26ª Reunión anual

Sociedad Española de Investigación sobre Cannabinoides

Noviembre 26-28, 2026

Santiago de Compostela

SEIC
SOCIEDAD ESPAÑOLA DE INVESTIGACIÓN SOBRE CANNABINOIDES



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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)

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PROGRAMA CIENTÍFICO

Jueves, 26 de noviembre

11:30-12:15 **Registro, entrega de documentación**

Salón de actos de la facultad de ciencias políticas

12:15-12:30 **Inauguración**

- Cristina Sánchez (Presidenta de la SEIC)
- Raquel Sendón García (Decana de la Facultad de Farmacia, Universidad de Santiago de Compostela)
- Eddy Sotelo (Comité organizador local, Universidad de Santiago de Compostela)

12:30-13:30 **Conferencia inaugural:**

Presentada por Eddy Sotelo

CANNABINOIDS AND LIPID MEDIATORS IN CANCER: METABOLIC VULNERABILITIES AND THERAPEUTIC OPPORTUNITIES

Alessia Ligresti

National Research Council of Italy, Institute of Biomolecular Chemistry, Italy

13:30-15:00 **Comida**

15:00-16:15 **1ª Sesión de comunicaciones orales:**

Química Médica (I)

Moderada por Nadine Jagerovic

15:00-15:15 **O1.1**

DISCOVERY OF PERIPHERALLY RESTRICTED CB1 RECEPTOR ANTAGONISTS THAT RESTORES INSULIN SIGNALING IN ADIPOCYTES.
Prieto-Díaz R, Azuaje J, Ortigueira S, Fojo H, Loza MI, Contino M, Perrone MG, Mangiatordi GF, Lago-Baameiro N, Estévez-López A, Pardo M, Stefanachi A, Graziano G, Brea JM, Majellaro M, and Sotelo E.

15:15-15:30 **O1.2**

DEVELOPMENT OF CANNABINOID SUBTYPE-2 RECEPTOR (CB2R) FLUORESCENT PROBES: USEFUL TOOLS FOR NON-RADIATIVES ASSAYS
Musillo GS,Graziano G, Mammone M, Gioè-Gallo C, Majellaro M, Alberga D, Mangiatordi GF, Stefanachi A, Liturri A, Miciaccia M, Ligresti A, Brea J, Loza M, Riganti C, A. Colabufo N, Sotelo E, Contino M, Abate C.

15:30-15:45 **O1.3**

DESIGN AND DEVELOPMENT OF NEW DUAL TARGET AGENTS, CANNABINOID SUBTYPE 2 RECEPTOR (CB2R) AGONISTS AND FATTY ACID AMIDE HYDROLASE (FAAH) INHIBITORS AGAINST NEURODEGENERATION
Graziano G, Mastropasqua F, Fanizzi A, Papadopoulos MGE, Laghezza A, Mammone M, Musillo GR, Mangiatordi GF, Esposito D, Brea J, Loza MI, van der Horst C, Riganti C, Perrone MG, Leonetti F, Heitman LH, Colabufo NA, Stefanachi A, Abate C, Ligresti L, and Contino M.

15:45-16:00 **O1.4**

DESIGN OF DUAL CANNABINOID SUBTYPE 2 RECEPTOR (CB2R) AND SIGMA 1 RECEPTOR (S1R) LIGANDS AS NEW TARGETS FOR A THERAPEUTIC STRATEGY IN NEURODEGENERATION

Mammone M, Lillo J., Graziano G, Xutglà M, Musillo GR, Garrigós C, Mangiatordi G, Perrone M., Maurice T, Navarro G, Abate C, Contino M.

16:00-16:15 **O1.5**

IDENTIFICATION AND EVALUATION OF POTENTIAL CANNABIDIOL-BASED MODULATORS FOR THE ORPHAN RECEPTOR GPR12

Fidalgo-Casares L., Porzio-González S., Montoliu, E., Madariaga A., Jagerovic, N., Gómez-Cañas M., Morales P.

16:15-17:15 Café y 1ª sesión de pósteres (del 01 al 10)

17:15-18:30 2ª Sesión de comunicaciones orales:
Química médica (II), cáncer e inflamación

Moderada por María Majellaro

17:15-17:30 **O2.1**

SHEDDING LIGHT ON GPR18: NOVEL ASSAYS AND FLUORESCENT LIGANDS FOR AN ELUSIVE IMMUNE RECEPTOR

Porzio-Gonzalez S., Rodríguez Fernández A., Madariaga A., Ulloa García L., Sykes A., Veprintsev D., Morales P., Jagerovic N.

17:30-17:45 **O2.2**

IN SILICO SCREENING AND QM/MM EVALUATION OF CHEMICAL LIBRARIES TO IDENTIFY NOVEL CB2/GPR18 DUAL AGONISTS

Rodríguez A., Porzio-González S., Ruano L., Jagerovic N., Nogueira J.J., Morales P.

17:45-18:00 **O2.3**

CANNABINOID CB_2 RECEPTOR IS DIFFERENTIALLY EXPRESSED IN SPLENIC MURINE NK CELL POPULATIONS AND MODULATES ANTITUMOR EFFECTOR FUNCTION

Balsinde S.A., Rubert-Hernández M., Seijo-Vila M., Palomino-Duque F., Pérez-Gómez E., Sánchez C.

18:00-18:15 **O2.4**

TOWARDS THE IDENTIFICATION OF NOVEL BIOMARKERS OF SENSITIVITY AND RESISTANCE TO THE ANTICANCER ACTIVITY OF CANNABINOIDS AND CHEMORADIOTHERAPY IN GLIOBLASTOMA

Sanz-Gálvez, A; Salvador-Tormo, N; Tovar-Ambel, E; Mugica-Urruzola, E; Sánchez-Gómez, P, Sánchez-Parcerisa, D; Lorente, M; Sepúlveda, JM; Gutiérrez-Uzquiza, Á; Bragado, P and Velasco, G.

18:15-18:30 **O2.5**

GLOBAL AND BRAIN REGION-SPECIFIC ENDOCANNABINOID SYSTEM DYSREGULATION IN PRIMARY MITOCHONDRIAL DISEASE

Van der Walt G., Kouchaeknejad A., Yu J., Reynoso I., Yu L., Luna M., Quintana A., Gertsch J., Puighermanal E.

Viernes, 27 de noviembre

10:00-11:00 **3ª Sesión de comunicaciones orales: Neurodesarrollo, aprendizaje y envejecimiento**
Moderada por María Ceprian

10:00-10:15 **O3.1**
ASTROGLIAL CONTROL OF 2-ARACHIDONOYLGLYCEROL DRIVES CB1-DEPENDENT OLIGODENDROGENESIS DURING DEVELOPMENTAL MYELINATION AND DURING REMYELINATION
Ezquerro-Herce S., Sánchez-de la Torre A., Blázquez C., Arroyo-Gascón E., Monreal P., Muñoz-Canales V., Lutz B., Grabner G., Zimmermann R., Ortega-Gutierrez S., Guzmán M., Aguado T. & Palazuelos J.

10:15-10:30 **O3.2**
ENDOCANNABINOIDS SIGNALING REGULATES SHARP-WAVE RIPPLES FUNCTION ACROSS LEARNING
Martínez-Felix A, Abad-Pérez P, del Castillo-Berges I, Ramon-Duaso C, Busquets-García B, Martin-Fernández M, Mederos S, Valero M.

10:30-10:45 **O3.3**
EXPLORING COGNITIVE ALLEVIATION THROUGH THE MODULATION OF THE ENDOCANNABINOID SYSTEM DURING EARLY PHYSIOLOGICAL AGING IN MICE
Ciaran-Alfano L., Galera-López L., Ozaita A.

10:45-11:00 **O3.4**
MEDICAL CANNABIS IN OLDER ADULTS: REAL-WORLD EVIDENCE FROM A RETROSPECTIVE OBSERVATIONAL STUDY
Moreno-Monroy CS, Díaz-Moreno ID, Maiorana S, López-Ejeda N, Pineda P, Sánchez C.

11:00-12:00 **Café y 1ª sesión de pósteres** (del 11 al 20)

12:00 -13:00 **4ª Sesión de comunicaciones orales: Alteraciones del neurodesarrollo y enfermedades neurológicas/psiquiátricas**
Moderada por Susana Mato

12:00 -12:15 **O4.1**
CB_1 CANNABINOID RECEPTOR NEGATIVE MODULATION REGULATES CORTICAL AND BEHAVIORAL ALTERATIONS INDUCED BY SATB2 DEFICIENCY
Gavaldà-Vives T, Cassaro PH, Torres-Román AL, Simón-Sánchez S, Apostolova G, Guzmán M, Aguado T, Galve-Roperh I.

12:15 -12:30 **O4.2**
ASSESSMENT OF MOLECULAR SIGNATURES SENSITIVE TO CANNABIDIOL TREATMENT IN FRAGILE X SYNDROME
Galera-López L, de los Reyes-Ramírez L, Martínez-Cerdeño V, Eyras E, Ozaita A.

12:30 -12:45 **O4.3**
PATIENT-DERIVED NEURONS AS A PLATFORM TO INVESTIGATE THE EFFECTS OF CANNABINOIDS ON NEUROINFLAMMATORY SIGNALING
Unzueta-Larrinaga P., Barrenechea N., Cuesta E., Callado L.F., Horrillo I., Urigüen L.

12:45 -13:00 **O4.4**

DISENTANGLING THE LINK BETWEEN CANNABIS AND PSYCHOSIS: FROM MEDIATED LEARNING TO REALITY TESTING

Manzanares-Sierra I, Pinho J, Ramón-Duaso C, Joaquín Piriz, Busquets-Garcia A.

13:00-14:00 Comida

14:00-15:00 5ª Sesión de comunicaciones orales: Enfermedades neurodegenerativas

Moderada por Irene Reyes

14:00-14:15 **O5.1**

AGE-ASSOCIATED ALTERATIONS IN MESOACCUMBAL DOPAMINE DYNAMICS AND THEIR CANNABINOID MODULATION IN APP/PS1 MICE

P Subirana, D Antelo, J Bonaventura, E Aso.

14:15-14:30 **O5.2**

OX_2R ACTIVATION ENHANCES CB_2R-MEDIATED ANTI-INFLAMMATORY EFFECTS IN A 5xFAD MOUSE MODEL OF ALZHEIMER'S DISEASE

P Badia, JB Rebassa, T Capó, M Santandreu, X Antúnez, A Bellver-Sanchis, M Pallàs, C Griñán-Ferré, J Lillo, I Reyes-Resina, G Navarro.

14:30-14:45 **O5.3**

GPR12 EXPRESSION IN HUMAN TISSUE AND MURINE MODELS IN PARKINSON'S DISEASE

Madariaga A., Martín-Jiménez V., Akan E., Yunta M., Fernández-Ruíz J., Jagerovic N., Morales P. and Gómez-Cañas M.

14:45-15:00 **O5.4**

CANNABINOID MODULATES HIPPOCAMPAL ASTROCYTES IN HUNTINGTON'S DISEASE VIA MITOCHONDRIAL FUNCTION?

N Di Franco, C Blazquez Ortiz, A Castellanos, A Sánchez-Ruiz, A Pereda-Velarde, B Rebellato, C Gónzalez-Arias, L María Miquel Rio, A Bortolozzi, X Gasull, M Guzmán, G Perea, S Ginés.

15:00-16:00 Mesa redonda:

Moderada por Cristina Sánchez

"INVESTIGACIÓN CLÍNICA EN CANNABINOIDES"

José Martínez Orgado (Hospital Clínico San Carlos, Madrid), Guillermo Velasco (Universidad Complutense de Madrid) y Adán de Salas Quiroga (Consultor independiente)

Retransmitida en directo a través del canal de YouTube @SEIC_ES

16:00-17:00 Café, 3ª sesión de pósteres (del 21 al 31)

17:00-18:00 Asamblea General Ordinaria de socios/as de la SEIC

21:00 Cena del congreso

San Francisco Hotel Monumento

Sábado, 28 de noviembre

10:00-11:00 **6ª Sesión de comunicaciones orales:**
Daño cerebral neonatal
Moderada por Daniel Alonso-Alconada

10:00-10:15 **O6.1**

CELLULAR MECHANISMS OF THE NEUROPROTECTIVE EFFECT OF CANNABIDIOL IN A RAT MODEL OF INTRAVENTRICULAR HEMORRHAGE–INDUCED BRAIN INJURY IN EXTREMELY PRETERM INFANTS

M. De Hoz-Rivera, A. Romero, M. Martínez-Vega, L. Silva, S. Herrero, B. Pérez-Calero, G. Harry, C. Mitroi, A. García-Fernández, J. Martínez-Orgado.

10:15-10:30 **O6.2**

CHARACTERIZATION AND MODULATION OF THE ENDOCANNABINOID SYSTEM IN AN ANIMAL MODEL OF INTRAVENTRICULAR HAEMORRHAGE OF EXTREME PRETERM NEWBORNS

A. Romero, M. de Hoz-Rivera, M. Martínez-Vega, L. Silva, S. Herrero, B. Pérez-Calero, C. Mitroi, G. Harry, F. Piscitelli, R. Verde, J. Martínez-Orgado.

10:30-10:45 **O6.3**

CARDIOPROTECTIVE EFFECTS OF CANNABIDIOL FOLLOWING NEONATAL
HYPOXIC-ISCHEMIC INJURY

B. Pérez-Calero, M. De Hoz-Rivera, A. Romero, M. Martínez-Vega, L. Silva, S. Herrero-Escudero, J. Martínez-Orgado, L. Arruza

10:45-11:00 **O6.4**

LONG-TERM BEHAVIORAL AND HISTOLOGICAL OUTCOMES AFTER URB447
ADMINISTRATION IN A PRECLINICAL MODEL OF NEONATAL HYPOXICISCHEMIC BRAIN INJURY

Chillida M., Cantalapiedra P., Alart JA., Álvarez A., Herrero de la Parte B., Arango JC., Abarrategi A. and Alonso-Alconada D

11:00-11:15 **Premio a la mejor publicación predoctoral 2026**
Presentado por Olga Valverde

CANNABINOID TOLERANCE RELIES ON CB_1 RECEPTOR UBIQUITINATION BY NEDD4L

Álvaro-Blázquez A, Rodrigues R.S, Montero-Fernández C, Isasa M, Cannich A, Gisquet D, Rodríguez-Crespo I, Bellocchio L, Marsicano G, Costas-Insua C, Guzmán M.

11:15-11:30 **Premio a la mejor publicación postdoctoral 2026**

Presentado por: pendiente de conformar

CANNABINOID CB2 RECEPTOR DRIVES TRASTUZUMAB RESISTANCE AND PREDICTS DURABLE ANTI-HER2 RESPONSE

Seijo-Vila M., Balsinde S. A., Blasco-Benito S., Tundidor I., Rubert-Hernández M., Montero-Calle A., Barderas R., Kilpatrick L. E., Platt S., Karsai N., Philps I., Antón O. M., Gómez-Domínguez D., Pérez de Castro I., González-Lois C., García-Fresnadillo D., Silvestre-Egea G., Sánchez-López A. J., Ramírez-Medina E. , Rivas M. C., Almoguera B., Manso L., Zazo S., López-Ejeda N., Palomino-Duque F., Turienzo-Durán M., Martínez-Illescas N. G., Salazar-Roa M., Castillo-Lluva S., Hill S. J., Guzmán M., Pérez-Gómez E., Sánchez C.

11:30-12:30 **Café**

12:30-13:00 **Entrega de premios y clausura**

PRIMERA SESIÓN DE PÓSTERES

P1. PERIPHERAL EXPRESSION OF ENDOCANNABINOID AND INFLAMMATORY BIOMARKERS IN COMPLETED SUICIDE: A POSTMORTEM FORENSIC STUDY
Marino R, Martín-Cazorla F, Martín-de-las-Heras S, Rubio L, Suárez J.

P2. CONTRIBUTION OF THE A_{2A} RECEPTOR TO THE EFFECTS OF Δ^9-THC EXPOSURE DURING ADOLESCENCE
Antelo D., Subirana P., Gómez-Acero L., Deka L., Gomes F.V., Aso E.

P3. CANNABINERGIC REGULATION OF HABENULAR CIRCUITS
Sangroniz-Beltrán L., Rodriguez-Cedrés C., López N., Ceprián M., Egaña-Huguet J., Fedorov D., Santas-Martin J.A., Bonilla del Río I., Reyes P., Ducourneau E., Ramos-Miguel A., Piriz J., Grandes P., Marsicano G., Ferreira G., Mato S. and Soria-Gómez E.

P4. DIFFERENTIAL MODULATION OF CB1 AND CB2 RECEPTORS SIGNALING BY PHYTOCANNABINOIDS: RELEVANCE FOR NEURODEGENERATIVE DISEASES
Shi X, Badia P, Capó T, Sanchez de Medina V, Ferreiro-Vera C, Lillo J, Navarro G, Reyes-Resina I.

P5. CANNABIGEROL RESCUES COGNITIVE DEFICITS AND REDUCES AMYLOID, NEUROINFLAMMATORY, AND OXIDATIVE PATHOLOGY IN 5XFAD MICE THROUGH CB_1/CB_2 RECEPTOR ENGAGEMENT
Jaume Lillo, Aina Bellver-Sanchis, Gemma Navarro, Rafael Rivas-Santisteban, Carlos Ferreiro-Vera, Verónica Sánchez de Medina, Mercè Pallàs, Christian Griñán-Ferré, Rafael Franco.

P6. CAN CBD AMELIORATE BEHAVIORAL AND MOLECULAR ALTERATIONS ASSOCIATED WITH SATB2 DEFICIENCY?
Lirio PHC, Gavaldà-Vives T, Apostolova G, Campos AC., Galve-Roperh I.

P7. SEX-DEPENDENT EFFECTS OF MATERNAL DEPRIVATION AND ADOLESCENT ALCOHOL EXPOSURE ON STRESS-RELATED BEHAVIOR AND LIPID SIGNALING IN RATS
Sánchez-Marín L, Castro-Zavala A, Flores-López M, Gavito A, Reviriego R, Marco EM, Pavón-Morón FJ, Rodríguez de Fonseca F and Serrano A.

P8. CB1 RECEPTOR ACTIVATION DOWNREGULATES NEUROTROPHIC AND PKA-DEPENDENT SIGNALING AT THE NEUROMUSCULAR JUNCTION
Jami-El Hirchi M., Díaz-Wallach M., Polishchuk A., Pau-Butiñá A., Silvera C., Tomàs J., Just-Borràs L., Hurtado E., Lanuza MA.

P9. EXPLORING THE ANTI-INFLAMMATORY AND NEUROPROTECTIVE POTENTIAL OF THE ACIDIC AND VARINIC FORMS OF PHYTOCANNABINOIDS
Reyes-Resina I, Shi X, Badia P, Capó T, Sanchez de Medina V, Ferreiro-Vera C, Lillo J, Navarro G.

P10. AN IMAGING-BASED ASSAY KIT FOR SCREENING NOVEL LIGANDS AT THE HUMAN CB2 RECEPTOR
Camino T., Or-gueira S., Gioé-Gallo C., Robles A., Des-to P., Bernardez R., Sotelo E., Majellaro M.

P.11 A CLINICAL STUDY OF CANNABIDIOL AS ADDITIONAL TREATMENT FOR REFRACTARY EPILEPSY IN DOGS
Galán-Rodríguez A., Rodríguez Camacho A., Ferreiro Vera C., Sánchez de Medina V

P.12 BIOAVAILABILITY AND NEUROPROTECTIVE EFFICACY OF AN ORAL FORMULATION OF PURIFIED CANNABIDIOL IN DIFFUSE AND FOCAL HYPOXIC-ISCHEMIC BRAIN DAMAGE IN NEWBORN RATS
S. Herrero, A. García-Fernández, A. Mora, M. Martínez-Vega, M. De Hoz-Rivera, A. Romero, B. Pérez-Calero, G. Harry, C. Mitroi, J. Martínez-Orgado.

P.13 CONFORMATIONAL RESTRICTION OF DESIGNER DRUGS REVEALS SUBTYPE-SELECTIVE AND BIASED CB_2 AGONISTS WITH NEUROPROTECTIVE EFFECTS
Ortigueira S, Gioé-Gallo C, Prieto-Díaz R, Contino M, Stefanachi A, Loza, M. Brea J, Navarro G, and Sotelo E.

P.14 DEVELOPMENT OF AN INTEGRATED CELL-BASED WORKFLOW FOR THE CHARACTERIZATION OF CB2 RECEPTOR LIGANDS
Camino T., Ortigueira S., Gioé-Gallo C., Robles A., Destito P., Bernardez R., Sotelo E., Majellaro M.

P.15 CANNABINOIDS PROMOTE NEUROPROTECTION THROUGH CB_1R SIGNALING IN ALZHEIMER'S DISEASE
Garrigós C., Cubeles E., Capó T., Badia P., Shi X., Lillo J., Reyes-Resina I., and Navarro G.

P.16 EXPLORING CANNABINOID RECEPTOR HETEROMERS IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM MULTIPLE SCLEROSIS PATIENTS
Menéndez-Pérez C, Villar-Conde S, Peláez R, Oliva P, Martínez-Pinilla E.

P.17 A UGI-BASED APPROACH LED TO THE DISCOVERY OF A NEW FAMILY OF CB_2 RECEPTOR AGONISTS
Carmen Varrella, Hugo Fojo, Annalisa Fanizzi, Jhonny Azuaje, José M. Brea, Maria I. Loza, and Eddy Sotelo.

P.18 CB1R–OX1R HETEROMERS PRESERVE CB1R-MEDIATED NEUROPROTECTION DESPITE RECIPROCAL CROSS-ANTAGONISM IN ALZHEIMER'S DISEASE MODELS
Toni Capó, Joan Biel Rebassa, Claudia Garrigós, Martí Xutglà, Iu Raïch, Irene Reyes-Resina, Christian Griñán-Ferré, Mercè Pallàs, Jaume Lillo and Gemma Navarro.

P.19 CANNABINOID CB_1 RECEPTORS IN THE HABENULAR COMPLEX: IMPLICATIONS FOR PSYCHIATRY COMORBIDITIES ASSOCIATED TO MULTIPLE SCLEROSIS
Uribe-Irusta A, Iriarte-Sarria A, Carmona-Molina N, Bernal-Chico A, Soria-Gómez E, Baraibar AM, Elezgarai I, Mato S.

P.20 CB1 RECEPTORS IN PREFRONTAL PARVALBUMIN INTERNEURONS DRIVE SEX-DEPENDENT EMOTIONAL AND COGNITIVE DEFICITS IN MALE MICE
Ceprián M, Egaña-Huguet J, Godoy A, Pelaez A, Sangroniz-Beltrán L, Reyes-Velasquez P, Godoy L, Santas-Martín JA, Ramos-Miguel A, Bulnes S, Tonnesen J, Mato S, Soria-Gomez E.

P.21 ROLE OF P-GLYCOPROTEIN IN THE PATHOPHYSIOLOGY OF INFLAMMATION-ENHANCED HYPOXIC-ISCHEMIC BRAIN INJURY IN A NEONATAL RAT MODEL
Harry G, C. Mitroi, Romero A, de Hoz-Rivera M, Martínez-Vega M, Silva L, Herrero S, Pérez-Calero B, Martínez-Orgado J.

P.22 MEDIAL SEPTUM CB1R TUNES SEPTAL CONTROL OFLOCOMOTION AND EXPLORATION WITHOUT BEING NECESSARY FOR SPATIAL LEARNING
Egaña-Huguet J, Ceprián M, Torres-Maldonado P, Pelaez A, Sangroniz-Beltrán L, Reyes-Velasquez P, Rodriguez-Cedrés C, Soria-Gomez E.

P.23 CANNABIDIOL AS A MULTIFACETED THERAPEUTIC AGENT: MITIGATING ALZHEIMER'S DISEASE PATHOLOGY AND ENHANCING COGNITIVE FUNCTION
Navarro G, Raïch I, Lillo J, Rebassa J.B., Griñán-Ferré C, Bellver-Sanchis A, Reyes-Resina I, Franco R, Pallàs M.

P.24 A CONFORMATIONALLY CONSTRAINED ANANDAMIDE ANALOG COMBINES POTENT CB1 AGONISM WITH PRESERVATION OF MITOCHONDRIAL FUNCTION UNDER OXIDATIVE STRESS
Carandente Sicco S, Esposito D, de Bourg M, Gani O, Vik A and Ligresti A.

P.25 CANNABIDIOL TARGETS A VDAC1-ASSOCIATED MITOCHONDRIAL VULNERABILITY IN INVASIVE MELANOMA CELLS
Esposito D, Carandente Sicco S, Szász I, Koroknai V and Ligresti A.

P.26 THC IMPAIRS HIPPOCAMPAL MEMORY FORMATION BY DISRUPTING CA1 GLUTAMATERGIC SIGNALING.
Kouchaeknejad A, Yu J, Molina M, van der Walt A, Lago N, Albero R, Biever A, Valjent E, Cutando L, Puighermanal E.

P.27 THE IMPACT OF CANNABIDIOL ON NEUROPATHOLOGY IN A HUNTINGTON'S DISEASE MOUSE MODEL
Bonet-Freixinet J., DiFranco N., Ginés S.

P.28 HYPOTHALAMIC POMC NEURONS GATE THE FEAR-FEEDING TRADE-OFF.
Miralpeix C, Eraso-Pichot A, Simon V, Jouque V, Sadallah M, Clark S, Zizzari P, Skupio U., Covelo A, Dupuy N, Nicolas JC, Cannich A, Leste-Lasserre T, Laplagne G, Quarta C, Marsicano G, Cota D.

P.29 LOCOMOTOR RESPONSE FOLLOWING ORAL ADMINISTRATION OF CANNABIDIOL, PARACETAMOL, AND PHENYLBUTAZONE IN AN EXPERIMENTAL MODEL OF EQUINE LAMENESS
Miraz, R; Serrano-Rodríguez, JM; Sánchez de Medina, V; Ferreio, C; Mendoza, N; Muñoz, A; Sánchez de Medina, A

P.30 ASTROCYTIC MORPHOLOGY IN THE MEDIAL HABENULA: SEX DIFFERENCES AND MODULATORY FACTORS
Rodríguez-Cedrés C, Sangroniz-Beltrán L, Delgado-Martín N, López N, Ducourneau E, Ospital P, Beriain S, Piriz J, Marsicano G, Ferreira G, Mato S and Soria-Gómez E.

P.31 CBD-BASED MOLECULAR HYBRIDS INCORPORATING A SELECTIVE CB2 RECEPTOR AGONIST: LINKER-DEPENDENT MODULATION OF MICROGLIAL ACTIVATION
A. A. Corallo, N. Galeotti, F. Manetti, F. Pellati, P. Governa, A. Ligresti, C. Mugnain

CANNABINOIDS AND LIPID MEDIATORS IN CANCER: METABOLIC VULNERABILITIES AND THERAPEUTIC OPPORTUNITIES

Ligresti Alessia

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Cannabinoids and endogenous lipid mediators are emerging as promising pharmacological tools to investigate and potentially target mechanisms underlying cancer progression and therapeutic resistance. Their biological effects extend beyond canonical cannabinoid receptor signalling and involve multiple molecular pathways whose relevance depends on the tumour type, cellular phenotype and metabolic context.

This lecture will bring together findings from our research on phytocannabinoids, synthetic cannabinoids and endogenous lipid signalling across different cancer models, including breast, prostate, gastric and colorectal cancers, as well as melanoma. Rather than focusing exclusively on tumour cell proliferation and survival, these studies explore how cannabinoid-based pharmacological interventions can interfere with processes underlying tumour aggressiveness, invasive behaviour and resistance to treatment. Particular attention will be given to the identification of mitochondrial bioenergetic vulnerabilities, the ability of cannabinoid combinations to restore drug responsiveness in preclinical models, and the functional interplay between cannabinoid receptors and specialized pro-resolving lipid mediators. The latter reveals distinct mechanisms through which cannabinoid signalling can influence tumour angiogenesis and other malignant properties.

Together, these findings illustrate that the anticancer actions of cannabinoids cannot be attributed to a single molecular target or mechanism. Instead, they emerge from the interaction between pharmacological properties and tumour-specific biological vulnerabilities. Understanding this complexity is essential to move beyond the observation of anticancer effects and towards the identification of mechanistically informed therapeutic opportunities.

INVESTIGACIÓN CLÍNICA EN CANNABINOIDES

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Cannabinoid research has grown remarkably over the last decades, and the Spanish scientific community has contributed significantly to this progress, particularly at the preclinical level. However, this knowledge has not reached the clinic at the same pace. The typical challenges associated to any clinical research (difficulty securing funding, complex regulatory procedures, logistical complexity, etc.) are compounded by others specific to this field (additional administrative requirements arising from the controlled-substance status of many cannabinoids, limited availability of standardized, pharmaceutical-grade formulations, the social stigma that still surrounds cannabis, even among healthcare professionals, etc.).

The goal of this round table is to give visibility to the clinical research on cannabinoids that is already in progress in Spain, and to open a discussion on how to strengthen the still fragile bridge between the lab and the clinic.

The round table will feature our fellow SEIC members José Martínez Orgado, Head of the Neonatology Department at Hospital Clínico San Carlos (Madrid) and Principal Investigator of a study focused on developing an oral CBD formulation for the prevention of neonatal hypoxic-ischemic encephalopathy; Guillermo Velasco, Full Professor of Biochemistry and Molecular Biology at Complutense University of Madrid, involved in a phase Ib clinical trial aimed at assessing the safety and early signs of antitumor efficacy of a cannabinoid combination in patients with glioblastoma; and Adán de Salas Quiroga, neuroscientist and independent consultant, who will broaden the perspective on clinical research towards complementary approaches based on the analysis of real-world evidence obtained from routine clinical practice.

Keywords: Medical cannabis, clinical research

CANNABINOID TOLERANCE RELIES ON CB_1 RECEPTOR UBIQUITINATION BY NEDD4L

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Cannabinoids, the active components of cannabis, exert numerous acute effects in the brain by engaging cannabinoid CB_1 receptors (CB_1Rs). However, tolerance emerges rapidly after repeated drug exposure, undermining the efficacy of cannabinoid-based therapies and contributing to cannabis-associated adverse effects. Although the processes of CB_1R short-term desensitization (i.e., receptor uncoupling and internalization) are well characterized, the mechanisms underlying CB_1R long-term tolerance (i.e., downregulation of receptor protein levels) remain elusive. Here, we identify a ubiquitin-dependent pathway that couples CB_1R activation to its proteasomal degradation. We show that cannabinoids engage a $G_{q/11}$–PLC–PKC signaling cascade that phosphorylates and activates the E3 ubiquitin ligase NEDD4L, promoting its recruitment to CB_1R and the ubiquitination of four specific lysine residues. This modification targets the receptor for proteasomal clearance, reducing neuronal CB_1R abundance in vitro and in the mouse brain. Using molecular, pharmacological, and circuit-specific rescue approaches, we demonstrate that preventing CB_1R ubiquitination stabilizes receptor levels and abolishes behavioral cannabinoid tolerance in mice without impairing acute drug responses. These findings reveal a molecular mechanism that controls CB_1R stability and identify NEDD4L-mediated ubiquitination as a central driver of cannabinoid tolerance.

Keywords: CB_1 receptor; protein ubiquitination; drug tolerance

CANNABINOID CB2 RECEPTOR DRIVES TRASTUZUMAB RESISTANCE AND PREDICTS DURABLE ANTI-HER2 RESPONSE

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Acquired or innate lack of response to standard HER2-targeted therapies remains a clinical challenge in patients with HER2-positive breast cancer. Here, we investigated the role of the cannabinoid CB_2 receptor (CB_2R) in trastuzumab resistance. In human breast cancer samples, a decreased expression of HER2-CB_2R heterodimers following neoadjuvant treatment, due to CB_2R downregulation, was linked to poor long-term outcomes. Using various preclinical models, we demonstrate that CB_2R drives trastuzumab resistance. Mechanistically, CB_2R loss enabled cancer cells to evade antitumor IFN-γ signaling while promoting a shift from HER2-CB_2R to HER2-EGFR heterodimers, thus reducing dependence on HER2 and increasing reliance on EGFR-mediated pathways. Moreover, EGFR inhibition restored trastuzumab sensitivity. In summary, we reveal an unprecedented role for CB_2R as a key regulator of oncogenic and immune signaling in response to anti-HER2 therapy and its potential as a predictive biomarker of therapeutic efficacy. We also propose dual HER2/EGFR targeting and non-CB_2R-selective cannabinoid therapies as potential strategies to overcome CB_2R-mediated trastuzumab resistance. Together, these findings position the endocannabinoid system as a pivotal and actionable node to elucidate, anticipate, and counteract resistance to HER2-targeted therapies.

Keywords: breast cancer, receptor heterodimers, predictive biomarkers

DISCOVERY OF PERIPHERALLY RESTRICTED CB_1 RECEPTOR ANTAGONISTS THAT RESTORES INSULIN SIGNALING IN ADIPOCYTES

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Central CB_1R blockade can improve obesity-associated metabolic dysfunction, but first-generation antagonists such as rimonabant were limited by neuropsychiatric adverse effects.[1,2] We developed 53 rimonabant-inspired pyrazole derivatives using convergent Ugi-based multicomponent chemistry to combine CB_1R antagonism with physicochemical features favoring peripheral restriction. Several compounds showed potent, subtype-selective CB_1R binding. Most notably, the permanently charged piperazine-1-ium derivative 24a retained nanomolar affinity (K_i = 115 nM), high CB_1R/CB_2R selectivity, and an antagonist functional profile, while in silico and permeability analyses were consistent with limited blood-brain barrier penetration.

The metabolic relevance of **24a** was evaluated in differentiated C3H10T1/2 adipocytes with palmitate-induced lipid hypertrophy and insulin resistance. At 10-20 μM, 24a significantly restored insulin-stimulated Akt phosphorylation (Ser473) and increased HSL phosphorylation (Ser563) in a concentration-dependent manner, demonstrating recovery of insulin signaling and activation of lipolysis. These responses were comparable to or greater than those produced by rimonabant under identical conditions. Thus, permanent cationization can restrict CNS exposure without abolishing functionally relevant CB_1R pharmacology, identifying 24a as a promising peripherally directed lead for obesity and insulin resistance.

Keywords: CB_1 receptor, peripheral restriction, insulin resistance, obesity.

DEVELOPMENT OF CANNABINOID SUBTYPE-2 RECEPTOR (CB2R) FLUORESCENT PROBES: USEFUL TOOLS FOR NON-RADIATIVES ASSAYS

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Cannabinoid receptor subtype 2 (CB2R) is expressed at low levels in the CNS but is markedly upregulated under neuroinflammatory conditions associated with neurodegenerative diseases and cancer, making it an attractive therapeutic and diagnostic target. Radioligands have traditionally been employed to investigate CB2R biology; however, their application is constrained by safety and environmental considerations. Consequently, fluorescence-based techniques have rapidly emerged as safer and more versatile alternatives to radioligand-based approaches for investigating biological and pathological processes involving CB2R. Among the fluorescent compounds developed, two green-emitting ligands featuring an oxoquinolinonic scaffold, **SM15** (*N*-((3s,5s,7s)-adamantan-1-yl)-1-(5-(5-(dimethylamino)-1,3-dioxoisoindolin-2-yl)pentyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, K_i CB2R = 130 nM; K_i CB1R = 14% at 1 mM) and **VM7** (*N*-((3s,5s,7s)-adamantan-1-yl)-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide, K_i CB2R = 209 nM; K_i CB1R = 1% at 1 mM), demonstrated promising CB2R affinity and selectivity.[1,2] These probes were successfully applied in fluorescence-based saturation and competition binding assays using flow cytometric analysis. With the aim of developing red-emitting fluorescent probes for CB2R, structural modifications at the C-6 position were explored, including the replacement of the 7-nitrobenzofurazan (NBD) in VM7, group with its Se-containing isostere (Se-NBD).[3] However, the resulting derivative retained green emission, likely due to the direct conjugation of the NH group with the aromatic system. Nevertheless, the newly developed derivative combines nanomolar CB2R affinity with good fluorescence across different organic solvents and enables the discrimination of cellular models expressing different levels of the receptor in flow-cytometry assays. Overall, these findings establish the Se-bearing compound as a promising tool for CB2R detection and provide a rational basis for the development of optimized fluorescent ligands for receptor imaging, target validation, and related translational applications.

Keywords: cannabinoid receptor subtype 2; fluorescent probes; flow cytometry.

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DESIGN AND DEVELOPMENT OF NEW DUAL TARGET AGENTS, CANNABINOID SUBTYPE 2 RECEPTOR (CB2R) AGONISTS AND FATTY ACID AMIDE HYDROLASE (FAAH) INHIBITORS AGAINST NEURODEGENERATION

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Dual modulation of Cannabinoid subtype 2 Receptor (CB2R) and Fatty Acid Amide Hydrolase (FAAH) is an emerging strategy for the treatment of neuroinflammation. Based on our previously work [1,2], we developed a series of *N*-adamantyl-benzamides with an anthranilic or salicylic scaffold. Among them, salicylic derivatives **18**, **22,** and **25** displayed an optimal balance of CB2R potency, CB2R/CB1R selectivity, and FAAH inhibition [3]. These compounds were characterized for their functional selectivity, binding kinetics, CNS accessibility, and anti-inflammatory activity. Compounds **18** and **25** showed an unbiased CB2R agonism and FAAH inhibitory effect, whereas compound **22** showed signaling bias, behaving as a CB2R antagonist in the cAMP pathway and as a partial agonist in β-arrestin-2 recruitment. All three compounds significantly reduced inflammatory responses in monocytes, macrophages, and in a human activated microglia model. Overall, these results support dual CB2R/FAAH targeting as a promising approach for neuroinflammatory disorders and provide chemical tools to probe CB2R binding, kinetic profile, and biased signalling.

Keywords: *N*-adamantyl-benzamides, Dual target compounds, functional selectivity.

DESIGN OF DUAL CANNABINOID SUBTYPE 2 RECEPTOR (CB2R) AND SIGMA 1 RECEPTOR (S1R) LIGANDS AS NEW TARGETS FOR A THERAPEUTIC STRATEGY IN NEURODEGENERATION

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Cannabinoid subtype 2 receptor (CB2R), a key component of the endocannabinoidome, is predominantly expressed in immune cells, offering the chance to act on immunomodulation while excluding the psychoactive effects associated with the other subtype CB1R. While primarily peripheral, CB2R overexpression in neurodegenerative diseases highlights its potential as a pivotal biomarker for inflammation-based pathologies at earlier stages (1, 2). Similarly, Sigma-1 receptors (S1Rs) are pivotal players in inflammation. S1Rs are chaperone proteins that modulate many different cell survival mechanisms in response to oxidative stress, mitochondrial disfunction, accumulation of unfolded proteins and neuroinflammation, given their strategic location at the interface between mitochondrial and endoplasmic reticulum membranes (*MAMs*). S1Rs activation has proven to be essential to neuroprotection, synaptic plasticity and inhibition of cellular stress responses, making S1R a promising target to tackle neurodegenerative diseases (3). Noteworthy, our preliminary data showed that these two receptors, CB2R and S1R, may also co-localize in certain inflammatory states, paving the way to the design of dual drugs capable of simultaneously modulating both receptors as an effective strategy in the treatment of neurodegenerative disorders. Aim of this project is the development and the *in vitro* and *in vivo* biological evaluation of our dual targeting CB2R/S1R ligands as potential neuroprotective agents.

Keywords: multitarget, inflammation

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IDENTIFICATION AND EVALUATION OF POTENTIAL CANNABIDIOL-BASED MODULATORS FOR THE ORPHAN RECEPTOR GPR12

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GPR12 is a Class A orphan G protein-coupled receptor (oGPCR) considered to be constitutively active. Together with GPR3 and GPR6, it preferentially signals through Gs proteins and constitute a subgroup of homologous orphan receptors that belongs to the class A MECA cluster. GPR12 is mainly expressed in the central nervous system, where it has been associated with cognition and memory processes. Although the endogenous ligand of GPR12 remains unknown, its similarity to cannabinoid receptors CB1 and CB2 and its relationship with the endocannabinoid system have prompted the investigation of endogenous, phytogenic and synthetic cannabinoids as potential GPR12 modulators. Among them, cannabidiol (CBD) has attracted particular interest. Despite the limited knowledge of GPR12 pharmacology, its high constitutive activity highlights the potential of inverse agonists as receptor modulators. Therefore, this work aimed to identify and evaluate novel CBD-based GPR12 inverse agonists.

As no experimentally resolved inactive structure of GPR12 is currently available, an inactive-state model was generated using different approaches. A homology model based on the inactive GPR6 structure (PDB id 8T1W) was selected as the most reliable one, for which the inactive orthosteric binding pocket was defined. This structure was used for virtual screening of a library containing over 8,000 CBD-based compounds. A two-step molecular docking protocol, combining High-Throughput Virtual Screening and Extra Precision docking, was performed. Following successive filtering steps, two potential hits sharing the same core scaffold were selected for the design of a family of structural analogues.

The synthesis was proposed through a two-step retrosynthetic strategy involving Friedel-Crafts acylation of a carboxylic acid with a resorcinol derivative,followed by carbonyl reduction. To date, a family of 18 carbonyl-containing compounds were obtained. Their potential binding modes were assessed by molecular docking into the inactive GPR12 model.

The synthesised compounds are currently being evaluated through functional assays, including cAMP accumulation and β-arrestin recruitment assays. Their interaction with CB1 and CB2 is also being investigated to assess their selectivity towards GPR12. Future work will include completion of the reduction step, analysis of the biological evaluation data, and investigation of potential biased agonism.

Keywords: GPR12, inverse agonist, cannabidiol

SHEDDING LIGHT ON GPR18: NOVEL ASSAYS AND FLUORESCENT LIGANDS FOR AN ELUSIVE IMMUNE RECEPTOR

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GPR18 is an orphan G protein–coupled receptor (GPCR), predominantly expressed in lymphoid tissues, that remains poorly characterized despite increasing evidence supporting its relevance in immune regulation and cancer-related processes. It has attracted interest as a drug target[1] both in oncology — being overexpressed in several tumour types[2] and recently exploited as a metabolite-sensing receptor engineered into NK and T cells for solid tumour immunotherapy[3] — and in inflammation, where growing evidence places it in the resolution phase as a proposed receptor for resolvin D2.[4-7]
To date, only a limited number of GPR18 ligands have been identified, and the literature around this topic remains conflicting: endogenous lipid mediators, phyto- and synthetic cannabinoids, and a restricted set of synthetic small molecules.[8] Among the latter, our research group previously identified a series of pyrazolylresorcinol derivatives, structurally inspired by cannabidiol, as effective modulators of GPR18.
Here we summarize our efforts to characterize the receptor and to develop GPR18-targeting molecules. These include the first BRET-based G protein recruitment assay for GPR18, which revealed coupling to G_i, G_s and G_q proteins and allowed the agonist activity of cannabinoid ligands to be quantified; and the design and synthesis of the first fluorescent GPR18 probes, intended as pharmacological tools for binding studies and imaging for this receptor, and for the discovery and optimization of new ligands.

Keywords: GPR18, fluorescent ligands, BRET

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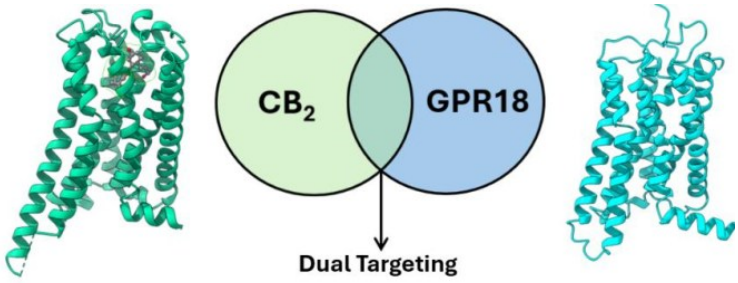
IN SILICO SCREENING AND QM/MM EVALUATION OF CHEMICAL LIBRARIES TO IDENTIFY NOVEL CB2/GPR18 DUAL AGONISTS

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Neuroinflammation is a complex pathological process where the cannabinoid receptor type 2 (CB2) and the orphan receptor **GPR18** play complementary immunomodulatory roles [1]. Both receptors are upregulated in activated microglia to promote a tissue-reparative state [2]. Identifying a polypharmacological ligand capable of simultaneously engaging both targets, **CB2 and GPR18,** could offer a highly synergistic strategy [3].

To identify novel **dual-modulators**, we deployed an advanced in silico **Structure-Based Drug Design** (SBDD) pipeline. Because GPR18 lacks an experimentally resolved structure, we generated an active-state model using **AlphaFold 3**. This model, alongside the active CB2 cryo-EM structure, was embedded in explicit lipid bilayers and subjected to 1 µs **Molecular Dynamics** (MD) simulations to capture physiological conformational ensembles. A curated database of structural analogues was then evaluated through a hierarchical **virt ual screening workflow** (HTVS, SP, XP) and thermodynamic **MM-GBSA** rescoring to identify promising dual-binding chemotypes.



To rigorously validate the atomic interactions governing dual-target recognition, top candidates were subjected to hybrid **Quantum Mechanics / Molecular Mechanics** (QM/MM) calculations and **Energy Decomposition Analysis** (EDA-NCI) [4]. This quantum-level evaluation revealed that successful dual-agonism relies on massive, dispersion-driven lipophilic anchoring to disrupt the deeply buried toggle switches (W6.48 in CB2; F6.48/M7.42 in GPR18). Simultaneously, the ligand must present a highly adaptable polar network to satisfy radically distinct electrostatic anchors: the rigid T3.33/S7.39 axis in CB2 and the basic R5.42/H6.52 cluster in GPR18. Together, these findings establish a computational and biophysical framework to guide the identification of novel chemotypes for next-generation neuroprotective therapeutics.

Keywords: Dual Agonists, Computational Chemistry, Virtual Screening, QM/MM

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CANNABINOID CB_2 RECEPTOR IS DIFFERENTIALLY EXPRESSED IN SPLENIC MURINE NK CELL POPULATIONS AND MODULATES ANTITUMOR EFFECTOR FUNCTION

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Breast cancer (BC) is the most frequently diagnosed malignancy in women. Triple negative breast cancer (TNBC) is an especially aggressive type of BC associated with a high mortality rate and metastatic disease development. Tumors develop within a complex environment, termed tumor microenvironment, made up of cancer, stromal and immune cells. Among the immune component, Natural Killer (NK) cells stand out for producing inflammatory cytokines. These have a direct cytotoxic effect over tumor cells inhibiting their proliferation and leading to apoptosis. Although breast tumors are usually characterized as immune-desert, TNBC has been found to be the most immune-inflamed BC subtype, making it a good candidate for immunotherapy-based treatment. Cannabinoid CB_2 receptor (CB_2R) is mainly expressed in the immune system and its activation is associated with suppression of immune function. In NK cells, CB_2R seems to enhance migration and modulate cytokine production. However, the exact implication of CB_2R in regulating NK cell effector function in the microenvironment of TNBC and its impact in disease progression has yet to be characterized. Thus, we aimed to decipher the role of CB_2R in regulating NK cell antitumor response. Here we describe a differential expression of CB_2R in the four subpopulations of murine NK cells determined by CD27 and CD11b staining, being almost restricted to double-negative NK cells. Moreover, NK cell CB_2R expression undergoes short-term changes upon stimulation with IL-2. Regarding modulation of antitumor activity, when presented with the TNBC cell line AT-3, CB_2R-KO NK cells show decreased killing capacity as opposed to WT cells. All in all, these results suggest an implication of CB_2R in the activation dynamics and effector function of NK cells and hint to its potential as a therapeutic target for development of NK cell-based immunotherapy against TNBC.

Keywords: Breast cancer, Tumor microenvironment, Natural Killer cells.

TOWARDS THE IDENTIFICATION OF NOVEL BIOMAKERS OF SENSITIVITY AND RESISTANCE TO THE ANTICANCER ACTIVITY OF CANNABINOIDS AND CHEMORADIOTHERAPY IN GLIOBLASTOMA

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Introduction

Glioblastoma (GBM) is the most frequent primary brain tumor and one of the most aggressive cancer types. However, these tumors display high resistance to conventional radiotherapy (RT) and temozolomide (TMZ) 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 CBD, another major ingredient of the hemp plant) together with TMZ or RT targets the population of GICs *in vitro* and strongly reduces the growth of GBM xenografts *in vivo*. These observations settled the bases for the development of clinical studies where the combined effect of THC:CBD + RT + TMZ was analyzed.

In this work we aimed at identifying novel biomarkers of resistance/sensitivity to the effect of cannabinoids in glioma models by using CRISPR/Cas9 libraries. The results were subsequently validated in preclinical models of glioma and correlated with a RNAseq analysis of samples from patients from the clinical study GEINOCANN.

CAS9-expressing U87MG and GIC primary cultures were infected with lentiviral vectors that containing genome-wide gRNA library and treated with THC, RT or TMZ. After DNA isolation genes associated with sensitivity and resistance to the different treatments were identified by NGS and subsequent bioinformatic analysis. In addition, the gene expression profile of patients from the GEINOCANN study was determined by RNAseq. By using the CRISPR/Cas9 library approach we identified a series of genes potentially associated with resistance and sensitivity to the different treatments. We also validate the participation of some of these genes in sensitivity and resistance to THC in GICs-derived models. Likewise, we identified gene expression profiles potentially associated to a worse and better response to the treatment with THC:CBD combined with RT and TMZ.

Our findings show the existence of gene expression profiles associated with resistance and sensitivity to the combination of THC:CBD + RT + TMZ.

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

GLOBAL AND BRAIN REGION-SPECIFIC ENDOCANNABINOID SYSTEM DYSREGULATION IN PRIMARY MITOCHONDRIAL DISEASE

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Primary mitochondrial diseases (MDs) are orphan conditions induced by inherited mutations in mitochondria-related genes, primarily presenting as progressive and fatal encephalomyopathies. We have previously shown that daily treatment with cannabidiol (CBD) extends survival and attenuates neuropathology in various MD models. While the expression of certain endocannabinoid system (ECS) components was altered in affected model brain regions, the exact contribution of the ECS to MD pathology and CBD's therapeutic mechanisms in these models remains unknown.

Here we present an integrated multi-omics study delineating a shared molecular signature across transcriptome and proteome datasets from six MD model tissues, showing a consistent downregulation in gene programs related to retrograde ECS signaling. The affected genes correlate with the core disease signature, characterized by exacerbated innate immune responses, altered ion homeostasis and dysregulated synaptic signaling. Targeted lipidomics confirms a global shift in endocannabinoid (eCB) tone in the whole brain of the Ndufs4KO mouse model of MD, while qPCR confirmation across various brain regions indicates that cannabinoid receptor-level responses to the global eCB shifts induced by MD and CBD treatment are markedly brain-region specific.

This study presents the first clear evidence of ECS involvement in MD pathology while reinforcing the notion that eCB-related mechanisms partially mediate the broad therapeutic benefits of CBD observed in various MD models. These findings underline the need for exploring the tissue- and cell type-specific activity profiles of ECS components towards establishing the ECS as a promising therapeutic target in MD.

Keywords: Mitochondrial disease, Endocannabinoid system, Cannabidiol, Multi-omics

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ASTROGLIAL CONTROL OF 2-ARACHIDONOYLGLYCEROL DRIVES CB_1-DEPENDENT OLIGODENDROGENESIS DURING DEVELOPMENTAL MYELINATION AND DURING REMYELINATION

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Efficient myelin formation and regeneration require coordinated communication between oligodendrocyte precursor cells (OPCs) and their surrounding glial niche, yet the extracellular signals that orchestrate this crosstalk remain incompletely understood. Cannabinoid CB1 receptor signaling modulates oligodendrogenesis, but the cellular source and regulation of the endogenous ligand that activates OPC CB1 receptors have not been identified. Here we show that astrocytes are an important cellular source of extracellular 2-arachidonoylglycerol (2-AG) and identify astroglial monoacylglycerol lipase (MGL) as a critical regulator of this paracrine pathway controlling developmental myelination and remyelination. Pharmacological MGL blockade at early `postnatal ages increased 2-AG-dependent oligodendrocyte (OL) differentiation, requiring diacylglycerol lipase (DGL)-mediated 2-AG synthesis and CB1 receptors expression in OPCs. Astrocyte-specific MGL gene inactivation elevated brain 2-AG levels selectively, and enhanced developmental oligodendrogenesis and myelination in vivo and in cerebellar organotypic cultures. Under toxin-induced demyelination, astroglial MGL deficiency similarly enhanced OL regeneration and remyelination in the cuprizone model in vivo and the lysophosphatidylcholine model ex vivo. Mechanistically, cocultures of astrocytes and organotypic slices showed that astrocytes generate a DGL-dependent 2-AG pool sufficient to activate CB1 receptors in neighboring OPCs and to promote myelin formation and regeneration. Together, these findings identify astrocytes as a source of 2-AG and establish an astrocyte-to-OPC lipid signaling axis that regulates CNS myelination and remyelination, nominating astroglial MGL as a potential target for promoting endogenous myelin regeneration in neurological disorders.

Keywords: 2-arachidonylglycerol, oligodendrocytes, (re)myelination.

ENDOCANNABINOIDS SIGNALING REGULATES SHARP-WAVE RIPPLES FUNCTION ACROSS LEARNING

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Learning and memory critically depend on hippocampal network dynamics. Sharp-wave ripples (SPW-Rs) are brief hippocampal network events essential for memory consolidation, characterized by highly synchronous neuronal firing and increased population spiking. Endocannabinoids (eCBs) are released during periods of elevated neuronal activity and act primarily through type-1 cannabinoid receptors to regulate presynaptic neurotransmitter release, particularly at inhibitory synapses formed by CCK/Id2 interneurons. Here, we hypothesize that elevated neuronal activity associated with SPW-Rs constitutes a key trigger for endocannabinoid release in CA1, positioning eCB signaling as a regulator of SPW-R–induced plasticity. To test this, we chronically implanted high-density silicon probes coupled with optical fibers in CA1, enabling simultaneous recording of extracellular neuronal activity and eCB dynamics using the GRABeCB3.0 sensor expressed in hippocampal neurons. Mice were recorded over a two-week period, encompassing sleep sessions and learning of a rewarded spatial alternation task. Furthermore, we performed pharmacological interventions using JZL, DIO, and rimonabant to manipulate eCB signaling, observing distinct changes including altered spike timing variability, phase modulation, and weaker synchronous population bursts. Notably, baseline eCB levels increased during SPW-R events and were further modulated in a learning-dependent manner. Consistent with their prominent expression of CB1 receptors, increased eCB signaling was associated with a reduction in CCK/Id2–pyramidal cell coupling. In turn, SPW-Rs with higher eCB levels were accompanied by a modest increase in the firing rates of superficial pyramidal cells. Together, these results reveal a ripple-associated endocannabinoid mechanism that selectively modulates inhibitory control onto pyramidal neurons during learning. Future experiments will knock down CB1 receptors in CCK/Id2 interneurons to directly test their causal role in inhibitory circuit plasticity.

EXPLORING COGNITIVE ALLEVIATION THROUGH THE MODULATION OF THE ENDOCANNABINOID SYSTEM DURING EARLY 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 cannabinoid type 1 receptors (CB1R) with the peripherally restricted 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 cognitive domains affected by aging including short-term and long-term spatial memory, recognition memory, and motor learning. To integrate the results of an individual across the various behavioral tests we developed an aging score. Using this framework, we investigated the potential of chronic peripheral CB1R inhibition with AM6545 in mitigating cognitive decline in male and female aged mice. Notably, chronic treatment with AM6545 promoted cognitive recovery in 18-month-old male mice, whereas no cognitive improvement was observed in females. Transcriptomic profiling of hippocampal synaptoneurosomes followed by Gene Set Enrichment Analysis (GSEA) demonstrated that treatment activated biological processes centered on mitochondrial respiration, oxidative phosphorylation, and ATP synthesis, while suppressing processes related to tRNA aminoacylation and regulation of microtubule-based processes. In addition, at the intestinal level, the treatment modified the expression of genes relevant for intestinal permeability. These results highlight a sex-dependent response to peripheral CB1R blockade and suggest this strategy may attenuate age-related cognitive impairment. Furthermore, they provide key mechanistic insights into the interplay between synaptic bioenergetics, gut-brain axis alterations, and healthy cognitive preservation during aging.

Keywords: aging, CB1 inhibition, cognition, sex-difference

MEDICAL CANNABIS IN OLDER ADULTS: REAL-WORLD EVIDENCE FROM A RETROSPECTIVE OBSERVATIONAL STUDY

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Older adults remain underrepresented in clinical trials despite their healthcare needs related to multimorbidity, polypharmacy, frailty, and symptom burden. Their participation in medical cannabis research has been further limited due to concerns regarding their increased susceptibility to cannabinoid-related adverse effects. Consequently, evidence to guide clinical decision-making in this population remains limited. Real-world observational studies can help address this gap by providing complementary evidence on the safety and clinical outcomes of medical cannabis in routine practice.

We conducted a retrospective observational descriptive study using medical records from a specialized medical cannabis practice in Colombia. Patients aged ≥65 years who initiated physician-supervised treatment with standardized cannabis between January 2024 and September 2025 were eligible. Baseline analyses included all eligible patients (n = 124); longitudinal analyses included those with at least one follow-up visit ≥3 months after treatment initiation (n = 44). Outcomes included safety, prescribing patterns, symptom evolution, clinically effective doses, and changes in concomitant medication.

Median age was 74 years, and women accounted for more than two-thirds of the cohort. The most frequent diagnoses were pain-related and musculoskeletal disorders, cancer and neurodegenerative and non-degenerative neurological disorders, and the predominant symptoms were pain, insomnia, and mood/behavioral. Oral oil-based formulations were the most prescribed. During follow-up, symptom severity decreased significantly across the three main symptom domains, with improvement in approximately 90% of patients. Documented clinically effective doses remained low, typically within a few mg/day of THC, CBD and/or CBG. Overall medication burden remained stable, although dose reductions or discontinuations of concomitant medications occurred in 25% of patients. Adverse events affected approximately one-third of patients, were mild or moderate, and were mainly somnolence and dizziness. No treatment-related serious adverse events, falls, or hospitalizations were recorded.

In this real-world cohort, medical cannabis was associated with favorable symptom evolution, low clinically effective doses, and an acceptable safety profile. Although causal inferences cannot be drawn, these findings add to the growing evidence that cannabis may represent a therapeutic option for selected older adults under specialist supervision. Prospective multicenter studies are needed to further define its benefit-risk balance.

Keywords: Medical cannabis, older adults, real-world evidence

CB_1 CANNABINOID RECEPTOR NEGATIVE MODULATION REGULATES CORTICAL AND BEHAVIORAL ALTERATIONS INDUCED BY SATB2 DEFICIENCY

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The disruption of the balance between the generation of superficial and deep-layer cortical neurons contributes to the etiopathology of neuropsychiatric disorders, including SATB2-associated syndrome (SAS). This syndrome is a rare and incurable disease characterized by intellectual disability and behavioral traits, such as autistic features, and is caused by mutations in the SATB2 gene locus. SATB2 encodes a transcription factor essential for the specification of interhemispheric projection neurons predominant in superficial cortical layers. Since the endocannabinoid system (ECS) regulates the differentiation and migration of pyramidal neurons and modulates synaptic activity, we hypothesize that regulating CB_1 cannabinoid receptor (CB_1R) during adolescence may attenuate some of the behavioral alterations associated to SAS.

We generated a conditional knockout mouse model lacking SATB2 in forebrain pyramidal neurons from early embryonic development (*Satb2$^{ff/}$;Emx-Cre*). Behavioral characterization shows that adult *Emx1-Satb2* deficient mice exhibit hyperactivity, increased impulsivity, reduced anxiety and impaired social interaction. Immunofluorescence studies confirmed that *Emx1-Satb2* deficient mice present a loss of superficial layer callosal neurons concomitant with an expansion of subcortical projecting deep layer neurons and revealed an increased expression of CB_1R. Hence, we administered between postnatal days 22-36 the CB_1R inverse agonist SR141716 or the CB_1R signaling-specific inhibitor AEF0117 and determined their impact on *Emx1-Satb2* deficient mice behavioral alterations. CB_1R negative modulation induces changes in postnatal mortality and weight gain during development in a sex-dependent manner. These were followed by rescue of some behavioral alterations in adulthood. At the cellular level, CB_1R inhibition induced changes in interneuron-specific synaptic markers, pointing to modulation of E/I balance as the mechanism underlying the cannabinoid-mediated regulation of SAS-like symptoms in females. Together, these findings highlight that modulation of endocannabinoid signaling constitutes a potential therapeutic strategy for managing specific symptoms of neurodevelopmental disorders such as SAS.

Keywords: cortical development, endocannabinoid system, SAS.

ASSESSMENT OF MOLECULAR SIGNATURES SENSITIVE TO CANNABIDIOL TREATMENT IN FRAGILE X SYNDROME

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Fragile X syndrome (FXS) is the principal monogenic cause of inherited intellectual disability and autism. It is caused by the CGG expansion in the Fragile X messenger ribonucleoprotein 1 (FMR1) gene leading to the hypermethylation and subsequent loss of its related protein, FMRP. The most prominent phenotypes of FXS include intellectual disability, susceptibility to epileptic seizures, hyperactivity and synaptic alterations. *Fmr1* knockout (FX) mouse model is a suitable tool in the preclinical evaluation of treatments because it recapitulates the cognitive impairment and altered synaptic plasticity observed in FXS. There are no approved pharmacological treatments for intellectual disability in FXS although promising targets have been described. Among the treatments under study, cannabidiol (CBD), given it is well-tolerated, continues under scrutiny at the clinical level to reduce FXS clinical features.
We set to assess the efficacy of CBD in improving memory performance in FX mice, and the outcome this treatment may have at gene and alternative splicing regulation in hippocampal synaptoneurosomes. We compared the results with CBD with those obtained for two other relevant drugs under study in FXS: the inhibitor of phosphodiesterase 4D (PDE4D) BPN14770 (BPN) and the metabotropic glutamate receptor 5 (mGluR5) antagonist CTEP. All three drugs were tested in novel object-recognition (NOR) memory after a single administration and seven-day later after seven days of treatment. CBD and BPN improved NOR memory in FX mice to levels similar to WT controls receiving vehicle while treatment resistance in terms of memory performance was developed after CTEP repeated administration. Since CBD and BPN approaches shared the alleviation of the cognitive phenotype in FX mice, we hypothesized that both drugs could result in comparable molecular effects. Paradoxically, we found that few synaptoneurosomal genes were differentially expressed in FX mice after treatments with CBD and BPN. Instead, alternative splicing landscape was highly modified in FX mice and also by the different effective treatments in FX hippocampal synaptoneurosomes including alterations in microexons. By comparing the common alternative splicing reversion of FX genotype by effective treatments (CBD and BPN) with those not modified in treatment resistance conditions (CTEP), we pinpointed potential molecular signatures linked to treatment efficacy including relevant genes such as *Gatad1*, *Rtkn*, *Med23* and *Pax6*. To assess the translational relevance of these signatures, we compared this set of splicing events sensitive to effective treatment in mice with *in silico* alternative splicing alterations in *post-mortem* hippocampal synaptoneurosomes from FXS human subjects. As a result, we identified 2 orthologous genes presenting conserved synaptic splicing alterations in both species: Rhotekin (RTKN) and Paired box 6 (PAX6). Together, we identified molecular signatures that may predict cognitive treatment efficacy in the context of FXS.

Keywords: Fragile X syndrome; cannabinoid type-1 receptor; cannabidiol, alternative splicing

PATIENT-DERIVED NEURONS AS A PLATFORM TO INVESTIGATE THE EFFECTS OF CANNABINOIDS ON NEUROINFLAMMATORY SIGNALING

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The endocannabinoid system has emerged as a key regulator of neurodevelopment, neuronal activity, and neuroimmune responses, making it an attractive target for the development of novel therapeutics for neuropsychiatric disorders. Among phytocannabinoids, Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most extensively investigated compounds, yet their direct effects on neuronal inflammatory signaling remain poorly understood. This is particularly relevant in schizophrenia, a chronic and severe psychiatric disorder in which neuroinflammation has been proposed as a contributing pathogenic mechanism.

To investigate cannabinoid-mediated modulation of neuronal inflammatory responses, we employed neuron-enriched cultures derived from the olfactory neuroepithelium (OE) of schizophrenia patients and matched controls. The OE represents a unique and minimally invasive source of patient-derived neural cells, providing an accessible platform for translational studies of neuronal function and disease-related mechanisms. Cytokine secretion profiles were assessed under basal conditions and following acute exposure to THC and CBD.

Neuron-enriched cultures isolated via nasal exfoliation from human olfactory neuroepithelium of schizophrenia patients and healthy controls successfully secreted detectable levels of inflammatory cytokines into the culture media, quantified via the Ella Automated Immunoassay System. Total protein quantification and MTT experiments confirmed no significant cell death or neurotoxicity following 24-hour acute exposure to varying doses of phytocannabinoids (CBD: 0.1, 10, 50 μM; Δ^9-THC: 0.1, 1, 10 μM) or antipsychotics (clozapine: 0.1, 1, 25 μM; aripiprazole: 0.1, 1, 10 μM).

Following cannabinoid treatment, a decrease in pro-inflammatory IL-6 levels was detected across control subjects for both CBD and THC after 24 hours, with THC maintaining a sustained effect. A similar downward trend in IL-6 was observed in the schizophrenia subject. However, no significant alterations were observed in IL-10 or IL-1β concentrations across doses or time points, whereas TNF-α levels remained below the immunoassay detection limit.

This study establishes OE-derived neuronal cultures as a valuable human model for investigating cannabinoid-neuron interactions and neuroinflammatory mechanisms. Furthermore, it supports the use of patient-derived neuronal systems to explore the cellular and molecular actions of phytocannabinoids and their potential relevance in neuropsychiatric disorders.

DISENTANGLING THE LINK BETWEEN CANNABIS AND PSYCHOSIS: FROM MEDIATED LEARNING TO REALITY TESTING

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Cannabis is the most cultivated, trafficked and used illicit drug worldwide, and its use, particularly during adolescence, has been associated with an increased risk of psychotic symptoms. However, the neurobiological mechanisms underlying positive psychotic-like states remain poorly understood, limiting the pharmacological options for non-responders. Recently, higher-order conditioning has been proposed as a translational model of positive psychotic-like behaviors.

In rodents, these processes can be investigated using sensory preconditioning paradigms, in which associations between neutral stimuli later evoke conditioned responses through mediated learning (ML). Increasing these associations during the preconditioning phase promotes a transition from ML to reality testing (RT), a process that improves discrimination between real and inferred stimuli and that is commonly impaired in psychotic-like states.

Here, we established a light–tone sensory preconditioning task in male mice, along with a RT protocol involving extended preconditioning associations. Acute administration of Δ9-tetrahydrocannabinol (THC) (1 mg/kg) disrupted RT, indicating altered processing of internal sensory representations. Notably, this impairment was reversed by the cannabinoid receptor type-1 (CB1R) inverse agonist rimonabant (2 mg/kg), demonstrating the CB1R role in this effect.

Interestingly, using the TRAP2 mice, which allow you to permanently tag neurons that were active during a specific time window, we collected brains from mice that experienced a moderate versus an extended preconditioning session of light-tone pairings. Preliminary data shows how the number of the pairings during the preconditioning shapes brain activity in different brain regions, which might indicate their involvement during light-tone associations. Furthermore, we found that the activity of specific regions seems to be specifically modulated not only by the extension of the pairings but also by the effect of THC in RT.

This whole brain cfos-based activity analysis will allow us to study which are the key brain regions involved in the RT mental process and how they are specifically affected by THC. Future experiments will try to manipulate specific cell-types from these regions trying to establish new causal links between THC and its psychotic-like effects.

Keywords: THC, higher order conditioning, TRAP2 mice

AGE-ASSOCIATED ALTERATIONS IN MESOACCUMBAL DOPAMINE DYNAMICS AND THEIR CANNABINOID MODULATION IN APP/PS1 MICE

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Behavioral and psychological symptoms of dementia substantially contribute to the clinical burden of Alzheimer's disease (AD), yet their neurobiological substrates remain poorly understood. Alterations in mesolimbic dopaminergic function may contribute to these manifestations and represent a potential target for cannabinoid modulation. Here, we characterized evoked dopamine (DA) dynamics in the nucleus accumbens (NAcc) of APP/PS1 mice at 6 and 12 months of age using the genetically encoded fluorescent sensor dLight1.3b and investigated their modulation by low-dose Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). At 6 months, APP/PS1 mice exhibited shorter NAcc DA transients following ventral tegmental area (VTA) stimulation, whereas responses to direct NAcc stimulation and VTA TH+ cell numbers were preserved, indicating an early functional alteration in the absence of detectable dopaminergic cell loss. At 12 months, reduced DA responses emerged following direct NAcc stimulation and coincided with decreased VTA TH+ cell numbers. Acute Δ9-THC + CBD administration enhanced VTA-evoked DA responses in 6-month-old APP/PS1 mice, an effect no longer significant following CB1R antagonist pretreatment. However, chronic Δ9-THC + CBD treatment from 6 to 12 months did not produce detectable changes in mesoaccumbal DA dynamics or consistently improve social behavior or sensorimotor gating, despite improving recognition-memory performance. These findings reveal distinct stage-associated patterns of mesoaccumbal DA dysfunction in APP/PS1 mice and indicate that acute cannabinoid sensitivity does not necessarily translate into sustained modulation after chronic treatment, highlighting the importance of disease stage, treatment duration, and symptom domain when evaluating cannabinoid-based approaches in AD.

Keywords: Alzheimer, dopamine, cannabinoids, non-cognitive symptoms of dementia

OX_2R ACTIVATION ENHANCES CB_2R-MEDIATED ANTI-INFLAMMATORY EFFECTS IN A 5xFAD MOUSE MODEL OF ALZHEIMER'S DISEASE

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The endocannabinoid receptor CB_2 is primarily expressed in glial cells within the Central Nervous System. Its expression increases further when these cells are activated during inflammation. Among the conditions that induce inflammation, Alzheimer's disease is prominent. This disease is also marked by changes in the sleep-wake cycle, which is regulated by the orexinergic system. Activation of the OX_1R receptor appears to promote beta-amyloid peptide accumulation due to sleep disturbances. However, the role of the OX_2R receptor remains uncertain.

In this study, we evaluated the roles of CB_2R and OX_2R receptors in Alzheimer's disease. Our results indicate that both receptors exert beneficial effects by reducing neuronal death and oxidative stress in primary cultures of activated microglia with LPS and IFN-γ and in 5xFAD mice model of AD. Furthermore, activation of the CB_2R receptor polarized microglia toward a neuroprotective M2 phenotype. These effects were even more pronounced when activated microglia were co-treated with agonists for both receptors, leading to decreased ROS production, reduced microglial inflammation, and lower levels of cell death. These outcomes can be explained by direct interaction between the two receptors, resulting in a positive cross-talk.

In conclusion, these findings present the CB_2R–OX_2R heteromer as a novel therapeutic target for combating inflammation and reducing the symptomatology of Alzheimer's disease.

Keywords: Alzheimer's disease, cannabinoids, OX_2R.

GPR12 EXPRESSION IN HUMAN TISSUE AND MURINE MODELS IN PARKINSON'S DISEASE

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GPR12 is an orphan G-protein-coupled receptor (GPCR) that appears to be related to the cannabinoid receptor family, based on its close phylogenetic relationship and substantial structural similarity to CB1 and CB2, approximately 40% sequence identity within their transmembrane regions. As well as the reported ability of cannabinoids such as cannabidiol (CBD) to modulate its activity. GPR12 also shares substantial sequence similarity with GPR6, a closely related GPCR implicated in Parkinson's disease (PD) and currently being investigated as a potential therapeutic target in clinical trials. Importantly, GPR12 is highly expressed in the basal ganglia, particularly in the substantia nigra and striatum, two brain regions critically affected during PD progression. Together, the structural relationship of GPR12 with the cannabinoid receptors and GPR6 and its expression in PD-relevant brain regions suggest that GPR12 may play a role in PD-related pathology.

In this study we investigated GPR12 expression in three murine models that display distinct pathological events of PD: neuroinflammation, oxidative stress and α-synuclein aggregation. GPR12 expression was assessed by immunofluorescence, including colocalization with astrocytic markers. To determine whether these alterations were also present in human disease, GPR12 genic expression and localization through immunofluorescence was analysed in post-mortem human samples from PD patients.

In murine models, GPR12 immunoreactivity showed a significant increase in the lesioned substantia nigra, with partial colocalization with astrocytic markers. Interestingly, this pattern contrasted with the human samples, in which GPR12 mRNA levels were significantly reduced in the midbrain. These findings suggest that GPR12 expression is altered in PD-related pathological conditions and support further investigation into its relevance in Parkinson's disease.

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Keywords: GPR12 expression; Parkinson's Disease; murine models, human

CANNABINOID MODULATES HIPPOCAMPAL ASTROCYTES IN HUNTINGTON'S DISEASE VIA MITOCHONDRIAL FUNCTION?

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Huntington's disease (HD) is a rare genetic disorder classically considered a striatal neuron-centered neurodegenerative disease, with motor symptoms being one of its main clinical manifestations. HD is caused by an aberrant expansion of CAG repeats in exon 1 of the huntingtin gene, leading to a wide range of symptoms, including hippocampal-memory dysfunctions. Unfortunately, there are currently no effective treatments for the cognitive deficits that severely affect patients' daily lives, including hippocampal-dependent memory functions. Recent evidence has shown that HD also affects other brain regions, such as the hippocampus, where non-neuronal cells may play a crucial role in the hippocampal memory deficits observed in HD, potentially even before the onset of motor symptoms.

Interestingly, we found that cannabinoid receptor 1 (CB1) is decreased in HD mouse models and in post-mortem human HD brains, particularly in the hippocampus, in both neurons and astrocytes. Restoring CB1 specifically in hippocampal astrocytes improves memory functions in HD mice. In this project, we aim to: 1) characterize CB1 expression and function in astrocytes, both in vivo and in vitro; 2) investigate the effects of CB1 modulation on gliotransmission and calcium release; and 3) assess the effects of astrocytic CB1 modulation on mitochondrial respiration and mitochondrial health.

We found that increasing CB1 expression specifically in hippocampal astrocytes was sufficient to improve memory function in HD mice. Furthermore, the cannabinoid agonist WIN was able to modulate neurotransmission, calcium signaling, and mitochondrial respiration in hippocampal astrocytes from HD mice. These findings suggest that, although CB1 expression is reduced in HD, the remaining receptor is still functional and its modulation plays a crucial role in hippocampal astrocyte function.

Overall, our results suggest that cannabinoid signaling in astrocytes may regulate hippocampal function in HD, potentially through the modulation of mitochondrial respiration. These 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.

Elucidating the contribution of cannabinoid-induced mitochondrial signaling in hippocampal astrocytes could be useful for developing therapeutic strategies to rescue cognitive deficits in HD.

CELLULAR MECHANISMS OF THE NEUROPROTECTIVE EFFECT OF CANNABIDIOL IN A RAT MODEL OF INTRAVENTRICULAR HEMORRHAGE– INDUCED BRAIN INJURY IN EXTREMELY PRETERM INFANTS

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Intraventricular hemorrhage (IVH) is the most common neurological condition in preterm newborns. The onset of IVH triggers a cascade of pathophysiological events that lead to inflammation, oxidative stress, and excitotoxicity, along with disruption of the blood–brain barrier (BBB) and cell death. These processes result in cognitive and motor sequelae such as Cerebral Palsy. Cannabidiol (CBD) has shown neuroprotective effects by reducing lesion volume and improving both pathophysiological outcomes and motor function in an experimental neonatal rat model of IVH; however, the cellular mechanisms underlying these effects remain uncharacterized. To explore these mechanisms, it was studied the involvement of four cellular receptors proposed as potential CBD targets: CB_2, 5-HT_{1A}, PPARγ, and TRPV1.

IVH was induced in P1 Wistar rats via paraventricular injection of 0.2 U of *Chlostridium collagenase* into the germinal matrix. Animals exposed to anaesthesia but not to surgery remained as controls (SHAM). Vehicle (VEH) or CBD were administered prenatally (10mg/kg i.p.) and post-insult (5mg/kg i.p. 1, 24 and 48h post-HIV). Some rats received CB_2, 5-HT_{1A}, PPARγ or TRPV1 antagonists AM630 (5mg/kg), WAY100635 (5mg/kg), GW9662 (5mg/kg) or SB366791 (1mg/kg), respectively. The antagonists were injected 1h pre-insult and 1h prior to VEH or CBD administration during the next 2 days. Brain injury was assessed at P6 using Nuclear Magnetic Resonance (NMR) image and at P14 exploring motor coordination (negative geotaxis) and grip strength, as well as grip and grasp reflexes. Inflammation and BBB impairment were analysed using Immunofluorescence (IBA1, GFAP), Western blot (TLR4, MMP9, MFSD2A) and RT-qPCR of several cytokines as well as microglial and vascular genes. Oxidative stress was evaluated through protein carbonylation (OxyBlot), and metabolic damage was assessed by NMR spectroscopy. The analyses were performed at P6 and P14.

IVH led to brain injury accompanied by motor deficits, microglial and astrocytic activation, increased proinflammatory response, BBB disruption, oxidative stress, and metabolic changes associated with excitotoxicity and neuronal damage. CBD treatment was neuroprotective in all the parameters assessed. Co-administration of CBD with the CB_2, 5-HT_{1A}, PPARγ, or TRPV1 antagonists resulted in loss of CBD neuroprotection. CB_2 and PPARγ blockade abolished CBD effects on inflammation, gliosis, oxidative stress and BBB, whereas 5-HT_{1A} and TRPV1 antagonism blunted CBD action on excitotoxicity, neuronal integrity, and BBB preservation; although all the receptors were involved in improvements of structural damage and motor function.

CBD exerts protection against neonatal IVH-induced brain injury through the coordinated interaction of CB_2, 5-HT_{1A}, PPARγ, or TRPV1. No single receptor accounted for all of CBD's effects, suggesting that its neuroprotective response relies on complementary mechanisms, with each receptor involved in distinct biological processes.

Key words: Cannabidiol, neuroprotection, CB_2, 5-HT_{1A}, PPARγ, TRPV1

CHARACTERIZATION AND MODULATION OF THE ENDOCANNABINOID SYSTEM IN AN ANIMAL MODEL OF INTRAVENTRICULAR HAEMORRHAGE OF EXTREME PRETERM NEWBORNS.

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Intraventricular Hemorrhage (IVH) is a frequent complication in extremely low gestational age newborns, increasing the risk of Cerebral Palsy (CP), due to White Matter injury after enhanced neuroinflammation. The endocannabinoid system (ECS), in particular CB_2 receptors (CB2R) and some of its ligands as 2-AG, plays an important role–the modulation of neuroinflammation in newborn rat brain after hypoxic-ischemic brain damage. However, there is no information regarding ECS response to acquired brain damage in very immature rat brain.

To assess ECS responses to IVH-induced brain damage in the area of damage (Striatum) of immature newborn rats, and the effects of its modulation.

IVH was induced in immature Wistar rats (P1) by injecting *Chlostridium* collagenase into the left Germinal Matrix by stereotactic surgery (GMH). Non-injected animals served as controls (SHAM). Temporal analysis were assessed, all experiments were performed at: 3HPI, 12HPI, PND2, PND5 and PND7. RNAseq and Western Blot was performed on samples from ipsilateral striatum to determine CB1R, CB2R, NAPE-PLD, DAGLα, DAGLβ, FAAH and, MAGL expression. Brain damage was assessed in that area by determining Caspase 3-Activated, TLR-4 and COX-2 expression, AA concentration, protein carbonylation expression and microglial phenotype. Brain concentration of endocannabinoids (eCBs) such as AEA, 2-AG, 2-DHG and DHEA were quantified using mass spectrometry. Cell localization of CB2R was carried out by Immunofluorescence co-localization of CB2R marker together with Iba-1, GFAP, Sox-10 and NeuN. To modulate the enzyme MAGL, the inhibitor JZL184 was used (3 doses), and results as inflammation, oxidative stress, microglial phenotype and CB2R-2-AG pathway were assessed as in the previous step.

GMH triggered a rapid but transient endocannabinoid response in the core of the injury. This response was dominated by an early 2-AG peak that fails to be sustained (though 2-DHG and DHEA present a transient compensatory activation) collapsing into a state of net endocannabinoid depletion, associated with sustained high MAGL activity and a metabolic shift toward AA-derived, pro-inflammatory eicosanoid signalling. In this context, CB2R overexpression emerged as a delayed, partially effective compensatory mechanism in M1 microglia, engaged once the TLR4/COX-2 inflammatory cascade and oxidative damage were established, and closely linked to the recovery of the neuronal population via direct antiapoptotic action in surviving neurons. Targeting MAGL in GMH effectively reduced the inflammatory and apoptotic components of the damaging process in the injury site changing microglial phenotype to M2, and preventing the activation of the 2AG/CB2R axis, but was insufficient to completely neutralise oxidative damage. The 2-AG/CB2R/MAGL axis emerged as a genuine, endogenous defence mechanism against GMH in the very immature brain.

Keywords: Intraventricular hemorrhage, cannabinoid system, 2-AG, CB2R, MAGL, newborn rats.

CARDIOPROTECTIVE EFFECTS OF CANNABIDIOL FOLLOWING NEONATAL HYPOXIC-ISCHEMIC INJURY

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hypoxic-ischemic encephalopathy (HIE) is a major cause of neonatal morbidity and mortality and frequently involves multiorgan dysfunction, including cardiac injury. Cardiovascular dysfunction is a clinically relevant determinant of outcome in infants with HIE, with myocardial dysfunction and heart failure being independently associated with increased mortality and adverse neurological outcomes. Moreover, impaired cardiac function may further aggravate brain injury by compromising cerebral perfusion. Despite therapeutic hypothermia, the current standard of care, a substantial proportion of infants with HIE continue to experience death or long-term neurological impairment. Cannabidiol (CBD) has shown promising neuroprotective effects against hypoxic-ischemic brain injury in experimental neonatal HIE; however, whether these benefits extend to the cardiovascular system remains largely unexplored.

To investigate the potential cardioprotective effects of CBD following neonatal HI.

Acute HI was induced in 2–3-day-old newborn piglets by bilateral carotid artery occlusion combined with exposure to hypoxia (10% O_2) for 30 min. Thirty minutes after the end of the HI insult, piglets were randomly administered intravenous CBD (1 mg/kg) or vehicle. Non-manipulated 3-day-old piglets served as controls. Piglets were monitored for 6 h following the intervention, with continuous assessment of hemodynamic parameters (heart rate and arterial blood pressure), tissue perfusion and oxygenation (near-infrared spectroscopy, NIRS; fractional tissue oxygen extraction, FTOE), and metabolic status (blood gas analysis), together with cerebral activity using amplitude-integrated electroencephalography (aEEG). At the end of the experimental period, the heart was collected for histological and biochemical analyses. Cardiac apoptosis was assessed by TUNEL immunohistochemical staining and Western blot quantification of cleaved caspase-3, a marker of apoptotic activation. Myocardial injury and inflammatory response were also evaluated by Western blot analysis of cardiac troponin I (cTnI), a marker of myocardial injury, and TNF-α, a pro-inflammatory cytokine, respectively.

From a clinical perspective, CBD prevented HI-induced hypotension and preserved systemic tissue perfusion, as assessed by NIRS. CBD also attenuated the systemic metabolic derangement associated with HI, as evidenced by normalization of blood pH and lactate levels. These findings were further supported by biochemical analyses, showing that CBD attenuated cardiac apoptosis, as evidenced by normalization of cleaved caspase-3 expression and TUNEL-positive cells. This was associated with reduced myocardial injury, reflected by decreased cTnI expression, and attenuation of the inflammatory response, as indicated by reduced TNF-α expression.

In a neonatal HI piglet model, CBD exerts cardioprotective effects by attenuating myocardial ischemic injury, resulting in improved hemodynamic stability.

These findings suggest that CBD may represent a promising therapeutic strategy to protect the cardiovascular system following neonatal HI and potentially contribute to improved neurological prognosis.

Keywords. Hypoxic-ischemic injury, cardioprotection, cannabidiol, newborn piglets.

LONG-TERM BEHAVIORAL AND HISTOLOGICAL OUTCOMES AFTER URB447 ADMINISTRATION IN A PRECLINICAL MODEL OF NEONATAL HYPOXIC-ISCHEMIC BRAIN INJURY

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Neonatal hypoxic-ischemic encephalopathy (HIE) remains a major cause of infant mortality and long-term neurodevelopmental disability. Although therapeutic hypothermia is the standard of care, its neuroprotective efficacy is limited. Therefore, alternative strategies are critically needed to treat neonatal HIE. Here, we evaluated the long-term neuroprotective capacity of the synthetic cannabinoid URB447, a simultaneous CB1-antagonist/CB2-agonist, in mitigating functional deficits and reducing brain injury in a preclinical model of neonatal HIE.

Unilateral hypoxic-ischemic (HI) brain injury was induced in postnatal day 7 (P7) Sprague Dawley rats using the Rice-Vannucci model. Then, 3 hours after HI, rats received either vehicle or 1 single dose of URB447 (i.p., 1 mg/kg). Long-term motor and cognitive outcomes were evaluated at P42 (young adulthood) and P90 (adulthood) using a battery of four behavioral tests. Then, the extent of the brain lesion, specifically in the cortical and hippocampal regions, was analyzed by histological studies at P90.

HI animals showed progressive motor and cognitive impairments at both P42 and P90. URB447 treatment significantly attenuated the behavioral deficits after HI brain injury. Histological evaluation at P90 demonstrated that URB447 administration markedly reduced brain damage, specifically in the cortical and hippocampal regions, obtaining more than 80% of neuroprotection within these regions. In addition, histological damage correlated with the overall behavioral burden, confirming that functional outcomes were reflected in the neuropathology.

In conclusion, URB447 administration provides long-lasting neuroprotective effects by promoting behavioral improvement and reducing brain injury following neonatal HIE, supporting the potential of targeting the endocannabinoid system as a viable neuroprotective strategy for neonatal HIE.

Keywords: Neonatal brain injury, neuroprotection, behavior, histology.

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PERIPHERAL EXPRESSION OF ENDOCANNABINOID AND INFLAMMATORY BIOMARKERS IN COMPLETED SUICIDE: A POSTMORTEM FORENSIC STUDY

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Suicide is a multifactorial phenomenon involving neurobiological, inflammatory, and psychosocial mechanisms. Increasing evidence suggests that the endocannabinoid system plays a relevant role in mood regulation, stress response, and suicidal behavior. However, the forensic utility of endocannabinoid biomarkers in completed suicide remains poorly understood.

A cross-sectional postmortem study was conducted on 66 forensic cases, including completed suicide (n = 25) and controls who died from natural causes (n = 14), accidental causes (n = 12), or agitated delirium (n = 15). Gene expression was quantified in peripheral blood samples using RT-qPCR. Targets included cannabinoid receptors (CNR1, CNR2, GPR55), cannabinoid enzymes (DAGLA, DAGLB, FAAH, MGLL), inflammatory mediators (CX3CL1, CX3CR1, CCL2, CXCL12, IL4, IL10, TNFα, IL6) and neurobiological markers (BDNF, HTR3A, TLR4). Statistical analyses included ANOVA, ANCOVA adjusted for age, post hoc comparisons, and binary logistic regression models.

Several genes showed significant alterations according to cause of death. Suicide cases exhibited reduced expression of CNR1, DAGLA, DAGLB, MGLL and CX3CR1, together with increased expression of CX3CL1 and CXCL12, suggesting dysregulation of both immune and endocannabinoid pathways. Post hoc analyses revealed significant differences in CNR1, CNR2 and FAAH expression between suicide and agitated delirium groups. Logistic regression identified DAGLA as the most relevant predictor distinguishing suicide from accidental causes of death, as well as DAGLB, MGLL and GPR55 as the most relevant predictors distinguishing suicide from natural death. Models comparing suicide with natural and accidental deaths achieved excellent discrimination, reaching AUC values of 1.0 and very high sensitivity and specificity.

Alterations in peripheral DAGLA and DAGLB expression and its interaction with inflammatory biomarkers support a role for the endocannabinoid system in the biological signature of completed suicide. These findings suggest that endocannabinoid-related biomarkers, particularly those cannabinoid enzymes that produce monoacylglycerols (2-AG, 2-LG, 2-OG), may contribute to forensic identification and risk stratification models of suicidal behavior.

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Keywords: Endocannabinoid system; CNR2; CB2 receptor; suicide; postmortem blood; forensic biomarkers; neuroinflammation.

CONTRIBUTION OF THE A_{2A} RECEPTOR TO THE EFFECTS OF Δ^9-THC EXPOSURE DURING ADOLESCENCE

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Adolescence is a period of increased vulnerability to the adverse effects of cannabis, yet the neurobiological mechanisms underlying this susceptibility remain poorly understood. Previous studies have suggested a reciprocal antagonistic functional interaction between the adenosine A_{2A} receptor ($A_{2A}R$) and the cannabinoid type 1 receptor (CB_1R), raising the possibility that $A_{2A}R$ activation may modulate the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive component of cannabis and a partial CB_1R agonist. In this context, the present study aimed to evaluate the impact of $A_{2A}R$ activation on the acute and long-term effects of Δ^9-THC exposure during adolescence.

In vivo electrophysiological recordings showed that acute Δ^9-THC administration (2 mg/kg, i.v.) increased the firing activity of ventral tegmental area (VTA) dopaminergic neurons in late, but not early, adolescent male rats. This effect was prevented by the $A_{2A}R$ agonist CGS21680 (0.05 mg/kg, i.v.). To determine whether developmental changes in receptor expression could underlie these age-dependent effects, we quantified $A_{2A}R$ and CB_1R mRNA levels using RNAscope® in male mice across developmental stages, from early adolescence to adulthood, in brain regions involved in the modulation of VTA dopaminergic activity. We found a main effect of age on $A_{2A}R$ expression and the $CB_1R/A_{2A}R$ ratio in the VTA, but not in the prelimbic cortex, nucleus accumbens, or hippocampus. Specifically, late-adolescent male mice showed the lowest $A_{2A}R$ expression and the highest $CB_1R/A_{2A}R$ ratio in the VTA.

We then tested whether pharmacological stimulation of $A_{2A}R$ could modulate long-term Δ^9-THC-induced deficits in social interaction, a behavior known to involve VTA dopaminergic signaling. Chronic co-treatment with CGS21680 (0.1 mg/kg/day, i.p.) failed to prevent the social interaction deficits induced by adolescent Δ^9-THC administration (5 mg/kg/day, i.p.; PND28–60) in adult mice. Moreover, chronic CGS21680 treatment during adolescence impaired memory and sociability in adulthood, independently of Δ^9-THC exposure.

Together, these findings suggest that $A_{2A}R$ contributes to the age-dependent regulation of the acute effects of Δ^9-THC on VTA dopaminergic activity during adolescence. However, $A_{2A}R$ activation did not prevent the long-term social deficits induced by adolescent Δ^9-THC exposure. Furthermore, the behavioral alterations induced by chronic $A_{2A}R$ stimulation suggest that $A_{2A}R$ signaling during adolescence may contribute to the maturation of cognitive and social functions.

Keywords: Δ^9-tetrahydrocannabinol (Δ^9-THC), adolescence, adenosine A_{2A} receptor ($A_{2A}R$)

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 such as anxiety or depression. The Medial Habenula (MHb) is a sub-ventricular structure coding stress-related stimuli. 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. Previously, we 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).

DIFFERENTIAL MODULATION OF CB1 AND CB2 RECEPTORS SIGNALING BY PHYTOCANNABINOIDS: RELEVANCE FOR NEURODEGENERATIVE DISEASES

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Neurodegenerative disorders such as Alzheimer's disease (AD) are characterized by progressive neuronal dysfunction and remain without sufficiently effective disease-modifying therapies. Phytocannabinoids derived from Cannabis sativa have attracted increasing attention due to their potential neuroprotective properties.

In this study, we systematically evaluated nine phytocannabinoids to characterize their signaling profiles at CB_1R and CB_2R and investigated their neuroprotective effects in primary cortical neurons. In HEK-293T cells expressing CB_1R or CB_2R, phytocannabinoids differentially inhibited cAMP production and promoted β-arrestin II recruitment. Distinct efficacy profiles were observed between receptors and signaling pathways, supporting ligand-dependent functional selectivity.

To assess their relevance in AD-related pathology, primary mouse cortical neurons were exposed to $A\beta_{1-42}$ oligomers (500 nM) for 48 h, followed by treatment with phytocannabinoids (200 nM) for an additional 48 h. Phytocannabinoid treatment attenuated $A\beta_{1-42}$-induced neuronal death, with particularly marked neuroprotective effects observed for Δ^9-THCA, Δ^9-THCV, CBD, CBDA, and CBDV. Moreover, phytocannabinoids reduced $A\beta_{1-42}$ aggregate formation in cortical neurons, although the effect of Δ^9-THC did not reach statistical significance.

Altogether, these findings demonstrate that phytocannabinoids display distinct CB_1R- and CB_2R-mediated signaling profiles and exert neuroprotective effects against $A\beta_{1-42}$-induced pathology in primary cortical neurons. These results support further investigation of phytocannabinoid signaling and its potential therapeutic relevance in neurodegenerative diseases.

Keywords: Cannabinoid receptors; Phytocannabinoids; Neurodegenerative diseases.

CANNABIGEROL RESCUES COGNITIVE DEFICITS AND REDUCES AMYLOID, NEUROINFLAMMATORY, AND OXIDATIVE PATHOLOGY IN 5XFAD MICE THROUGH CB_1/CB_2 RECEPTOR ENGAGEMENT

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This study explores the neuroprotective potential of cannabigerol (CBG), a non-psychotropic phytocannabinoid from *Cannabis sativa L.*, in Alzheimer's disease (AD). While cannabidiol (CBD) has been widely studied, CBG, the biosynthetic precursor of major cannabinoids, remains less explored despite its promising pharmacological profile. Using in vitro neuronal cultures and in vivo 5XFAD transgenic mice, we evaluated CBG's eRects on amyloid toxicity, oxidative stress, neuroinflammation, and cognitive function. CBG protected primary cortical neurons from amyloid-β–induced neurite damage through CB_1 receptor activation and improved spatial and recognition memory after four weeks of oral administration (5 mg/kg). Molecular analyses revealed restoration of synaptic plasticity (PSD95, cFos), reduced proinflammatory and oxidative stress markers (Il-6, Tnf-α, iNos, Aox1), and upregulation of antioxidant defenses (Gpx1, Sod1). CBG also decreased amyloid-β plaque burden in hippocampus and cortex, likely via BACE1 downregulation. Collectively, CBG exerts multitarget neuroprotective actions, anti-amyloidogenic, anti-inflammatory, antioxidant, and synaptogenic, highlighting its promise as a therapeutic candidate for AD.

CAN CBD AMELIORATE BEHAVIORAL AND MOLECULAR ALTERATIONS ASSOCIATED WITH SATB2 DEFICIENCY?

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The potential use of cannabinoid-based products for therapeutic applications in neurodevelopmental disorders (NDDs) has gained attention in recent years. Beyond the efficacy of CBD in the management of drug-resistant childhood epilepsy, phytocannabinoid extracts, mostly enriched in CBD, have been reported to modulate symptoms in patients with autism spectrum disorders. The present study aims to determine the impact of oral CBD administration during adolescence on the behavioral and molecular alterations of a syndromic intellectual disability mouse model induced by the ablation of the SATB2 transcription factor (SATB2-associated syndrome, SAS). CBD has been shown to modulate glial activity and inflammatory and oxidative pathways, suggesting that its effects on glial homeostasis may contribute to the behavioral improvements observed in NDDs. However, this potential mechanism remains largely unexplored in syndromic forms of intellectual disability

Using a conditional knockout mouse model lacking SATB2 in forebrain pyramidal neurons from early embryonic development (*Emx1^{Cre}-Satb2$^{f/f}$*), we are characterizing the behavioral alterations of adult SATB2-deficient mice following oral administration of CBD-loaded nanoparticles (5 mg/kg) between postnatal days 22–36. Ongoing analyses will determine the effects of CBD on hyperactivity, impulsivity, anxiety-like behavior, cognitive performance, and social interaction, including the persistence of behavioral effects following treatment cessation. CBD exposure will be assessed in brain and plasma at selected time points to provide an initial characterization of its distribution and brain bioavailability. Additionally, changes in brain metabolites and myelin integrity associated with the SAS phenotype, as well as their modulation by CBD, will be explored using MRI and HRMAS. Importantly, glutamatergic metabolites and potential metabolic alterations selectively associated with the SAS genotype will be assessed. At the cellular level, confocal immunofluorescence analysis of GFAP and IBA1, complemented by morphological analyses will characterize neural cells phenotypes. In parallel, gene expression analyses will investigate molecular pathways modulated by CBD administration in oxidative stress, mitochondrial dysfunction and neuroinflammation. These approaches will determine whether modulation of glial homeostasis contributes to the behavioral effects of CBD in the SAS model.

Results from this study will provide insight into the therapeutic properties and mechanisms of action of CBD nanosuspension in a model of syndromic intellectual disability. By integrating behavioral characterization, glial population and in vivo metabolic analyses, and cell signaling studies this work will provide mechanistic insights of CBD effects in the neurodevelopmental SAS disorder.

Keywords: cannabidiol, intellectual disability, phytocannabinoid

SEX-DEPENDENT EFFECTS OF MATERNAL DEPRIVATION AND ADOLESCENT ALCOHOL EXPOSURE ON STRESS-RELATED BEHAVIOR AND LIPID SIGNALING IN RATS

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Early-life stress is a major risk factor for neuropsychiatric and substance use disorders and can induce long-lasting alterations in stress responsivity and emotional behavior. Maternal deprivation (MD) is a well-established model of early-life adversity. Alcohol exposure during adolescence represents an additional challenge that may interact with the consequences of early-life stress, with potentially different effects in males and females. Here, we investigated the effects of MD and adolescent alcohol exposure on stress-related behavior and lipid signaling in male and female rats.

Male and female Wistar rats underwent a single 24-h MD episode on postnatal day 9 (PND9). During adolescence (PND31-55), animals received intermittent alcohol exposure (3 g/kg) or saline. Stress-related behavior was assessed using the forced swim test and elevated plus maze. Plasma corticosterone, monoacylglycerols, N-acylethanolamines, LPA, and autotaxin were measured, and gene expression related to ECS and LPA signaling was assessed in the medial prefrontal cortex (mPFC). Data were analyzed using three-way ANOVA with sex, MD, and adolescent alcohol exposure as factors.

MD induced long-lasting metabolic alterations and sex-dependent differences in alcohol pharmacokinetics, with higher blood alcohol concentrations in MD females. MD produced sex-dependent effects on stress-related behavior, increasing active coping in males and decreasing it in females, and increased open-arm exploration independently of adolescent alcohol exposure. Adolescent alcohol exposure increased plasma corticosterone. MD and sex significantly altered circulating lipid mediators, with distinct patterns in males and females. In the mPFC, MD and adolescent alcohol exposure differentially regulated ECS- and LPA-related gene expression, indicating sex-dependent molecular adaptations.

Overall, MD produces persistent sex-dependent alterations in behavioral, endocrine, and lipid-related responses to stress, while adolescent alcohol exposure modifies these adaptations. These findings support a role for lipid signaling in the long-term consequences of early-life adversity and adolescent alcohol exposure, with distinct adaptations in males and females.

Keywords: maternal deprivation, adolescent alcohol exposure, sex differences.

CB1 RECEPTOR ACTIVATION DOWNREGULATES NEUROTROPHIC AND PKA-DEPENDENT SIGNALING AT THE NEUROMUSCULAR JUNCTION

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The endocannabinoid system is increasingly recognized as an important modulator of synaptic function and neurotransmitter release. Although cannabinoid signaling has been extensively studied in the central nervous system, its role in peripheral neuromuscular communication remains less well characterized. The neuromuscular junction (NMJ) is a highly specialized synaptic region containing signaling proteins that are selectively enriched and localized within the synaptic compartment. Among these, neurotrophic signaling through BDNF/TrkB, intracellular protein kinase pathways, muscarinic acetylcholine receptors, and proteins of the SNARE machinery are key molecular systems involved in the regulation of neurotransmitter release and synaptic plasticity at the NMJ.

In this study, we investigated whether acute activation of cannabinoid CB1 receptors modulates these molecular pathways under basal conditions. Paired hemidiaphragms from the same animal were incubated ex vivo for 1 h in Ringer solution or with 10 μM arachidonyl-2′-chloroethylamide (ACEA), a highly selective CB1 receptor agonist. Afterwards, muscle samples were processed by Western blotting to determine the changes in proteins involved in neuromuscular synaptic signaling. Acute ACEA exposure reduced BDNF protein levels and TrkB receptor phosphorylation, indicating a selective reduction in TrkB activation. ERK phosphorylation was also reduced, while PKC levels remained unchanged. In addition, ACEA decreased PKA catalytic subunit levels and reduced phosphorylation of its downstream specific target SNAP-25 Thr138, suggesting impaired PKA-dependent regulation of the neurotransmitter release machinery.

Overall, these findings indicate that acute CB1 receptor activation selectively modulates molecular pathways involved in neuromuscular function, particularly neurotrophic and PKA-dependent signaling. These results support a role for the endocannabinoid system in the fine molecular regulation of neurotransmission at the NMJ and highlight the neuromuscular synapse as a target of cannabinoid signaling modulation.

Keywords: Neuromuscular junction; CB1 receptor; Endocannabinoid system

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EXPLORING THE ANTI-INFLAMMATORY AND NEUROPROTECTIVE POTENTIAL OF THE ACIDIC AND VARINIC FORMS OF PHYTOCANNABINOIDS

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Alzheimer's Diseases (AD), the most common form of dementia worldwide, is characterized by the deposition of amyloid-β (Aβ) plaques and neurofibrillary tangles formed by hyperphosphorylated Tau protein. These aberrant protein aggregates lead to microglial activation, inflammation and oxidative stress, los of synaptic contacts and neuronal death, finally provoking a progressive cognitive impairment. Nowadays, this pathology has no cure, only treatments to counteract the symptomatology. Cannabinoids such as cannabidiol (CBD), which lacks psychoactive effects, have widely demonstrated anti-inflammatory and neuroprotective properties, and medicines containing Δ^9-tetrahidrocannabinol (Δ^9-THC) and/or CBD have been recently approved for specific indications. This opens new avenues for other phytocannabinoids present in Cannabis sativa L. varieties. In fact, the varinic and acidic forms of phytocannabinoids have gained interest, as they shown to be beneficial for a variety of diseases. We have previously described these compounds to act as partial agonists of cannabinoid CB_1 and CB_2 receptors.

Here we aimed to determine the anti-inflammatory and neuroprotective potential of the acidic and varinic forms of Δ^9-THC, CBD and cannabigerol (CBG) in an in vitro model of AD. Thus, Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabidiolic acid (CBDA), cannabidivarin (CBDV), cannabigerolic acid (CBGA), and cannabigerivarin (CBGV) were assayed in primary cultures of neurons and microglia treated with Aß and pTau aggregates.

Our results demonstrate the ability of the different phytocannabinoids to combat various AD hallmarks, such as pTau aggregation, ROS formation, synaptic loss, pro-inflammatory M1 microglia activation. Moreover, the tested compounds were able to increase the activation of the M2 neuroprotective microglial phenotype. In conclusion, phytocannabinoids appear as promising tools for the treatment of AD.

Keywords: Acidic and varinic phytocannabinoids, Alzheimer's disease, neuroprotection

AN IMAGING-BASED ASSAY KIT FOR SCREENING NOVEL LIGANDS AT THE HUMAN CB2 RECEPTOR

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The cannabinoid CB2 receptor is a relevant target in drug discovery because of its involvement in immune modulation and cancer-related processes.[1] The discovery and characterisation of novel CB2 ligands may therefore support the development of new therapeutic strategies. Fluorescent ligands provide valuable tools for investigating CB2 receptor binding and visualising receptors in living cells.[2]
Building on this approach, high-content imaging can integrate binding measurements with the assessment of cellular features.Here, we present the development, validation and application of a fluorescence-based kit for measuring ligand binding to human CB2 receptors using high-content screening. The assay combines cell cycle-arrested HEK293 cells stably overexpressing human CB2 receptors with the CB2-selective fluorescent tracer CELT-331 and automated imaging using the Operetta CLS system. Binding affinity is assessed through competitive tracer displacement, while nuclear staining enables concurrent evaluation of cell number and nuclear morphology. The protocol was developed and validated using reference CB2 ligands, yielding affinity estimates consistent with their expected pharmacological profiles. The validated assay was subsequently applied to a series of synthetic cannabinoid receptor agonists (SCRAs), demonstrating its utility in a research setting. Optimisation of SCRAs aims to combine suitable CB2 receptor affinity with favourable distribution profiles, particularly for therapeutic applications in which peripheral activity is preferred to minimise unwanted psychotropic effects associated with CB1 receptor activation in the central nervous system. The assay supported compound prioritisation by differentiating CB2 binding profiles and identifying candidates for further pharmacological characterisation. A key advantage of the kit is the integration of quantitative binding measurements with cellular observations within a single, non-radioactive assay, combined with a validated protocol. This approach provides a practical tool for screening novel CB2 ligands and guiding compound optimisation. A complementary kit targeting CB1 receptors is currently under development to extend the platform to both cannabinoid receptor subtypes.

Keywords: CB2 receptor; high-content imaging; screening; binding affinity

A CLINICAL STUDY OF CANNABIDIOL AS ADDITIONAL TREATMENT FOR REFRACTARY EPILEPSY IN DOGS

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Idiopathic epilepsy (IE) is the second most commonly diagnosed neurological disorder in small animal clinical practice. Currently, six to seven antiepileptic drugs (AEDs), administered either as monotherapy or in combination, are commonly used for its management. However, these drugs may cause adverse effects that can substantially affect the quality of life of both patients and their families. Furthermore, an increasing number of patients develop drug resistance.. Cannabidiol (CBD) modulates neuronal excitability by reducing presynaptic intracellular calcium concentrations, thereby limiting the excessive release of glutamate and other excitatory neurotransmitters (Potschka et al., 2022). In addition, CBD promotes postsynaptic hyperpolarization, reduces glutamatergic activity (Potschka et al., 2022), and increases cerebral GABA concentrations, thereby contributing to a reduction in seizure activity (Mehr et al., 2026).

Based on these premises, we conducted a prospective clinical study to evaluate the antiepileptic effect of 99% CBD in sesame oil in patients with IE in whom, for various reasons, the addition of further AEDs and/or an increase in the doses of their current AEDs was to be avoided. The study included 20 patients, comprising 10 females and 10 males of different breeds, with body weights ranging from 5 to 34.5 kg and ages ranging from 2 to 15 years. All patients were presented to the Neurology Service of the Veterinary Teaching Hospital of the University of Córdoba for epileptic seizures. Inclusion criteria were treatment with at least one AED, a minimum treatment duration of six months, and the absence of any concurrent disease.

Potential changes in seizure frequency (Sf), duration and intensity (Sd), as well as the duration and severity of the postictal period (Pd), were assessed. Of the 20 patients treated, four were receiving a single AED, twelve were receiving two AEDs, and four were receiving three or more AEDs. Patients were treated with CBD oil for 90 days. The treatment period was shortened to 50 days in cases in which no signs of clinical improvement were observed or in patients that experienced relapse following an initially favourable response. Patients received CBD oil at a dose of 10 mg/kg/day administered as a single daily dose, except in four cases in which the dose was increased to 15 mg/kg/day.

Of the 20 patients, five showed no clinical improvement effects after 50 days of treatment. Twelve patients showed a reduction in Sf, seven of whom also showed a reduction in Sd, eleven showed a reduction in Pd and two patients remained seizure-free throughout the treatment period. In five patients receiving combination therapy, treatment with CBD allowed at least one AED to be discontinued, while in two cases the doses of the concomitant AEDs could be reduced.

These results suggest that CBD oil may be a useful adjunctive therapeutic option in dogs with idiopathic epilepsy receiving antiepileptic combination therapy. Controlled studies with larger populations are required to confirm its efficacy and define optimal dosing protocols.

Keywords: Cannabidiol (CBD); Epilepsy; Pharmacoresistance.

BIOAVAILABILITYAND NEUROPROTECTIVE EFFICACY OFAN ORAL FORMULATION OF PURIFIED CANNABIDIOLIN DIFFUSEAND FOCAL HYPOXIC-ISCHEMIC BRAIN DAMHE N NEWVORN RATS

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Cannabidiol (CBD) administered by parenteral route exerts robust neuroprotective effects in experimental models of neonatal diffuse and focal hypoxic-ischemic (HI) brain injury. However, parenteral CBD administration in human newborn can induce hemodynamic side effects and is not safe in underdeveloped environments.There is no information so far about the bioavailability and efficacy of CBD administered by oral route in neonatal HI conditions. For this purpose, we studied in the present work the effects of the only commercial oral formulation suitable so far for use in newborns, Convupidiol® (CVP), a purified CBD formulation.

To evaluate the bioavailability and neuroprotective effects of CVP in newborn rats after diffuse HI brain injury (DHI) or stroke.

In a first phase, the appropriate volumes of CVP were selected to administer 1 to 50 mg/kg of CBD to P7 rats; CVPwas administered using a 2.5 F orogastric catheter and mixed with 2 mLof veterinary milk. The effects of hypothermia or DHI (unilateral carotid artery cauterization followed by 90 min of 8% O_2) were also assessed. Brain concentration of CBD was determined by LC-MS three or six hours after treatment. The optimal oral dose was defined as the one leading to a brain concentration of CBD of 40-60 ng/g, according to previous experiments by our group. The neuroprotective efficacy of the optimal oral dose was tested using two experimental models of neonatal brain injury in P7-P10 Wistar rats: the DHI model (with normo- or hypothermia) and a neonatal stroke model [MCAO (3 h-long carotid artery occlusion with and occluder)]. In both models CVP or placebo (both supplied by Alef Medical) were administered 30 min after the end of the insult; non-HI and non-MCAO rats served as controls. Seven days after the HI or MCAO insult (P14), neurobehavioral performance was assessed using negative geotaxis (coordination test), grip (gross motor reflex) and grasp (fine motor reflex) tests.
Although oral CBD at 10 mg/kg led to appropriate CBD brain concentration 6 h after the administration to healthy rats, the optimal dose after DHI was 20 mg/kg. Hypothermia did nor modify the bioavailability or oral CBD. Oral administration by gavage was well tolerated in all animals, with no remarkable treatment-associated adverse effects. Although at P14 DHI and MCAO animals receiving placebo showed impaired motor test performance, the administration of CVP (CBD 20 mg/kg single dose) after DHI (in normo- or hypothermia) or MCAO restored the motor performance at that timepoint.

Conclusions: The use of an oral CBD purified formulation (Convupidiol) in newborn rats was feasible and well-tolerated. Treatment with oral CBD 20 mg/kg after diffuse or focal HI injury led to medium-term neuroprotective effects as robust as those previously observed after parenteral administration of CBD. These findingssupport the oralroute as

a feasible,safe andeffective way forCBD asa treatment ofneonatal brain injury.

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Keywords: Convupidiol, hypoxic-ischemic brain injury, neonatal stroke, neuroprotection, oral administration, newborn rats.

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 signaling 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.

DEVELOPMENT OF AN INTEGRATED CELL-BASED WORKFLOW FOR THE CHARACTERIZATION OF CB2 RECEPTOR LIGANDS

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The cannabinoid type 2 receptor (CB2) is a G protein-coupled receptor (GPCR) involved in the modulation of several physiological and pathological processes and represents an attractive target for the discovery and characterization of novel cannabinoid-related compounds. Reliable in vitro characterization of CB2 ligands requires the integration of complementary approaches to assess receptor binding, functional activity and potential compound-related effects on cell viability.
Here, we describe the development of an integrated cell-based workflow for the characterization of CB2 ligands using HEK293 cells stably expressing the human CB2 receptor. The workflow combines a fluorescence-based binding assay, functional characterization of receptor activity and complementary cell viability measurements.
Ligand binding is evaluated using a fluorescent CB2 tracer and high-content imaging on an Operetta CLS platform, allowing the assessment of ligand displacement and the identification of compounds interacting with the receptor. Compounds showing CB2 binding are subsequently evaluated in a functional cAMP assay to characterize their ability to modulate receptor-mediated signalling and to distinguish different pharmacological profiles.
In parallel, cell viability is assessed using metabolic assays to identify potential cytotoxic or metabolically disruptive effects that could interfere with the interpretation of binding or functional responses. The combination of these complementary readouts provides a sequential strategy to discriminate receptor-specific pharmacological activity from nonspecific cellular effects.
Overall, this workflow provides an integrated approach for the in vitro characterization of CB2 ligands, combining receptor engagement, functional response and cellular viability within the same experimental model. This strategy may facilitate the selection and further characterization of novel compounds targeting the CB2 receptor.

Keywords: CB2 receptor; ligand characterization; cell-based assays

CANNABINOIDS PROMOTE NEUROPROTECTION THROUGH CB_1R SIGNALING IN ALZHEIMER'S DISEASE

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Alzheimer's Disease (AD) has gradually emerged as a significant challenge in our society, lacking efficient treatment. It is a highly complex condition involving multiple molecular pathways. According to the World Health Organization, over 50 million adults worldwide are affected by this disease, and this number is expected to rise annually due to the increasing lifespan worldwide. Moreover, Alzheimer's Disease International Federation estimated that approximately 1.7 million deaths around the world can be directly attributable to this disease.
Currently, there are several treatments aimed at slowing AD symptoms but no treatment to cure the pathology. Despite the vast amount of information accumulated since the first reported case and the multifactorial nature of the disease, the cause remains unknown. Nowadays, research is mainly focused on amyloid-beta (Aβ) plaques, but new studies suggest combining antibodies against Aβ and Tau proteins to mitigate AD progression. On the other hand, cannabinoids binding to CB_1 and CB_2 receptors have shown a neuroprotective role in the Central Nervous System. Then, the aim of the present study is to analyse the role of cannabinoids in the pathophysiology of AD, highlighting the main differences between endocannabinoids, phytocannabinoids, and synthetic cannabinoids. In parallel, it was also analysed the impact of Aβ and pTAU proteins expression on cannabinoids binding to CB_1R and induced signalling.
Throughout the experiments, it has been observed that cannabinoids can induce protection in front of several features of AD such as neuronal plasticity, the formation of ROS species, microglia polarization to a neuroprotective phenotype, Aβ1-42 and pTAU protein aggregates axonal transport or neuronal death. These beneficial effects could be explained due to the capacity of cannabinoids to decrease protein aggregates accumulation. In conclusion, after further experimental research, cannabinoids binding on CB_1R could become a new therapeutic target to combat AD.

Keywords (2-3): Cannabinoid 1 Receptor (CB_1R) signaling, Alzheimer's disease, Cannabinoids.

EXPLORING CANNABINOID RECEPTOR HETEROMERS IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM MULTIPLE SCLEROSIS PATIENTS

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Multiple sclerosis (MS) is a chronic autoimmune, inflammatory and neurodegenerative disease of the central nervous system (CNS). The endocannabinoid system (ECS) plays an important role in the pathophysiology of MS and has emerged as a potential source of disease biomarkers. In recent years, cannabinoid receptor heteromers have been identified *in vitro* and *in vivo*, and alterations in their expression have been associated with neurodegenerative diseases. In particular, CB_1R-GPR55 and CB_2R-GPR55 heteromers have been demonstrated in post-mortem brain sections from patients with MS, showing increased expression compared with control subjects. However, whether these receptor heteromers are also detectable in peripheral blood cells remains unknown. Given the accessibility and minimally invasive nature of peripheral blood sampling, their detection in peripheral blood mononuclear cells (PBMCs) could provide a valuable approach for investigating their potential as peripheral biomarkers of MS.
The aim of this study was to investigate the presence of CB_1R-GPR55, CB_2R-GPR55 and CB_1R-CB_2R heteromers in PBMCs from patients with MS and control subjects. PBMCs were isolated from peripheral blood samples by Ficoll density-gradient centrifugation and subsequently cytocentrifuged onto microscope slides using the *Cytospin* technique. The expression of CB_1R, CB_2R and GPR55 was evaluated by immunofluorescence. In addition, *Proximity Ligation Assays* (PLA) were performed to detect receptor-receptor interactions. Samples were subsequently analysed by confocal microscopy.
Immunofluorescence analysis demonstrated the expression of CB_1R, CB_2R and GPR55 in PBMCs from both control subjects and patients with MS. Red fluorescence signals detected by PLA were consistent with CB_1R-GPR55, CB_2R-GPR55 and CB_1R-CB_2R receptor complexes in both groups. These PLA signals were predominantly observed in cells displaying a monocyte-like morphology.
These findings demonstrate that cannabinoid receptor heteromers can be detected in PBMCs from both control subjects and patients with MS, suggesting that these receptor complexes may contribute to the peripheral ECS. Further characterization of their expression and distribution may help elucidate their potential role in MS and, given the accessibility and minimally invasive nature of peripheral blood sampling, their utility as peripheral biomarkers of the disease.

Keywords: Multiple Sclerosis, Cannabinoid Receptor Heteromers, PBMCs, biomarkers

A UGI-BASED APPROACH LED TO THE DISCOVERY OF A NEW FAMILY OF CB_2 RECEPTOR AGONISTS

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Keywords: CB_2 receptor, Ugi reaction, multicomponent chemistry, pyrazoles.

Cannabinoid receptor 2 (CB_2R), one of the two main receptors of the endocannabinoid system, has emerged as a therapeutic target of considerable interest, particularly in cancer and neurodegenerative disorders, where modulation of immune responses and neuroinflammation may offer relevant therapeutic opportunities. Unlike CB_1R, CB_2R is mainly expressed in immune cells and is upregulated under pathological conditions, supporting its potential for selective pharmacological intervention. [1]

Despite this promise, no CB_2R-targeting compounds have yet reached clinical use, and the discovery of potent and selective ligands based on novel chemical scaffolds remains a major challenge in the field. In this context, we herein report the discovery of a new family of pyrazole-based CB_2R agonists with excellent potency and selectivity. The compound library was generated through an efficient synthetic strategy based on multicomponent chemistry. The route involves the preparation of hydrazone precursors, followed by an Ugi four-component reaction (U-4CR) and subsequent cyclization to afford the target pyrazole derivatives. [2] This strategy enabled the discovery of a novel family of potent and selective CB_2R ligands and facilitated the rapid diversification of this new chemotype, underscoring the potential of Ugi-based multicomponent chemistry for cannabinoid receptor ligand discovery.

CB_1R–OX_1R HETEROMERS PRESERVE CB_1R-MEDIATED NEUROPROTECTION DESPITE RECIPROCAL CROSS-ANTAGONISM IN ALZHEIMER'S DISEASE MODELS

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CB_1R exerts neuroprotective effects in Alzheimer's disease (AD), whereas OX_1R signaling has been associated with AD-related pathological processes. Here, we investigated the functional interaction between CB_1R and OX_1R. We identified CB1R–OX1R heteromeric complexes in transfected cells and primary neurons, associated with reciprocal negative cross-talk affecting cAMP and MAPK signaling. This interaction was maintained under Aβ-induced pathological conditions and in the 5xFAD model. Our findings identify CB_1R–OX_1R heteromerization as a regulatory mechanism modulating CB1R signaling in AD-related conditions.

CANNABINOID CB_1 RECEPTORS IN THE HABENULAR COMPLEX: IMPLICATIONS FOR PSYCHIATRY COMORBIDITIES ASSOCIATED TO MULTIPLE SCLEROSIS

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Psychiatric comorbidities such as depression and anxiety are common in multiple sclerosis (MS) and lead to significant distress, poor quality of life, and reduced adherence to disease-modifying therapy. The habenular complex is a highly conserved brain structure composed by lateral (LHb) and medial (MHb) subnuclei critically involved in the (patho)biology of emotional states. Specifically, hyperactivity of the LHb has been consistently associated with depression and deficient emotional control. Cannabinoid CB_1 receptors (CB_1R) fine-tune synaptic inputs and astrocyte-to-neuron communication mechanisms that determine the output function of the habenular complex, with emerging implications in depression and anxiety disorders. Nevertheless, the functional roles of CB_1R within habenular subnuclei in the pathophysiology of psychiatric comorbidities associated to MS remain unknown.

Here, we investigated the expression and function of habenular CB_1R in the cuprizone mouse model of primary demyelination. Immunofluorescence experiments targeting APC, GFAP, S100β and Iba1 demonstrated that CPZ intoxication induces oligodendrocyte loss and glial inflammatory responses that affect habenular subnuclei in sex-specific manner. These cellular adaptations were encompassed by anxiety- and/or depression-like behaviors in the open field and tail suspension tests. CB_1R immunolabeling revealed by DAB immunohistochemistry showed a heterogeneous pattern of receptor expression in the habenular complex with the highest levels corresponding to the LHb. Whole-cell patch-clamp recordings from LHb neurons were then used to evaluate the modulatory role of CB_1R on excitatory synaptic transmission. Bath application of the CB_1R agonist WIN55,212-2 inhibited both spontaneous and evoked excitatory postsynaptic synaptic currents (EPSCs) in the LHb. The inhibitory effect of WIN55,212-2 on evoked excitatory transmission was encompassed by an increase in the paired pulse ratio thus pointing to the involvement of the presynaptic receptor pool. Cuprizone administration induced a marked increase in the frequency of spontaneous EPSCs in LHb neurons together with sex-specific reductions in the expression and/or function of CB_1R that affected predominantly male mice. These results demonstrate that the habenular complex is sensitive to toxin-induced demyelination and points to specific roles for CB_1R within the LHb in the pathophysiology of psychiatric comorbidities associated to MS.

Keywords: CB1 receptors, habenular complex, excitatory transmission, multiple sclerosis.

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CB1 RECEPTORS IN PREFRONTAL PARVALBUMIN INTERNEURONS DRIVE SEX-DEPENDENT EMOTIONAL AND COGNITIVE DEFICITS IN MALE MICE

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Excitation/inhibition imbalance in the prefrontal cortex (PFC) underlies the hallmark cognitive and behavioural deficits of Schizophrenia (SCZ), a devastating psychiatric disorder that affects 24 million people worldwide. Cannabis use further elevates SCZ risk, symptom severity, and relapse, with cannabinoid receptor 1 (CB1) levels decreased in the PFC of SCZ patients.

Previously, we showed that CB1 deletion in PFC GABAergic neurons alters PFC function, leading to emotional impairment similar to that observed in SCZ patients (Ceprian M., 2026). Recent studies have shown that CB1 is expressed in a subset of Parvalbumin-positive (PV) interneurons across brain regions. These interneurons comprise about 40% of medial PFC GABAergic cells and are central to maintaining this cortical excitation/inhibition balance. In this work, **we hypothesise that prefrontal CB1-PV signalling is key to modulating emotional and cognitive behaviour**.

For this, we studied CB1 expression in PV neurons in mice and human PFC by immunofluorescence. We characterised CB1 function in these cells by deleting CB1 from PV and GABAergic PFC interneuron populations in adult CB1-flox male and female mice. One month later, mice were subjected to different behavioural tests to evaluate their emotional and cognitive performance. GABAergic and PV neuronal activity was studied via calcium imaging by fiber photometry. CB1 expression in PV neurons was studied in mice and human PFC by immunofluorescence.

PV and GABAergic CB1 deletion in the PFC altered the innate emotional behaviour exclusively in males. Interestingly, the impact of the loss of CB1 induced opposite behaviours in both groups. In the same line, a memory impairment in the NORT was only found in PV(CB1)-KO animals but not in Dlx(CB1)-KO ones. PV CB1 deletion was also linked to an alteration of PV cells and global GABAergic population activity.

To summarise, 1) the CB1 receptor was found in a population of PV neurons of the PFC, and 2) its CB1 deletion induced a distinct emotional and cognitive impairment in a sex-dependent manner. This was linked to a different activity pattern, not only of PV cells but also in the global PFC-GABAergic population.

Keywords: CB1, Prefrontal cortex, Parvalbumin.

ROLE OF P-GLYCOPROTEIN IN THE PATHOPHYSIOLOGY OF INFLAMMATION-ENHANCED HYPOXIC-ISCHEMIC BRAIN INJURY IN A NEONATAL RAT MODEL

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Neonatal hypoxic-ischemic (HI) brain injury is a major cause of neurological morbidity, and a preceding inflammatory exposure can increase the susceptibility of the immature brain to subsequent HI damage. Alterations in the blood-brain barrier (BBB) may further influence disease progression and the cerebral disposition of pharmacological treatments. P-glycoprotein (P-gp), an ATP-dependent efflux transporter expressed at the BBB, is a critical determinant of central nervous system (CNS) drug penetration. However, the regulation of P-gp following inflammation-sensitised HI injury during early brain development remains poorly characterised. Cannabidiol (CBD), a pleiotropic compound with neuroprotective and anti-inflammatory properties, may modulate BBB transporter function and consequently influence drug brain availability (Topiramate).

To characterise alterations in P-gp expression and functional activity following inflammation-sensitised HI brain injury in neonatal rats, and to investigate the effects of CBD on P-gp function and the cerebral disposition of the P-gp substrate topiramate, compared to a known inhibitor, tariquidar (TRQ).

Post-natal day 7 Wistar rats underwent the Rice–Vannucci model of HI injury, with or without prior inflammatory sensitization with lipopolysaccharide (LPS; 100 µg/kg, i.p.). Animals were allocated to SHAM, HI, LPS-HI, LPS-HI-vehicle, and LPS-HI-CBD groups. Functional and structural outcomes were evaluated at medium- and long-term time points using neurobehavioral and neuroimaging assessments. P-gp expression was assessed by Western blot, whereas transporter activity was evaluated by measuring brain rhodamine 123 distribution by measuring fluorescence intensity. The effects of repeated CBD administration (5 mg/kg, i.p.) in LPS-HI animals were investigated and compared with pharmacological P-gp inhibition using TRQ. Cerebral topiramate concentrations were quantified by liquid chromatography–tandem mass spectrometry (LC–MS/MS).

LPS administration alone did not induce detectable structural or functional brain injury. However, prior inflammatory exposure exacerbated the long-term functional consequences of HI without increasing macroscopic lesion volume. P-gp expression was increased following both LPS exposure and HI, whereas P-gp functional activity was selectively enhanced following the combined exposure, indicating a dissociation between transporter expression and function. In HI-LPS animals, repeated CBD treatment improved functional outcomes and reduced lesion volume, while normalising P-gp expression and attenuating its functional activity. These findings suggest that CBD modulates P-gp at the BBB through mechanisms beyond direct transporter inhibition. Pharmacological characterisation of tariquidar further revealed a distinct inhibitory profile in neonatal animals compared with that reported in adult models. Preliminary LC-MS/MS analyses also indicated increased cerebral topiramate concentrations following CBD or tariquidar administration, although these findings remain exploratory given the limited sample size.

Inflammation sensitises the immature brain to hypoxic-ischemic injury and alters the neurovascular response at the level of P-gp, particularly its functional activity. CBD modulates this response while exerting neuroprotective effects, suggesting that regulation of BBB transporters may represent an additional mechanism influencing therapeutic drug availability after neonatal brain injury. These findings provide a rationale for further investigating P-gp as a pharmacological target to optimise CNS drug exposure in neonatal HI.

Keywords: neonatal hypoxic-ischemic brain injury; inflammation; blood-brain barrier; P-glycoprotein; cannabidiol; topiramate; tariquidar.

MEDIAL SEPTUM CB1R TUNES SEPTAL CONTROL OF LOCOMOTION AND EXPLORATION WITHOUT BEING NECESSARY FOR SPATIAL LEARNING

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The medial septum (MS) is a forebrain nucleus in charge of connecting limbic cortex to the hippocampus. It participates in the synchronization of hippocampal theta oscillations, the main electrophysiological signature of hippocampal-driven functions (e.g., active exploration and memory encoding), mainly due to its GABAergic, cholinergic, and glutamatergic outputs. It is known that the disruption of septo-hippocampal communication impacts on hippocampal theta rhythms and the cognitive operations it supports. As one of the most abundant GPCRs in the brain, the CB1R is also expressed in MS, where its co-localizes with GABAergic and cholinergic neurons, nevertheless, the functional and specific contribution of MS-CB1R to its functions remains unexplored, as constitutive knockout approaches cannot distinguish septal from widespread hippocampal or cortical effects. In line with this, we previously showed that neuronal and glial CB1R in the hippocampus specifically modulate spatial navigation and innate emotional responses in a sex-dependent manner, and here we extend this approach to the septal hub that gates information into hippocampus, therefore governing theta-activity and related behaviors.

On this project, we combined AAV-mediated deletion of CB1R from all MS neurons in CB1-floxed mice, with expression of a Gi/o-DREADD selectively in DLX-positive GABAergic neurons of the MS, enabling acute chemogenetic inhibition of the septal GABAergic population with CNO. Preliminary results show that deleting CB1R from all MS neurons reduced open-field general locomotion while altering aversive-zone exploration, dissociating medial septum CB1R contributions by stage. Moreover, chemogenetic inhibition of DLX-positive septal GABAergic neurons increased immobility in the Light and dark box (LDB) irrespective of CB1R genotype, identifying septal GABAergic output as a determinant for anxiogenic-zone exploration. Finally, septal CB1R deletion does not impact spatial learning in the Barnes maze in both sexes.

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

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Background: Cannabidiol (CBD), the second most abundant phytocannabinoid in Cannabis sativa, has garnered significant interest due to its non-psychoactive nature and diverse receptor interactions. **Methods:** 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. **Results:** Our findings demonstrate CBD's ability to decrease pTau and Aβ aggregation and to mitigate their axonal transport between cortical and hippocampal neurons. Moreover, CBD treatment was shown to reduce neuroinflammation, as CBD was able to skew microglia towards a neuroprotective M2 phenotype while attenuating proinflammatory cytokine release in the 5xFAD AD mouse model. 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. The study also 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. Additionally, CBD treatment led to a reduction in reactive oxygen species (ROS) formation and increased neuronal viability in the presence of AD-associated protein aggregates. **Conclusions**: 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.

A CONFORMATIONALLY CONSTRAINED ANANDAMIDE ANALOG COMBINES POTENT CB1 AGONISM WITH PRESERVATION OF MITOCHONDRIAL FUNCTION UNDER OXIDATIVE STRESS

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Anandamide (AEA) is an endogenous cannabinoid receptor type 1 (CB1) agonist whose pharmacological exploitation is limited by its high conformational flexibility and rapid metabolic degradation. To overcome these limitations, we characterized a novel series of conformationally constrained AEA analogues and investigated whether optimization of their pharmacological profile could be associated with preservation of mitochondrial function under oxidative stress.

The compounds were characterized by integrating computational modelling with competitive radioligand binding at CB1 and CB2 receptors and functional assessment of CB1-mediated inhibition of cAMP production. The lead compound, MDB265, was then investigated in retinoic acid-differentiated SH-SY5Y cells, in which cytotoxicity and mitochondrial respiratory function under H_2O_2-induced oxidative stress were assessed by SRB assay and high-resolution respirometry (Oroboros O2k), respectively.

MDB265 showed the most favourable pharmacological profile, displaying subnanomolar affinity for CB1 (Ki = 0.071 nM) and approximately 35-fold selectivity over CB2 (Ki = 2.52 nM). Consistent with its high receptor affinity, MDB265 behaved as a CB1 agonist with an EC50 of 91 nM, approximately 14-fold lower than that of AEA (1.31 μM). MDB265 did not significantly affect cell viability up to 15 μM. Exposure of differentiated SH-SY5Y cells to H_2O_2 (350 μM) markedly compromised mitochondrial respiration, with significant reductions in OXPHOS I+II, OXPHOS II, and maximal ETS capacity. Notably, while MDB265 (5 μM) had no significant effect on mitochondrial respiration under basal conditions, pretreatment with the compound significantly preserved OXPHOS I, OXPHOS I+II, OXPHOS II and maximal ETS capacity following oxidative stress.

Overall, these results identify MDB265 as a high-affinity CB1 agonist and provide evidence that its pharmacological activity is accompanied by preservation of mitochondrial respiratory function under oxidative stress. Preliminary findings further suggest a contribution of CB1 signalling to this effect, although additional studies are required to establish receptor dependency and define the underlying molecular mechanisms.

Keywords: CB1 receptor; anandamide analogues; mitochondrial bioenergetics

CANNABIDIOL TARGETS A VDAC1-ASSOCIATED MITOCHONDRIAL VULNERABILITY IN INVASIVE MELANOMA CELLS

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Melanoma progression involves phenotypic plasticity and metabolic rewiring. Cannabidiol (CBD) exerts anti-tumour effects in melanoma through multiple molecular targets and can interfere with mitochondrial function in cancer cells. We investigated whether residual mitochondrial activity of invasive melanoma cells represents an exploitable vulnerability and whether CBD can target this dependency to impair their pro-metastatic behaviour.
To address this, WM1366 melanoma cells were co-cultured in transwell with human hepatic sinusoidal endothelial cells (HHSEC) and separated into invasive and non-invasive fractions. Respiration was assessed by high-resolution respirometry (Oroboros O2k) in digitonin-permeabilised cells using a SUIT protocol resolving complex I- and complex II-linked OXPHOS and maximal electron transfer capacity. Cell viability was assessed after 24 h CBD exposure (5, 10, 25 μM). 5 μM CBD, which did not significantly affect cell viability after 24 h, was selected to investigate acute mitochondrial effects by direct titration into the respirometry chamber after cell permeabilisation. A higher concentration (10 μM) was also tested to assess the consistency of the respiratory response. Migration was quantified by a transwell assay after 24 h. VDAC1 involvement was probed with its inhibitor 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), alone and combined with CBD, in both settings.
Invasive $WM1366^{HHSEC}$ cells showed lower respiration than their non-invasive counterparts across all states ($p \leq 0.05$), indicating a broad reduction in oxidative capacity. Acute intra-chamber exposure to CBD (5 μM) significantly reduced complex I-linked OXPHOS, maximal electron transfer capacity and complex II-linked OXPHOS ($p \leq 0.001$), an effect preserved at 10 μM, supporting a direct mitochondrial action independent of plasma membrane signaling. At 5 μM, CBD markedly impaired migration ($p < 0.05$). DIDS alone was inactive, but partially restored respiration in CBD-treated cells ($p < 0.01$) and almost completely abolished the anti-migratory effect of CBD ($p < 0.05$), supporting VDAC1 involvement in both the bioenergetic and functional responses to CBD.
Invasive melanoma cells adopt a low-OXPHOS phenotype yet remain dependent on residual mitochondrial output for motility. CBD further compromises this residual mitochondrial function without affecting cell viability, impairing migration through a mechanism involving VDAC1. These findings indicate that the metabolic adaptation associated with melanoma invasiveness retains a pharmacologically exploitable dependency, which CBD targets to interfere with the invasive melanoma phenotype.

Keywords: Cannabidiol; Mitochondrial bioenergetics; Melanoma invasiveness.

THC IMPAIRS HIPPOCAMPAL MEMORY FORMATION BY DISRUPTING CA1 GLUTAMATERGIC SIGNALING

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The hippocampus plays a pivotal role in memory formation, and cannabinoids, including Δ⁹-tetrahydrocannabinol (THC), 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 and reduced dendritic spine density. Here, we sought to identify the cell type–specific molecular mechanisms underlying these alterations following chronic THC exposure, with the goal of developing pharmacological strategies that preserve the therapeutic potential of THC while preventing memory impairment. To decipher the THC-induced translatome, we used Wfs1-CreERT2:RiboTag mice expressing 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 RNAseq. Bioinformatic analyses of differentially translated transcripts revealed significant enrichment of Gene Ontology and KEGG categories related to memory, synaptic organization, structural plasticity, and, notably, glutamatergic transmission, including altered expression of glutamate receptor subunits in THC-treated mice. Guided by these molecular signatures, we tested whether pharmacological modulation of the glutamatergic system could normalize circuit function and rescue behavior. Restoring E/I signaling recovered performance in hippocampus-dependent memory tasks after repeated THC exposure, while preserving THC-induced analgesia. Collectively, our data identify CA1 pyramidal neuron–specific pathways dysregulated by chronic THC, link these changes to glutamatergic dysfunction and memory deficits, and provide proof-of-concept that targeting synaptic balance can dissociate the therapeutic and adverse cognitive effects of cannabinoids.

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

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THE IMPACT OF CANNABIDIOL ON NEUROPATHOLOGY IN A HUNTINGTON'S DISEASE MOUSE MODEL

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Huntington's Disease (HD) is a neurodegenerative disorder characterized by progressive motor, cognitive and psychiatric symptoms. It is caused by a CAG trinucleotide expansion in the HTT gene that is dominantly inherited and has full penetrance, which leads to a mutant and aggregating form of the protein (mHTT). Despite extensive research, effective disease-modifying treatments have long remained elusive. This project investigates the therapeutic potential of cannabidiol (CBD), a non-psychotropic plant-derived cannabinoid, and the inflammatory pathways derived from its administration.
R6/1 transgenic mice were used as an HD model to study the mechanisms underlying the therapeutic potential of CBD administration. Building on previous data from the group showing that daily CBD administration (100mg/kg) starting at early stages of disease development alleviates cognitive decline and depressive-like behaviour, this research focused on the molecular changes in neuroinflammation, mHTT burden and cannabinoid receptor 1 (CB1R) expression, which is underexpressed in both mouse models and HD patients alike. Immunohistochemistry and biochemical analyses were conducted in key brain regions for these symptoms: hippocampus and frontal cortex.
Our data revealed that CBD-treated R6/1 mice exhibit reduced mHTT aggregation in hippocampus, while also partially restoring the expression of CB1R and decreasing several neuroinflammation markers in this region.
These findings suggest that CBD may offer neuroprotective benefits in HD by reducing mHTT aggregation and inflammation in hippocampus. This research contributes to the growing body of evidence that supports the endocannabinoid system as a promising target for therapeutic intervention in neurodegenerative diseases, while at the same time highlighting the need for further research on the topic.

Keywords: Huntington's Disease (HD), Cannabidiol (CBD), neuroinflammation

HYPOTHALAMIC POMC NEURONS GATE THE FEAR-FEEDING TRADE-OFF.

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Survival in natural habitats forces animals to constantly adapt their behavior according to their intrinsic needs and environmental conditions. For instance, a hungry animal in a threatening situation will favor fear responses over their motivation to eat to ensure survival. However, how the brain senses the inner state and a threatening situation to orchestrate an optimal survival response has been poorly studied. Within the hypothalamus, pro-opiomelanocortin (POMC) expressing neurons classically promote satiety during energy surfeit and have a role in the adaptations to stressful and fearful events. We hypothesized that POMC neurons are at the intersection of fear and feeding responses. Therefore, we have used the fear-suppression of feeding paradigm where hungry mice must choose between eating or displaying fear responses in the presence of a tone associated with a foot shock. Conditioned control mice had suppressed eating behavior compared to non-conditioned mice due to increased time immobile. C-fos expression and chemogenetic studies showed that POMC activation is necessary for suppressing eating behavior in a threatening situation. POMC neurons directly project to hypothalamic CRH neurons which are key modulators of the stress response. Fear-suppression of feeding recruited CRH neurons and increased plasma corticosterone levels, indicating that feeding and fear behaviors might be controlled simultaneously by engaging POMC neurons and the stress response. In addition, POMC neurons activity is regulated by cannabinoid type 1 (CB1) receptors, key physiological determinants of synaptic and behavioral functions. Mice lacking CB1 in POMC neurons displayed higher motivation for eating and decreased fear response than their control littermates due to altered stress response. Collectively, these results suggest that POMC neurons are key to balancing fear and feeding behaviors by probably modulating CRH neurons in a CB1-dependent manner.

Keywords: POMC, competing behaviors, feeding, fear, CRH, HPA axis, CB1R

LOCOMOTOR RESPONSE FOLLOWING ORAL ADMINISTRATION OF CANNABIDIOL, PARACETAMOL, AND PHENYLBUTAZONE IN AN EXPERIMENTAL MODEL OF EQUINE LAMENESS

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Musculoskeletal pain and lameness are common in horses and may negatively affect performance and welfare. Phenylbutazone and paracetamol are commonly used analgesic drugs in equine medicine, although the search for alternative therapeutic options has increased interest in cannabidiol (CBD). This study aimed to evaluate changes in locomotor asymmetry following oral administration of two CBD formulations, compared with phenylbutazone and paracetamol, in an experimental model of reversible lameness. Five adult horses were included in a crossover study. Moderate lameness was induced using a reversible frog-pressure model with a modified horseshoe. Each horse received phenylbutazone (2.2 mg/kg), paracetamol (20 mg/kg), CBD in sesame oil (10 mg/kg), and micellar CBD (10 mg/kg). Locomotion was objectively assessed using EquiGait® inertial sensors before treatment and at 2, 4, and 6 hours after administration. Due to the small sample size, data were analysed descriptively.All treatments were associated with a reduction in locomotor asymmetry (reflecting an improvement in lameness). Phenylbutazone showed a progressive improvement, reaching its greatest reduction at 6 hours (−18.20 ± 10.73 mm), while paracetamol showed its greatest effect at 4 hours (−12.60 ± 10.73 mm). Both CBD formulations produced an early improvement at 2 hours, with changes of −11.40 ± 8.11 mm for micellar CBD and −11.60 ± 10.53 mm for the oil-based formulation. The micellar formulation showed a further improvement at 6 hours (−13.40 ± 11.71 mm), whereas the effect of the oil-based formulation decreased after 4 hours, reaching −10.00 ± 11.81 mm at 6 hours.These preliminary findings suggest that both CBD formulations produced reductions in locomotor asymmetry within the range observed with commonly used equine analgesics in this experimental model. Notably, micellar CBD showed a sustained response over the 6-hour observation period. These findings support further investigation of CBD, particularly micellar formulations, as a potential analgesic option for lameness in horses. Larger studies incorporating pharmacokinetic and pharmacodynamic assessments are warranted.

ASTROCYTIC MORPHOLOGY IN THE MEDIAL HABENULA: SEX DIFFERENCES AND MODULATORY FACTORS

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The medial habenula (MHb) is an epithalamic structure involved in aversive processing and emotional regulation, notable for its marked cellular heterogeneity and high astrocyte density. This cellular composition suggests that astrocytes may play an important role in MHb structure and plasticity, potentially contributing to the regulation of emotional states. The aim of this study is to characterize sex-dependent astrocytic morphology in the MHb and determine how it is modulated by peripheral alterations and direct central manipulations. At the peripheral level, a high-fat diet (HFD) was used as a model of metabolic stress, and systemic lipopolysaccharide (LPS) administration was used to induce a peripheral inflammatory challenge. At the central level, MHb astrocytes were targeted through two complementary approaches: conditional deletion of the cannabinoid *CB1* receptor in MHb astrocytes (MHb-(GFAP)*CB1*-KO mice), and a chemogenetic approach using Gi-DREADDs under the GFAP promoter, which allowed selective modulation of astrocytic intracellular signalling independently of peripheral influences. To explore potential interactions between central and peripheral challenges, LPS was additionally administered in MHb-(GFAP)*CB1*-KO mice. Preliminary results indicate sex-dependent morphological differences in MHb astrocytes across all these experimental conditions, supporting the idea that MHb astrocytes are sensitive to both peripheral and central disturbances and may represent a key cellular substrate linking body-brain interactions with emotional regulation.

Keywords: Medial Habenula (MHb); Astrocytes; *CB1* receptor.

CBD-BASED MOLECULAR HYBRIDS INCORPORATING A SELECTIVE CB2 RECEPTOR AGONIST: LINKER-DEPENDENT MODULATION OF MICROGLIAL ACTIVATION

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Neuroinflammation involves complex signalling pathways associated with microglial activation, making the modulation of complementary molecular targets an attractive pharmacological strategy. Cannabidiol (CBD) is a non-psychoactive phytocannabinoid with a broad pharmacological profile, while cannabinoid CB2 receptor activation represents a potential approach to controlling neuroinflammatory responses. β-Caryophyllene (BCP), a naturally occurring sesquiterpene, was initially described as a selective CB2 receptor agonist,1 although its receptor affinity and functional activity remain controversial. Here, we investigated whether the combined effects of CBD and BCP on microglial activation could be reproduced using COR167, a potent and selective synthetic CB2 receptor agonist (Ki = 6.3 nM; SI = 194), and explored the potential of integrating CBD and COR167 into single molecular hybrids.

In LPS-stimulated BV2 microglial cells, co-administration of CBD (5 μM) and BCP (20 μM) reduced cell length and the percentage of cells displaying a pro-inflammatory morphology, with greater effects than either compound alone. Molecular docking suggested the possibility of simultaneous interactions of CBD and BCP with distinct CB2 binding sites, raising the hypothesisof an allosteric contribution of CBD. To investigate this possibility using a pharmacologically well-characterized CB2 agonist, BCP was replaced with COR167. Co-administration of COR167 and CBD at submaximal concentrations (5 μM each) significantly counteracted LPS-induced morphological changes, achieving effects comparable to those observed with higher individual concentrations of COR167 (10 μM). These findings guided the design and synthesis of molecular hybrids linking COR167 to CBD or selected CBD analogues through linkers of varying lengths, to accommodate simultaneous interactions with the putative orthosteric and allosteric CB2 binding sites. Preliminary evaluation in LPS-stimulated BV2 cells revealed a linker-dependent response: shorter-linker hybrids showed no appreciable inhibition of LPS-induced NF-κB activation or reduction in CD11b expression, whereas a longer-linker derivative retained the ability to modulate microglial activation through inhibition of LPS-induced NF-κB activation at 0.1 μM. The shorter-linker hybrids also lacked detectable CB2 binding affinity (Ki > 10 μM), while the affinity of the biologically active longer-linker derivative is currently under investigation.

These preliminary findings identify linker length as an important structural parameter influencing the biological activity of CBD-based molecular hybrids. Although the proposed allosteric mechanism of CBD remains to be experimentally validated, the observed structure-dependent responses provide a rationale for further optimization of these compounds and investigation of their interactions with CB2.

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