol (2-AG) in the CNS. In turn, 2-AG has been shown to exert anti-inflammatory and neuroprotective effects in the brain. The present study aimed to evaluate the protective effect of MAGL inhibition in a murine model of Parkinson's disease by assessing its impact on motor function maintenance and neuroinflammation induced by 1-metil-4-fenil-1,2,3,6-tetrahydropyridine (MPTP). The study was conducted in male C57BL6/J mice of twelve weeks-old (25-30 g) that received intraperitoneal (i.p.) injections of MPTP (30 mg/kg, one injection per day for five consecutive days). The MAGL inhibitor, JZL184 (20 mg/kg) was administered i.p. (1x day) for 6 days, starting 1 day prior to MPTP treatment. Control mice received vehicle only. Motor activity of the subjects was evaluated using several behavioral tests, and the concentration of the proinflammatory cytokines TNFα, IL1-β and IL-6 was quantified in substantia nigra and striatum using ELISA assay. Results show that treatment with JZL184 improved the MPTP-induced motor alterations, including bradykinesia, loss of balance, and deficient muscle strength. JZL184 also decreased TNFα, IL1-β and IL-6 expression in MPTP-treated mice compared to control mice. These preliminary findings suggest that the inhibition of MAGL constitutes a protective strategy in the murine model of Parkinson's disease that deserves more detailed characterization.

Keywords: Parkinson's disease; 2-Arachidonoyl glycerol; Monoacylglycerol lipase

THERAPEUTIC POTENTIAL OF CB2 RECEPTOR ACTIVATION OR SEROTONERGIC MODULATION IN A MOUSE MODEL OF DRAVET SYNDROME

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Dravet Syndrome (DS) is a rare and severe form of childhood epilepsy caused by mutations in the *SCN1A* gene, leading to abnormal neuronal hyperexcitability through Nav1.1 sodium channels, resulting in severe epileptic seizures and various associated comorbidities. This disease is refractory to current pharmacological treatments, highlighting the urgent need for the identification and validation of novel therapeutic targets. A few years ago, Epidiolex, an oral formulation of cannabidiol (CBD), and fenfluramine (FFA), which acts on the serotonergic system, were approved in both the United States and Europe for the reduction of epileptic seizures in patients with Dravet Syndrome (DS). The aim of this study is to evaluate the disease-modifying effects of compounds targeting the endocannabinoid and serotonergic systems. To this end, we employed RO-6866945 (RO-686), a CB2 receptor agonist, and FFA, which modulates the serotonergic system. Furthermore, we evaluated whether the combined administration of FFA and RO-686 enhanced the effects observed with each drug when administered individually.

Conditional knock-in mutant mice (Syn1-Cre/Scn1a$^{WT/A1783V}$) generated by Cre-loxP technology were used. Treatments were administered intraperitoneally every two days between PND10 and PND24, and motor reflexes and body weight were recorded on alternate days. Behavioural tests were conducted on PND23 and PND24 to evaluate motor deficits, cognitive impairment, and autistic traits. Finally, on PND25, the animals were euthanized to analyse glial reactivity using immunohistochemical techniques.

Treatment with RO-686, as well as with FFA, improved survival, the acquisition of motor reflexes, and the performance in locomotion and social interaction tests. Additionally, a reduction in astrogliosis (GFAP) in the hippocampus and prefrontal cortex was observed. However, the combined administration of both compounds did not provide additional benefits beyond those obtained with the individual administration of each drug.

In conclusion, both FFA and Ro686 exert beneficial effects on several neurological and behavioural outcomes in the DS model. Although their combined administration did not enhance the individual effects, these findings indicate that independent modulation of either endocannabinoid or serotonergic systems may represent a promising strategy to alleviate some DS-associated comorbidities. Moreover, they underscore the importance of further investigating the cellular and molecular mechanisms underlying these neuroprotective effects.

Keywords: Dravet, CB2, fenfluramine, epilepsy.

NEUROINFLAMMATION DISRUPTS ENDOCANNABINOID-MEDIATED CONTROL OF ASTROCYTIC LACTATE SUPPLY

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Astrocytes are primarily glycolytic cells that produce and release lactate to support neuronal energy demands during neurotransmission. This astrocyte-neuron lactate shuttle is driven by neuronal cues that stimulate aerobic glycolysis and/or lactate efflux in astrocytes, including K^+, NH_4^+, and endocannabinoids acting on CB_1 receptors (CB_1R). Current hypothesis postulate that deficits in astrocyte-neuron metabolic coupling encompass the pathogenic activation of these cells under neuroinflammation and may contribute to neurodegeneration and disease progression in multiple sclerosis (MS). However, the specific features of CB_1R-mediated astrocytic lactate supply in MS remains unclear.

Here, we combined biochemical and imaging approaches applied to *in vitro* and *in vivo* models to interrogate astrocyte lactate efflux with particular emphasis on endocannabinoid signaling during neuroinflammation. Seahorse analysis of cultured astrocytes activated with pro-inflammatory factors revealed increased basal glycolysis but reduced compensatory glycolysis. Astrocytes expressing the intracellular lactate reporter Laconic displayed reduced intracellular lactate levels together with blunted responses to the cannabinoid agonist WIN55,212-2 as well as to high extracellular K^+ and NH_4^+. Two-photon *ex vivo* imaging of the extracellular lactate biosensor eLACCO2.9 targeted to astrocytes in mouse somatosensory cortex slices showed positive responses to K^+, NH_4^+ and to pharmacogenetic activation of G_q-mediated signaling that were significantly reduced in the experimental autoimmune encephalomyelitis (EAE) model of MS. Notably, electron microscopy analysis showed reduced expression levels of CB_1R in cortical astrocytes from EAE mice.

Together, these observations identify endocannabinoids and CB_1R as key regulators of astrocytic lactate supply in the somatosensory cortex and highlight maladaptive astrocyte-neuron metabolic coupling during autoimmune inflammation.

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NEUROPROTECTIVE AND NEUROIMMUNOMODULATORY POTENTIAL OF MAGL INHIBITION IN A TDP-43-BASED ALS MURINE MODEL

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive motor neuron loss, muscle atrophy, weakness, paralysis, and ultimately respiratory failure. The absence of effective treatments has encouraged the exploration of the endocannabinoid system (ECS) as a therapeutic target, given its ability to modulate multiple pathogenic mechanisms in ALS, including excitotoxicity, neuroinflammation, oxidative stress, and protein aggregation. Elevated endocannabinoid tone has been described in ALS models, and its potentiation through inhibition of the degrading enzymes FAAH or MAGL has previously shown beneficial effects, including motor neuron preservation, delayed disease onset, and extended survival.

To further assess this therapeutic strategy in the context of ALS heterogeneity, we investigated the effects of enhancing 2-AG levels through MAGL inhibition in a TDP-43-driven ALS model (Prp-TDP-43^{A315T}). This mouse model ubiquitously overexpresses human TDP-43 carrying the A315T mutation and develops motor deficits, motor neuron loss, and glial reactivity. To this end, male Prp-TDP-43^{A315T} were treated daily by intraperitoneal injection from postnatal day (PND) 65 (presymptomatic stage) to PND95 (symptomatic stage) with either the reversible MAGL inhibitor RO7232432 (RO-432, 10 mg/kg) or the irreversible inhibitor RO7278391 (RO-391, 3 mg/kg).

MAGL inhibition did not significantly improve motor function at treatment endpoint, although RO-432 delayed clasping onset and rotarod deficits. Despite the limited behavioral effects, both inhibitors restored nerve conduction velocity and normalized compound muscle action potential responses during high-frequency stimulation, demonstrating an effect on motor neuron and peripheral nerve integrity. These functional improvements were accompanied by up to 80% motor neuron survival and reduced astroglial and microglial reactivity. Transcriptomic analyses revealed that MAGL inhibition attenuated the shift toward a maladaptive pro-inflammatory microglial phenotype, without inducing major adaptations in ECS components.

In conclusion, MAGL inhibition in the Prp-TDP-43^{A315T} model confers partial but meaningful neuroprotective and anti-inflammatory effects, improving motor nerve conduction and delaying motor phenotype onset. Therefore, these findings reinforce the therapeutic promise of MAGL inhibition as a potential strategy for ALS.

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Keywords: ALS, MAGL, neuroprotection

MULTIMODAL IMMUNOREGULATORY MECHANISMS OF CANNABIDIOL IN A MITOCHONDRIAL EPILEPSY MODEL

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Inherited defects in mitochondria-related genes cause mitochondrial disease (MD) that often involves treatment resistant epilepsy (mitochondrial epilepsy, ME). Mice with conditional knockout of the respiratory chain protein NDUFS4 in *Gad2*-positive GABAergic neurons (GABA-cKO) effectively model ME, with a severe epileptic phenotype correlated to progressive neuroinflammation in the external globus pallidus (GPe). We have previously shown that cannabidiol (CBD) extends lifespan, fully prevents fatal spontaneous seizures and attenuates gliosis in the GPe of GABA-cKO animals. However, the molecular determinants of CBDs anti-inflammatory action in this brain region remain incompletely understood. Therefore, this study aims to elucidate gene expression and lipidome alterations in the GPe of ME model mice after chronic CBD treatment.

RNA Sequencing of GABA-cKO GPe revealed >200 differentially expressed genes across the relevant genotype-treatment comparisons. Genes related to the innate immune response were markedly upregulated in cKO GPe and partially normalized by CBD, that also implicated lipid signaling-related gene ontology. Protein-level analyses confirmed that CBD attenuates the expression and/or activation levels of multiple, elevated inflammatory signaling proteins in GABA-cKO GPe. A pilot, LC-MS lipid profiling study revealed oxylipin, phospholipid and endocannabinoid species as discriminatory between the experimental groups, with CBD attenuating the cKO-induced lipidome shifts.

Our study provides the first molecular characterization of CBD’s anti-inflammatory mechanisms in a brain region critically affected by ME. These findings support a model in which CBD alters broad protein and lipid immunoregulatory signalling cascades, offering novel insights into both disease mechanisms and therapeutic strategies for seizure-related mitochondrial disorders.

Keywords: Cannabidiol, Mitochondrial Disease, Epilepsy, Neuroinflammation

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CANNABINOID TYPE-1 RECEPTOR GENETIC HEMIDELETION REMODELS SYNAPTIC TRANSCRIPTOME IN FRAGILE X SYNDROME MOUSE MODEL.

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Fragile X syndrome (FXS) is the most common monogenic cause of inherited intellectual disability and autism produced by the genetic silencing of the fragile X messenger ribonucleoprotein 1 (*FMR1*) gene and the subsequent loss of its related protein, FMRP. The *Fmr1* knockout (KO) (FX) mouse model of FXS shows cognitive impairment and synaptic alterations; phenotypes that were significantly prevented by both genetic and pharmacological inhibition of the cannabinoid type-1 receptor (CB1R). Specifically, we previously demonstrated that the genetic hemideletion of CB1R (*Cnr1* HZ) in FX mice (RES) rescues behavioral deficits in FX; however, the molecular implications of CB1R hemideletion have not been addressed. In this study, we assessed whether CB1R hemideletion modulates hippocampal synaptoneurosomes composition in FX mice. To this end, we first analyzed the main components of the endocannabinoid system (ECS) in FX and RES mice. We detected subtle alterations in ECS components in FX mice that were absent in RES animals. Then, we performed an unbiased high-throughput analysis of hippocampal synaptoneurosomal mRNA and analyze gene expression expanding our functional analysis of differentially expressed genes through the study of protein interaction databases. Our findings revealed that synaptic transcriptome is modestly modified at the gene expression level in FX mice compared to wild-type (WT) mice, while RES mice showed relevant modifications in gene expression compared to FX mice. Particularly, we found the upregulation of diacylglycerol kinase eta and the downregulation of some genes related with RNA metabolism and mitochondrial bioenergetics in RES mice. Finally, we also analyzed the synaptic transcriptome of genetic hemideletion of CB1R (HZ) mice that were WT for the *Fmr1* gene) and we found the upregulation of genes related with synapse organization and channel activity, and downregulation of genes associated to translation and RNA regulation. Interestingly, when comparing the effect of CB1R hemideletion in both FX mice and WT mice for *Frm1*, we observed that more than half of the differentially modulated genes in both comparisons were commonly upregulated and that these genes encoded proteins mainly related with RNA translation, ATP synthesis and the proteasome. Overall, we reveal a profound effect of CB1R hemideletion in hippocampal synaptoneurosomes independent of FMRP expression.

Keywords: cannabinoid type-1 receptor, synaptoneurosomes, fragile X syndrome

CNR1 RS806371 POLYMORPHISM, COGNITIVE FUNCTION, EMPATHY AND CORTISOL LEVELS IN OLDER ADULTS

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The endocannabinoid system contributes to the regulation of stress responses and cognitive processes, both of which are vulnerable to age-related decline. Genetic variations in the CNR1 gene, which encodes the cannabinoid receptor type 1, may influence these mechanisms through modulation of neural and neuroendocrine pathways. We examined the CNR1 promoter polymorphism rs806371 (T/G) in a sample of 189 adults aged over 60 years. Participants completed a comprehensive neuropsychological assessment covering executive function, memory, attention, and processing speed. Salivary cortisol was collected at 6 time points to compute three indices of diurnal secretion: the cortisol awakening response (AUCiCAR), the total cortisol output across the day (AUCtotal), and the diurnal cortisol slope.

Carriers of the G allele exhibited poorer executive and memory performance compared with TT homozygotes, as well as higher empathy scores on the Pictorial Empathy Test (PET). They also showed lower cortisol levels across two indices (AUCiCAR and AUCtotal), suggesting a blunted hypothalamic–pituitary–adrenal (HPA) axis response. These findings indicate that the CNR1 rs806371 polymorphism may contribute to individual differences in cognitive and socioemotional aging and neuroendocrine regulation. Overall, the results support the involvement of the endocannabinoid system as a biological pathway linking genetic susceptibility, stress regulation, empathy, and cognitive function in older adults.

Keywords: endocannabinoid system, cortisol, executive function, empathy

EXPLORING COGNITIVE ALLEVIATION THROUGH THE MODULATION OF THE ENDOCANNABINOID SYSTEM DURING PHYSIOLOGICAL AGING IN MICE

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The global increase in life expectancy necessitates focusing on ensuring aging occurs under optimal health conditions. A critical aspect of health, particularly vulnerable to the aging process, is cognitive function. Age-related cognitive decline is a significant concern, detrimental to quality of life and independent living. These deficits tend to emerge subtly in early adulthood and progress with age. Despite this general trend, significant interindividual differences exist due to factors like cognitive reserve and resilience mechanisms. Sex differences also influence the manifestation of cognitive aging. Studies have reported steeper declines in general cognitive functions in males compared to females, and transcriptomic analyses of the hippocampus in aging mice reveal more extensive gene expression changes in females. Research shows that cognitive decline is closely associated with synaptic changes, such as synaptic loss or altered plasticity. These synaptic alterations directly affect learning and memory, with the hippocampus, a region with a central role in memory formation, being particularly susceptible. The endocannabinoid system (ECS) is an endogenous neuromodulatory system highly expressed in the central nervous system and peripheral tissues and plays a relevant role in modulating learning and memory processes. Previous research by our group has shown that inhibiting CB1R with the neutral antagonist AM6545, improves memory and synaptic plasticity in young adult mice, as well as in models of intellectual disability. In our laboratory, we have established a one-month behavioral testing pipeline which studies domains affected by aging including long-term and spatial memory, anxiety and locomotion. To integrate the results across the various behavioral tests we developed an aging score. Comparison of young adult (3 months), middle-aged (10 months) and old (18 months) male and female mice revealed an age-associated cognitive decline from middle age onward, with a steeper decline in males than in females. Transcriptomics analyses of hippocampal synaptoneurosomes indicated an enrichment of terms related to synapse modification and the immune system during aging. Using this framework, we investigated the potential of chronic peripheral CB1R inhibition with AM6545 in mitigating cognitive decline in male and female aged mice. This approach will provide mechanistic insights into how peripheral CB1R blockade may influence hippocampal synaptic transcripts and cognition during aging.

Keywords: aging, CB1 inhibition, cognition

CANNABINOIDS ADMINISTRATION ENHANCES THE ANTICANCER ACTIVITY OF BEVACIZUMAB IN PRECLINICAL MODELS OF GLIOMA

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1. Introduction and objectives: Glioblastoma (GB) is one of the most aggressive forms of cancer. This situation is due, at least in part, to the presence within these tumors of a population of cells with stem-like characteristics, termed glioma-initiating cells (GICs), which may be responsible for the relapses in GB patients. In addition, GB are highly angiogenic and vascularized tumors. Different anti-angiogenic therapies, including Bevacizumab, have been used in GB patients although this treatment does significantly modify the survival of GB patients. Previous observations by our group have shown that Δ9-Tetrahydrocannabinol (THC) and cannabidiol (CBD) have anticancer activity in GB models due to their ability to target the GIC population through autophagy-mediated apoptosis and to inhibit tumor angiogenesis.

Thus, the main objective is to investigate the effect of the combined administration of BVZ and cannabinoids in preclinical models of GB, specifically targeting the population of GICs.

2. Material and Methods: In this study we explored the combinational effect of a cannabinoids and BVZ targeting GICs *in vitro* and *in vivo* in GICs-derived xenografts.

3. Results: Our results show that the combined administration of cannabinoids and BVZ: i) decreased the proliferation and self-renewal capacity of GICs; (ii) enhanced autophagy-associated GIC death; (iii) interfered with the vessel-forming capacity of endothelial cells; (iii) presented an antiangiogenic and an antitumoral effect in GICs-derived xenografts.

4. Conclusion: Our findings support the idea that the combined administration of cannabinoids and BVZ could be explored as a potential therapeutic strategy for the management of GB.

CANNABINOID ADMINISTRATION AS A THERAPEUTIC STRATEGY TO IMPROVE GLIOBLASTOMA RESPONSE TO RADIOTHERAPY.

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Introduction

Glioblastoma (GBM) is the most frequent primary brain tumor and one of the most aggressive cancer types. The standard treatment for patients consists in the administration of conventional radiotherapy (RT) and temozolomide (TMZ). However, these tumors display high resistance to radio-chemotherapy and undergo recurrence shortly after surgical resection. These features are due, at least in part, to the presence within the tumor mass of a population of cells with stem-like features termed Glioma Initiating Cells (GICs).

Cannabinoids, the main active components of marijuana, are being investigated as potential therapeutic agents. Specifically, previous observations from our group showed that the combination of THC (or of THC and cannabidiol, CBD another major ingredient of the hemp plant) together with TMZ targets the population of GICs *in vitro* and strongly reduces the growth of GBM xenografts *in vivo*. Strikingly, in addition to these anticancer actions, THC and CBD have also demonstrated to protect non transformed cell populations of the central nervous system (and specifically, astrocytes) from different cytotoxic insults.

In this work we aimed at analyzing the effect of the combined administration of cannabinoids (THC:CBD 1:1) and conventional Radiotherapy on the population of GICs as well as in astrocytes.

Methods

In this study, we used primary cultures of GICs and astrocytes, that were treated with THC:CBD at 1:1 ratio and/or irradiated with conventional radiotherapy in GammaCell Cs-137 irradiator. Assays with GICs were also performed *in vivo*, carrying out chicken embryo chorioallantoic membrane assays (CAM) and orthotopic xenografts in nude mice.

Results

Our data show that administration of THC:CBD (1:1): enhances the inhibitory effect of RT on: (i) the proliferation of GICs *in vitro*; and (ii) the capacity of GICs to generate tumors *in ovo* and in murine orthotopic xenografts. In addition, we found that cannabinoids protected primary cultures of astrocytes from radiotherapy-induced cytotoxicity. Moreover, THC:CBD (1:1) administration abolished the capacity of irradiated astrocytes conditioned medium to enhance radio-resistance of GICs.

Main conclusions

These observations suggest that THC and CBD enhance GICs sensitivity to conventional radiotherapy and reduce radiotherapy-induced damage in non-transformed glial cells.

Keywords: Glioblastoma, THC:CBD 1:1 ratio, radiotherapy.

THERAPEUTIC POTENTIAL OF HER2-CB_2R HETEROMERS IN HER2 POSITIVE BREAST CANCER

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Breast cancer is the most frequent malignancy diagnosed in women. HER2+ breast cancer subtype is characterized by the overexpression of the human epidermal growth factor family member HER2. Usually treated with the humanized monoclonal antibody trastuzumab, and despite its efficacy, treatment still presents a challenge due to innate and acquired resistance to therapy. In the past, our group demonstrated that cannabinoid receptor CB_2R associates with HER2 forming heteromers which promote the oncogenic activity of the latter. Here we report an association between decreased HER2-CB_2R heteromer expression after neoadjuvant treatment and lower disease-free survival, meaning resistance to treatment. Experiments conducted in various cell lines showed a decrease in CB_2R to be involved in trastuzumab resistance. A loss of the cannabinoid receptor prompts a signaling switch which drives HER2 to form alternative heteromers with other members of the HER family, specifically EGFR. Blocking EGFR signaling with erlotinib, a selective tyrosine kinase inhibitor, decreases cell viability in trastuzumab resistant cells and restores sensitivity to trastuzumab both *in vitro* and *in vivo.* These results highlight the pivotal role of CB_2R in trastuzumab resistance and establish CB_2R-HER2 and EGFR as a biomarker with predictive value. Moreover, our findings reveal the potential of targeting EGFR and HER2 simultaneously as a promising treatment strategy to overcome trastuzumab resistance mediated by CB2R loss.

Keywords: breast cancer, trastuzumab resistance, receptor heteromers

THE INTERPLAY BETWEEN CB_2 AND NMDA RECEPTORS IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is a progressive neurological disorder that affects movement, causing symptoms such as tremors, stiffness, slowness, and balance problems due to the degeneration of dopamine-producing neurons in the brain. Nowadays there is no cure for PD. Alpha synuclein (α-syn) aggregates, which are a hallmark of PD, are known to induce microglial activation, specifically the detrimental M1 microglial phenotype, which contributes to neuroinflammation and disease progression. Cannabinoid receptor 2 (CB_2R) activation has been shown to counteract neuroinflammation. CB_2R is able to interact with N-methyl-D-aspartate (NMDA) receptors (NMDAR), which has also attracted attention in PD research due to its role in excitotoxicity. Here we aimed to study the interaction between CB_2R and NMDAR in a PD context. We observed that α-syn fibrils alter CB_2R activation and CB_2R-NMDAR heteromerization in a heterologous expression system. Furthermore, activation of CB_2R counteracted NMDAR signaling. In rat microglia α-syn fibrils decreased CB_2R-NMDAR heteromer expression, while increasing CB_2R signaling. Importantly, CB_2R activation counteracted the α-syn fibrils-induced increase in M1 activated microglia, while it favored the polarization of microglia to the beneficial M2 phenotype. These results reinforce the idea of using cannabinoids for treating PD, as they provide not only the anti-inflammatory effects of cannabinoids but also counteract the detrimental increase in NMDAR signaling present in this disease.

Keywords: Cannabinoid receptor 2, NMDA receptor, Parkinson's disease.

CANNABINOID CB_1 RECEPTOR ACTIVATION MITIGATES N- METHYL-D-ASPARTATE RECEPTOR (NMDA) -MEDIATED NEUROTOXICITY

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Alzheimer's disease (AD) is characterized by synaptic dysfunction and excitotoxicity, yet effective therapeutic strategies remain limited. This study explores the functional and physical interplay between cannabinoid CB1 receptors (CB1Rs) and N-Methyl-D-Aspartate receptors (NMDARs), which are implicated in AD pathology.

Using bioluminescence resonance energy transfer and imaging assays in HEK-293T cells, we demonstrate a direct interaction between CB1R and the N1 subunit of NMDAR, supporting the formation of receptor complexes. Functional assays further reveal a bidirectional negative crosstalk: NMDAR activation attenuates CB1R-mediated cAMP inhibition, while CB1R activation reduces NMDA-induced calcium influx and mitogen activated protein kinase signaling pathway activation. This negative crostalk suggests the existence of receptor–receptor interactions with functional consequences.

Complexes of CB1Rs and N1 subunits of NMDARs are present in both neurons and microglia, and their expression is upregulated in response to Aβ1-42 and in cells derived from the APPSw/Ind AD model mice. However, upregulation did not always correlate with stronger CB1R-NMDAR cross-modulation, suggesting that cell-specific signalosome composition shapes the signaling outcome.

Functionally, CB1R activation confers neuroprotection: it rescues neurite loss induced by NMDA and Aβ1-42, highlighting the therapeutic potential of modulating CB1R–NMDAR interactions. These findings support a model in which CB1R–NMDAR interactions, through dynamic functional cross-modulation, finely tune excitotoxic and inflammatory signaling pathways. This mechanism offers therapeutic prospects for addressing cannabinoid-glutamatergic interactions.

Keywords: NMDA receptor; Receptor–receptor interaction, Excitotoxicity, Functional selectivity, Neuroprotection, Alzheimer's disease, Signalosome

NEUROGLIAL CB1 RECEPTORS CONTROL NAVIGATION STRATEGIES

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Navigation and memory functions are essential for survival and are regulated by the hippocampus. One of the key modulators of hippocampal activity is the endocannabinoid system through the cannabinoid receptor type-1 (CB1). CB1 is widely expressed in various types of hippocampal cells. While it is known that CB1 participates in memory processes, its specific roles in different cell types and how these roles may differ between sexes remain unclear. This study investigates the cell- and sex-specific modulation of navigation and memory by CB1 receptors. To this end, we selectively deleted CB1 receptors from neurons or astrocytes from the hippocampus of adult male and female mice. We then assessed its effect on a comprehensive range of behaviors, including innate emotional responses, memory, navigation, and nesting.

Specific deletion of CB1 in CAMKII-expressing neurons produced a pronounced effect in males, leading to increased anxiety and impairments in both reference and spatial memory. These mice also showed altered performance in the Barnes maze, relying less on spatial strategies. By contrast, females were less affected by this specific deletion. Interestingly, deletion of CB1 from astrocytes led to spatial memory impairments in both males and females, which also showed reduced LTP and a decreased reliance on Spatial strategies in the Barnes maze. In conclusion, our findings show that neuronal CB1 receptors are critical for the spatial navigation strategy in males, while astrocytic CB1 receptors play a key role in memory processes both in males and females.

CANNABIDIOL AS A MULTIFACETED THERAPEUTIC AGENT: MITIGATING ALZHEIMER'S DISEASE PATHOLOGY AND ENHANCING COGNITIVE FUNCTION

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Cannabidiol (CBD), the second most abundant phytocannabinoid in *Cannabis sativa*, has garnered significant interest due to its non-psychoactive nature and diverse receptor interactions. This study employs in vitro and in vivo methodologies to validate CBD's potential as a treatment for Alzheimer's disease (AD) by addressing key hallmarks of the condition and promoting neuroprotective effects on spatial memory. The study reveals CBD's capacity to partially revert neurite formation loss induced by Aβ, Tau, and pTau proteins, suggesting a potential role in promoting neuronal plasticity as well as an increased neuronal viability in the presence of AD-associated protein aggregates. Our findings also demonstrate CBD's ability to decrease pTau and Aβ axonal transport between cortical and hippocampal neurons. Notably, daily CBD injections (10 mg/Kg) for 28 days in 5xFAD mice resulted in significant improvements in both short- and long-term spatial memory and better motility in *C. elegans* CL2006 strain. These multifaceted effects of CBD, ranging from molecular-level modulation to behavioral improvements, underscore its potential as a comprehensive therapeutic approach for AD. The findings not only support CBD's neuroprotective properties but also highlight its ability to target multiple pathological processes simultaneously, offering a promising avenue for future AD treatment strategies.

Keywords: CBD, Alzheimer’s disease, β-amyloid

METABOLISM, PHARMACOKINETICS, AND BIOAVAILABILITY OF CANNABIGEROL IN HORSES FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION WITH MICELLAR AND OIL FORMULATIONS.

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Introduction: Cannabigerol (CBG) is a non-psychoactive cannabinoid with growing interest in veterinary medicine, however its pharmacokinetics profile in horses remains unknown. Understanding its absorption, distribution, metabolism, and elimination is essential to optimize dosing strategies and evaluate its potential for clinical use in equine patients.

Methods: A prospective crossover study was conducted in eight healthy adult horses to assess the *in vivo* metabolism, the pharmacokinetics after intravenous (IV) administration at 1 mg/kg and oral administrations at 10 mg/kg with two formulations (micellar and oil). Plasma concentrations of CBG and its main metabolite, CBG-glucuronide (CBG-G), were analyzed by LC-MSMS and modelled using a non-linear mixed effects model with MonolixSuite®. The model estimated bioavailability, metabolic conversion, and absorption parameters. Additionally, Monte Carlo simulations were performed to predict and evaluate the drug exposure after multiple doses regimen.

Results: High *in vivo* metabolism was observed with the formation of epoxy and hydroxy metabolites via phase I reactions, and CBG-G as the main metabolite from phase II reactions (75% of biotransformation). After IV administration, CBG showed a high volume of distribution (V_{ss} = 74 L/kg) and systemic clearance (Cl = 1.67 L/h/kg), with a terminal half-life of approximately 29 hours. The oral bioavailability was estimated at 28% between formulations, and an extensive presystemic metabolism was obtained with metabolite/parent AUC ratios exceeding 50. The micellar formulation showed a shorter time to achieve maximum concentration (T_{max}) and faster absorption compared to the oil formulation. The Monte Carlo simulations of multiple oral doses (10 mg/kg q24 hours for 14 days) predicted differences between formulations. No adverse clinical effects were observed during the study.

Discussion: This study shows the first evaluation of the *in vivo* metabolism and pharmacokinetics of CBG in horses after IV and oral administratione. The findings highlight extensive metabolite formation with a significant glucuronidation, a large distribution volume and high clearance. While both oral formulations produced similar systemic exposure, the faster absorption with the micellar formulation may inform clinical decisions depending on therapeutic goals. These data support the potential use of CBG in horses and offer a foundation for further studies in equine medicine.

Keywords: metabolism, pharmacokinetics, bioavailability, cannabigerol, horses, micellar, oil

HETEROMERIZATION OF OLFACTORY AND CANNABINOID RECEPTORS: IMPLICATIONS FOR NEUROPROTECTIVE GPCR SIGNALING

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Alzheimer's disease is an emerging health problem worldwide, with olfactory dysfunction, specifically anosmia (loss of smell), being one of its earliest and most significant symptoms. This highlights the critical role of olfactory receptors (ORs), which represent 400 out of the ~800 total GPCRs, in neurobiology. G protein-coupled receptors (GPCRs) constitute the largest family of therapeutic targets in neuroscience, with cannabinoid receptors emerging as particularly promising in the context of neuroinflammation and neurodegenerative diseases. Among these, the cannabinoid receptor type 2 (CB_2R) is of special interest due to its neuroprotective role. We hypothesized that interactions could exist between CB_2R and ORs. Here, we present preliminary evidence suggesting novel GPCR interactions relevant to cannabinoid biology and olfactory receptors. Using Bioluminescence Resonance Energy Transfer (BRET) in HEK-293T cells, we observed signals consistent with heteromer formation between CB_2R and two specific olfactory receptors, OR51E2 and OR2V1.

Another GPCR of major therapeutic relevance is the adenosine A_{2A} receptor ($A_{2A}R$), widely studied for its implication in neuroprotection, including the involvement of $A_{2A}R$-D_2R heteromers targeted by the $A_{2A}R$ antagonist, istradefylline, for Parkinson's disease treatment. We further hypothesized that $A_{2A}R$ could form heteromeric complexes with ORs. Indeed, BRET assays demonstrated heteromerization between $A_{2A}R$ and OR51E2. While heteromerization is increasingly recognized as a mechanism modulating GPCR function, the potential influence of ORs on cannabinoid and adenosine signaling remains largely unexplored. Importantly, functional assays revealed that the OR51E2–$A_{2A}R$ heteromerization reduced $A_{2A}R$ signaling, whereas the CB_2R–$A_{2A}R$ interaction did not alter the canonical $A_{2A}R$ response to its selective agonist.

These findings highlight a previously unrecognized layer of complexity in cannabinoid receptor biology, positioning olfactory GPCRs as potential modulators of other therapeutically relevant GPCRs. This work opens new perspectives at the intersection of the endocannabinoid system, GPCR heteromerization, and neurodegeneration.

Keywords: CB_2 receptor, GPCR heteromerization, Olfactory receptors.

DIFFERENTIATION-STATE DEPENDENT METABOLIC PROFILES OF OLIGODENDROCYTES: MODULATION BY CANNABINOID CB_1 RECEPTORS

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Oligodendrocyte precursor cells (OPCs) respond to neuronal signals and give rise to myelinating oligodendrocytes (OLs) during development and adulthood. In turn, mature OLs provide lactate and/or pyruvate that support the axonal compartment in an activity-dependent manner. OLs and OPCs are involved in a growing number of neurological disorders and current evidence suggests that deficits in OL bioenergetic supply leads to neuroaxonal pathology. Nonetheless, the specific features of OL-axonal signaling during OL lineage progression remain poorly understood. Endocannabinoids modulate brain energy metabolism and regulate a myriad of brain functions through the activation of cannabinoid CB_1 receptors (CB_1R). OL populations express CB_1R that promote lineage progression but the metabolic implications of endocannabinoid-to-OL signaling remain uncertain. Here, we compared the bioenergetic profiles of OPCs and OLs differentiated *in vitro* and investigated the role of CB_1R in modulating mitochondrial respiration *in vitro* and *ex vivo*.

Extracellular flux analysis of primary oligodendrocytes *in vitro* showed reduced basal OCR/ECAR ratios and mitochondrial ATP production in OLs *versus* OPCs. OLs displayed higher ability to release monocarboxylates than OPCs in basal conditions as determined by the analysis of extracellular lactate content. Time-lapse imaging with the intracellular lactate sensor Laconic unveiled fluorescence decreases in response to extracellular K^+ in OLs but not in OPCs, thus suggesting a differentiation-stage dependent lactate export upon membrane depolarization in mature cells. The CB_1R agonist ACEA inhibited mitochondrial respiration in OL cultures and this effect was prevented by the cell permeable selective antagonist AM251, but not by the membrane impermeable peptide hemopressin, pointing to the involvement of intracellular CB_1R pools. Consistently, acute exposure to ACEA inhibited oxygen consumption in $O4^+$ cells from CB_1-WT and this effect was absent in cells isolated from CB_1-KO mice. Furthermore, ACEA inhibited respiration in purified oligodendrocyte mitochondria from newly generated *Plp*-MITO-Tag mice. These results suggest that maturation modulates the bioenergetic profile of oligodendrocyte lineage cells and fine-tunes their ability to release lactate in response to neuronal activity, and highlight modulatory roles of CB_1R in oligodendrocyte bioenergetics that may be relevant to high metabolic demands during myelin formation, maintenance and repair.

Keywords: oligodendrocytes, oligodendrocyte precursors, mitochondria respiration, lactate

OX_1R ACTIVATION NEGATIVELY REGULATES CB_1R EFFECTS IN ALZHEIMER'S DISEASE

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The cannabinoid type 1 receptor (CB_1R) is the most abundant membrane receptor in the central nervous system (CNS). Recent studies have shown that CB_1R exerts neuroprotective effects in Alzheimer's disease. Alzheimer's is characterized by the formation of neurofibrillary tangles of hyperphosphorylated tau protein and beta-amyloid plaques, which lead to an inflammatory process and ultimately neuronal death. Recently, it has been described that this neurodegenerative disease is accompanied by sleep disturbances, and these alterations further aggravate disease progression. Orexin A is a hormone that acts as a neuropeptide in the CNS and actively participates in the regulation of the sleep–wake cycle. This hormone binds to orexin receptors OX_1 and OX_2. Specifically, the OX_1 receptor has been linked to increased accumulation of protein aggregates. Thus, the main objective of our study was to analyze the role of cannabinoid CB_1 and orexin OX_1 receptors in Alzheimer's disease.

In this article, we demonstrated a negative regulation between CB_1 and OX_1 receptors in primary cortical and hippocampal neuronal cultures, both control and treated with $Aß_{1-42}$ aggregates, in the cAMP pathway and MAPK phosphorylation. Similar results were observed in brain slices from adult 5xFAD mice, a model of Alzheimer's disease. To explain this regulation, it has been described that both receptors are capable of interacting by forming heteromeric complexes, both in co-transfected HEK-293T cells and in primary cultures of microglia and neurons.

CB_1R is mainly responsible for the psychoactive effects caused by cannabinoid compounds. Currently, the CB_1 receptor is considered a potential key element to slow down the progression of Alzheimer's disease due to its neuroprotective effects. The results presented in this study indicate that it is necessary to take into account the negative regulation exerted by the OX_1R receptor on CB_1R in order to better understand the role of the cannabinoid receptor in Alzheimer's disease and to attempt to discover new compounds that are more effective and with fewer side effects.

THE CANNABIS RESEARCH COMMUNITY AND PALESTINE GENOCIDE

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Let's talk frankly and without harshness about some ethics on cannabinoid research. The discovery of THC and endocannabinoids are one on the numerous examples of the seminal contributions to *Cannabis* research by Israel scientists (Mechoulam, 2016). Israel has developed an active program of clinical trials, a *Cannabis* industry ecosystem with efficient plant growing systems, and modern methods for the isolation and formulations of active compounds. The ongoing genocide of innocent Palestinians forces us to position ourselves in this horrific scenario. Israel is considered for the European Union as a privileged partner, in fact makes part of most academic and scientific European societies and receives funding by EU programs. Is it acceptable to contribute with normality with Israel partners within our research, development and academic activities while human rights are destroyed and the education, health systems are eradicated, not talking about the use of famine as war weapon? This is not a religious issue, nor a pro-terrorist justification. As academics or professionals our activities, what we say and don't say, what we write and how we interpretate and "sell" our findings are also intermingled with many political factors and beliefs. Of course, Israel is only one example, and many other cases shall be faced and considered. Indigenous lands in Africa or South-central America appropriated by international big companies, or by the national oligarchies, responsible of extractive colonization, cannabis producers that use slavery labor force, racial discrimination, stigma of pregnant woman … (Hart, 2020; Howe et al., 2025; Prakash et al., 2023). The list is large, but the intimate link between the cannabis research and industry with Israel, considered as European country, urges the discussion. This poster contribution aims to trigger the debate into consideration in all the sectors involved in cannabis products: recreational users, medical cannabinoid-benefited patients, the industry, academicians and clinical scientists. Academic boycott has demonstrated its efficacy previously to refrain crimes against humanity, and it must be clarified once again that boycott is focused on institutions, not individuals.

Keywords: genocide, research ethics, academic boycott

SEX-DEPENDENT MODULATION OF EMOTIONAL AND COGNITIVE PROCESSES BY PREFRONTAL CB1 RECEPTORS

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The prefrontal cortex (PFC) integrates emotional and cognitive functions. CB1 receptors are widely expressed in this region, principally on GABAergic interneurons, and participate in the Excitation-Inhibition (E-I) balance. E-I alterations are described in psychiatric disorders, including depression, anxiety, and schizophrenia, where a reduction of CB1 has also been described. Both the endocannabinoid system and mood disorders display important sex differences.

In this work, we aim to **elucidate the role of prefrontal CB1 receptors in the development of behavioral disorders in a sex-dependent way**. For this, we deleted CB1 from 1) all PFC neurons and 2) the GABAergic population of adult CB1-flox male and female mice. One month later, mice were subjected to different behavioral tests to evaluate their emotional and cognitive performance. The GABAergic neuronal activity was studied via calcium imaging by fiber photometry. Parvalbumin neurons were also modulated using an optogenetic approach in a cohort of animals.

GABAergic CB1 deletion in the PFC induced an anxiety-like effect in both sexes but was much more pronounced in females, with no impact on memory. This effect correlated with lower activation of GABAergic neurons. Fear conditioning test revealed an impairment in the learned-aversive responses in males and females.

Immunofluorescence studies showed that global PFC CB1 deletion induced GABAergic-parvalbumin interneurons modulation with a sex-dimorphic pattern. Interestingly, the optogenetic modulation of these interneurons produced effects resembling those observed following CB1 deletion in PFC GABAergic neurons.

To summarize, PFC-GABAergic CB1 deletion induces an anxiogenic state and an exacerbated response to conditioned cues in a sex-dependent manner, potentially mediated by parvalbumin neuron activity. Thus, maladaptive prefrontal CB1 signaling could participate in the development of behavioral disorders.

THE THERAPEUTIC EFFECT OF THE SYNTHETIC CANNABINOID URB447 IN INFECTION-SENSITIZED NEONATAL RATS MAY BE MEDIATED BY A DECREASE IN NEUROINFLAMMATION

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Background: Perinatal asphyxia in newborns can cause severe brain damage, which is often exacerbated by systemic inflammation. Although hypothermia is standard treatment, it can be detrimental in inflammation-sensitized babies. As cannabinoids provide neuroprotection by attenuating neuroinflammation, we aimed to evaluate whether URB447, a CB1 antagonist and CB2 agonist, modulates the inflammatory response and limits brain injury after hypoxia-ischemia (HI) in infection-sensitized neonatal rats.

Methods: Fifty-eight 7-day-old neonatal rats were exposed to 0.1 mg/kg lipopolysaccharide (LPS) injection 4h prior to unilateral HI (50 minutes). Pups were randomly assigned to LPS+HI (n=19) or LPS+HI+URB447 (n=25, a single URB447 i.p. dose of 1 mg/kg) groups. 7 days after HI, brains were collected and sliced to evaluate brain infarct, neuropathological score and severity of damage. In another subset of pups sacrificed at 24h after HI, we analyzed the inflammatory response in the hippocampus, cortex and striatum by using a Proteome Profiler antibody array against 29 markers. HI-only (n=7) and Sham (n=7) animals served as controls. A $p<0.05$ was considered statistically significant.

Results: Using a moderate (50 min of HI) infection-sensitized model of neonatal brain injury, URB447 reduced total, hippocampal and cortical neuropathological score, together with decreased brain infarct ($p<0.001$ vs LPS+HI). Further, the treatment was especially effective with severe forms of damage, being able to reduce them mostly (from 65% to 10%, $p<0.01$ vs LPS+HI). This effect was preceded by a strong modulation in the inflammatory response: URB447 significantly ameliorated the proteomic changes in key inflammatory mediators in the hippocampus (it reduced CNTF, fractalkine, ICAM1, thymus and TIMP-1) and cortex (decreasing CNTF, thymus and TIMP-1).

Conclusions: Systemic inflammation-exacerbating neonatal brain injury was reduced by URB447 administration in neonatal rats subjected to HI by reducing the expression of key proteins involved in inflammatory pathways, decreasing infarct size, and improving neuropathological outcomes. These effects support the potential of CB1/CB2-targeted cannabinoids as anti-inflammatory neuroprotective agents in perinatal brain injury.

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Keywords: Neonatal brain, hypoxia-ischemia, neuroprotection, neuroinflammation

DEVELOPMENT OF GRAB SENSORS FOR UNRAVELING THE MOLECULAR DYNAMICS OF THE GLIAL PURINERGIC SYSTEM AND THE ECS IN FAAH KNOCKOUT CONTEXT

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The endocannabinoid system (ECS), comprising receptors, ligands, and enzymes, acts as an important neuromodulator in the central nervous system, since it is implicated in neuroprotection and regulation of key physiological processes such as cognition, memory, and inflammation. Consequently, the ECS has been considered as a promising target for pharmacological intervention in neurodegenerative pathologies. In the case of Alzheimer's Disease (AD), enhancing the endogenous endocannabinoid tone has been proposed as a possible therapeutic alternative. Specifically, the enzyme fatty acid amide hydrolase (FAAH), responsible for AEA degradation, is of particular interest. Studies in AD mouse models suggest that the genetic inactivation of FAAH elevates endogenous AEA and paradoxically modifies the neuroinflammatory profile, leading to significant beneficial outcomes, including the prevention of memory decline, enhanced synaptic plasticity, and a reduction in beta-amyloid deposition. The mechanisms underlying these results suggest that the role of glial cells, such as astrocytes and microglia, is critical in achieving these beneficial effects, but they need to be further characterized.

To directly evaluate the molecular mechanisms involved in this neuroprotective phenotype, we applied genetically encoded fluorescent sensors, such as G protein-coupled receptor activation-based (GRAB) sensors, to study the role of FAAH in a mouse model of AD. These biosensors can be used to track, in real-time, the molecular dynamics of the two main endocannabinoids (ECBs), AEA and 2-AG, and ATP, which signaling pathways are known to be tightly linked to glial cell activation and function. In this study, we have developed and optimized several viral vectors for the expression of GRAB sensors targeting both ECBs and ATP. We have evaluated their functionality *in vitro* in the GL261 glioma cell line as a controlled environment model, and, critically, primary astrocyte cultures derived from both wild type (WT) and FAAH knockout ($FAAH^{-/-}$) mice. Results from these *in vitro* studies in GL261 cells and primary astrocytes will serve as a crucial validation step for future *in vivo* experiments involving viral injection into live mice to track real-time molecular dynamics of the purinergic system and ECBs involving WT and $FAAH^{-/-}$ glial cells in the context of AD.

Keywords: GRAB Sensors, endocannabinoids, astrocytes.

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EFFECTS OF ARACHIDONIC AND DOCOSAHEXAENOIC PUFA ENRICHMENT IN THE CELL MEMBRANE ON CB_1 RECEPTOR FUNCTION

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Cell membranes are complex lipid–protein assemblies where the composition of polyunsaturated fatty acids (PUFAs) exerts a critical influence on the activity of GPCRs. The CB_1 receptor is particularly sensitive to lipid microenvironmental changes, since the endocannabinoid (eCB) precursors would be generated from these membrane PUFA-containing phospholipids. Moreover, this interaction could be important in pathological conditions such as in Alzheimer's disease (AD), where alterations in membrane composition, particularly in PUFAs like arachidonic acid (AA) and docosahexaenoic acid (DHA), have been consistently observed.
In this study, we focused on the impact of two physiologically relevant PUFAs for the eCB system: AA and DHA. CB_1-expressing HEK-293 cells were incubated with AA or DHA, and the effects on receptor binding, G protein activation, and lipid composition were evaluated.

Lipidomic analysis performed by MALDI mass spectrometry confirmed the efficient incorporation of both fatty acids, predominantly within phosphatidylcholine (PC), phosphatidylinositol (PI), and phosphatidylethanolamine (PE) lipid classes. In addition, significant alterations were detected in diacylglycerol (DAG) species, suggesting remodeling of lipid intermediates relevant to receptor signaling.

Radioligand binding assays using [^{3}H]CP55,940 and [^{3}H]SR141716A revealed that PUFAs enrichment modified both receptor affinity and density. In [^{3}H]CP55,940 experiments, AA decreased the total number of binding sites (B_{max}), while DHA increased them. In contrast, [^{3}H]SR141716A binding showed the opposite effect, with DHA decreasing and AA increasing receptor density. These opposite trends indicate that changes in membrane lipid composition contribute to stabilize different CB_1 receptor conformations. Therefore, functional assessment through [^{35}S]GTPγS binding showed that both AA and DHA also modified CB_1-mediated G protein activation. AA showed a trend toward increased E_{max}, while DHA slightly reduced it, consistent with opposing effects on receptor signaling efficiency.
Together, these results demonstrate that modifying the lipid microenvironment with AA or DHA alters CB_1 receptor affinity, density, and activity. This lipid-dependent modulation provides new insight into how reported PUFAs dysregulation in AD could contribute to alter eCB signaling and highlights the potential of targeting membrane lipid composition for these receptors.

Keywords: polyunsaturated fatty acids; MALDI lipidomics; radioligand binding assays

FATTY ACID AMIDE HYDROLASE REGULATES THE MICROGLIAL RESPONSE TO ACUTE CORTICAL INJURY IN AN ALZHEIMER'S DISEASE MOUSE MODEL

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Microglia, the resident immune cells of the CNS, are central to the neuroinflammation associated with Alzheimer's disease (AD). Fatty acid amide hydrolase (FAAH), the enzyme that degrades anandamide, is upregulated in astrocytes adjacent to β-amyloid (Aβ) plaques in AD. Previous evidence indicates that deleting FAAH paradoxically augments glial activation while preserving synaptic function and exerting neuroprotective effects. In this study, we assessed whether FAAH deletion regulates the microglial response to acute brain injury within the context of AD.
Using *in vivo* two-photon microscopy, we tracked microglial dynamics in awake 5xFAD mice with or without genetic ablation of FAAH. Focal cortical lesions were induced to evaluate the capacity of microglia to adapt their morphology and motility in response to localised tissue damage.

Preliminary observations show that the absence of FAAH alters microglial morphology and motility, suggesting a modified ability to respond to acute injury under chronic Aβ-driven neuroinflammatory conditions.

These data support that FAAH influences the regulation of microglial responses to acute injury in AD. Ongoing experiments seek to further delineate how FAAH deletion shapes glial dynamics and contributes to the balance between neuroinflammation and neuroprotection.

Keywords: neuroinflammation, Alzheimer's disease, microglial activity.

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EXPLORING CANNABINOID RECEPTOR EXPRESSION IN THE OLFACTORY NEUROEPITHELIUM OF INDIVIDUALS WITH MAJOR PSYCHIATRIC DISORDERS

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The endocannabinoid system (ECS) constitutes a key neuromodulatory network within the central nervous system (CNS), comprising the cannabinoid receptors CB1R, CB2R, and GPR55, along with their endogenous ligands and the enzymes responsible for their synthesis and degradation. Activation of the ECS regulates essential processes such as neurotransmission, synaptic plasticity, stress response, neurogenesis, and neuronal survival, positioning it as a potential therapeutic target in various neurological and psychiatric disorders. In this context, the olfactory neuroepithelium represents a particularly valuable model, as it is located in the nasal mucosa, contains stem and progenitor cells capable of differentiating into sensory neurons, and exhibits remarkable regenerative capacity. Its accessibility through minimally invasiveprocedures enables the isolation of viable neuronal cells from patients, facilitating the study of cellular and molecular mechanisms involved in CNS pathophysiology.

This study aimed, for the first time, to characterize the expression of cannabinoid receptors in olfactory neuroepithelium samples obtained via deep nasal exfoliation from patients diagnosed with schizophrenia and bipolar disorder, as well as from healthy control subjects. Immunocytochemistry was employed to assess the presence of CB1R, CB2R, and GPR55 receptors, and confocal laser microscopy confirmed their expression in all groups analyzed.

Our findings demonstrate that the olfactory neuroepithelium expresses key components of the ECS and constitutes a valuable model for investigating its involvement in neuropsychiatric disorders. Moreover, these results open new avenues for research into the identification of novel biomarkers and personalized therapeutic strategies for conditions such as Alzheimer’s disease, multiple sclerosis, and other neurodegenerative disorders, all of which are characterized by early alterations in the olfactory system.

Keywords: Olfactory neuroepithelium, cannabinoid receptors, mental disorders

SEX-DEPENDENT EFFECTS OF CANNABIDIOL ON NEUROTRANSMITTER RECEPTORS AND PKA/MTOR SIGNALING IN OFFPRING´S HIPPOCAMPUS AFTER PERINATAL ETHANOL EXPOSURE.

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Alcohol intake during pregnancy disrupts neurodevelopment by exploiting vulnerability and adaptive changes of the immature brain, leading to significant impairments in hippocampal-dependent behavior. Cannabidiol (CBD), a phytocannabinoid with established neuroprotective properties, has not been extensively studied yet for its effects on hippocampal neurotransmission and key intracellular pathways in the context of perinatal ethanol exposure (PEE). This study investigates the impact of PEE (20% v/v, administered orally *ad libitum* for 2 or 4 hours/day, 4 days/week) on offspring neurobiology, and evaluates the modulatory effects of prior cannabidiol (CBD; 20 mg/kg, subcutaneously) administered throughout gestation and lactation. This study evaluated gene and protein expression related to endocannabinoid, glutamatergic, and dopaminergic systems—key components of synaptic transmission modulation—in the hippocampus of male and female mouse offspring at postnatal day (PND) 21. Our previous findings showed that CBD decreased NOR preference ratio, an effect that was accompanied by reduced SGZ cell proliferation, in male offspring born to control dams, but not after PEE. The present study indicated that CBD decreased the mRNA levels of cannabinoid receptor 2 (*Cnr2*) and fatty acid amide hydrolase (*Faah*), showing a higher reduction in PEE female offspring. Concerning the glutaminergic system, CBD decreased mRNA levels of glutaminase 2 (*Gls2*) and two NMDA receptor subunits (*Grin1* and *Grin2b*), as well as increased mRNA levels of *Grin2a* and the AMPA receptor subunit *Gria2* in female offspring with PEE. Furthermore, regarding dopaminergic signaling, CBD increased protein levels of tyrosine hydroxylase (TH) in PEE offspring of both sexes, as well as dopamine receptor D2 and dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32) in PEE female offspring. These changes in neurotransmitter/neuromodulator receptors were accompanied by increased PKA/mTOR signaling in PEE female offspring. Our study suggests that CBD alters hippocampal glutamatergic and dopaminergic receptor expression, potentially via cannabinoid receptor 2 activation. This modulation influences the PKA/mTOR signaling pathway and affects SGZ cell proliferation and memory performance in a sex-dependent manner.

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Keywords: Pregnancy, ethanol, cannabidiol, neurotransmitter receptors, intracellular pathways

CELL TYPE-SPECIFIC ROLE OF CB1 RECEPTORS IN THE ANTERIOR INSULAR CORTEX: IMPLICATIONS FOR EMOTIONAL AND COGNITIVE REGULATION

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The insular cortex (IC) serves as a critical hub integrating interoceptive, emotional, and cognitive information, and is implicated in several psychiatric disorders, including anxiety, addiction, depression, and autism spectrum disorders (ASD). Among its neuromodulatory systems, the endocannabinoid system, particularly CB1 receptors (CB1R), plays a key role in regulating synaptic transmission and network excitability. We hypothesize that CB1R signaling in the anterior IC (aIC) might play a pivotal role in modulating emotional and cognitive behaviors, and its dysregulation may contribute to the development of pathological conditions.

To test this, we employed a combinatorial viral vector strategy to manipulate CB1R expression in a cell type- and compartment-specific manner in CB1 floxed mice. Using AAV-Cre and AAV-DIO-Flex vectors under CAMKII promoters, we selectively modified CB1R expression bilaterally in the aIC in glutamatergic neurons. A comprehensive behavioral battery was performed in both male and female mice, assessing anxiety-like behavior, social interaction, impulsivity, cognitive flexibility, stereotypy, operant learning, and sensorimotor gating.

Our results revealed that CB1R signaling in glutamatergic neurons of the aIC critically influences anxiety and cognition. These findings underscore the specific contribution of glutamatergic CB1Rs in regulating aIC-dependent behaviors, pointing to a cell type-specific mechanism by which endocannabinoid signaling modulates emotional and cognitive processes relevant to psychiatric disorders.

Keywords: Insular cortex, cannabinoid receptors, anxiety

RANDOMIZED CONTROLLED TRIAL OF HEMP-DERIVED CANNABIDIOL WITH AND WITHOUT LOW-DOSE THC FOR THE TREATMENT OF CHRONIC PAIN

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In the U.S. and worldwide, chronic pain affects approximately 20–25% of the population (CDC, 2023). Traditional treatment approaches using opioids have produced mixed results in terms of efficacy and are associated with serious side effects, including opioid use disorder. Recent reviews suggest that formulations containing THC, or combinations of THC and CBD, may help reduce chronic pain, although higher doses of THC are linked to adverse effects (e.g., Bell et al., 2024). Evidence for a positive effect of CBD alone, however, remains mixed.

Most previous studies have evaluated higher doses of THC with or without small amounts of CBD. The present study was designed to test whether a high dose of CBD (200 mg), with or without a low dose of THC (7 mg), could reduce chronic pain and sleep disruption in a randomized, placebo-controlled trial. We hypothesized that the formulation combining CBD and THC would improve both pain and sleep compared to CBD alone or placebo.

A total of 150 chronic pain patients were recruited through clinics and social media and randomized to 12 weeks of treatment in one of three conditions: 200 mg CBD (using hemp derived broad spectrum CBD; bsCBD), 200 mg CBD + 7 mg THC (using hemp derived full spectrum CBD; fsCBD), or placebo. Assessments of pain and sleep were conducted at baseline, 6 weeks, 12 weeks, and 16 weeks (four weeks post-treatment). Outcomes were analyzed using residualized-change linear mixed-effects models with restricted maximum likelihood estimation (REML). Analyses included all randomized participants, consistent with intent-to-treat (ITT) principles. Each model included a random intercept for participant and fixed effects for time, treatment condition (placebo, bsCBD, fsCBD), the time-by-condition interaction, and covariates (age and baseline score for the outcome).

Results indicated that both fsCBD and bsCBD were well tolerated with no significant side effects. Moreover, fsCBD produced significant reductions in pain and sleep disruption relative to bsCBD and placebo across follow-up assessments ($p < .05$). There were no significant treatment effects on comorbid anxiety or depression. These findings suggest that high doses of CBD combined with low doses of THC may be effective for managing chronic pain and improving sleep without producing notable adverse effects.

EFFECTS OF A Δ^9-TETRAHYDROCANNABINOL AND CANNABIDIOL COMBINATION ON THE DYSREGULATED DOPAMINERGIC SYSTEM IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is characterized not only by progressive cognitive decline but also by a range of non-cognitive symptoms, including impairments in sociability, anxiety and psychotic symptoms in the advanced stages of the neurodegenerative process. These behavioral and psychological symptoms of dementia (BPSD) are highly prevalent and are associated with a more rapid progression to severe dementia and earlier mortality. However, current pharmacological treatments show limited efficacy and are often associated with adverse side effects, highlighting the need for alternative therapeutic strategies. In this context, growing evidence suggests that the endocannabinoid system (ECS) could be considered as a multifaceted target to mitigate both cognitive and non-cognitive symptoms in AD.

Given the established link between dopaminergic dysfunction and many of the BPSD, the aim of this study was to evaluate the effects of a 1:1 combination of Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) on dopamine (DA) dynamics in the mesolimbic system in APP/PS1 mice, a transgenic AD model that replicates both cognitive and non-cognitive symptoms. By using viral vectors encoding fluorescent biosensors for dopamine and fiber photometry techniques, we evaluated dopamine release in the nucleus accumbens (NAcc) following electrical stimulation of the ventral tegmental area (VTA) in 6-months-old APP/PS1 and wild-type (WT) mice. Our results reveal that APP/PS1 mice exhibit reduced DA release in the NAcc in response to VTA stimulation. Notably, acute administration of THC and CBD at a non-psychoactive dose previously demonstrated to reduce cognitive impairment in this AD model (0.5 mg/kg each compound, i.p.) partially reversed this DA release deficiency in APP/PS1 mice. This effect was blocked by pretreatment with rimonabant (1 mg/kg), indicating a crucial role for CB_1 receptor.

Altogether, these findings suggest that chronic administration of this THC and CBD combination might reverse the dysregulation of the midbrain DA system, potentially leading to a reduction in non-cognitive symptoms in this AD model. Ongoing experiments are being conducted to test this hypothesis, and if successful, they would support the potential therapeutic utility of these natural cannabinoids for alleviating both cognitive and non-cognitive symptoms occurring in AD.

Keywords: Alzheimer's disease, dopamine, THC, CBD

PKC-GAMMA DELETION DISRUPTS CB1R SIGNALLING MECHANISMS RELEVANT FOR COGNITIVE PERFORMANCE

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Cannabinoid receptor type-1 (CB1R) is among the most abundant G protein coupled receptor in the mammalian brain, with the highest expression in several brain regions that controls learning and memory including the hippocampus. CB1R stimulation causes the modulation of a wide variety of cellular functions through the activation of different signal transduction pathways including the mammalian target of rapamycin (mTOR). While several CB1R interacting proteins have been described, recent studies focusing in the hippocampus have identified the protein kinase C (PKC)-signalling and specifically PKC-gamma isoform as a relevant CB1R interactor. In this study we investigated whether mice lacking PKC-gamma (PKC-gamma KO) would show altered CB1R expression, signalling and function. Since the hippocampal distribution of PKC-gamma is subregion specific, with the highest expression in *Cornu ammonis* (CA) fields and residual expression in the dentate gyrus (DG), we first analysed CB1R density and activity in PKC-gamma KO mice in comparison with wild-type (WT) mice in the different hippocampal subregions. We found that CB1R density and activity were significantly modified specifically in CA1-3 subregion of the hippocampus and unaltered in DG in accordance with PKC-gamma distribution. We also performed a lipidomic MALDI mass spectrometry analysis in the hippocampus of PKC-gamma KO mice in which we observed a higher number of specific lipid species modified in CA1-3 fields compared with DG in PKC-gamma KO mice. Moreover, endocannabinoid system components related with 2-AG signalling, including protein levels of diacylglycerol lipase (DAGL) and monoacylglycerol lipase (MAGL), were altered in the hippocampus of mice lacking PKC-gamma. To further characterize the role of PKC-gamma isoform in CB1R signalling we determined the levels of phosphorylated CB1R in S317 residue and the status of mTOR signalling pathway. We found decreased levels of phosphorylated CB1R in S317 residue and altered expression in the mTOR signalling components involving S6 in PKC-gamma KO mice. Since the involvement of serotonergic system in cannabinoid effects has been extensively studied and a crosstalk between 5-HT2A receptors (5-HT2AR) and PKC-gamma isoform has been reported, next we studied the density of CB1R/5-HT2AR heterodimer. We observed a significant up-regulation of CB1R/5-HT2AR heterodimers only in CA1-3 subregion of the hippocampus again in accordance with PKC-gamma distribution. Finally, we set to assess the effect of Δ9-tethahydrocannabinol (THC) in PKC-gamma KO mice. We found that PKC-gamma KO mice is less sensitive to hippocampal-dependent THC amnesic-like effects while other pharmacological effects of THC such as analgesia or hypothermia are not altered. Together, these results reveal the significance of PKC-gamma isoform in CB1R expression, signalling and function relevant for cognitive performance.

Keywords: PKC-gamma, Cannabinoid type-1 receptor, Δ9-tetrahydrocannabinol

CAN CANNABINOID ADMINISTRATION REDUCE THE IMMUNOSUPPRESSIVE MICROENVIRONMENT IN GLIOBLASTOMA?

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Glioblastomas (GBM), the most aggressive subtype or primary brain tumours exhibit a median patient survival lower than 15 months. The standard therapy against GBM consists of the surgical tumour resection preceded by radiotherapy with simultaneous and adjuvant administration of temozolomide (TMZ) One of the reasons for the aggressive behavior of GBM is the existence of an immunosuppressive microenvironment that facilitates the evasion of the antitumoral immune response. Thus, clinical studies based on the use of immune checkpoint inhibitors [ICIs, for example anti–programmed cell death protein 1 (α-PD-1)] did not show in GBM the encouraging results obtained in other cancer types.

Δ^9-tetrahydrocannabinol (THC) the most important active ingredient of *Cannabis Sativa*, has been shown to exhibit anticancer activity in preclinical models of Glioma. This effect relies on a mechanism based on cannabinoid receptor activation and the subsequent stimulation of autophagy-mediated apoptotic cell death. Besides, the combination of THC (or THC and cannabidiol, CBD) and TMZ produces a very strong anticancer activity in animal models of cancer. Likewise, the combination of THC and inhibitors of the Midkine/Anaplastic Lymphoma Kinase axis (MDK/ALK) have also been shown to strongly reduce tumor growth and overcome the resistance of glioma cells to other anticancer treatments in preclinical models of GBM. However, the impact of cannabinoids (or cannabinoid-based combinational therapies) on the immune microenvironment in GBM and the potential efficacy of ICIs remains unexplored.

In this work, we analyzed the effect of cannabinoids in a syngeneic murine GBM model. Our results showed that the administration of THC:CBD (1:1) together with TMZ increased survival, reduced tumor volume, and decreased the production of immunosuppressive factors as well as the M2 anti-inflammatory phenotype of tumor associated macrophages and microglial cells; this supports the idea that cannabinoid administration facilitates a less immunosuppressive GBM microenvironment. Ongoing work is analyzing the effect of the combination of THC:CBD together with α-PD1 antibodies in this preclinical model of GBM.

Keywords: glioblastoma, cannabinoids

EXPLORING PYRIMIDINE-2-ONE SCAFFOLDS AS INNOVATIVE POSITIVE ALLOSTERIC MODULATORS OF THE CB2 RECEPTOR

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Allosteric modulation of the cannabinoid type 2 receptor (CB2R) has emerged as a promising avenue in the development of next-generation therapies for a wide range of diseases. Among the two main cannabinoid receptor subtypes, CB2R—unlike the centrally expressed CB1R—is primarily found in immune cells and peripheral tissues, where it plays crucial roles in immunoregulation and inflammation control.

The concept of allosteric modulation has gained increasing relevance in drug discovery, as it enables fine-tuned regulation of receptor function. For CB2R, allosteric modulators provide a particularly attractive strategy to enhance or modulate receptor activity with improved selectivity and fewer off-target effects. Such ligands have demonstrated potential anti-inflammatory and neuroprotective actions, supporting their investigation in inflammatory, neurodegenerative, and autoimmune disorders. Remarkably, only one synthetic positive allosteric modulator (PAM) of CB2R has been identified so far.

Within the framework of a project aimed at discovering new cannabinoid receptor modulators using multicomponent reaction (MCR)-based methodologies, we report the identification and optimization of a novel family of CB2R positive allosteric modulators (PAMs) synthesized via the Biginelli reaction. These compounds exhibit high potency and excellent efficacy, representing a valuable addition to the expanding repertoire of CB2R-targeted modulators. Furthermore, preliminary data indicate that selected ligands modulate microglial polarization and exert notable anti-inflammatory effects.

Keywords: Allosterism, Immunomodulation, Neurodegeneration

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CANNABIDIOL MODULATES THE INFLAMMATORY CARGO OF EXTRACELLULAR VESICLES TO CONFER NEUROPROTECTION IN A MURINE MODEL OF NEUROLOGICAL EFFECTS OF LONG-COVID

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SARS-CoV-2 primarily causes respiratory symptoms, but one-third of patients experience neurological issues, known as long COVID. Extracellular vesicles (EVs) contribute to viral spread and neuroinflammation, potentially worsening neurological symptoms. Cannabidiol (CBD) may reduce neuroinflammation and modulate EV content, potentially alleviating long COVID's neurological effects. In this study, CALU-3 cells were infected with SARS-CoV-2 and treated with CBD (0.3 and 3 μM). EVs were isolated by ultracentrifugation and analyzed by NanoSight and ELISA forACE-2, BDNF, TNF-alpha, IL-6, CCL-3, andIL-1β. SH-SY5Y cell viability was assessed via MTT assay. Male C57BL/6 mice were administered EVs intranasally (4x10^7 per nostril, 20 μL total), and memory was assessed 14 days later using the Novel Object Recognition Test (NORT) and Barnes Maze. Data were analyzed with One-wayANOVA, Tukey post-hoctest, andPCA.No significantdifference was found in nanoparticle size between EVs from uninfected and infected cells, or between infected cells treated with different CBD concentrations ($F(3,8) = 1.210$, $p = 0.3667$). However, nanoparticle concentration was reduced in EVs from infected cells treated with CBDcompared tocontrols ($F(3,8) = 4276$, $p < 0.0001$).EVs frominfected cells had higher TNF-α, IL-6, and IL-1β concentrations, which were reduced by CBD (TNF-α: $F(3,8) = 39.69$, $p < 0.0001$; IL-6: $F(3,8) = 63.40$, $p < 0.0001$; IL-1β: $F(3,8) = 14.47$, $p = 0.0014$). BDNF concentration was higher in infected cells, and CBD reversed this increase ($F(3,8) = 8.834$, $p = 0.0064$). ACE-2 concentration increased with CBD treatment ($F(3,8) = 8.514$, $p = 0.0072$). PCA showed 84% of the variance explained by two components, with PC1 reflecting pro-inflammatory content and PC2 reflecting BDNF and ACE-2. Intranasal EVs from infected cells impaired spatial memory in mice (Barnes Maze: $F(3,12) = 6.585$, $p = 0.007$), and CBD partially reversed this impairment. CBD also improved object discrimination in the NORT ($F(3,12) = 30.16$, $p < 0.0001$). These results suggest that EVs from SARS-CoV-2-infected CALU-3 cells induce cognitive deficits, potentially mitigated by CBD through reducing pro-inflammatory content in vesicles.

INTRANASAL ADMINISTRATION OF FLUORESCENT CANNABIDIOL-LOADED NANOPROBE EXHIBITS ANTIDEPRESSANT-LIKE AND NEUROPROTECTIVE EFFECTS IN NEUROINFLAMMATORY AND ALZHEIMER'S DISEASE MOUSE MODELS

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Introduction: Cannabidiol (CBD), the main non-psychotomimetic phytocannabinoid from Cannabis sativa, exhibits neuroprotective, anti-inflammatory, anxiolytic, and antidepressant-like effects. However, its clinical translation is limited by poor pharmacokinetics, including low bioavailability, first-pass metabolism, and high lipophilicity. Nanotechnology-based delivery systems offer strategies to enhance brain targeting, stability, and safety, while intranasal administration provides a direct "nose-to-brain" route, bypassing the blood–brain barrier. This study evaluated the antidepressant-like effects of hybrid CBD-loaded nanoparticles (CBD-NP) in an LPS-induced neuroinflammation model and cognitive outcomes in 3xTg-AD mice.

Methods: CBD-NP and Reference Nanoparticles (REF) were characterized by Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscopy (TEM), and Dynamic Light Scattering (DLS) to determine size, concentration, and polydispersity index (PdI). Zeta potential and encapsulation efficiency (EE%) were measured by electrophoretic mobility and UPLC. In the LPS-induced neuroinflammation model, male C57BL/6 mice (8–12 weeks; CEUA-FMRP/USP #1251/2023R) received 0.83 mg/kg (i.p.), and formulations were administered intranasally before behavioral testing. Sickness behavior was assessed with the Irwin scale during the first 4 h, and antidepressant-like activity evaluated 24 h later in the Tail Suspension Test (TST). After euthanasia, brains were collected for ELISA of BDNF, CCL2, and CCL3, and fluorescence microscopy for CBD-NP visualization. For the 3xTg-AD model, wild-type (WT) and 3xTg mice were treated intranasally with CBD-NP or REF once or twice weekly for 8 weeks. Behavioral assessments included the Barnes Maze for spatial learning and memory and the Novel Object Recognition (NOR) test for recognition memory. Results: NTA revealed modal diameters of 203.2 nm (REF) and 187.1 nm (CBD-NP), with span indexes of 0.97and0.89, indicating homogeneous distributions.

Concentrations were 1.14×10^{11}(REF) and 1.13×10^{11} particles/mL (CBD-NP). DLS showed PdI values of 0.111 (REF) and 0.065 (CBD-NP), confirming monodispersity. CBD encapsulation efficiency was 98–99% ($r^2 = 0.996$), and zeta potentials were −29.2 mV (REF) and −36.2 mV (CBD-NP), indicating high colloidal stability and enhanced electrostatic repulsion conferred by CBD. In the LPS model, CBD-NP markedly reduced sickness behavior (Irwin score: 3 ± 0.42 vs. 13 ± 3.5; $p < 0.001$) and immobility in the TST ($F(3,22)=9.628$; $p=0.0003$). Fluorescence microscopy confirmed CBD-NP distribution in the dentate gyrus 10 min after intranasal delivery. In the 3xTg-AD model, CBD-NP improved learning and memory performance. In the Barnes Maze, WT and 3xTg mice treated once or twice weekly with CBD-NP learned faster and showed reduced latency to locate the target hole compared with 3xTg + REF ($F(5,47)=4.534$; $p=0.0019$). During the probe test, 3xTg + REF mice spent less time in the target quadrant, while CBD-NP (1x or 2x/week) prevented these deficits ($F(5,47)=3.234$; $p=0.0137$). In the NOR test, 3xTg + REF mice exhibited a reduced discrimination index, reversed by twice-weekly CBD-NP treatment ($F(5,47)=4.154$; $p=0.0033$).

Conclusion: Intranasal CBD-NP exhibited antidepressant-like and pro-cognitive effects in LPS-induced neuroinflammation and 3xTg-AD models. Its neuroprotective and plasticity-enhancing properties, combined with efficient nose-to-brain delivery, may overcome CBD's pharmacokinetic limitations. The formulation's high encapsulation efficiency, colloidal stability, and CNS penetration highlight its potential as a therapeutic nanoplatform for neuropsychiatric and neurodegenerative disorders.

Keywords: Cannabidiol, Nanoprobe, Behavior.

UNRAVELLING WOMEN'S FERTILITY: ENHANCING THE PERFORMANCE OF UHPLC-HESI-MS/MS FOR PROFILING ENDOCANNABINOIDS IN FOLLICULAR FLUID

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The endocannabinoid system plays a crucial role in human fertility, influencing key processes from gametogenesis to childbirth, as well as mechanisms underlying endometriosis. Most endocannabinoids exist in trace amounts (ng g^{-1} levels) within biofluids, including follicular fluid (FF). FF is the liquid within the antral follicle that bathes the oocyte and granulosa cells, facilitating nutrient exchange. Therefore, this unique microenvironment offers a valuable opportunity to study the intricate links between these compounds and fertility alterations. Given the diverse and complex nature of the matrix, developing methodologies to eliminate interferences and enhance ionization efficiency is essential.

Despite significant efforts in the detection and quantification of endocannabinoids such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), others, including N-arachidonoyl dopamine (NADA), remain almost unexplored in FF. Thus, the aim of this work was to develop a fast and reliable method based on the ultra-high-performance liquid chromatography–heated electrospray ionization–tandem mass spectrometry (UHPLC-HESI-MS/MS) for the simultaneous analysis of eight endocannabinoid compounds in FF. To achieve this, comparisons between two columns (C18 vs Biphenyl) and two mobile phase additives (formic acid vs ammonium fluoride) were carried out to improve both chromatographic separation and ionization efficiency.

Using the biphenyl column, complete separation of all endocannabinoids, including the isomers 1-AG and 2-AG, was achieved in less than 14 minutes. Moreover, ammonium fluoride emerged as a promising additive in LC-MS, enhancing sensitivity, peak shape, and ionization efficiency in both positive and negative electrospray ionization modes. Its use resulted in up to a 3.4-fold improvement in signal intensity on average compared to formic acid.

Under optimized conditions, UHPLC–HESI–MS/MS method, preceded by liquid–liquid extraction (LLE), was validated at 5, 25, 100, and 400 ng g^{-1} (the latter only for 1-AG and 2-AG). Adequate trueness (apparent recoveries in the 70–130% range) and repeatability (relative standard deviation among replicates <30%) were demonstrated, with limits of quantification ranging from 0.1 to 6.7 ng g^{-1}.

Finally, the method was applied to 62 FF samples from volunteer women obtained at Cruces University Hospital (Bizkaia, Spain), including individuals with endometriosis (n = 28) or polycystic ovary syndrome (PCOS, n = 10), and healthy volunteers as controls (n = 24). All eight endocannabinoids were quantified in the samples, with concentrations mostly below 10 ng g^{-1}, except for 1-AG, which showed levels approximately 10 times higher. Statistical analysis further indicated that palmitoylethanolamide (PEA) concentrations were elevated in women with PCOS at a 90% confidence level (Kruskal–Wallis test, $p < 0.1$) since the median PEA concentration was 2.8 ng g^{-1} in controls, 2.3 ng g^{-1} in endometriosis cases, and 3.4 ng g^{-1} in PCOS cases.

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Keywords: follicular fluid, endocannabinoids, mass spectrometry**.**

ENDOCANNABINOIDS SIGNALING REGULATES SHARP-WAVE RIPPLES FUNCTION ACROSS LEARNING

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The hippocampus is crucial for learning and memory. Within this structure, inhibitory interneurons play a fundamental role in shaping network activity by regulating neuronal excitability and synchronizing oscillatory patterns, such as sharp-wave ripples (SPW-Rs), which are strongly linked to memory. Endocannabinoids (eCBs), acting primarily through type-1 cannabinoid receptors, have been shown to involve memory and neuronal activity. However, their precise role in hippocampal network dynamics, particularly in relation to SPW-Rs and memory function, remains poorly understood. Therefore, to elucidate the role of eCBs in hippocampal network dynamics, we chronically implanted high-density silicon probes coupled with optic fibers in CA1 to simultaneous record extracellular recordings and endocannabinoid dynamics using the GRABeCb2.0 sensor expressed in all hippocampal neurons (n = 6 mice). This approach enabled the measurement of the temporal relationship between eCBs release and hippocampal dynamics, including SPW-Rs. Mice were exposed to a rewarded spatial alternation task across 10 days. Data showed a strong correlation between eCBs fluctuations and SPW-Rs events which were modulated across learning. These results suggest a fundamental role of eCBs in shaping memory consolidation and provide a novel framework for studying eCB-related cognitive impairments.

Keywords: Endocannabinoids, Sharp-wave ripples, memory

COMPUTATIONAL EXPLORATION OF GPR12 MODULATORS: A STRUCTURE-BASED APPROACH

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GPR12 is a class A orphan GPCR that shares high sequence homology with lipid-sensing GPCRs such as the cannabinoid receptors CB1 and CB2. It is further distinguished by its constitutive activity and its selective coupling to G_s protein–mediated pathways —similar to its close relatives GPR3 and GPR6.[1,2] Despite structural and functional parallels within this subfamily, GPR12 remains poorly characterized pharmacologically, largely due to the absence of selective ligands. Its predominant expression in the central nervous system and emerging links to neurodegenerative disorders highlight its potential as a therapeutic target.[1]

This study focused on identifying novel GPR12 modulators—both agonists and inverse agonists—using a structure-based computational approach. An active-state cryo-EM structure of GPR12 was analysed[3] alongside homologous receptors to define a putative agonist binding site. The lack of a resolved inactive-state structure led us to develop a homology inactive model based on a GPR6–inverse agonist cryoEM complex to pinpoint key residues involved in inverse agonist interactions.

In silico screenings were performed using curated libraries derived from oleic acid and tyrosol (agonists), and cannabidiol and triazolopyrimidine derivative (inverse agonists). A High-Throughput Virtual Screening including an Extra Precision Docking was applied, followed by ADME profiling, receptor interaction analysis, and off-target assessment—particularly on cannabinoid receptors.

The outcome was a diverse set of candidate compounds with favourable pharmacokinetic properties, distinct binding profiles, and high predicted selectivity for GPR12. The most promising hits are currently undergoing synthesis and will be evaluated through G-protein and β-arrestin functional assays.

Keywords: GPR12, agonists and inverse agonists, virtual screening

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THC IMPAIRS MEMORY CONSOLIDATION THROUGH GLUTAMATERGIC DYSREGULATION IN THE CA1 REGION

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The hippocampus plays a pivotal role in memory formation, and cannabinoids, including Δ⁹-tetrahydrocannabinol (THC), are known to modulate hippocampus-dependent memory, alter multiple forms of synaptic and structural plasticity, and disrupt the excitatory/inhibitory (E/I) balance. In particular, the CA1 region has been identified as a critical locus for these effects, where repeated THC exposure has been associated with impaired long-term potentiation (LTP) and reduced dendritic spine density. In this study, we sought to identify cell type–specific molecular mechanisms underlying these effects after repeated THC exposure, with the goal of enabling the development of pharmacological strategies that preserve the therapeutic potential of THC without impairing memory consolidation.

To decipher the THC-induced translatome, we used Wfs1-Cre:RiboTag mice expressing hemagglutinin (HA)-tagged ribosomes selectively in CA1 pyramidal neurons. Mice received daily intraperitoneal injections of THC (10 mg/kg) or vehicle for six days, after which HA-associated mRNAs were isolated and analyzed by RNA sequencing. Gene Ontology and KEGG pathway analyses revealed enrichment of terms related to memory, synaptic organization, and structural plasticity, with several categories linked to glutamatergic transmission, and altered expression of glutamate receptor subunits in THC-treated mice.

Finally, we tested whether pharmacological modulation of the glutamatergic system could reverse the THC-induced cognitive deficits. Behavioral assays demonstrated restored memory performance, indicating that targeting E/I balance can counteract the neurophysiological disruptions caused by repeated cannabinoid exposure.

In summary, our findings reveal CA1-specific molecular pathways dysregulated by repeated THC exposure and demonstrate that the resulting memory impairments can be pharmacologically prevented. These results provide mechanistic insight into how THC disrupts hippocampal glutamatergic signaling and highlight the therapeutic potential of restoring synaptic balance to counteract cannabinoid-induced cognitive deficits.

Keywords: hippocampus, THC, memory, RNAseq, glutamatergic transmission, synaptic plasticity, translatome.

MONOCYTE CB2R EXPRESSION IN ISCHEMIC STROKE: PRELIMINARY RESULTS OF A CLINICAL STUDY

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Stroke is the leading cause of acquired disability in adults and the second leading cause of death worldwide. Despite advances in the treatment of ischemic stroke (IS), it is estimated that between 20-35% of patients die, and up to a third of survivors suffer irreversible neurological dysfunction. Therefore, it is imperative to continue seeking new therapeutic tools that improve the secondary management of these patients.

In recent years, the inflammatory response has gained considerable relevance in IS, and particularly the possible critical role of circulating monocytes. Therefore, given the important role that the endocannabinoid system plays in modulating the immune system, we have conducted a study with patients in the stroke unit at Hospital Universitario Fundación Alcorcón with the aim of determining the role of the CB2R receptor in the post-stroke inflammatory response. Monocyte subpopulations (classical, non-classical, and intermediate) were determined by flow cytometry from peripheral blood samples. In addition, CB2R expression was analyzed using a specific probe in every subpopulation. Cell sorting was used to separate the three phenotypes for the determination of the expression of different elements of the endocannabinoid system by RT-qPCR.

Changes in CB2R expression appear to be associated with different etiologies of IS. Furthermore, sex-related changes have been observed. These results, although preliminary, provide new therapeutic opportunities for personalized treatment based on the patient's sex and/or etiology.

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Keywords: ischemic stroke, monocytes, inflammation.

CANNABINOID TYPE-1 RECEPTOR SIGNALLING AND ITS ROLE IN REGULATING BRAIN LACTATE DYNAMICS IN FREELY MOVING MICE

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The brain, though representing only ~2% of body weight, consumes nearly 20% of total energy, mainlythrough glucose metabolism that produces ATP and lactate.
In the CNS, astrocytes generate most lactate via aerobic glycolysis, which is then shuttled to neurons as an energy source in the astrocyte-to-neuron lactate shuttle (ANLS).

Beyond its metabolic role, lactate also acts as a signaling molecule regulating neuronal plasticity and behaviour.

However, how its production and release are controlled remains poorly understood. Recent evidence implicates the cannabinoid receptor type 1 (CB1R) in astrocytic metabolic regulation.

Persistent activation of mitochondrial CB1Rs reduces lactate production, where astransient stimulation induces the opposite effect. Yet, the impact of CB1R activation on brain lactate dynamics in vivo has remained unexplored. In this thesis, using fiber photometry and the fluorescent biosensor eLACCO2.1, we established a protocol to monitor extracellular lactate in freely moving mice.

We found that lactate levels are modulated by both cannabinoid exposure and locomotor state.

Specifically, Δ^9-THC altered lactate dynamics during immobility in a region-dependent manner, suggesting that CB1R signaling couples metabolic regulation to behavioural state.

Finally, pharmacological experiments in cultured astrocytes revealed that protein kinase C (PKC) is required for both the transient and persistent CB1R-mediated effects on lactate, acting as a key molecular switch in this pathway. Together, these findings uncover a novel mechanism by which CB1R activity regulates lactate metabolism and link cannabinoid signaling, astrocyte function, and locomotion through shared neurometabolic processes.

THE ROLE OF THE ENDOCANNABINOID SYSTEM UN INCIDENTAL ASSOCIATIONS: FOCUS ON INTERACTIONS WITH DOPAMINE SIGNALING

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Reinforced conditioning is a powerful learning mechanism that allows individuals to predict future events. However, many daily behaviors rely on unreinforced connections of low-salience stimuli called Incidental Associations (IAs). IAs enhance predictive capacity in unstable environments and are observed across species. To study them in rodents, sensory preconditioning tasks are used, where two low salience stimuli (S1/S2) are presented together in a preconditioning phase, followed by classical conditioning of S1 with a potent reinforcer. As a result, subjects present a direct response to the S1 stimulus, but also display a mediated response to the S2 stimulus never explicitly reinforced, indicating IA formation. Using a sensory preconditioning task with light (S1) and sound (S2) as sensory cues, we showed that mice exhibit both direct and mediated responses, indicating IA formation. Previous work demonstrated that endocannabinoid (eCB) system and type-1 cannabinoid receptors (CB1R) are critical for this process. Global CB1R knock-out mice did not show mediated response to S2 while direct response to S1 was unaltered. *In vivo* fiber photometry recordings revealed higher hippocampal eCB signaling when the light and sound are simultaneously presented compared to unpaired light and sound presentations. Recent studies also demonstrated that dopamine signaling played a pivotal role in IAs. In line with these findings, we found that mice lacking CB1R specifically in D1-receptor (D1R) positive cells (D1R-CB1R-KO) fail to exhibit mediated responses, indicating that this specific CB1R population is essential for the process. Future experiments will determine whether hippocampal dopaminergic activity is enhanced during paired presentations of S1 and S2 and where these D1R-CB1R positive cells are located. Additionally, we showed that exogenous CB1R activation, using THC administration, during short preconditioning induces IAs under conditions that are insufficient for controls. Similar IA facilitation was found by boosting endogenous cannabinoid levels through blockade of eCB degradation using JZL195. This effect of JZL195 was also observed in D1R-CB1-KO mice that exhibited mediated responses upon JZL195 administration during preconditioning. However, this effect was abolished when THC was administered, meaning that D1R-CB1R positive cells are essential for physiological but not pharmacologically induced IAs. Further studies will unveil the exact location of the CB1R subpopulation underlying THC's effects.

Keywords: Incidental Associations, Endocannabinoid System, Dopamine.

THE ENDOCANNABINOID SYSTEM MODULATES THE MICROGLIAL RESPONSE AGAINST AN ACUTE BRAIN INJURY IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE

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Microglia, as resident immune cells of the CNS, are involved in a wide variety of processes. Their ability to dynamically shift between different polarization states is thought to contribute to the onset and progression of various neurodegenerative diseases, such as Alzheimer's disease (AD). One of the main features of this disease is the presence of protein deposits in the brain parenchyma, known as β-amyloid (Aβ) plaques, primarily composed of amyloid peptides. These plaques cause profound alterations in the brain environment and, specifically, induce an intense neuroinflammation. Among other effects, Aβ plaques trigger the activation of microglial cells, which play a relevant role in the neuroinflammatory response. We have previously shown that the endocannabinoid system is involved in this microglial response and in the neuromodulation of inflammation. Using *in vivo* two-photon microscopy, we aimed to elucidate the effects of the genetic deletion of cannabinoid system components on the ability of microglial cells to respond to an acute brain injury in the presence or absence of chronic inflammation. For this purpose, the 5xFAD mouse model of AD, combined with CB2 knockout (CB2KO) were used. Preliminary quantitative 2D and 3D image analyses suggest that the lack of CB2 modifies the microglial response to environmental stimuli, presenting different capacity to extend and retract cell projections, as well as a different response to acute injury. The absence of CB2 resulted in decreased mean fluorescence intensity following injury, suggesting a reduced microglial responsiveness. These findings indicate that CB2 receptor signaling contributes to proper microglial activation and motility in response to acute brain injury, and its absence may compromise the microglial response in neurodegenerative contexts such as AD.

Keywords: neuroinflammation, Alzheimer's disease, cannabinoid system, microglial activity, two-photon microscopy, acute brain injury.

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ADOLESCENT CANNABINOID VAPOR EXPOSURE HAS SEX-DEPENDENT EFFECTS ON THE RELATIONSHIP BETWEEN VULNERABILITY TRAITS AND ETHANOL SELF-ADMINISTRATION AND AFFECTS NALTREXONE EFFICACY IN RATS

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Objectives: Cannabis use during adolescence, particularly by the actions of Δ9-tetrahydrocannabinol (THC), may alter the normal role of the endocannabinoid system (eCBs) which is involved in psychological development and brain maturation. This could have effects on psychological processes predicting addiction-like behaviours and alcohol use disorder per se during the adulthood. The present study examined whether adolescent vaporised THC or THC:CBD combinations influence behavioural vulnerability traits, compulsive ethanol self-administration in adulthood, and the therapeutic efficacy of naltrexone, an opioid receptor antagonist widely used to reduce alcohol intake and prevent relapse. Moreover, we studied how the linear relationship between these predictors and ethanol use could be affected by early cannabinoid exposure.

Methodology: Male and female rats were exposed to different THC and CBD concentrations (THC alone, THC/CBD 33:1, THC/CBD 1:33) or vehicle on alternate days from postnatal day (PND) 28 to 44 using an in-house designed vapour chamber. We evaluated the effects of these treatments on body temperature and plasma THC and CBD concentrations 30 minutes after exposure. Adult behavioural assessments (PND70-95) included locomotor activity, novelty and saccharine preference, sign/goal-tracking behaviours and anxiety (or disinhibition) in an elevated plus maze. Alcohol self-administration was studied under different conditions, including acquisition, progressive ratio, and punished seeking. Subsequently, naltrexone was administered (i.p.) at a dose of 0.3 mg/kg.

Results: Cannabis use during adolescence did not produce robust alterations in baseline behavioural traits or ethanol self-administration. Nevertheless, clear sex-dependent patterns were identified, with females displaying heightened vulnerability to ethanol consumption and seeking behaviours. In males previously exposed to THC, sucrose preference was inversely related to compulsive ethanol seeking—a relationship not observed in vehicle-treated controls. Among females, THC exposure disrupted the correlation between novelty preference and ethanol intake, while promoting a negative relationship between goal-tracking tendencies and compulsive alcohol seeking. Naltrexone showed the strongest efficacy in reducing ethanol intake in THC-exposed animals, suggesting that prior cannabinoid experience modulates pharmacological responsiveness to this opioid antagonist.

Keywords: Alcohol use disorder; adolescence; cannabinoid vaporisation; Sex difference

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INVOLVEMENT OF PPARγ RECEPTOR IN NEUROPROTECTIVE EFFECT OF CANNABIDIOL AFTER INTRAVENTRICULAR HEMORRHAGE IN IMMATURE NEWBORN RATS

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Background: Intraventricular hemorrhage (IVH) is a frequent complication in extremely low gestational age newborns increasing the risk of Cerebral palsy development. Cannabidiol (CBD) has shown neuroprotective effects in models of neonatal brain injury such as IVH; however, the molecular mechanisms underlying its effects are not fully understood, especially in immature brain. Peroxisome proliferator-activated receptor gamma (PPARγ) is known to modulate inflammatory and oxidative pathways in the developing brain. This study explores whether CBD's neuroprotective actions following IVH involve PPARγ activation.

Methods: IVH was induced in 1-day-old Wistar rats via paraventricular injection of 0.2 U of *Chlostridium collagenase* into the left Germinal Matrix. Animals exposed to anaesthesia but not to surgery remained as controls (SHAM), vehicle (VEH) or CBD were administered prenatally (10 mg/kg i.p. to pregnant rats at E21) and post-insult (5 mg/kg i.p. 1, 24 and 48h post-insult). Some rats received PPARγ antagonist GW9662 (5 mg/kg) i.p. 1h pre-insult and 1h prior to VEH or CBD administration during the next 2 days. Brain injury was assessed at PND6 using magnetic resonance image (MRI) and at PND14 exploring motor coordination (negative geotaxis) and grip strength, as well as grip and grasp reflexes. Inflammation and Blood Brain Barrier (BBB) impairment were analysed using Western Blot studies to determine the expression of TLR4, indicative of the main biochemical pathway of neuroinflammation in immature brain IVH-induced damage; and Mfsd2a, a membrane transport protein expressed in the endothelium of the BBB. Oxidative stress was evaluated through protein carbonylation by OxyBlot, while microglial and astroglial activation were assessed using Iba1 and GFAP immunohistochemistry. All the analyses were performed at PND6 and PND14.

Results: IVH led to brain damage as observed in MRI studies, along with functional impairment demonstrated by poorer performances in neurobehavioral tests. Those effects were associated with neuroinflammation, oxidative stress and BBB disruption, evidenced by increased TLR4, Iba1, GFAP and carbonylated proteins, and diminished Mfsd2a expression. CBD treatment reduced both anatomical and functional brain damage in a manner linked to the modulation of inflammation, oxidative stress and BBB integrity. Co-administration of CBD with the PPARγ antagonist resulted in loss of CBD protective effects on damage volume, neurobehavioral tests and oxidative status, as well as TLR4, Iba1, GFAP and Mfsd2a expression.

Conclusions: CBD treatment attenuated IVH-induced brain damage in immature rats modulating inflammation, oxidative stress and BBB permeability. Blunting of CBD-mediated neuroprotection by PPARγ antagonism suggests the involvement of the receptor in CBD mechanisms of action in this IVH rat model.

Funding: Supported by PI23/00733 grant

Keywords: Cannabidiol, intraventricular hemorrhage, PPARγ, neuroprotection, rat

MODULATION OF IMPULSIVITY BY INDUCED BEHAVIORS DURING THC WITHDRAWAL

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Research on impulsivity seeks strategies to enhance self-control, a key factor implicated in disorders such as addiction. Schedule-induced behaviors (SIB) have been proposed as a potential approach to enhance self-control. This study evaluated whether SIB modulates delay discounting performance in rats undergoing THC withdrawal. Forty male Wistar rats were trained under a fixed-interval schedule with water access to induce SIB and then received escalating THC doses for 11 days (2,5 mg/kg for 3 days, 5 mg/kg 4 days, and 10 mg/kg for 4 days). Subsequently, animals performed a delay discounting task offering choices between small immediate and large delayed reward. Among THC-treated rats, those engaged in SIB exhibited significantly greater self-control compared to their non-SIB controls. These findings suggest that SIB may attenuate the temporal devaluation of delayed reinforcers and mitigate THC-induced impulsivity.

Keywords: Impulsivity, Cannabis, delay discounting.

FUNCTIONAL CROSSTALK BETWEEN CB_2R AND OX_2R IN MICROGLIA: CB_2R-OX_2R HETEROMER AS A NOVEL THERAPEUTIC TARGET IN ALZHEIMER'S DISEASE

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The endocannabinoid receptor CB_2 is characterized by exhibiting very low expression levels in hippocampal neurons. In fact, many authors doubt its expression. Conversely, in microglia its expression is well stablished and moreover, it is increased when these cells are activated due to an inflammatory process. Among the pathologies that involve an inflammatory process, we find Alzheimer's disease. The orexinergic system has become an object of study due to its participation in the disruption of the sleep-wake cycle, characteristic of Alzheimer's patients. It seems that the OX_1 receptor could have a main role in the accumulation of beta-amyloid peptide due to sleep alteration in Alzheimer's disease. However, the role of the OX_2 receptor remains unclear.

In this study we focused on attempting to evaluate the roles of CB_2R and OX_2R in Alzheimer's disease. The results obtained indicate that both receptors present a beneficial effect in reducing neuronal death and oxidative stress in primary cultures of microglia activated with LPS and IFN-γ. Moreover, activation of the CB_2 receptor polarized microglia toward a neuroprotective M2 phenotype. These effects were more potent when activated microglia were co-treated with agonists of both receptors, decreasing ROS formation, microglial inflammation, and cell death. These effects are explained by the direct interaction between both receptors that provides a phenomenon of positive cross-talk.

In conclusion, these results present the CB_2R-OX_2R heteromer as a new therapeutic target to combat inflammation and reduce the symptomatology of Alzheimer's disease.

MÜLLER GLIA: A SOURCE OF ENDOCANNABINOIDS IN THE RETINA

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The retina is a highly specialized tissue that, as part of the central nervous system, can be modulated by the endocannabinoid system (ECS), which regulates neuronal activity, synaptic plasticity and visual processing. Different components of the ECS have been described in the retina: for instance, cannabinoid type-1 (CB1) receptors are widely expressed across neuronal layers, and both endocannabinoids, 2-arachidonoylglycerol (2-AG) and anandamide (AEA), have been measured. Despite the impact of the ECS in retinal physiology, most studies have focused on neurons, leaving the role of glial cells, particularly Müller glia, poorly understood. Müller glia provide structural and metabolic support, regulate ion balance and contribute to retinal neuroprotection. Similar to astrocytes in the brain, which have recently been shown to possess an active ECS involved in synaptic regulation, metabolism and behavior, Müller cells may also be active players of the retinal ECS. Here, we investigated the presence and functionality of the ECS in Müller glia. Both acutely isolated Müller glia or pure Müller culture express ECS-related genes, with particularly high levels of the endocannabinoid metabolic enzymes MAGL, NAPE-PLD, and DAGLβ. CB1 receptors mRNA was also detected, though at low levels. Furthermore, using the endocannabinoid sensor (GRABeCB2.0) and mass spectrometry for endocannabinoid quantification, we found that Müller cells in pure culture produce 2-AG and not AEA in response to ATP stimulation. However, when co-cultured with neurons, Müller glia seem to produce AEA as well. These results indicate that Müller glia possess a functional ECS *in vitro* and can actively synthesize endocannabinoids. Our results suggest that Müller cells are active participants in retinal endocannabinoid communication and may represent a novel target in retinal pathologies.

Keyword: retina, Müller cell, endocannabinoids

SEX DEPENDENT CANNABINOID EFFECTS ON HIPPOCAMPAL FUNCTION

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Tetrahydrocannabinol (THC), the main psychoactive compound in cannabis, has well-documented detrimental effects on cognition, particularly memory impairment. Its amnestic effects are primarily mediated by CB1 receptor interactions in the hippocampus, a key region for memory formation, spatial navigation, and learning. However, recent research suggests that THC's impact on memory differs between sexes, likely due to variations in CB1 receptor expression in different cell types and neural pathways. In this work, we study hippocampal-dependent cognitive processes using a plethora of behavioral tasks aiming at revealing potential sex differences and the participation of neurons and glial cells.

In wild-type male and female mice we used Novel Object Recognition (NOR) and the Barnes Maze to assess non-spatial and spatial memory, respectively. Our results show that THC impairs Barnes Maze performance in males but not females, suggesting sex-dependent pathways for spatial memory with CB1 involvement in males only. In contrast, in the NOR test, THC affects both sexes. However, calcium imaging (fiber photometry) recordings reveal distinct hippocampal activity patterns between sexes during NOR, suggesting different underlying mechanisms despite CB1 involvement in both cases. In parallel, CB1-floxed mice with CB1 deletion from hippocampal principal cells or astrocytes were tested in the cheeseboard maze, an apparatus that allows the study of different types of memory while recording hippocampal cellular activity. Here we observed that neuronal and glial activity behave differently in males and females depending of the cognitive processes measured by the maze. Thus, these results add an extra dimension to the role of hippocampal circuits in the control of memory in males and females. Current experiments are being carried to evaluate the effect of THC on the hippocampal CB1-KO in order to elucidate the cell-specific participation of the receptor in such cognitive processes.

EVALUATING THE EXPRESSION OF ORPHAN RECEPTOR GPR12 IN A NEUROINFLAMMATORY MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disease characterized by the progressive loss of dopaminergic neurons in the substantia nigra, leading to striatal denervation and the onset of motor and non-motor symptoms. The main pathological events of PD include neuroinflammation, protein aggregation (Lewy bodies) and mitochondrial dysfunction.
PD is the second most prevalent neurodegenerative disorder, with incidence and prevalence rising sharply in the recent years. However, there is currently no cure, so it is critical to explore new therapeutic approaches that offer better outcomes for patients. Given all the new therapeutic strategies that have arised from class A orphan GPCRs, we explored GPR12 as a potential target for PD treatment

GPR12 is an orphan receptor highly expressed in the central nervous system, especially in the basal ganglia, and shares structural similarity with other receptors involved in PD, such as GPR6 and the cannabinoid receptors CB1 and CB2. GPR12 is a cannabinoid-like receptor since it shares a 35% sequence homology with the canonical cannabinoid receptors in the transmembrane regions, responds to lipidic ligands and is phylogenetically related. The mentioned receptors are all part of the MECA cluster. In this context, we decided to study the potential of the orphan G protein-coupled receptor GPR12 as a possible mechanism for the treatment of PD.

To explore GPR12's role in PD, we examined its expression in an inflammatory murine model. Using qPCR, western blot, and immunohistochemistry, we analyzed GPR12 levels in the striatum and mesencephalon of mice subjected to unilateral intrastriatal LPS injection. We observed a downregulation of GPR12 on the lesioned side. These results could suggest a potential neuroprotective function for GPR12, which could be significant in the search for therapeutic targeting strategies in PD.

Keywords: GPR12; New targets; Parkinson's Disease.