ICRS

# 35TH ANNUAL SYMPOSIUM ON THE CANNABINOIDS

July 6-10, 2025
Bloomington, IN

# CONFERENCE PROGRAM

# 35TH ANNUAL

# SYMPOSIUM OF THE

# INTERNATIONAL CANNABINOID

# RESEARCH SOCIETY

## BLOOMINGTON, IN

JULY 6 – 10, 2025

Biddle Hotel & Conference Center
Indiana Memorial Union Building
900 E 7th St, Bloomington, IN 47405

International Cannabinoid Research Society

Winston Salem, NC, USA



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# 35th ANNUAL SYMPOSIUM ON THE CANNABINOIDS CONFERENCE AGENDA

Biddle Hotel & Conference Center
Indiana Memorial Union Building
900 E 7th St, Bloomington, IN 47405

**Important locations during the conference (July 7-10)**
Breakfasts/Lunches/Coffee Breaks – Alumni Hall/Solarium
Meeting Room – Alumni Hall
Poster Sessions – Solarium
Dinners/Banquet – Tudor Room

| **Sunday, July 6, 2025** | |
|---|---|
| 16:00 | Arrivals, ICRS Registration (Alumni Hall) |
| 18:00-20:00 | Welcome reception (Solarium) |

| **Day 1, Monday July 7** | | | |
|---|---|---|---|
| 7:15 | Breakfast | | |
| 8:30-8:45 | Welcome and Opening Remarks | | |
| **Session 1. Pain, Inflammation and Neuroimmune Disorders**<br>Chairs: Martin Kaczocha & David Finn | | | |
| 8:45-9:00 | INHIBITORS OF ENDOCANNABINOID DEACTIVATION DIFFERENTIALLY SUPPRESS PACLITAXEL-INDUCED NEUROPATHY AND TUMOR GROWTH IN A MOUSE MODEL OF BREAST CANCER | Jonah Wirt, Emily Fender-Sizemore, Mirjam Huizenga, Mario Van der Stelt and Andrea G. Hohmann | 39 |

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| 9:00-9:15 | STRUCTURE-GUIDED OPTIMIZATION OF MAGL INHIBITORS FOR THE TREATMENT OF NEUROPATHIC PAIN | Mirjam Huizenga, Jonah Wirt, Richard van den Berg, Darcy Reynolds, Joey Reverey, Daan van der Vliet, Diep Vu, Roger Buijsman, Antonius Janssen, Andrea Hohmann and Mario van der Stelt | 40 |
| 9:15-9:30 | CB2 CANNABINOID RECEPTOR-SPECIFIC THERAPEUTIC ANTIBODY AGONISTS FOR TREATMENT OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY | Carlos Henrique Alves Jesus, Raghavender Gopireddy, Swastik Sen, Richard Yu, Toshihiko Takeuchi, Lauren Schwimmer and Andrea Hohmann | 41 |
| 9:30-9:45 | THE NAAA INHIBITOR ARN19702 SUPPRESSES INFLAMMATORY AND NEUROPATHIC PAIN IN MICE | Laura Boullon, Luana Assis Ferreira, Danielle Piomelli and Andrea G Hohmann | 42 |
| 9:45-10:00 | VALIDATION OF A RAT MODEL OF INCISIONAL WOUND-RELATED PAIN: EFFECT OF ENDOCANNABINOID SYSTEM MODULATION | Catherine R. Healy, Maria C. Redmond, Mary Hopkins, Georgina Gethin, Abhay Pandit and David P. Finn | 43 |
| 10:00-10:15 | SEX-STRATIFIED ANALYSIS OF SPINAL ENDOCANNABINOID SYSTEM AND NEUROIMMUNE INTERACTIONS IN NEUROPATHIC PAIN | Marta Kaminska, Natalia Stelmach, Magdalena Maciuszek, Katarzyna Popiolek-Barczyk and Natalia Malek | 44 |
| 10:15-10:30 | CANNABIDIOL (CBD) IMPROVES ALLODYNIA AND COGNITIVE FUNCTION ALONG WITH SYNAPTIC PLASTICITY IN A MOUSE MODEL OF SPARED NERVE INJURY | Maciej Degutis, Magdalena Białoń, Katarzyna Popiołek-Barczyk, Michela Perrone, Antimo Fusco, Roozbe Bonsale, Ida Marbese, Sabatino Maione, Katarzyna Starowicz | 45 |
| 10:30-11:00 | Coffee Break | | |
| 11:00-12:00 | **Presidential Plenary Lecture 1:**<br><br>NEUROINFLAMMATION IN PAIN – HAVE WE BEEN OVERLOOKING THE MESENCHYME? | Franziska Denk | 46 |

| Session 2. Neurodevelopmental Consequences of Cannabinoid Exposure During Early Life and Adolescence<br>Chairs: Nephi Stella & Istvan Katona | | | |
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| 12:00-12:15 | REVERSIBLE BEHAVIORAL DEFICITS INDUCED BY PRENATAL THC EXPOSURE BECOME IRREVERSIBLE AFTER POSTNATAL ISOLATION | Diana Dimen, Petra Aradi, Caroline Shumaker, Lisette Gold, Joseph Kimmel Leffel, Miklos Zoldi, Laszlo Barna, Hui-Chen Lu, Kenneth Mackie and Istvan Katona | 47 |
| 12:15-12:30 | STRESS RESILIENCE/VULNERABILITY PHENOTYPE DETERMINES BEHAVIORAL AND MOLECULAR EFFECTS OF PRENATAL AND ADOLESCENT THC EXPOSURE IN MICE | Dilorom Begmatova, Mohamed Mari, Albert Pinhasov and Natalya Kogan | 48 |
| 12:30-12:45 | ADOLESCENT CO-EXPOSURE TO ETHANOL AND CANNABINOID PRODUCED SUSTAINED IMPAIRMENT OF NEUROGENESIS AND HIPPOCAMPUS-DEPENDENT FUNCTIONS DURING ADULTHOOD | Somnath Mukhopadhyay, Dal Khatri and Madhungi Vaidyanathan | 49 |
| 12:45-13:00 | FUNCTIONAL, STRUCTURAL, AND BEHAVIORAL IMPACTS OF ADOLESCENT CANNABIS VAPOR EXPOSURE IN ADULT MALE AND FEMALE RATS | Sara Westbrook, Riana Abeshima, Leisa Uelese, Matteya Proctor, Sara Burres, Zachary Fisher, Travis Brown, Kristen Delevich and Ryan McLaughlin | 50 |
| 13:00-14:30 | Lunch | | |
| **Session 3. Neuroimmune Modulation and Organ Protection: Cannabinoids and Beyond**<br>Chairs: Radka Kocavarova & Resat Cinar | | | |
| 14:30-14:45 | LOW-DOSE ALCOHOL ENHANCES THE BENEFICIAL EFFECTS OF CANNABIDIOL IN REDUCING NEUROINFLAMMATION IN A MURINE MODEL OF MULTIPLE SCLEROSIS VIA GUT MICROBIOTA MODULATION | Rahul Pushparaj, Jingdan Pei, Xing Gao, Md Manirujjaman, Panpan Huang, Guihua Pan, Lixian Chen, Chunbao Sun, Lihua Zhang and Wenke Feng | 51 |

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| 14:45-15:00 | GDE1-ASSOCIATED SPONGIFORM ENCEPHALOPATHY AND MALE INFERTILITY | Zsolt Lele, Benjámin Barti, Christina Miskolczi, Zsófia László, Eszter Horváth, Dániel Nagy, Sámuel Szabó, Susanne Prokop, Miklós Zöldi, László Barna, Máté Kisfali, Anikó Reichart, László Biró, Zoltán Varga, Bíborka Bruzsik, Huba Szebik, Máté Tóth, Mária Baranyi, Flóra Gölöncsér, Levente Zsichla, Kata Nagy, Csaba Cserép, Imre Kacskovics, Benjamin Cravatt, Ádám Dénes, Viktor Müller, Beáta Sperlágh, Éva Mikics and István Katona | 52 |
| 15:00-15:30 | **ICRS Mid-Career Awardee**<br>FROM MOLECULES TO NETWORKS:<br>ADVANCING CANNABINOID-BASED STRATEGIES FOR COMPLEX DISEASE MODULATION | Alessia Ligresti | 53 |
| **15:30-16:15** | **Data Blitz 1**<br>Chairs: Bogna Ignatowska-Jankowska & Mario van der Stelt | | |
| | INACTIVATION OF MONOACYLGLYCEROL LIPASE IN ASTROCYTES ATTENUATES SEIZURE SEVERITY AND POST-SEIZURE INJURY | Chudai Zeng, Fei Gao, Jian Zhang, Mingzhe Pan and Chu Chen | P1-16 |
| | SENSORY PROCESSING DYNAMICALLY MODULATES ENDOCANNABINOID LEVELS IN THE PRIMARY SOMATOSENSORY CORTEX | Jui-Yen Huang and Hui-Chen Lu | P1-23 |
| | CHRONIC MICRODOSING OF LSD IN MICE CAUSES INCREASES IN ENDOGENOUS LIPID SIGNALING MOLECULES IN THE CORTEX WITH LITTLE EFFECT IN THE PLASMA AND HIPPOCAMPUS 24 HOURS AFTER FINAL TREATMENT | Noah Brauer, Ashley Xu, Wenwen Du, Taylor Woodward and Heather Bradshaw | P1-21 |

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| | SEX-SPECIFIC EFFECTS OF PRENATAL THC AND EARLY LIFE STRESS ON ANXIETY AND DEPRESSIVE-LIKE BEHAVIOUR: THE IMPACT ON ELECTROPHYSIOLOGICAL PROPERTIES IN THE VENTRAL TEGMENTAL AREA AND BASOLATERAL AMYGDALA | Enzo Perez-Valenzuela, Taygun Uzuneser, Marieka DeVuono, Daniel Hardy, Walter Rushlow and Steven Laviolette | P1-24 |
| | CB1R MEDIATED REGULATION OF CHRONIC ETHANOL-INDUCED NEUROIMMUNE GENE EXPRESSION IN HIPPOCAMPUS AND PREFRONTAL CORTEX OF ADULT RAT BRAIN | Somnath Mukhopadhyay, Dal Khatri and Genevieve Laroche | P1-20 |
| | TEMPORAL AND SPATIAL CHARACTERIZATION OF THE VACUOLIZATION PHENOTYPE OF MICE LACKING GDE1 | Eszter Horvath, Daniel Nagy, Zsofia Laszlo, Susanne Prokop, Balazs Pinter, Kata Nagy, Imre Kacskovics, Benjamin Cravatt, Zsolt Lele and Istvan Katona | P1-17 |
| | DAILY CANNABIS USE LINKED TO DAILY ANXIETY REDUCTIONS IN INDIVIDUALS USING CANNABIS FOR ANXIETY COPING | Luiza Rosa, Jonathon K. Lisano, Carillon J. Skrzynski, Angela D. Bryan and L. Cinnamon Bidwell | P1-25 |
| **16:15-18:15** | **Poster Session 1**<br>*Odd numbered posters will be presented in the first hour (16:15) of the poster session and even numbered posters will be presented in the second half (17:15) of the session | | |
| 18:15 | Dinner/Trainee mentorship event | | |

# Day 2, Tuesday July 8

| | | | |
|---|---|---|---|
| 7:30 | Breakfast | | |
| 8:25-8:30 | Opening remarks | | |
| **Session 4. Advances in Cannabinoid Receptor Pharmacology and Endocannabinoid Pathway Modulation**<br>Chairs: Chris Breivogel & Steve Alexander | | | |
| 8:30-8:45 | CELLULAR AND BEHAVIORAL INVESTIGATION OF ZCZ011 ENANTIOMERS | Mohammed Mustafa, Giulia Donvito, Zhixing Wu, Sri Sujana Immadi, Rachel Dopart, Kristen Trexler, Steven Kinsey, Debra Kendall, Dai Lu and Aron Lichtman | 54 |
| 8:45-9:00 | UNCOVERING THE ROLE OF DAGLα IN ACETAMINOPHEN'S ACTION MECHANISM: A NEW PERSPECTIVE ON ENDOCANNABINOID INTERACTIONS | Michaela Dvorakova, Taryn Bosquez-Berger, Jenna Billingsley, Natalia Murataeva, Wenwen Du, Taylor Woodward, Emma Leishman, Anaëlle Zimmowitch, Anne Gibson, Jim Wager-Miller, Ruyi Cai, Shangxuan Cai, Yulong Li, Heather Bradshaw, Ken Mackie and Alex Straiker | 55 |
| 9:00-9:15 | AN ENDOCANNABINOID SENSOR-BASED ASSAY OF NAPE-PLD ACTIVITY | Jim Wager-Miller, Connor Schmitt, Ru-Yi Cai, Shangxuan Cai, Yulong Li, Ken Mackie and Alex Straiker | 56 |
| 9:15-9:30 | DEUTERIUM-LABELED ENDOCANNABINOIDS ARE ABUNDANTLY PRESENT IN CENTRAL NERVOUS SYSTEM TISSUE MINUTES AFTER PERIPHERAL INJECTION | Heather Bradshaw, Taylor Woodward, Wenwen Du | 57 |

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| 9:30-9:45 | WATCHING THE CLOCK: CANNABINOID REGULATION OF CIRCADIAN TEARING | Natalia Murataeva, Sam Mattox and Alex Straiker | 58 |
| 9:45-10:00 | DEUTERIUM-LABELED PALMITOYLETHANOLAMIDE (D5-PEA) SHOWS RELIABLE BRAIN PENETRANCE IN A DOSE DEPENDENT MANNER AND EXOGENOUS PEA SHOWS CHANGES IN CNS CONNECTIVITY AND LIPIDOME | Taylor Woodward, Wenwen Du, Shreyas Balaji, Praveen Kulkarni, Craig Ferris and Heather Bradshaw | 59 |
| 10:00-10:30 | Coffee break | | |
| 10:30-11:30 | **ICRS Lifetime Achievement Awardee** ENDOCANNABINOIDS AND THEIR INTERACTION WITH BIOACTIVE LIPIDS: THE NEXT FRONTIER | Mauro Maccarrone | 60 |
| **Session 5. Organ-Specific Cannabinoid Modulation (Liver, Kidney, Gut)** Chairs: Tony Jourdan & Ozge Gunduz Cinar | | | |
| 11:30-11:45 | HEPATIC CB1 RECEPTOR SIGNALING TRIGGERS $G_{i/o}\alpha$-MEDIATED LIPOLYSIS IN LEAN MICE BUT $G_s\alpha$-MEDIATED LIPOGENESIS IN OBESE MICE | Jie Liu, Grzegorz Godlewski, Radka Kocvarova, Muhammad Arif, Abhishek Bisu, Malliga R. Iyer, Resat Cinar, Joseph Tam and George Kunos | 62 |
| 11:45-12:00 | PHYTOCANNABINOIDS REPROGRAM LIPID METABOLISM TO REVERSE FATTY LIVER DISEASE | Radka Kocvarova, Shahar Azar, Ifat Abramovich, Bella Agranovich, Eyal Gottlieb, Liad Hinden and Joseph Tam | 63 |
| 12:00-12:15 | ENDOCANNABINOID SYSTEM DYNAMIC CHANGES IN A POLYCYSTIC KIDNEY DISEASE MOUSE MODEL | Shridhar Betkar, Liad Hinden and Joseph Tam | 64 |

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| 12:15-12:30 | ANTI-FIBROTIC AND RENOPROTECTIVE SYNERGY THROUGH CB1R/SGLT2i COMBINATION | Océane Pointeau, Abhishek Basu, Julia Leemput, Romain Barbosa, Patricia Passilly-Degrace, Laurent Demizieux, Hélène François, Bruno Vergès, Resat Cinar, Pascal Degrace and Tony Jourdan | 65 |
| 12:30-12:45 | DYNAMIC ECS/CB1R ALTERATIONS GUIDE THERAPEUTIC TARGETING IN ACUTE KIDNEY INJURY AND ITS MALADAPTIVE REPAIR | Ariel Rothner, Liad Hinden, Aviram Kogot-Levin, Elisheva Benkovitz, Amani Zoabi, Anna Permyakova, Asaf Kleiner, Vladislav Nesterenko, Alina Nemirovski, Ifat Abramovich, Bella Agranovich, Inbar Plaschkes, Eyal Gottlieb, Katherine Margulis, Gil Leibowitz and Joseph Tam | 66 |
| 12:45-14:00 | Lunch | | |
| 14:00-14:30 | **ICRS Lifetime Achievement Awardee**<br><br>TWO DECADES OF RESEARCH EXAMINING THE OCULAR ENDOCANNABINOID SYSTEM AS A TARGET FOR OCULAR THERAPEUTICS | Melanie Kelly | 67 |
| **Session 6. Psychedelics and Broader Neuropharmacology**<br><br>Chairs: Heather Bradshaw & Alex Straiker | | | |
| 14:30-14:45 | PSILOCYBIN AS A TREATMENT FOR REPETITIVE MILD HEAD INJURY: EVIDENCE FROM NEURORADIOLOGY AND MOLECULAR BIOLOGY POINTING TO INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM | Reagan Walhof, Wenwen Du, Taylor Woodward, Praveen Kulkarni, Craig Ferris and Heather Bradshaw | 68 |

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| 14:45-15:00 | CANNABIS-PSILOCYBIN CO-USE AND ARTERIAL STIFFNESS: PRELIMINARY FINDINGS FROM YOUNG ADULTS IN THE HERBAL HEART STUDY | Bria-Necole Diggs, Amrit Baral, Ranya Marrakchi El Fellah, Sarah Messiah, Girardin Jean-Louis, Michelle Weiner, Marvin Reid, Marilyn Lawrence-Wright, Winston de la Haye, Barry Hurwitz, Claudia Martinez and Denise Vidot | 69 |
| **15:00-15:45** | **Data Blitz 2**<br>Chairs: Steven Kinsey & Andrea Hohmann | | |
| | THE FATTY ACID BINDING PROTEIN 5 INHIBITOR ART26.12 ALLEVIATES OSTEOARTHRITIS PAIN | Kai Bou, Adam Bruzzese, Chris Gordon, Kaitlin Farrell, Saoirse O'Sullivan, David Komatsu and Martin Kaczocha | P2-5 |
| | ASSESSMENT OF ANTINOCICEPTION AND TOLERANCE TO INHIBITORS OF FATTY ACID AMIDE HYDROLASE AND MONOGLYCERIDE LIPASE IN A MURINE MODEL OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY | America Alanis, Robert Barnes, Mikaela Aleman, Henry Blanton, Boyd Rorabaugh, Daniel Morgan and Josee Guindon | P2-6 |
| | TARGETING ENDOCANNABINOID SYSTEM DEFICITS AS A THERAPEUTIC STRATEGY FOR GULF WAR ILLNESS IN A MOUSE MODEL | Kwang-Mook Jung, Erica Squire, Hye-Lim Lee, Vipan Parihar and Daniele Piomelli | P2-30 |
| | CANNABINOID 2 RECEPTOR INCREASES EXPRESSION IN THE PERIPHERY AND BRAIN OF AGED MICE | Ann Titus, Kaylin Falkner, Danielle Robins, Cassandra Cole, Janna Jernigan and Valerie Joers | P2-31 |
| | INVESTIGATING THE ANALGESIC, ABUSE-RELATED, AND THC SPARING EFFECTS OF MYRCENE, A CANNABIS-BASED TERPENE, IN A WITHIN-SUBJECTS PLACEBO-CONTROLLED STUDY | Samantha L. Baglot, Stephanie Lake, Conor H. Murray, Elisa Pabon, Alisha Eversole, Katherine Hampilos, Timothy Fong and Ziva D. Cooper | P2-34 |
| | UK MEDICAL CANNABIS REGISTRY: AN UPDATED CLINICAL OUTCOMES ANALYSIS ACROSS ALL CONDITIONS | Simon Erridge, Evonne Clarke, Katy McLachlan, Ross Coomber, Sushil Beri, Shaheen Khan, Mark Weatherall, Michael Platt, James Rucker and Mikael Sodergren | P2-16 |

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| | IMPACT OF CANNABIS EXPOSURE ON GUT BARRIER FUNCTION IN METABOLIC HEALTH AND DISEASE | Martin Olmos and Nicholas DiPatrizio | P2-45 |
| **15:45-17:45** | **Poster Session 2**<br>*Odd numbered posters will be presented during the first hour (16:00) of the poster session and even numbered posters will be presented in the second half (17:00) of the poster session | | |
| 17:45-18:15 | **Business meeting** | | |
| 18:15 | Dinner | | |

# Day 3, Wednesday July 9

| | | | |
|---|---|---|---|
| 7:30 | Breakfast | | |
| 8:25-8:30 | Opening remarks | | |
| **Session 7. Cannabis in Healthy Populations: Effects on Cognitive, Metabolic, and Cardiovascular Systems**<br>Chairs: Matthew Hill & Carrie Cuttler | | | |
| 8:30-8:45 | MAPPING ACUTE EFFECTS OF CANNABIS ON MULTIPLE MEMORY DOMAINS: A DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY | Carrie Cuttler and Ryan McLaughlin | 70 |
| 8:45-9:00 | INVESTIGATING 'THE MUNCHIES' IN RODENTS AND HUMANS; EFFECTS OF Δ-9-THC VAPOUR INHALATION ON FEEDING PATTERNS, MACRONUTRIENT PREFERENCE, SATIETY AND REWARD VALUE | Catherine Hume, Carrie Cuttler, Samantha Baglot, Lucia Javorcikova, Ryan McLaughlin and Matthew Hill | 71 |

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| 9:00-9:15 | THE EFFECT OF VAPORIZED & ORAL ∆8-TETRAHYDROCANNABINOL VS ∆9-TETRAHYDROCANNABINOL ON SIMULATED DRIVING PERFORMANCE IN HEALTHY ADULTS | Lakshmi Kumar, Austin Zamarripa, Tory Spindle, Edward Cone, Ruth Winecker, Ronald Flegel and Ryan Vandrey | 72 |
| 9:15-9:30 | CANNABIS AND ARTERIAL STIFFNESS: DIFFERENCES BY ROUTE OF CONSUMPTION AMONG HEALTHY YOUNG ADULTS IN THE HERBAL HEART STUDY | Amrit Baral, Ranya Marrakchi El Fellah, Bria-Necole Diggs, Sarah Messiah, Girardin Jean-Louis, Marvin Reid, Marilyn Lawrence-Wright, Lisa Reidy, Barry Hurwitz, Claudia Martinez and Denise Vidot | 73 |
| 9:30-9:45 | CANNABIS AND VASCULAR FUNCTION: ENDOTHELIAL DYSFUNCTION AND ARTERIAL STIFFNESS AMONG YOUNG ADULTS IN THE HERBAL HEART STUDY | Denise C. Vidot, Amrit Baral, Kylee Krivijanski, Bria-Necole A Diggs, Sarah E. Messiah, Girardin Jean-Louis, Marvin Reid, Marilyn Lawrence-Wright, Lisa Reidy,Barry Hurwitz, Claudia Martinez | 74 |
| 9:45-10:00 | COMPREHENSIVE POPULATION PHARMACOKINETIC ANALYSIS OF TETRAHYDROCANNABINOL AND ITS ACTIVE METABOLITE ACROSS INTRAVENOUS, ORAL, VAPED, AND SMOKED ROUTES | Babajide Shenkoya, Michael Tagen, Linda Klumpers, Ryan Vandrey and Mathangi Gopalakrishnan | 75 |
| 10:00-10:15 | THE IMPACT OF PRODUCT FORMULATION ON THE PHARMCOKINETICS AND PHARMACODYNAMICS OF CANNABIS EDIBLES | Lakshmi Kumar, Austin Zamarripa, Elise Weerts, David Wolinsky, Jost Klawitter, Uwe Christians, Ryan Vandrey and Tory Spindle | 76 |
| 10:15-10:30 | IMPACT OF ACUTE Δ9-TETRAHYDROCANNABINOL (THC) ON PHARMACOKINETICS, SUBJECTIVE EFFECTS AND COGNITIVE FUNCTION IN HEALTHY VOLUNTEERS | Nadia Leen, Henrika van Hell, Gerry Jager, Nick Ramsey and Matthijs Bossong | 77 |
| 10:30-11:00 | Coffee break | | |

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| 11:00-11:30 | RESOURCE CENTER FOR CANNABIS & CANNABINOID RESEARCH (R3CR): A NEWLY FUNDED CENTER | Ikhlas Khan, Mahmoud ElSohly, Mary F. Paine, Donald Stanford, Nandakumara Sarma, Robert Welch, Patrick C. Still, Inna Belfer, Heather L. Kimmel, Alexis Bakos, Kathleen Castro | 78 |
| **Session 8. Metabolic Determinants of Organ Dysfunction**<br>Chairs: Yossi Tam & Resat Cinar | | | |
| 11:30-11:45 | PARTIAL INVERSE AGONISM OF CB1 AS A DE-RISKED STRATEGY FOR OBESITY | Lucas Laudermilk, George Amato, Vineetha Vasukuttan, Andrew Harris, Sheryl Moy, Elaine Gay and Rangan Maitra | 79 |
| 11:45-12:00 | ENDOTOXEMIA ALTERS PLASMA ENDOCANNABINOID AND ETHANOLAMIDE PROFILES IN DAIRY COWS | Madison N. Myers, Miguel Chirivi, Jair Parales-Girón, Jose M. dos Santos Neto, Lynn C. Worden, Adam L. Lock and G. Andres Contreras | 80 |
| 12:00-12:15 | ENDOCANNABINOIDS PRODUCED BY VISCERAL ADIPOSE TISSUE MAY LIMIT THE FORMATION OF NEW ADIPOCYTES | Romain Barbosa, Patricia Passilly-Degrace, Julia Leemput, Océane Pointeau, Tony Jourdan, Laurent Demizieux, Bruno Vergès and Pascal Degrace | 81 |
| 12:15-12:30 | CNS-SPARING CANNABINOID THERAPEUTICS: A DUAL CB1R/INOS BLOCKADE APPROACH TO TREAT METABOLIC SYNDROME DISORDERS | Pinaki Bhattacharjee, Szabolcs Dvorácskó, Paul Volesky, Nick Rutland, Luca Maccioni, Sergio A. Hassan, Resat Cinar and Malliga R. Iyer | 82 |
| 12:30-13:30 | **Kang Tsou Memorial Lecture**<br>MEDICINAL EVOLUTION OF NATURAL HORMONES TO TRANSFORM DRUG TREATMENT OF OBESITY | Richard Di Marchi | 83 |
| 13:30-15:00 | Lunch | | |
| 15:00 | Free time | | |

# Day 4, Thursday, July 10

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| 7:30 | Breakfast | | |
| 8:25-8:30 | Opening remarks | | |
| 8:30-9:30 | **Presidential Plenary Lecture 2:**<br><br>1992-2025: FROM ENDOCANNABINOID-MEDIATED INTER-CELLULAR SIGNALING TO ENDOCANNABINOIDOME-MEDIATED INTER-KINGDOM CHEMICAL COMMUNICATION | Vincenzo Di Marzo | 84 |
| **Session 9. Medical Cannabis in Clinical Practice: Emerging Evidence and Indications**<br><br>Chairs: Ziva Cooper & Linda Klumpers | | | |
| 9:30-9:45 | ASSOCIATIONS BETWEEN CANNABIS USE FREQUENCY, CIRCULATING CANNABINOID AND ENDOCANNABINOID LEVELS, AND PAIN SENSITIVITY | Elisa Pabon, Adren Tran, Alexa Torrens, Anna Hilger, Conor H. Murray, Stephanie Lake, Timothy Fong, Daniele Piomelli and Ziva D. Cooper | 85 |
| 9:45-10:00 | ENDOCANNABINOID SYSTEM ALTERATIONS IN PATIENTS WITH CHRONIC LOW BACK PAIN | Mary Hopkins, Stephanie Bourke, Caroline Mitchell, Mairead Finn, David O'Gorman, Chris Maharaj, David Cosgrave, Brian McGuire and David Finn | 86 |
| 10:00-10:15 | A RANDOMIZED PHASE II TRIAL OF MEDICAL CANNABIS TO REDUCE SYMPTOM BURDEN IN PATIENTS WITH ADVANCED PANCREATIC CANCER | Dylan Zylla, Elle Chrenka, Grace Gilmore, Jordan Cowger, David Rak and Arjun Gupta | 87 |

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| 10:15-10:30 | UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS OF MEDICAL CANNABIS FOR FIBROMYALGIA | Madhur Varadpande, Simon Erridge, Arushika Aggarwal, Evonne Clarke, Katy McLachlan, Ross Coomber, Shelly Barnes, Alia Darweish Medniuk, Rahul Guru, Wendy Holden, Mohammed Sajad, Robert Searle, Azfer Usmani, Sanjay Varma, James Rucker, Michael Platt and Mikael Sodergren | 88 |
| 10:30-10:45 | CANNABIS WITHDRAWAL SYNDROME AT ILLNESS ONSET IN FIRST-EPISODE PSYCHOSIS AND THE RISK OF SUBSEQUENT RELAPSE: A NESTED CASE-CONTROL STUDY | Edward Chesney, Dominic Oliver, Samuel Atkinson, Yasamine Farahani-Englefield, Fraser Scott, Amelia Jewell, Thomas J Reilly, Diego Quattrone, Robin Murray, Marta Di Forti, Daniel Stahl, Philip McGuire and Edoardo Spinazzola | 89 |
| 10:45-11:00 | PREDICTIVE ROLE OF INCREASED BLOOD ANANDAMIDE AT HOSPITAL ADMISSION IN ACUTE COVID PNEUMONIA RELATED RESPIRATORY FAILURE ON THE POST-COVID LUNG FIBROSIS | Abhishek Basu, Lenny Pommerolle, Muhammad Arif, Angelo Meliton, Inemesit Udofia, David Wu, Gökhan Mutlu, Bernadette Gochuico, Ross Summer, Ayodeji Adegunsoye, Ellen Burnham and Resat Cinar | 90 |
| 11:00-11:30 | Coffee break | | |
| **Session 10. Sex-Specific Cannabinoid Effects on Neurobiology, Behavior, and Inflammation**<br><br>Chairs: Cecilia Hillard & Natalia Malek | | | |
| 11:30-11:45 | MODELING ADOLESCENT EDIBLE THC CONSUMPTION TO EXPLORE THE INFLUENCE OF SEX AND DOSE ON LONG-TERM NEURODEVELOPMENTAL OUTCOMES | Marieka DeVuono, Samantha Anderson, Amanda Alcaide, Mathusha Pusparajah, Jaun-Pablo Galindo Lazo, Mohammed Sarikahya, Hanna Szkudlarek, Marta De Felice, Ken Yeung, Walter Rushlow and Steven Laviolette | 91 |

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| 11:45-12:00 | INVESTIGATING THE RESPONSE OF THE ENDOCANNABINOID SYSTEM FOLLOWING REPETITIVE MILD TRAUMATIC BRAIN INJURY IN ADOLESCENT MALE AND FEMALE SPRAGUE-DAWLEY RATS | Lucia Javorcikova, Samantha L Baglot, Catherine Hume, Thomas Carr, Aly Muhammad Salim, Jessica Scheufen, Alexander W Lohman and Matthew N Hill | 92 |
| 12:00-12:15 | EFFECTS OF ESTROUS CYCLE ON BEHAVIOR AND NEUROINFLAMMATION IN D9-THC-TREATED HIV-1 TAT TRANSGENIC FEMALE MICE | Barkha Yadav-Samudrala, Karenna Barmada, Havilah Ravula and Sylvia Fitting | 93 |
| 12:15-12:30 | SEX DIFFERENCES IN STRESS RESPONDING IN A CLINICAL TRIAL OF PROGESTERONE IN CANNABIS USE DISORDER | Erin Martin, Nathaniel Baker, Brian Neelon, Zoe Watson, Michael Saladin, Rachel Tomko, Brian Sherman, Kevin Gray and Aimee McRae-Clark | 94 |
| 12:30-12:45 | CANNABINOID REGULATION OF MURINE VAGINAL SECRETION | Natalia Murataeva, Sam Mattox, Kyle Yust and Alex Straiker | 95 |
| 12:45-13:00 | Δ9-TETRAHYDROCANNABINOL AND ITS EFFECTS IN A NEUROHIV MOUSE MODEL | Havilah Ravula, Barkha Yadav-Samudrala, Laith Sawaqed, Sarah Arteaga, Essie Acquah and Sylvia Fitting | 96 |
| 13:00-14:30 | Lunch | | |
| 14:30-15:30 | **Mechoulam Awardee**<br><br>A NEW MECHANISM OF CANNABINOID TOLERANCE | Manuel Guzman | 97 |

| Session 11. Phytocannabinoids: Pharmacology, Therapeutic Potential, and Behavioral Effects<br>Chairs: Ethan Russo & Hunter Land | | | |
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| 15:30-15:45 | DEVELOPMENT OF A RAPID LC-MS/MS QUANTIFICATION METHOD IN MICE TO STUDY THE PHARMACOKINETICS AND ANXIOLYTIC EFFECTS OF CANNABIGEROL | Alex Mabou Tagne, Faizy Ahmed, Adren Tran, Lana Debbaneh and Daniele Piomelli | 98 |
| 15:45-16:00 | ANTINOCICEPTIVE EFFECTS OF CANNABIDIOL AND CANNABIGEROL IN AN INCISIONAL WOUND MODEL | Maria Redmond, Catherine Healy, Rosmara Infantino, Mary Hopkins, Georgina Gethin, Abhay Pandit and David Finn | 99 |
| 16:00-16:15 | EVALUATING THE THERAPEUTIC POTENTIAL OF CBD-CORTICOSTEROID COMBINATION THERAPY ON OSTEOARTHRITIS-RELATED CELL LINES IN VITRO | Tim Lefever, Natalia Malek, Julia Jarco and Hunter Land | 100 |
| 16:15-16:30 | Body Break | | |
| 16:30-16:45 | EXPLORING THE PHARMACOKINETICS OF PHYTOCANNABINOIDS AND THEIR METABOLITES: AN ASSESSMENT OF ACUTE ORAL CANNABIGEROL (CBG) AND CANNABICHROMENE (CBC) | Ee Tsin Wong, Antasha Zainal, Eric Salazar, Jenny Ho, Blaine Phillips, Wenhao Xia, David Bovard, Elizabeth Cairns and Julia Hoeng | 101 |
| 16:45-17:00 | EFFECTS OF CANNABIDIOL (CBD) ON SPONTANEOUS OPIOID WITHDRAWAL IN MALE AND FEMALE RATS | Bryan Jenkins, Cerina Pang, Travis Wilberger, Robbie Kuang, Won Park, Allie Hausker, Tylaah George, Tom Wang, Elise Weerts and Catherine Moore | 102 |
| 17:00-17:15 | Δ9-TETRAHYDROCANNABINOL EFFECTS ON PHYSIOLOGY AND BEHAVIOR ARE ALTERED IN MICE LACKING REGULATOR OF G PROTEIN SIGNALING 12 | Antonio Reck, David Siderovski and Steven Kinsey | 103 |

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| 17:15-17:45 | **ICRS Early Career Awardee**<br><br>PAMTASTIC VOYAGE: EXPLORING CB1 PHARMACOLOGY WITH POSITIVE ALLOSTERIC MODULATOR (PAM)-ANTAGONISTS | Thomas Gamage | 104 |
| 19:00 | **Banquet (Tudor Room)** | | |

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| P1-2 | ASSESSMENT OF ACEA ANTINOCICEPTIVE SEX DIFFERENCES AND TOLERANCE DEVELOPMENT IN MURINE MODELS OF INFLAMMATORY AND NEUROPATHIC PAIN | Robert Barnes, Dakota Robison, America Alanis, Mikaela Aleman, Satish Banjara and Josee Guindon | 107 |
| P1-3 | NOVEL CANNABICHROMENE DERIVATIVE LACKING ABUSE LIABILITY EFFECTIVELY REDUCES PAIN IN MICE | Miguel De Leon, Rebecca Ozborn, Hannah Harris, Iram Shahzadi, Waseem Gul, Mahmoud ElSohly and Nicole Ashpole | 108 |
| P1-4 | ALLOSTERIC LIGANDS TARGETING THE CB1 CANNABINOID RECEPTOR SUPPRESS INFLAMMATORY PAIN WITHOUT PRODUCING RESPIRATORY DEPRESSION OR MOTOR IMPAIRMENT | Ifeoluwa Solomon, Ganesh Thakur, Sumanta Garai and Andrea Hohmann | 109 |
| P1-5 | ANTINOCICEPTIVE EFFECTS OF FAAH OR MGL INHIBITION IN A PRECLINICAL MODEL OF LOW BACK PAIN | Mary Hopkins, Maria Redmond, Catherine Healy and David Finn | 110 |
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| P1-7 | THE ENDOCANNABINOID SYSTEM IN NEURAL STEM CELLS AS A BRIDGE AMONG ADULT HIPPOCAMPAL NEUROGENESIS, STRESS RESILIENCE AND ENVIRONMENTAL CHALLEGES | Mauro Maccarrone, Lucia Scipioni, Daniel Tortolani, Francesca Ciaramellano and Sergio Oddi | 112 |

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| P1-11 | SYNTHETIC CANNABINOID WIN-55 INCREASES MITOCHONDRIAL OXYGEN CONSUMPTION IN HEALTHY OPTIC NERVE HEAD ASTROCYTES WHILE DECREASING LACTATE RELEASE IN STRESSED ASTROCYTES | Olivia Young and Denise Inman | 116 |
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ICRS 2025

35th ANNUAL SYMPOSIUM

JULY 6-10 • BLOOMINGTON, IN

# ORAL PRESENTATION ABSTRACTS

# INHIBITORS OF ENDOCANNABINOID DEACTIVATION DIFFERENTIALLY SUPPRESS PACLITAXEL-INDUCED NEUROPATHY AND TUMOR GROWTH IN A MOUSE MODEL OF BREAST CANCER

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**Introduction:** Neuropathic pain is a dose-limiting side effect of chemotherapeutic treatment. Inactivation of enzymes that degrade endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA) suppress neuropathic pain behavior in rodent models of chemotherapy-induced peripheral neuropathy (CIPN). Our labs previously reported that global and peripherally-restricted inhibitors of either fatty-acid amide hydrolase FAAH) or monoacyglycerol lipase (MAGL) suppressed paclitaxel-induced neuropathic pain in a mouse model of chemotherapy-induced peripheral neuropathy. Impact of these treatments on CIPN in tumor-bearing animals remains poorly understood.

**Methods:** We evaluated impact of prophylactic dosing with the peripherally-restricted MAGL inhibitor LEI-515, global MAGL inhibitor JZL184, and peripherally-restricted FAAH inhibitor URB937 in Balbc mice inoculated with 4T1 breast cancer cells. Paclitaxel was administered every two days. Mechanical and cold hypersensitivities were evaluated using the von Frey test and the acetone test, respectively. Tumor volume was measured daily using calipers. Weight of colonic contents was measured at the terminal endpoint.

**Results:** Peripherally-restricted MAGL (LEI-515) and FAAH (URB937) inhibitors suppressed development of both mechanical and cold hypersensitivity induced by paclitaxel whereas the global MAGL inhibitor (JZL184) failed to do so, likely due to tolerance. Both LEI-515 and JZL184 enhanced ability of paclitaxel to reduce tumor volumes *in vivo*. The peripherally-restricted FAAH inhibitor URB937 did not alter ability of paclitaxel to reduce tumor volume *in vivo*. Paclitaxel-induced slowing of colonic transit was not exacerbated by any inhibitor administered prophylactically.

**Conclusions:** Peripherally-restricted MAGL inhibitor LEI-515 holds promise for suppressing neuropathic pain induced by chemotherapy while enhancing the anti-cancer effects of chemotherapy in a mouse model.

# STRUCTURE-GUIDED OPTIMIZATION OF MAGL INHIBITORS FOR THE TREATMENT OF NEUROPATHIC PAIN

Mirjam C.W. Huizenga[1], Jonah L. Wirt[2], Richard J.B.H.N. van den Berg[1], Darcy N. Reynolds[1], Joey Reverey[1], Anna F. Stevens[1], Daan van der Vliet[1], Diep Vu[3], Rogier Buijsman[3], Antonius P.A. Janssen[1], Andrea G. Hohmann[2], Mario van der Stelt[1]

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**Introduction:** Chemotherapy-induced peripheral neuropathy (CIPN) is a major dose-limiting side effect of anti-tumor agents, often leading to chemotherapy discontinuation. Current analgesic treatments show limited efficacy and cause central nervous system (CNS)-mediated side effects. Monoacylglycerol lipase (MAGL) inhibitors have emerged as promising alternatives, but their therapeutic potential is restricted by CNS-related adverse effects. Recently, our research led to the discovery of **LEI-515**, a peripherally restricted, reversible MAGL inhibitor that effectively suppressed CIPN in mice without inducing tolerance, physical dependence, or CNS side effects. To advance **LEI-515**-related MAGL inhibitors into the clinic, multiple properties, including cellular target engagement and selectivity, require optimization.

**Methods:** The co-crystal of **LEI-515** with MAGL was used to design and synthesize novel inhibitors. The compounds were evaluated on biochemical activity and cellular target engagement. Furthermore, the *in vitro* ADME properties were assessed as well as selectivity using activity-based protein profiling (ABPP) methods. The compound with the most promising profile was tested in a spared nerve injury (SNI) mouse model to assess *in vivo* efficacy in a robust preclinical pain model.

**Results:** Structure-guided optimization yielded a library of seventeen compounds with low nanomolar biochemical MAGL activity and excellent cellular target engagement. The inhibitors were selective and exhibited excellent *in vitro* ADME properties. The compound with the best overall profile was able to suppress mechanical allodynia in a SNI mouse model.

**Conclusion:** This study demonstrates the successful structure-guided optimization of a MAGL inhibitor with *in vivo* efficacy, which represents a promising candidate for the treatment of neuropathic pain.

# CB2 CANNABINOID RECEPTOR-SPECIFIC THERAPEUTIC ANTIBODY AGONISTS FOR TREATMENT OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

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**Introduction:** Chemotherapy-induced peripheral neuropathy (CIPN) is a common and debilitating complication of cancer treatment. CB2 receptors are highly expressed in immune cells where their activation can reduce inflammation. CB2 is an attractive G protein-coupled receptor (GPCR) target for drug development because activation does not produce adverse CNS side-effects. CB2 agonists suppress CIPN in preclinical studies. Antibodies of GPCRs offer high target specificity and peripheral restriction, thereby avoiding unwanted off-target activity. We partnered with Abalone Bio Inc., who used their Functional Antibody Selection Technology (FAST) platform to develop CB2-specific antibodies, to investigate the efficacy of CB2-specific antibody agonists in a mouse model of paclitaxel-induced peripheral neuropathy.

**Methods**: We assessed efficacy of CB2-specific antibodies (AB110 and AB120) and their isotype control (AB100) on mechanical and cold hypersensitivity in a mouse model of paclitaxel-induced peripheral neuropathy (CIPN). Impact on mouse and human macrophage activation was also assessed *in vitro.*

**Results**: AB100 and AB120 decreased M1 and M2 macrophage activation markers *in vitro*. Paclitaxel produced mechanical and cold hypersensitivity in female mice. AB110 and AB120 suppressed paclitaxel-induced hypersensitivities for ~48 hours after injection. Repeated daily dosing with AB110 and AB120 did not induce analgesic tolerance. Prophylactic dosing with AB110 and AB120 before and during paclitaxel dosing attenuated development of paclitaxel-induced mechanical and cold hypersensitivity. This therapeutic effect dissipated after cessation of treatment. AB100 did not alter mechanical or cold responsiveness in any study.

**Conclusion**: CB2 specific antibodies show therapeutic potential for the treatment of CIPN without development of analgesic tolerance to repeated dosing.

**Acknowledgements:** Supported by CA241513 (to LS and AGH)

# THE NAAA INHIBITOR ARN19702 SUPPRESSES INFLAMMATORY AND NEUROPATHIC PAIN IN MICE

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**Introduction**
The enzyme *N*-acyl ethanolamine acid amidase (NAAA), which degrades the anti-inflammatory lipid palmitoylethanolamide (PEA), is implicated in pain processing. We characterized effects of the NAAA inhibitor ARN19702 in inflammatory and neuropathic pain models and ascertained whether inhibition of NAAA could counteract development of morphine tolerance in mice.

**Methods**
Inflammatory pain was induced by intraplantar injection of complete Freund's Adjuvant (CFA). Neuropathic pain was induced by performing a unilateral spared nerve injury (SNI). Mechanical paw withdrawal thresholds were assessed before and after injury and following pharmacological manipulations. Vehicle or various doses (i.p.) of ARN19702 were administered acutely (CFA, SNI) to assess duration of action. Effective doses were tested in NAAA knockout (KO) mice. Mice with SNI received repeated injections of vehicle, morphine, ARN19702 or ARN19702 co-administered with morphine to ascertain whether ARN19702 would suppress the development of morphine tolerance.

**Results**
ARN19702 reversed CFA- and SNI-induced mechanical hypersensitivity but was inactive in NAAA KO mice. Ibuprofen attenuated CFA-induced mechanical hypersensitivity in NAAA KO mice. Males showed faster onset, whereas females showed more prolonged ARN19702-induced analgesia in CFA-injected mice. ARN19702 attenuated established neuropathic pain in the SNI model. Anti-allodynic efficacy of acute ARN19702 (30 mg/kg) lasted at least 24 hours in mice with SNI. Following chronic dosing in the SNI model, tolerance developed to the anti-allodynic effects of morphine, but not ARN19702. Moreover, a behaviorally inactive dose of ARN19702 attenuated development of morphine tolerance.

**Conclusions**
NAAA inhibitors represent a therapeutic strategy to suppress inflammatory and neuropathic pain and counteract opioid analgesic tolerance.

**Acknowledgments**
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# VALIDATION OF A RAT MODEL OF INCISIONAL WOUND-RELATED PAIN: EFFECT OF ENDOCANNABINOID SYSTEM MODULATION

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**Introduction:** There is no well-validated preclinical model to study incisional wound-related pain. Our previous experiments have highlighted the dorsum incision model as a model of wound-related pain. The endocannabinoid system (ECS) is involved in wound healing and pain modulation. Experimental aims were to investigate the effects of 1) morphine administration (3 mg/kg s.c.) or 2) inhibitors of the endocannabinoid-catabolising enzymes FAAH (URB597, 1mg/kg i.p.) and MGL (MJN110, 5 mg/kg i.p.) on pain-related behaviour following dorsum incision.

**Methods:** 36 male Sprague-Dawley rats (6-8 weeks, 180-200g on arrival) were used for each experiment. A 1.2 cm incision was made on the dorsum under isoflurane anaesthesia. Mechanical withdrawal thresholds were assessed at baseline, post-surgical day (PSD) 1, PSD 4, prior to drug administration (PSD 7 or 8) and 1 hour post-drug on PSD 8 via manual and electronic Von Frey test on the dorsum and hindpaws, respectively. Rats were euthanised 80 minutes post-morphine or 90 minutes post-URB597 or MJN110.

**Results:** There was robust primary (dorsum) and secondary (hindpaws) mechanical hypersensitivity following dorsum incision. Morphine significantly attenuated incision-related hypersensitivity at 1 hour post-administration. MJN110 significantly attenuated dorsum mechanical hypersensitivity 1 hour post-administration. MJN110 or URB597 administration significantly attenuated hindpaw mechanical hypersensitivity, 1 hour post-administration, and elevated endocannabinoid levels in the CNS. Morphine increased plasma levels of PEA and OEA.

**Conclusions:** The dorsum incision model of wound-related pain is sensitive to mu-opioid receptor agonism and elevation of endocannabinoids. These results provide a basis for further investigation into the ECS as a novel target for incision-related pain.

**Ethical Approval:** The experimental procedures were approved by the Animal Care and Research Ethics committee, University of Galway. The experiments were completed under licence from the Health Products Regulatory Authority in the Republic of Ireland, in accordance with the EU Directive 2010/63. The study was designed in accordance with the ARRIVE guidelines.

**Acknowledgements**: Funding provided by the Taighde Éireann - Research Ireland Postgraduate Scholarship, Hardiman Research Scholarship, Research Ireland and B. Braun Hospicare and co-funded under the European Regional Development Fund under Grant Number 13/RC/2073-P2

# SEX-STRATIFIED ANALYSIS OF SPINAL ENDOCANNABINOID SYSTEM AND NEUROIMMUNE INTERACTIONS IN NEUROPATHIC PAIN

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**Introduction:** Sex differences in pain pathophysiology and treatment efficacy have become a critical focus in biomedical research, with growing evidence that biological sex influences neuroimmune interactions and endocannabinoid system (ECS) regulation. While chronic pain models reveal sex-divergent ECS adaptations in the brain, spinal cord-specific changes remain poorly characterized. This study employs advanced spatial multi-omics to map sex differences in spinal ECS components and neuroimmune dynamics in a murine neuropathic pain model.

**Methodology:** We applied imaging mass cytometry (IMC) and single-cell spatial transcriptomics to compare spinal cord tissue from male and female mice subjected to chronic constriction injury (CCI). This approach enabled high-resolution quantification of ECS components, immune cell populations and spatial interactions across dorsal horn laminae.

**Results:** Females displayed distinct CB1 expression in dorsal horn interneurons and elevated CB2 levels in perivascular microglia, whereas males showed predominant CB2 expression in astrocytes. Chronic constriction injury (CCI) led to increased microglial clustering in males, while females exhibited heightened T lymphocyte infiltration. Additionally, CB2 co-localization with Iba1+ macrophages demonstrated clear sex-dependent patterns.
Conclusion: Our multi-omic atlas reveals profound sex differences in spinal ECS architecture and neuroimmune crosstalk during neuropathic pain. These findings underscore the necessity for sex-stratified therapeutic strategies targeting cannabinoid signaling pathways.

This work was supported by National Science Center grant 2023/49/B/NZ7/02172.

## CANNABIDIOL (CBD) IMPROVES ALLODYNIA AND COGNITIVE FUNCTION ALONG WITH SYNAPTIC PLASTICITY IN A MOUSE MODEL OF SPARED NERVE INJURY

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**Introduction:** Neuropathic pain contributes to hypocognition associated with hippocampal neuroinflammation. This study aimed to investigate the effect of chronic cannabidiol (CBD), a GPR55 antagonist, on allodynia, synaptic plasticity, and hippocampal neurogenesis in a mouse model of spared nerve injury (SNI), a model of neuropathic pain.

**Methods:** Male C57BL/6 mice underwent SNI and received CBD (5 mg/kg) or vehicle orally for 14 days, starting from the point at which neuropathic pain symptoms were well established. Pain sensitivity was assessed with Von Frey and cold plate tests, while cognition was evaluated with novel object recognition and Y-maze tests. Electrophysiological recordings of long-term potentiation (LTP) were performed in the lateral entorhinal cortex–dentate gyrus (LEC-DG) pathway. In vitro, hippocampal neural stem cells (P0-P1 mice) were exposed to CBD (0.1–5.0 μM) for 24 hours, followed by a 7-day differentiation period. Cells were then stained for astrocytic (GFAP) and neuronal (Tuj1) markers.

**Results:** CBD reduced allodynia, improved memory, and restored LTP in the LEC-DG pathway in SNI mice. Additionally, CBD increased Tuj1-positive neurons in hippocampal cell cultures.

**Conclusions:** Chronic CBD alleviates allodynia, restores synaptic function, and enhances neurogenesis, supporting its potential as a therapeutic agent. However, further studies are needed to elucidate its precise mechanisms.

**Acknowledgements:** The study was financed by the National Science Centre, Poland, under grant no. 2021/43/B/NZ7/01162.

## ICRS 2025 PRESIDENTIAL PLENARY: 11:00-12:00, Monday July 7, 2025

### NEUROINFLAMMATION IN PAIN – HAVE WE BEEN OVERLOOKING THE MESENCHYME?

Dr. Franziska Denk
Associate Professor, King's College London

**Biography**

Franziska Denk is an Associate Professor at King's College London, where she works on neuro-immune-stromal cell interactions in the context of chronic pain. Her lab uses transgenic models, stem cell derived human cell culture systems and high-throughput molecular analyses (https://www.franziskadenk.com/, @denklab.bsky.social).

Franziska's team is funded by the Wellcome Trust, the MRC, the European Union, industry and several charities. Her group is passionate about data sharing (Denk, Nature, 2017) and interdisciplinary research in an open, positive research culture (Soliman & Denk, Brain Behavior & Immunity, 2024).

# REVERSIBLE BEHAVIORAL DEFICITS INDUCED BY PRENATAL THC EXPOSURE BECOME IRREVERSIBLE AFTER POSTNATAL ISOLATION

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**Introduction:** As societal views on cannabis safety evolve, maternal cannabis use has seen a steady increase. However, longitudinal human studies provide compelling evidence that prenatal THC (pTHC) exposure significantly heightens the risk of mental health disorders in offspring. Unraveling the neurobiological and behavioral underpinnings of these effects is crucial. We propose that postnatal trauma, such as prolonged social isolation from weaning to adulthood, may exacerbate transient pTHC-induced changes, transforming them into persistent deficits.

**Method:** CD1 dams, including *cnr1* and *cnr2* heterozygotes, received THC (3 mg/kg, subcutaneously) from gestational day 5 until birth. Offspring were behaviorally assessed in adolescence (P28-35) and adulthood (P120-135). Littermate wild-type and $CB_1$ or $CB_2$ cannabinoid receptor knockout mice were compared in adolescence. Adult mice were analyzed under single-or group-housed conditions. Dopaminergic innervation in striatal regions was examined via immunolabeling and confocal microscopy.

**Results:** Prenatal THC exposure triggered marked anxiety- and depression-like behaviors in a $CB_1$ receptor-dependent, and $CB_2$ receptor-independent manner in adolescent mice. The behavioral deficits aligned with a sharp drop in neurochemical markers of dopaminergic fibers, especially in the Islands of Calleja region of the ventral striatum. These deficits faded in socially housed mice but became lifelong scars in pTHC-treated mice isolated from weaning until adulthood.

**Conclusions:** Our findings show that pTHC exposure leads to delayed maturation of dopaminergic afferents in the ventral striatum and elicits transient affective disorder-like behaviors in adolescent mice. Crucially, when combined with the trauma of postnatal social isolation, these effects persist into adulthood, highlighting the lasting impact of early-life exposures.

# STRESS RESILIENCE/VULNERABILITY PHENOTYPE DETERMINES BEHAVIORAL AND MOLECULAR EFFECTS OF PRENATAL AND ADOLESCENT THC EXPOSURE IN MICE

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**Introduction:** Cannabis consumption is rising globally, including during pregnancy and adolescence—two critical developmental windows. **Prenatal THC exposure (PTE)** has been linked to altered growth, neurodevelopment, and emotional regulation in offspring, while **adolescent THC exposure (ATE)** is associated with long-term cognitive impairments, anxiety, and social deficits. However, outcomes are highly variable, suggesting that individual traits, such as **stress-coping abilities**, may shape vulnerability or resilience to THC.

**Methods:** Dominant (Dom, stress-resilient) and Submissive (Sub, stress-vulnerable) mice were used. PTE model: Pregnant dams received THC (20 mg/kg, GD13, 15, 17) or vehicle. Adolescent THC model: Naïve mice received daily THC (8 mg/kg, PND30–51) or vehicle.
Behavioral tests: Marble Burying (MBT), Hole-Board Test (HBT), Novel Object Recognition (NORT), and Three-Chamber Test (TChT). mRNA levels of CB1R, CB2R, FAAH, D1R, D2R, and D5R were assessed in the hippocampus (HIP) and prefrontal cortex (PFC).

**Results:** PTE differentially affected growth and behavior depending on the animals' stress- coping phenotype. In Dom offspring, PTE led to a significant reduction in body weight, an effect not observed in Submissive Sub mice. Behavioral assessments revealed that PTE produced opposing effects on anxiety-like behavior in the MBT: Sub offspring exposed to PTE buried significantly fewer marbles compared to controls, suggesting reduced anxiety- like or compulsive behavior, while Dom offspring buried significantly more marbles, indicating increased anxiety-like behavior. Moreover, PTE enhanced sociability in Sub offspring in the TChT, while Dom offspring remained unaffected. At the molecular level, PTE altered the expression of key genes involved in endocannabinoid and dopaminergic signaling in brain regions, with distinct, phenotype-dependent patterns.

ATE also produced marked, phenotype-specific effects. Surprisingly, both Dom and Sub mice exhibited reduced weight gain during ATE. Interestingly, in the HBT, adolescent THC increased exploratory head-dipping behavior in Sub mice, with no effect in Dom.
Furthermore, while adolescent THC is typically associated with cognitive impairment, Sub mice exposed to THC demonstrated improved short-term memory in the NORT, an effect not seen in Dom. Social behavior was also differentially affected: ATE reduced sociability in Dom mice but, in contrast, enhanced social interaction in Sub mice.

**Conclusions:** Stress-coping phenotype shapes the behavioral and molecular consequences of both prenatal and adolescent THC exposure. These findings highlight the need for personalized risk assessment regarding cannabis exposure, that for some types of personalities, cannabis exposure could be beneficial, while harmful to others, and suggest that targeting the endocannabinoid system may offer stress-coping tailored interventions for neuropsychiatric conditions.

# ADOLESCENT CO-EXPOSURE TO ETHANOL AND CANNABINOID PRODUCED SUSTAINED IMPAIRMENT OF NEUROGENESIS AND HIPPOCAMPUS- DEPENDENT FUNCTIONS DURING ADULTHOOD

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**Introduction:** A large body of research has indicated the deleterious effects of both ethanol and cannabinoids on adolescent brain development and behavior. However, in spite of the increasing trend of coexposure to ethanol and cannabinoid during adolescence, very little is known about the effects of this combined exposure during adolescence in adult brain neurogenesis and hippocampal related learning and memory tasks in adulthood. The purpose of the current study is to determine a) if adolescence coexposure to ethanol and cannabinoid cause a significant change in neurogenesis and hippocampus related functions that persist during adulthood and b) the role of endocannabinoid system in the regulation of these processes.

**Method:** Adolescent male Wistar rats at post-natal day (PND) 28 (body wt. 200-300 gm) were treated with vehicle or ethanol (3 mg/kg/day, ig) or CB1R agonist ACEA (0.3 mg/kg/day, ip,) or JZL195 (0.3 mg/kg/day, ip; dual inhibitor of endocannabinoid degrading enzymes FAAH& MAGL), on a 2 day on/2 day off paradigm alone (AIE: adolescent Intermittent ethanol(AIE) and AIC : adolescent Intermittent cannabinoid) or in combination (AIE+AIC) till PND 48 (11 treatments) in the presence and absence of CB1R antagonist SR141716 (SR1;0.3 mg/kg/day, i.p).For combination treatment ethanol and cannabinoid drug doses were reduced to half and SR1 was administered 20 min prior to ethanol, ACEA or JZL195 treatment. Body weight was taken every alternate day and 24 hr. following the last treatment, animals were subjected to Y-maze or NOR (Novel Object Recognition) tasks respectively using standard protocols. The animals were then allowed to grow to adulthood (PND 90) without any drug treatment except food and water ad libitum. At PND 91, the animals were again subjected to Y-maze or NOR tasks.
After 24 hours of the last trial for both the tests, animals were sacrificed and brains were collected. Immunohistochemical analysis was carried out to assess for changes in neurogenesis, (DCX+IR), cell proliferation (Ki67) & apoptosis (cleaved caspase-3).

**Result:** We found that adolescent exposure to ethanol, cannabinoids or their combination significantly impaired both spatial memory performance (as indicated by the arm entry and dwell time in the novel arm in a Y-maze test) and novel object recognition ability (indicated by total exploration time) immediately following the test period during adolescence and these effects remain persistent when the same tests were performed during adulthood following a 6 weeks of abstinence. We also found that ethanol or CB1R agonist ACEA or endocannabinoid inactivation enzyme inhibitor alone or in combination significantly reduced doublecortin positive neurons (DCX+IR) with concomitant increase in cleaved caspase-3 positive cell in dentate gyrus of hippocampus. Pretreatment with CB1R antagonists SR141716 significantly blocked the ethanol/cannabinoid-induced impairment of spatial memory function and novel object recognition ability and reversed ethanol-induced inhibition of adult neurogenesis and cell death. Interestingly CB1R antagonist did not produced any effect when administered alone.

**Conclusion**: Together, the results from these studies revealed the following important phenomenon a) combined exposure to ethanol and cannabinoid during adolescence significantly blocked hippocampal- dependent spatial memory and object recognition function during adolescence, b) these effects persist even in the adulthood as evident from the reduced spatial memory and novel object recognition score measured during adulthood; c) the impairment of the behavioral functions may be correlated with the reduced hippocampal neurogenesis and increased cell death; d) combined exposure produced a significantly greater damage than the drugs alone as the reduced doses used during combined treatment produced almost the same deleterious effects in memory functions and neurogenesis and e) CB1R antagonist can attenuate the ethanol/ cannabinoid-mediated effects highlighting the important role of the eCB/CB1R system as the target for therapeutic intervention in these processes.

**FUNCTIONAL, STRUCTURAL, AND BEHAVIORAL IMPACTS OF ADOLESCENT CANNABIS VAPOR EXPOSURE IN ADULT MALE AND FEMALE RATS**

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**Introduction:** Cannabis is the most used illicit drug among adolescents, with a lifetime prevalence of nearly double that of all other illicit drugs combined. This is alarming as the long-term neurobehavioral consequences of adolescent cannabis use remain poorly understood. Recently, we reported that vaporized cannabis self-administration in adolescence led to long-lasting impairments in medial prefrontal cortex (mPFC)-dependent cognitive flexibility. Parvalbumin interneurons (PV) mediate cognitive flexibility as their inhibitory function tightly regulates mPFC output neurons, and PV interneuron function is supported by perineuronal nets (PNNs), which preferentially surround this cell type. Thus, we tested whether exposure to vaporized cannabis during adolescence induces long-lasting aberrations in PV function in the mPFC, possibly by altering PNNs, thereby leading to impairments in flexible decision making.

**Methods:** Adolescent male (n=27) and female (n=27) Sprague-Dawley rats received daily non-contingent vaporized cannabis extract (63.9% $\Delta^9$-tetrahydrocannabinol; extract diluted to 150 mg/ml) or vehicle (polyethylene glycol-400) exposure from postnatal day (P) 35-55 (3-s 'puff' every 2 min for 60 min). On ~P58, rats received bilateral microinfusions of a PV enhancer virus (AAV.PHP.eb-S5E2-dTom-nlsdTom; 300nl/side) into the prelimbic subregion of the mPFC. After a two-week washout period encompassing recovery from surgery, cognitive flexibility testing began on ~P70 using an operant-based attentional set-shifting task. After behavioral testing, the brains of littermates were either collected for immunohistochemistry or whole cell patch clamp slice electrophysiology to record from fluorescently tagged PV cells in the mPFC.

**Results:** Cannabis-exposed rats of both sexes were impaired in the set shifting but not reversal learning component of the task, requiring significantly more trials to reach criterion ($p$=0.0108), and had reduced fluorescent intensity of PNNs surrounding PV cells in the mPFC compared to vehicle-exposed rats ($p$=0.0450). Interestingly, *ex vivo* electrophysiology recordings revealed that PV cells from cannabis-exposed females were more excitable (increased max firing rate, $p$=0.0182, and lower rheobase, $p$=0.0041) than PV cells from vehicle vapor-exposed females, with no significant differences observed in males.

**Conclusions:** These findings support the hypothesis that adolescent vaporized cannabis exposure impairs mPFC-dependent cognitive flexibility in adulthood and that this coincides with increased intrinsic excitability of PV interneurons in females and alterations in PNNs that surround them. Thus, normalizing PNNs and PV cell function may be promising targets to alleviate adolescent cannabis-induced mPFC dysfunction.

# LOW-DOSE ALCOHOL ENHANCES THE BENEFICIAL EFFECTS OF CANNABIDIOL IN REDUCING NEUROINFLAMMATION IN A MURINE MODEL OF MULTIPLE SCLEROSIS VIA GUT MICROBIOTA MODULATION

Rahul Pushaparaj*, Jingdan Pei, Xing Gao, Md Manirujjaman, Panpan Huang, Guihua Pan, Lixian Chen, Chunbao Sun, Lihua Zhang and Wenke Feng

Department of Structural and Cellular Biology, Tulane University School of Medicine, New Orleans, LA.

**Introduction** Cannabidiol (CBD), a non-psychoactive compound derived from the Cannabis sativa plant, has demonstrated efficacy in treating various neuroimmune disorders. However, the effects of alcohol consumption on these conditions remain controversial. In this study, we investigate the role of CBD in modulating the gut-brain axis to alleviate symptoms of Multiple Sclerosis (MS) and assess the impact of low-dose ethanol in an experimental autoimmune encephalomyelitis (EAE) murine model of MS.

**Methods** Female C57BL/6 mice were divided into five groups: Healthy Control, EAE, EAE + 2% Ethanol, EAE + CBD (10 mg/kg), and EAE + Ethanol + CBD. Ethanol and CBD were administered via oral gavage for seven days, followed by EAE induction using $MOG_{35–55}$ antigen immunization on day 7. Paralysis scores were recorded daily. On day 17, mice were sacrificed, and brain and spinal cord tissues were collected for neuroinflammation analysis. Fecal samples were also collected for metagenomic analysis.

**Results** Mice receiving CBD exhibited a significant delay in disease onset and a trend toward reduced disease progression compared to the EAE group. Interestingly, 2% ethanol enhanced the protective effects of CBD, whereas ethanol alone had no significant impact. Myelin basic protein (MBP) staining revealed significantly reduced demyelination in CBD- and ethanol + CBD-treated groups. Additionally, spinal cord staining for F4/80 demonstrated reduced macrophage infiltration in these groups. 16S rRNA sequencing indicated significant gut dysbiosis in EAE mice, which was mitigated by CBD and ethanol + CBD treatments. At the genus level, we observed an increased abundance of *Akkermansia muciniphila* and enrichment of other beneficial commensal bacteria, such as *Roseburia* spp., suggesting a healthier gut microbiome in treated mice.

**Conclusions** Our findings indicate that oral administration of 2% ethanol combined with CBD modulates the gut microbiome, reduces neuroinflammation and demyelination, and ultimately alleviates disease severity in the EAE model of MS.

## GDE1-ASSOCIATED SPONGIFORM ENCEPHALOPATHY AND MALE INFERTILITY

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**Introduction**: Glycerophosphodiester phosphodiesterase1 (GDE1) is an enigmatic member of the endocannabinoid system. Although biochemical experiments demonstrated its role in the biogenesis of N-acylethanolamines including anandamide, the physiological function and pathological significance of GDE1 have remained elusive.

**Methods**: Immunohistochemistry, behavioral experiments, bioinformatics, confocal and electron microscopy, patch-clamp electrophysiology.

**Results**: We report that GDE1 has a fairly ubiquitous neuronal expression in the brain. It is primarily concentrated on the endoplasmic reticulum (ER) of the soma and related subcellular membrane compartments in the vicinity of chemical synapses. In mice lacking GDE1, a striking histopathological phenotype representing the hallmarks of spongiform encephalopathy emerges. A progressive dense vacuolization is associated with reactive astrogliosis, abnormal neuronal distribution and morphology, ultrastructural and functional synaptic deficits, as well as with age-dependent deterioration of fine motor skills and affective behaviors including a major defect in auditory startle response. Moreover, male GDE1 knockout mice are sterile due to the loss of DNA from their vacuolized sperm cells. In humans, allele frequency data in the UK Biobank indicate that ~50,000 patients carry two parental loss-of-function (pLOF) alleles or one pLOF and one missense variant which may result in neurological symptoms and infertility.

**Conclusion**: These observations demonstrate that GDE1 is essential for neuronal and sperm cell health. Impaired GDE1 activity and dysregulation of GDE1-associated lipid metabolism may account for hitherto undefined cases of combined spongiform encephalopathy and infertility. In addition, genomic analysis of GDE1 may help to diagnose rare disease patients who exhibit progressive neurological symptoms with indication for male fertility testing.

## ICRS 2025 MID-CAREER AWARDEE: 15:00-15:30, July 7, 2025

### FROM MOLECULES TO NETWORKS: ADVANCING CANNABINOID-BASED STRATEGIES FOR COMPLEX DISEASE MODULATION

Alessia Ligresti, Ph.D.
Research Director
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Over the past two decades, the endocannabinoid system (ECS) has emerged as a key regulator of physiological balance, shaping immune responses, metabolism, and cellular energy dynamics. This broader understanding has shifted the focus from classical pharmacology to a more nuanced approach, where the design of novel ligands depends not only on receptor selectivity but also on their ability to fine-tune downstream signaling in a context- and tissue-specific manner. Within this framework, medicinal chemistry and chemical biology play central roles in crafting small molecules—both natural and synthetic—with tailored functional profiles. This enables the exploration of cannabinoid receptor functions across subcellular compartments—including mitochondria—and within complex disease networks, particularly in oncology and neurodegeneration.

Our research integrates multidisciplinary expertise to develop multi-modal small molecules that selectively modulate ECS signaling, with a focus on their impact on complex diseases, including tumor metabolism and mitochondrial bioenergetics. We employ rational drug design, structure–activity relationship studies, and advanced pharmacological profiling, including binding kinetics, biased signaling evaluation, and allosteric modulation assays targeting key molecular players such as G protein-coupled receptors (GPCRs) and voltage-dependent ion channels (TRP). More recently, we have incorporated artificial intelligence tools to optimize ligand scaffolds and predict pharmacokinetic/pharmacodynamic profiles as well as synergistic drug combinations.

Ongoing work is exploring how cannabinoid receptor activation reprograms cellular metabolic states and immune functions through bioenergetic and immunometabolic cues. This includes investigating mitochondrial dysfunction in pathological contexts to identify targeted therapies capable of reshaping metabolic rewiring in cancer and neurodegenerative diseases. This lecture will offer a reflective journey through my scientific trajectory—from early investigations into natural compounds and cannabinoid pharmacology to current efforts in developing innovative therapeutic strategies at the interface of cannabinoids, metabolism, and systems pharmacology. It will highlight key milestones and outline future directions that reposition cannabinoids not merely as symptom modulators, but as strategic tools to rewire complex disease networks.

## CELLULAR AND BEHAVIORAL INVESTIGATION OF ZCZ011 ENANTIOMERS

Mohammed Mustafa*[1], Giulia Donvito[1], Zhixing Wu[2], Sri Sujana Immadi[2], Rachel Dopart[3], Kristen Trexler[4], Steven Kinsey[4], Debra Kendall[3], Dai Lu[2], and Aron Lichtman[1]

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**Background:** The CB1 allosteric modulator ZCZ011 shows efficacy in rodent models of neuropathic pain with negligible cannabimimetic *in vivo* effects. As this ligand elicits dual activity as a positive allosteric modulator (PAM) and as an allosteric-agonist at CB1, we separated its R-ZCZ011 and S-ZCZ011 enantiomers to discern between these actions.

**Methods:** We optimized the ZCZ011 synthesis, followed by separation of the enantiomers. R-ZCZ011 and S-ZCZ011 were characterized in PAM and allosteric-agonist mode (with and without CP55,940, respectively) in radioligand binding and functional (i.e., GTPγS binding, cAMP production, and β-arrestin recruitment) assays. The ligands were evaluated for antinociceptive effects in C57BL/6J mice that had undergone chronic constrictive injury (CCI) of the sciatic nerve. Finally, we examined whether each ligand augmented CP55,940 potency in the triad assay (catalepsy, thermal antinociception, temperature).

**Results:** Synthesis yield was 99% of product within 2 hours, compared to previous yields of 43-80% with 24+ hour reaction times. S-ZCZ011, but not R-ZCZ011, potentiated specific binding of CP55,940, while each ligand showed specific binding alone. In contrast, each ligand showed activity in both PAM and allosteric-agonist mode in the functional *in vitro* assays. Finally, both reversed CCI-induced mechanical hypersensitivity and increased the potency of CP55,940 in the triad assay.

**Conclusion:** Although we found enantioselectivity in PAM activity in the CP55,940 radioligand binding assay, R-ZCZ011 and S-ZCZ011 behaved as CB1 PAM/allosteric-agonists, elicited antinociception in the CCI neuropathic pain model, and augmented CP55,940 potency in the triad assay. These studies provide a template to discern PAM vs. allosteric-agonist effects of newly developed CB1 allosteric modulators.

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## UNCOVERING THE ROLE OF DAGL*α* IN ACETAMINOPHEN'S ACTION MECHANISM: A NEW PERSPECTIVE ON ENDOCANNABINOID INTERACTIONS

Michaela Dvorakova*[1,2], Taryn Bosquez-Berger[1], Jenna Billingsley[1], Natalia Murataeva[1], Wenwen Du[1], Taylor Woodward[1], Emma Leishman[1], Anaëlle Zimmowitch[1], Anne Gibson[1], Jim Wager-Miller[1,2], Ruyi Cai[3,4], Shangxuan Cai[3,4], Yulong Li[3,4,5], Heather Bradshaw[1], Ken Mackie[1,2], Alex Straiker[1,2]

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**Introduction:** Acetaminophen is a widely used medication for pain and fever relief, but its mechanism of action remains unclear. Despite causing approximately 500 deaths annually in the US due to liver toxicity, safer alternatives have not been developed. The endocannabinoid system, which regulates pain and inflammation through cannabinoid receptors and their ligands, has been suggested to influence acetaminophen's effects.

**Methods:** We examined the interaction between acetaminophen and endocannabinoid signaling using autaptic hippocampal neurons, lipidomics, lipase activity assays, cannabinoid receptor sensor assays, and a hot plate test for nociception. These methods allowed us to explore acetaminophen's effect on endocannabinoid pathways and pain perception.

**Results:** Acetaminophen inhibited endocannabinoid production in hippocampal neurons at concentrations as low as 10 μM, within the clinically relevant range. It selectively inhibited diacylglycerol lipase α (DAGLα), but not DAGLβ. This led to the hypothesis that DAGLα may have an antinociceptive role. Behavioral studies confirmed that acetaminophen's analgesic effects require CB1 receptor activation. Furthermore, DAGL inhibition by RHC80267 (20 mg/kg) was antinociceptive in wild-type mice, but not in CB1 knockout mice.

**Conclusions:** Based on these findings we propose 1) that DAGLα may play a counterintuitive role in some forms of nociception and 2) a novel mechanism for the antinociceptive actions of acetaminophen whereby acetaminophen inhibits a DAGLα/CB1-based circuit that plays a permissive role in at least one form of nociception. 3) DAGLα as a target for APAP is a novel mechanism that can have many unknown implications.

## AN ENDOCANNABINOID SENSOR-BASED ASSAY OF NAPE-PLD ACTIVITY

Jim Wager-Miller[1,2], Connor Schmitt[1,2], Ruyi Cai[3], YuLong Li[3], Ken Mackie[1,2], Alex Straiker[1,2]

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The endogenous cannabinoid signaling system consists of G protein-coupled receptors, lipid messengers, and the enzymatic machinery to synthesize and metabolize these messengers. Two lipid messengers, or endocannabinoids, have been identified: 2-arachidonoylglycerol (2-AG) and arachidonoylethanolamide (AEA), also known as anandamide. The synthesis and metabolism of 2-AG is relatively well understood, as is anandamide metabolism. Anandamide is implicated in important physiological systems -- particularly the sensation of pain – and several blockers of an anandamide-metabolizing enzyme FAAH have entered clinical trials. But the subject of anandamide synthesis has been the subject of some confusion and debate. At this point, convincing evidence points toward a phospholipase NAPE-PLD that cleaves anandamide from a N-arachidonoylphosphatidylethanolamide precursor. But our understanding of how NAPE-PLD is activated is limited as are available pharmacological tools. LEI401 has been proposed as a blocker of NAPE-PLD while cannabidiol and the sister compounds VU534/533 are proposed to activate NAPE-PLD.

We have developed a cellular assay that monitors NAPE-PLD activity in real-time in intact cells. In this assay an endocannabinoid sensor eCB3.0 that fluoresces when activated is co-transfected with NAPE-PLD in HEK293 cells. These cells natively express $G_q$-coupled muscarinic M3 receptors. We hypothesized that M3 activation by non-selective muscarinic agonist oxotremorine M (oxo-M) would activate anandamide synthesis. Testing this, we found that oxo-M stimulation induced a fluorescent signal that was absent if the NAPE-PLD was omitted or blocked by LEI-401. This suggests that NAPE-PLD can be activated by $G_q$-coupled receptors. Using this model, we tested CBD and VU534, finding that neither CBD nor VU534 directly activated NAPE-PLD, but interestingly, CBD reduced the subsequent effect of oxo-M.

These findings offer proof-of-concept that a novel fluorescence-based sensor may serve as a real-time assay of endogenously activated NAPE-PLD. They also show that $G_q$-coupled receptor activation can induce NAPE-PLD synthesis of acylethanolamines. This assay may improve our understanding of how this important enzyme is regulated and may facilitate the development of improved pharmacological tools.

**DEUTERIUM-LABELED ENDOCANNABINOIDS ARE ABUNDANTLY PRESENT IN CENTRAL NERVOUS SYSTEM TISSUE MINUTES AFTER PERIPHERAL INJECTION**

Heather B Bradshaw*, Taylor Woodward, Wenwen Du

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**Introduction***:* Decades of work has shown that endocannabinoids (eCBs) and related lipid signaling molecules are present in plasma and that their levels change with drug treatment, time of day, injury, and genetic sex. Importantly, the central hypothesis for eCB signaling is that these molecules are "made on demand" and act locally and primarily at neuronal synapses through neuronal CB1 receptors. While mounting evidence shows that eCB signaling is present in glia, vasculature, epithelium, muscle, endocrine glands, immune cells, and bone among other cell types and through additional receptors, the central dogma that eCBs are made exclusively on demand to signal locally at these sites remains the most accepted understanding. Therefore, a key question remains about the potential activity of circulating eCBs and how they could play a role in signaling, especially given the proposed therapeutic uses of eCBs as is the case with exogenously delivered *N*-palmitoyl ethanolamine (PEA). Of particular importance is to know whether peripherally circulating eCBs can enter the CNS in a level with the potential to effect signaling. Here, we test the hypothesis that if exogenously delivered eCBs readily enter the circulatory system then these lipids may be absorbed into the CNS at levels where they would be available to act as signaling molecules.

**Methods***:* 24, C57 male mice were injected with 3mg/kg of either deuterium-labeled PEA (d5), Anandamide (AEA-d8), Oleoyl ethanolamine (OEA-d4), or *N*-arachidonoyl glycine (NAGly-d8). Animals were sacrificed at either 15 or 120 minutes and blood and CNS tissue collected and stored prior to further dissection (8 CNS regions), lipid extraction, partial purification, and HPLC/MS/MS analysis.

**Results***:* All 4 deuterium-labeled eCBs (d-eCBs) were measured in plasma and in all brain regions after 15 minutes. The ratio for levels of d-eCBs in brain to plasma ranged from ~5-35% depending on the brain region and the specific lipid species (i.e. molarity CNS tissue/molarity plasma). At 120 minutes the levels in plasma and CNS were significantly lower (~10-fold) than at 15 minutes; however, the ratios of levels between plasma and CNS tissue were consistent overall. d5PEA and d4OEA had the highest overall penetrance into the CNS; though both d8AEA and d8NAGly were also readily measurable, especially at 15 minutes.

**Conclusions**: Circulating plasma eCBs penetrate CNS tissue in a rapid manner and at a similar rate regardless of plasma eCB molarity. The penetrance of specific eCBs into specific brain regions was dependent on both lipophilicity (shorter acyl chain and saturation length increased penetrance) and polarity (glycine moiety is more polar and had lowest percentage penetrance). These data provide a novel view that plasma eCBs may have a direct role in CNS eCB signaling and that exogenously delivered eCBs like PEA may act directly on the CNS.

# WATCHING THE CLOCK: CANNABINOID REGULATION OF CIRCADIAN TEARING

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**Introduction:** A great majority of animal species adapt to the daily cycle of light and dark both physiologically and behaviorally in order to thrive. The mechanics of the body's circadian clock have been the subject of intense study and much is now known about the clock genes that underlie this system. Often still missing is a detailed understanding of the peripheral effectors of this system, the messengers and receptors that translate this clock into specific physiological changes. We previously reported that GPR18 receptors contribute to the circadian cycling of ocular pressure in mice. Here we examine whether cannabinoid-related receptors similarly regulate circadian tearing.

**Methods:** We tested circadian regulation of tearing in mice using functional measures of tearing, lipidomics and immunohistochemistry.

**Results:** We report that basal tearing in mice varies substantially with the circadian cycle. This regulation differs by sex, most notably with a much higher amplitude in males. We find that circadian tearing is regulated in a sex-dependent manner by a combination of CB1 and GPR18 receptors. CB1 receptors account for the dampened oscillatory amplitude of circadian tearing in females by actively inhibiting production of tears. GPR18 receptors appear to underlie the oscillation in both males and females, actively increasing tearing.

**Conclusions:** Cannabinoid-family receptors play a central role as effectors of circadian regulation of tearing.

# DEUTERIUM-LABELED PALMITOYLETHANOLAMIDE (D5-PEA) SHOWS RELIABLE BRAIN PENETRANCE IN A DOSE DEPENDENT MANNER AND EXOGENOUS PEA SHOWS CHANGES IN CNS CONNECTIVITY AND LIPIDOME

Taylor Woodward*[1], Wenwen Du[1,2], Shreyas Balaji[3], Praveen P Kulkarni[3], Craig Ferris[3,4], Heather Bradshaw[1,2]

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**Introduction:** Palmitoylethanolamide (PEA) is an endogenous lipid that has developed into a broadly used nutraceutical for symptomatic relief for chronic pain and CNS disorders including anxiety. Because the PEA that is taken therapeutically is consumed orally and is, therefore, assumed to work primarily in the periphery, the potential effects on CNS function are unclear. Here, we test the hypothesis that if exogenously delivered PEA has effects on behavior and CNS function, then it will likewise drive changes in the CNS PEA levels and the broader CNS lipidome.

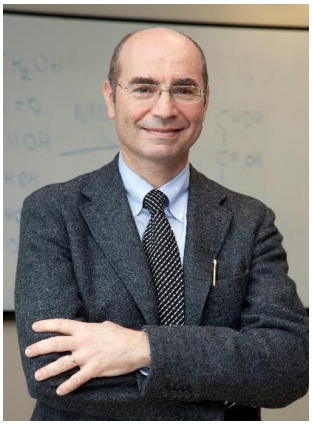
**Methods:** Awake rats were injected with PEA (30 mg/kg) or vehicle and imaged via MRI to evaluate changes in BOLD signal and functional connectivity. Plasma and CNS levels of PEA and over 80 endogenous lipids (endolipids) quantified post-treatment. An additional cohort of mice was received 30mg/kg deuterium-labeled PEA (d5-PEA) to evaluate plasma levels and CNS penetrance.

**Results:** Within 30 minutes post-PEA injection, there was an inverse dose-response for negative BOLD suggesting a decrease in brain activity affecting the prefrontal cortex, sensorimotor cortices, basal ganglia and thalamus. Overall levels of PEA in the CNS were significantly higher 30 minutes after 30 mg/kg treatment. However, levels of the endocannabinoid, Anandamide, and 20 additional endolipids, were significantly lower across the CNS ($p<.05$). Of the 78 endolipids that were detected in all CNS regions evaluated, 51 of them were modulated in at least one of the CNS regions evaluated. Importantly, d5-PEA was reliably measured in plasma and each CNS regions evaluated and the ratios of CNS tissue to plasma levels fell between 5-20% depending on the region (Hippocampus having ~5% and Striatum and Hypothalamus having the highest). This was calculated as levels in CNS/plasma levels. Therefore, even the 1% of capillary blood volume that may have been present after sacrifice and blood collection could not account for these levels of d5PEA in each CNS region.

**Conclusions:** Demonstrating that PEA taken acutely changes CNS function and CNS lipid signaling molecule regulation and that d5-PEA readily penetrates the CNS as early as 15 minutes post injection, we provide a novel insight into how peripherally administered endolipids like PEA can have a direct effect on CNS function.

## ICRS 2025 LIFETIME ACHIEVEMENT AWARDEE: 15:00-15:30, July 7, 2025

ENDOCANNABINOIDS AND THEIR INTERACTION WITH BIOACTIVE LIPIDS: THE NEXT FRONTIER



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**Biography**
Dr. Maccarrone is *Professor and Chair* of Biochemistry at the Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila (Italy). He is also *Head* of the Lipid Neurochemistry Unit at the European Center for Brain Research – IRCCS Santa Lucia Foundation, Rome. Dr. Maccarrone has published numerous highly cited papers (citations = 26615; h-index = 86 according to Scopus) and been an invited speaker at more than 110 international congresses, guest editor of 16 theme-issues of scientific journals, and is a holder of 10 granted patents. He has served as President of the International Cannabinoid Research Society (ICRS) in 2010-2011, *Chair* of the 2015 Gordon Research Conference on "Cannabinoid Function in the CNS" and *Visiting Professor* at Leiden University in 2017, University of Cambridge in 2019, and National University of Rosario in 2021.

Dr. Maccarrone has received various international awards, including the "2007 IACM (International Association for Cannabinoid Medicines) Award for Basic Research", the "2016 Mechoulam Award" for cannabinoid research, the "2020 Tu Youyou Award" for medicinal chemistry, and the "2024 Lifetime Achievement Award" by ICRS. He is included in the Stanford University "World Top 2% Scientists' List", the ScholarGPS "Top Scholars" (top 0.5%), the AD Scientific Index "World Top 100 Biochemists", and the "Top Italian Scientists".
Dr. Maccarrone is a *Secretary General* of the Italian Society of Biochemistry and Molecular Biology (2022-2024), *Fellow* and *Chartered Chemist* of the Royal Society of Chemistry, *Chair* of the FEBS Advanced Courses Committee, and *Director* and *Trustee* of FEBS since 2024.

**Abstract**
**Introduction**: In recent years different families of lipids able to act as authentic messengers between cells and/or intracellularly have been discovered and have been shown to exert their biological activity by triggering signal transduction pathways that regulate manifold pathophysiological processes in our body [1]. In addition to plant-derived compounds such as cannabinoids, our cells produce endogenous lipids – including endocannabinoids (eCBs), specialized pro-resolving mediators (SPMs) and shingosine-1-phosphate (S1P) – that support a complex network of molecular and cellular events responsible for several conditions, such as inflammation, cancer, autoimmune and neurodegenerative disorders, as well as neurogenesis, stress resilience and environmental

challenges, just to mention a few [2]. Unlike protein signals that can be stored in vesicles, lipid messengers are often synthesized “on demand”, i.e. when and where needed, and their endogenous content is tightly regulated by distinct biosynthetic and hydrolytic enzymes. Understanding mutual regulations of these enzymes, possibly through post-translational modifications, protein–protein and protein–lipid interactions, as well as via membrane and subcellular location, seems now urgent in order to exploit lipid signals as potential next-generation therapeutics. Here, I shall discuss recent data which show that the major eCB anandamide triggers efferocytosis - a hallmark of resolution of inflammation – via a $CB_2$ and GPR18-mediated mechanism [3]. In addition, I shall discuss how anandamide can reduce pro-inflammatory S1P levels by inhibiting sphingosine kinases 1 and 2 expression, again via $CB_2$ receptors [4].

**Conclusions**: It seems apparent that anandamide has pro-resolving properties, thus acting as a *bona fide* SPM, and that it is also able to promote an anti-inflammatory response by inhibiting S1P signaling. Thus, understanding how different classes of bioactive lipids crosstalk with each other seems urgent, because it represents a new layer of complexity in signal transduction. Of note, also plant-derived cannabidiol acts as a molecular switch in innate immune cells to promote the biosynthesis of SPMs [5], thus extending beyond eCBs its ability to modulate endogenous lipid signaling.

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# HEPATIC CB1 RECEPTOR SIGNALING TRIGGERS Gi/oα-MEDIATED LIPOLYSIS IN LEAN MICE BUT Gsα-MEDIATED LIPOGENESIS IN OBESE MICE

Jie Liu[1]*, Grzegorz Godlewski[1], Radka Kočvarová[2], Muhammad Arif[3], Abhishek Basu[3], Malliga R. Iyer[4], Resat Cinar[3], Joseph Tam[2] and George Kunos[1]

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**Introduction**: Obesity-induced steatotic liver disease (SLD) is driven by the uptake of adipocyte-derived fatty acids (FAs) into hepatocytes via the FA translocase CD36, which also prevents their elimination by inhibiting AMP kinase (AMPK)-mediated FA oxidation (FAO). We explored the role of hepatocyte CB1 receptors (hCB1R) in controlling hepatic triglyceride (TG) content by regulating CD36 and its downstream targets.

**Methods**: hCB1R knockout (hCB1Rko) mice and their control littermates maintained for 14 weeks on standard diet (lean mice), or on high-fat diet (DIO mice) and treated for 10 days with vehicle or a peripheral CB1R antagonist were used to analyze hCB1R-mediated hepatic gene expression profile and lipid metabolism in intact mice and in cultured hepatocytes.

**Results**: Multi-omics data obtained from liver tissue indicate that hCB1R target a distinct set of genes associated with SLD, including Cd36. In DIO mice, hCB1R signaling induces CD36 expression with consequent inhibition of the AMPK-FAO pathway, which is involved in both the development of SLD and its reversal by peripheral CB1R blockade. However, in lean mice hCB1R signaling inhibits CD36 expression and activates AMPK-mediated FAO. These opposite effects were replicated in AML12 mouse hepatocytes incubated with or without oleic acid (OA). OA, an endogenous ligand of GPR3, induced a switch in hCB1R signaling from a Gi/oα-mediated reduction in cAMP to a Gsα-mediated increase in cAMP in a GPR3/Gsα -dependent manner, a change paralleled by a robust increase in Gs and decrease in Gi/o proteins in the steatotic vs. lean liver.

**Conclusions**: In lean mice, endocannabinoid activation of hCB1R increases FAO, which protects against SLD, as found in chronic marihuana smokers, whereas in obese mice hCB1R activity tonically inhibits FAO, which promotes SLD and underlies the anti-steatotic effect of peripheral CB1R blockade.

# PHYTOCANNABINOIDS REPROGRAM LIPID METABOLISM TO REVERSE FATTY LIVER DISEASE

Radka Kočvarová*[1], Shahar Azar[1], Ifat Abramovich[2], Bella Agranovich[2], Eyal Gottlieb[2], Liad Hinden[1], Joseph Tam[1]

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**Introduction:** Metabolic dysfunction-associated steatotic liver disease (MASLD) is a major global health concern, affecting hundreds of millions worldwide. Despite its growing prevalence, no pharmacological treatment has been approved to date. Our work aims to address this unmet need. Previously, we demonstrated that cannabidiol (CBD) and cannabigerol (CBG) significantly reduced hepatic steatosis, restored glucose homeostasis, and improved insulin sensitivity in a high-fat diet (HFD)-induced MASLD mouse model. Metabolomic analysis indicated a metabolic shift toward the creatine-phosphocreatine system as an alternative energy source, while conventional AMPK-driven pathways (fat oxidation, glycolysis, and the TCA cycle) remained unchanged. These findings suggested profound metabolic remodeling, but the mechanisms by which phytocannabinoids influence hepatic lipid handling and clearance remained unclear. Here, we investigate how CBD and CBG alter hepatic lipid composition, structural lipid distribution, and lipid export capacity.

**Methods:** Male C57BL/6 mice were fed either a standard diet (STD) or an HFD for 14 weeks to induce hepatic steatosis, followed by 28 days of daily CBD or CBG administration. Lipidomic profiling via LC-MS identified over 2,100 lipids spanning more than 50 lipid classes. Data visualization included heatmaps, pie charts, and alluvial plots to map lipid class redistribution and molecular remodeling. Given the role of phosphatidylcholine (PC) subclasses were in very-low-density lipoprotein (VLDL) assembly and secretion, their composition was analyzed in detail. To assess whether these changes impact lipid export, direct VLDL quantification via fast protein liquid chromatography (FPLC) is currently underway.

**Results:** CBD and CBG treatment led to a significant reorganization of hepatic lipid composition. Triglycerides and ceramides—key markers of metabolic dysfunction—were markedly reduced, while lysosomal bis(monoacylglycerol) phosphate (LBPA) levels increased, suggesting enhanced lipid degradation and trafficking. Distinct shifts in PC subclasses were observed, potentially indicating an upregulation in VLDL-mediated lipid export. These lipidomic alterations were visualized using comprehensive analytical tools, revealing clear redistribution patterns across major lipid classes.

**Conclusions:** CBD and CBG modulate hepatic lipid metabolism, reducing steatogenic lipids while promoting lipid remodeling pathways. Alterations in PC subclasses suggest a shift toward enhanced VLDL-mediated lipid export, a hypothesis currently being tested via direct FPLC analysis. By elucidating the role of phytocannabinoids in hepatic lipid clearance, this research advances the potential for developing a pharmacological treatment for MASLD—one of the most prevalent metabolic diseases lacking an approved therapeutic intervention.

# ENDOCANNABINOID SYSTEM DYNAMIC CHANGES IN A POLYCYSTIC KIDNEY DISEASE MOUSE MODEL

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**Introduction**: Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder characterized by progressive kidney cyst formation and dysfunction. While the endocannabinoid system (ECS) is well established in chronic metabolic kidney diseases, its role in ADPKD remains largely unexplored. This study examines the temporal dysregulation of the ECS in a genetic ADPKD mouse model and its association with kidney function, gene expression, and endocannabinoid (eCB) levels.

**Methods**: PKD$^{rc/rc}$ knockout and wild-type (WT) mice were analyzed at 3, 6, 9, and 12 months. Kidney, serum, and urine samples were collected to assess ECS-related RNA and protein expression. eCB levels were quantified using LC-MS/MS and normalized to protein content. Kidney function was evaluated biochemically (Cobas C-111). Spearman's correlation analysis was performed to examine associations between ECS markers, eCB levels, and kidney function.

**Results:** PKD$^{rc/rc}$ mice exhibited significant kidney dysfunction, as indicated by an increased kidney-to-body weight ratio (Kid/BW), elevated blood urea nitrogen (BUN) levels, and reduced creatinine clearance (CCr). The AEA and OEA were significantly decreased in kidney at 6 and 9 months, while 2-AG levels showed an increasing trend, reaching statistical significance at 9 months. Dynamic changes in ECS-related gene and protein expression were observed in the PKD group. Correlation analysis revealed significant associations between eCB levels, ECS receptor and enzyme expression, and kidney dysfunction, suggesting a role for the ECS in ADPKD progression.

**Conclusions**: This study provides the first evidence of dynamic ECS alterations in ADPKD, emphasizing its potential role in disease progression and as a therapeutic target.

## ANTI-FIBROTIC AND RENOPROTECTIVE SYNERGY THROUGH CB1R/SGLT2i COMBINATION

Océane Pointeau*[1,2], Abhishek Basu[3], Julia Leemput[1,2], Romain Barbosa[1,2], Patricia Passilly-Degrace[1,2], Laurent Demizieux[1,2], Hélène François[4], Bruno Vergès[1,2], Resat Cinar[3], Pascal Degrace[1,2], and Tony Jourdan[1,2]

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**Introduction:** Diabetic nephropathy (DN) stands as one of the most prevalent complications of diabetes with a strong association with renal fibrosis. We investigated whether combining cannabinoid type 1 receptor (CB1R) blockade with sodium-glucose cotransporter type 2 inhibition (SGLT2i) enhances renoprotection compared to monotherapies.

**Methods:** 40 C57BLKS-Lepr$^{db/db}$ mice and 6 control mice were fed a high-protein diet for 9 weeks. After 5 weeks, diabetic mice were exposed to four different treatments: placebo, empagliflozin (SGLT2i), monlunabant (CB1R blocker), or their combination by oral gavage for 28 days. Renal fibrosis was evaluated through histological analysis, and Nanostring® technology was employed to identify key signaling pathways.

**Results:** The combination therapy demonstrated a significant reduction in tubulointerstitial fibrosis compared to monotherapies. Mechanistic studies revealed decreased STAT3 phosphorylation, indicating modulation of the JAK/STAT3 pathway. Further analysis suggests a complementary effect of both treatments: monlunabant appears to upregulate angiopoietin-1 (ANGPT1), while empagliflozin enhances fibroblast growth factor 20 (FGF20) expression. Both factors are associated with renoprotection and better prognosis in renal disease, supporting their involvement in the anti-fibrotic response. Ongoing *in vitro* studies aim to confirm their specific roles in JAK/STAT3 modulation, further refining the mechanistic understanding of this therapeutic synergy and reinforcing its potential as an innovative strategy for DN treatment.

**Conclusion:** Combining SGLT2i and CB1R blockade offers superior renoprotection by mitigating pro-fibrotic signaling and modulating the JAK/STAT3 pathway. Additionally, ANGPT1 and FGF20 emerge as potential key regulators, reinforcing the mechanistic understanding of this approach.

**Acknowledgements:** Funded by the French National Research Agency (ANR-22-CE14-0055- 01).

**DYNAMIC ECS/CB1R ALTERATIONS GUIDE THERAPEUTIC TARGETING IN ACUTE KIDNEY INJURY AND ITS MALADAPTIVE REPAIR**

Ariel Rothner[1], Liad Hinden[1], Aviram Kogot-Levin[2], Elisheva Benkovitz[3], Amani Zoabi[3], Anna Permyakova[1], Asaf Kleiner[1], Vladislav Nesterenko[1], Alina Nemirovski[1], Ifat Abramovich[4], Bella Agranovich[4], Inbar Plaschkes[5], Eyal Gottlieb[4], Katherine Margulis[3], Gil Leibowitz[2], Joseph Tam[1*]

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**Introduction:** Acute kidney injury (AKI) is a major risk factor for chronic kidney disease (CKD), largely due to maladaptive repair. While overactivation of the renal ECS/CB1R axis has been implicated in CKD pathogenesis, its role in AKI and its impaired healing remains less understood.

**Methods:** AKI progression and maladaptive repair was established in a murine folic acid (FA)-induced AKI model, assessing transcriptional, translational, and metabolic ECS alterations. ECS transcriptional changes were also examined in a murine ischemia-reperfusion injury (IRI)-induced model and kidney biopsies from human kidney transplant recipients (KTRs). Treatment with peripheral CB1R inverse agonist was evaluated in FA-induced AKI for effects on renal function, injury, inflammation, fibrosis, and metabolism at acute (2-day) and maladaptive repair (14-day) phases.

**Results:** FA-induced AKI displayed distinct phenotypic and metabolomic profiles across disease stages. ECS components exhibited dynamic, stage-specific changes, including fluctuations in endocannabinoid levels (AEA, 2-AG) and their enzymatic regulators. CB1R expression and activation increased at later disease stages in preclinical models and were elevated in KTRs with worse kidney function and fibrosis. While CB1R blockade did not prevent AKI onset, it improved kidney function, reduced injury and inflammation, and preserved glucose homeostasis during maladaptive repair. Metabolomic analysis implicated arginine metabolism in these therapeutic effects.

**Conclusions:** Our findings demonstrate dynamic shifts in endocannabinoid signaling during kidney injury and repair, underscoring the time-dependent relevance of ECS modulation in AKI therapy. While peripheral CB1R blockade does not prevent acute injury, it demonstrates therapeutic potential in mitigating post-AKI maladaptive repair.

## ICRS 2025 LIFETIME ACHIEVEMENT AWARDEE:
## 14:00-14:30, Tuesday July 8, 2025

TWO DECADES OF RESEARCH EXAMINING THE OCULAR ENDOCANNABINOID SYSTEM AS A TARGET FOR OCULAR THERAPEUTICS

Melanie Kelly, Ph.D.
Professor, Departments of Pharmacology,
Ophthalmology & Visual Science,
Faculty of Medicine,
Dalhousie University

**Biography**

Dr. Melanie Kelly (she/her) is a Professor in the Departments of Pharmacology, Ophthalmology & Visual Sci., Faculty of Medicine, Dalhousie University. Prof. Kelly is a Director for the Canadian Consortium for Investigation of Cannabinoids (CCIC) and President and Chief Scientific Officer for Altheda Wellness Innovation, a Canadian company operating in the natural products and health tech space. Professor Kelly has extensive research and leadership experience in academia as well as industry and is considered a key knowledge opinion leader in cannabinoid and phytochemical drug development. Her primary research expertise is in translational and ocular pharmacology. She has been funded for her research on the endocannabinoid system and lipid signaling by multiple international and national funding agencies and has successfully translated her team's research findings into novel therapeutics to improve health outcomes in age-related disease. Professor Kelly has over 135 peer reviewed publications and multiple patents for development and use of cannabinoid drugs and natural products.

# PSILOCYBIN AS A TREATMENT FOR REPETITIVE MILD HEAD INJURY: EVIDENCE FROM NEURORADIOLOGY AND MOLECULAR BIOLOGY POINTING TO INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM

Reagan Walhof*, Wenwen Du, Eric K. Brengel, Bryce Axe, Ashwath Maheswari, Muhammad I. Abeer, Richard J. Ortiz, Taylor J. Woodward, Rachel Utama, Courtney Sawada, Shreyas Balaji, Praveen P. Kulkarni, Michael A. Gitcho, Craig F. Ferris, and Heather B. Bradshaw

Indiana University, Bloomington IN and Northeastern University, Boston MA, USA

**Introduction***:* Repetitive mild head traumas (rmTBI) incurred while playing organized sports, during car accidents and falls, or in active military service are a major health problem. These rmTBIs can induce cognitive, motor, and behavioral deficits that can last for months and even years with an increased risk of dementia, Parkinson's disease, and chronic traumatic encephalopathy. There is no approved medical treatment for these rmTBIs. There is a growing literature suggesting that the natural product hallucinogen psilocybin (PSI) could be used to treat brain injury given its known anti-inflammatory effects and its action as a promoter of neuroplasticity and cell growth, though the mechanism of these outcomes is unknown. The Bradshaw group presented preliminary data at ICRS 2024 to show that PSI injection drives significant changes in plasma eCBs, which provides insights into potential mechanisms of action. Here, we test the hypothesis that if PSI was used as an acute therapeutic shortly after rmTBI events, then it will reduce both the short and long-term effects of rmTBI and that these changes will be associated with modulations in endocannabinoids (eCB) and/or their congeners. Data here will focus on the lipidomics outcomes.

**Methods***:* In two consecutive studies, three groups of SD female rats were randomly assigned into 3 groups (control, sham/vehicle group, the rmTBI/vehicle group, and the rmTBI/psilocybin). In study 1, rats were 9 months old, in study 2, rats were 18 months old. rmTBI groups were exposed to a minor traumatic brain injury once per day for a period of three days using a momentum exchange model procedure. After each injury (or sham procedure), rats in the rmTBI/psilocybin group received a single intraperitoneal injection of 3mg/kg psilocybin, whereas the rmTBI/vehicle and sham/vehicle groups were given a corresponding vehicle saline injection. Blood samples were taken after injection on the third day and plasma was extracted for lipidomics analysis as previously described by the Bradshaw group. Following the three days of mTBI, individuals underwent brain imaging and behavioral experiments.

**Results***:* PSI was shown to reduce vasogenic edema, restore normal vascular reactivity and functional connectivity. In addition, rmTBI alone was shown to significantly reduce specific lipid species in the plasma, whereas PSI treatment after rmTBI significantly increased selected lipids overall and reversed the reduction of two lipids, *N*-arachidonoyl methionine and *N*-stearoyl taurine. The eCB 2-AG was significantly increased after PSI treatment in the rmTBI subjects in the 18-month-old but not the 9-month-old subjects. The number of circulating lipids that increased after rmTBI with PSI treatment doubled in the 18-month-old subjects compared to the 9-month-old subjects.

**Conclusions**: These data show that PSI treatment following rmTBI in the rat are an effective therapeutic to reduce overall CNS injury and that these outcomes are associated with changes in eCBs and their endogenous congeners.

# CANNABIS-PSILOCYBIN CO-USE AND ARTERIAL STIFFNESS: PRELIMINARY FINDINGS FROM YOUNG ADULTS IN THE HERBAL HEART STUDY

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**Introduction:** Studies highlight increased prevalence of cannabis and psilocybin co-consumption, suggesting cannabis enhances psychedelic effects and eases the come-down phase. Research also links cannabis to increased arterial stiffness and altered cardiac mechanics. However, the impact of cannabis-psilocybin co-use on arterial stiffness remains unexplored.

**Methods:** Data are from the ongoing Herbal Heart Study on the impact of cannabinoids on cardiovascular risk in 18-to-35-year-olds. Cannabis consumption was self-reported and urine-confirmed, while psilocybin use was self-reported. Arterial stiffness was assessed using applanation tonometry with central augmentation index (cAIx). ANCOVA models were implemented using PROC GLM in SAS to assess cAIx across cannabis-only, cannabis+psilocybin (co-consumers), and non-consumers, adjusting for age, sex, and race/ethnicity. Least squares means were compared using the Tukey-Kramer adjustment for multiple comparisons.

**Results:** In the sample [N=189; age=25.1y (SD=4.8), 64.5% female, 54.5% Hispanic, 16.9% non-Hispanic Black], 47.1% were cannabis-only consumers, 32.8% co-consumers, 20.1% non-consumers. Co-consumers had higher cAIx (11.60 $\pm$ 1.51, p=0.01) than non-consumers (4.92$\pm$ 1.75); there was no difference between cannabis-only cAIx (8.81 $\pm$ 1.39) and non-consumers (4.92$\pm$ 1.75, p=0.28).

**Conclusion:** Findings suggest that co-consumption of cannabis and psilocybin may be linked to arterial stiffness, reflected in higher cAIx compared to non-consumers. Although no significant cAIx differences were observed among cannabis-only, results suggest that psilocybin may have a synergistic effect on arterial stiffness, warranting further investigation into its potential vascular implications and interactions with the endocannabinoid system.

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# MAPPING ACUTE EFFECTS OF CANNABIS ON MULTIPLE MEMORY DOMAINS: A DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

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**Introduction:** One of the most robust detrimental effects of acute cannabis intoxication is impairments in memory. However, memory is not a unitary construct and there are numerous domains of memory. The overwhelming bulk of previous research examining acute effects of cannabis on memory have focused on verbal memory. A paucity of research has considered impacts of cannabis on more ecologically valid domains of memory, such as prospective memory, source memory, false memory, and temporal order memory despite the high relevance of each of these memory domains to everyday life functioning. Moreover, to our knowledge no prior research has examined all these aspects of memory in a single study to determine which domains are impaired and which are spared under conditions of acute cannabis intoxication.

**Methods:** Cannabis-using participants in this ongoing study are randomly assigned to vaporize placebo, 20 mg delta-9-tetrahydrocannabinol (THC), or 40 mg THC using a Volcano Vaporizer. They subsequently complete a battery of memory tests designed to measure the following memory domains: prospective memory (the ability to remember to execute tasks at a later moment), verbal free recall (the ability to remember lists of words), verbal short-term memory (the ability to immediately recall verbal information), verbal working memory (the ability to store and manipulate verbal information), visuospatial memory (the ability to recall visual information), visuospatial short-term memory (the ability to immediately recall spatial information), visuospatial working memory (the ability to store and manipulate spatial information), source memory (the ability to remember the source of previously learned information), false memory (the recollection of items that were not previously presented), episodic memory (the ability to recall past events), and temporal order memory (the ability to recall the order in which past events occurred).

**Results:** Preliminary results from 99 participants ($n$ = 32-34/group) indicate that relative to the placebo group, participants administered 20 mg THC and those administered 40 mg THC demonstrated worse performance on tests of visuospatial memory, visuospatial short-term memory, verbal working memory, source memory, and false memory. Participants in the 20 mg group further demonstrated worse performance on tests of verbal free recall, prospective memory and temporal order memory relative to the placebo group. Only the episodic memory, spatial working memory, and verbal short-term memory tests failed to reveal significant effects of acute cannabis intoxication.

**Conclusion:** This is the first study to detect detrimental effects of acute cannabis intoxication on prospective memory and temporal order memory, which is critical as these tests better reflect everyday life memory and better predict daily functioning. Overall, results suggest that acute cannabis intoxication has broad impacts on multiple domains of memory rather than selectively impairing only a limited number of memory domains.

# INVESTIGATING 'THE MUNCHIES' IN RODENTS AND HUMANS; EFFECTS OF Δ-9-THC VAPOUR INHALATION ON FEEDING PATTERNS, MACRONUTRIENT PREFERENCE, SATIETY AND REWARD VALUE

Catherine Hume*[1], Carrie Cuttler[2], Samantha L. Baglot[1], Lucia Javorcikova[1], Ryan McLaughlin[2] & Matthew N. Hill[1]

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**Introduction:** With approximately 4% of the world's population using cannabis, there is need to fully understand its physiological effects. Increased food intake or 'the munchies' is a recognised side effect of cannabis use that is primarily driven by delta-9-tetrahydrocannabinol (THC), the major psychoactive cannabinoid in cannabis. These appetitive THC effects have been modeled in humans and rodents but ultimately remain understudied. Therefore, we characterised how vapour inhalation from a THC-dominant cannabis extract alters daily feeding patterns, macronutrient-specific food preferences and food reward value in a translational rat model alongside human participants.

**Methods:** Human participants inhaled vapourised placebo (n=28), low (20mg, n=26) or high (40mg, n=28) doses of THC-dominant cannabis (11.86% THC) in a controlled, double-blind manner, then were given access to a variety of snacks and beverages with varying energy densities and macronutrient profiles. Sprague-Dawley rats were exposed to vapourised vehicle or THC-dominant cannabis extract (10% THC, n=6-10/group/study) then given access to different foods (chow, high-carbohydrate, and/or high-fat food) or subjected to operant responding for sucrose. To assess satiety, rats were given pre-vapour access to a palatable preload. To assess aversion, the behavioural paradigm was paired with lithium chloride injections. To determine the necessity of cannabinoid 1 receptors (CB1R), rats were subjected to pre-vapour injections of the CB1R inverse agonist AM251, or the peripherally restricted CB1R antagonist AM6545.

**Results:** We showed that THC vapour inhalation increases energy intake on acute timeframes, irrespective of gender and dose in human participants ($p<.001$), and regardless of sex, food type and satiation in rats ($p<.05$). Within this timeframe, THC increased human participant carbohydrate, fat, and protein intake ($p<.05$) to the same extent so that the proportion of macronutrients consumed remained unchanged, and abolished pre-existing rat macronutrient preferences when given a food choice. In rats, these feeding effects were a result of THC vapour decreasing latency to eat ($p<.001$) and increasing feeding bout frequency ($p<.001$) and were compensated for through reductions in subsequent food intake so that THC vapour did not promote weight gain. Further, THC vapour exposure increased motivation for rats to acquire sucrose pellets ($p<.001$) and reduced food reward devaluation induced through satiety ($p<.001$) or aversion ($p=.02$). Finally, blocking peripheral CB1R in rats did not influence on the appetitive effects of THC vapour, but universally blocking CB1R did ($p<.001$), implying that THC acts through central mechanisms to mediate these effects.

**Conclusions:** Overall, this study complements and builds upon previous literature to characterise the appetitive effects of THC vapour inhalation and is the first to directly compare THC-driven feeding patterns and macronutrient-specific food preferences between humans and rodents. This research sheds light on whether cannabis use can have energy-balance effects, information which is highly beneficial for both recreational and medical cannabis users.

# THE EFFECT OF VAPORIZED & ORAL Δ8-TETRAHYDROCANNABINOL VS Δ9-TETRAHYDROCANNABINOL ON SIMULATED DRIVING PERFORMANCE IN HEALTHY ADULTS

Lakshmi Kumar*[1], C. Austin Zamarripa[1], Tory R. Spindle[1], Edward J. Cone[1], Ruth Winecker[2], Ronald Flegel[3], Ryan Vandrey[1]

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**Introduction:** Despite widespread availability of Δ8-Tetrahydrocannabinol (Δ8-THC), little controlled human research has been done. This controlled human laboratory study aims to characterize acute effects of vaporized and oral Δ8-THC on simulated driving performance compared to vaporized/oral Δ9-THC and placebo.

**Methods:** Healthy adults (N=4 per route of administration) completed four double-blind, outpatient drug administration sessions, self-administering either vaporized or oral Δ8-THC (30mg, 60mg), Δ9-THC (30mg), or placebo. Outcomes were assessed at baseline and over eight hours post-administration and included: self-reported drug effects, cognitive and psychomotor assessments (including field sobriety testing), simulated driving performance, and vital signs. Blood, urine, and oral fluid specimens were obtained to characterize pharmacokinetics (PK) of Δ8-THC, Δ9-THC, and their metabolites.

**Results:** Both Δ8-THC and Δ9-THC impaired driving performance, impaired cognitive and psychomotor function, and increased subjective drug effects and heart rate relative to placebo. Standard deviation of lane position (SDLP) was impacted during car following and divided attention tasks. Impairment of Δ9-THC was greater than Δ8-THC after oral dosing, but impairment was comparable after vaporized dosing. Two participants dropped out of the study related to adverse events, one after 60mg Δ8-THC and one after 30mg Δ9-THC. PK and field sobriety test results are pending.

**Conclusions:** This study extends prior research by examining higher doses of Δ8-THC and evaluating simulated driving performance. Preliminary findings indicate that Δ8-THC produces qualitatively similar impairment of driving ability, and that route of administration impacts relative magnitude of drug effects compared with Δ9-THC. Ongoing data collection and inferential analyses will clarify these effects.

**FINANCIAL SUPPORT:** Substance Abuse and Mental Health Services Administration (SAMHSA)

# CANNABIS AND ARTERIAL STIFFNESS: DIFFERENCES BY ROUTE OF CONSUMPTION AMONG HEALTHY YOUNG ADULTS IN THE HERBAL HEART STUDY

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**Introduction:**
Studies have identified a relationship between cannabis and arterial stiffness; however, the impact of route of consumption is unclear.

**Methods:**
Baseline data are from cannabis consumers (CB+) enrolled in the Herbal Heart Study cohort. Cannabis use was confirmed via urine. Arterial stiffness was assessed using applanation tonometry, measuring central augmentation index (cAIx). ANCOVA models evaluated routes of cannabis administration and arterial stiffness, adjusting for demographics. Least squares means (LSM) and standard errors were estimated with Tukey-Kramer adjustment for multiple comparisons to control for Type I error.

**Results:**
Among CB+ [N=126, 25.8y (SD=4.8), 57.1% female, 60.3% Hispanic/Latino]; 37.3% consumed joints, 35.7% vape, and 27.0% blunt. LSM values of vape consumers. There was a significant mean difference for cAIx between blunt (15.04% ± 2.04) and joint consumers (8.90% ± 1.89; $p=0.05$). Vape consumers presented with cAIx of 9.15% ± 2.08. Age was associated with cAIx ($\beta=0.587$, $p<0.01$); males had lower scores than females ($\beta=-8.86$, $p<0.0001$). The model significantly predicted cAIx ($F(7,113) = 8.44$, $p<0.0001$), with $R^2=0.343$, indicating that ~34.3% of the variance in cAIx was explained by the predictors.

**Conclusion:**
Blunt consumers show the highest cAIx, indicating greater arterial stiffness. Age and sex also influence stiffness, with older individuals and females affected more. These findings suggest a link between cannabis consumption methods and cardiovascular risk, warranting further research.

**Supported by:** NHLBI R01HL153467 (PI: Denise Vidot)

# CANNABIS AND VASCULAR FUNCTION: ENDOTHELIAL DYSFUNCTION AND ARTERIAL STIFFNESS AMONG YOUNG ADULTS IN THE HERBAL HEART STUDY

Denise C. Vidot, PhD*[1,2], Amrit Baral, MBBS, MPH[1,2], Kylee Krivijanski[1,2], Bria-Necole A Diggs, MSPH[1,2], Sarah E. Messiah, PhD[3], Girardin Jean-Louis, PhD[1,4], Marvin Reid, PhD[5], Marilyn Lawrence-Wright, MD[5], Lisa Reidy, PhD[1], Barry Hurwitz, PhD[1], Claudia Martinez, MD[1]

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**Introduction:**
Endothelial dysfunction and arterial stiffness are early markers of CVD; understanding their relationship with cannabis is essential for assessing potential CVD risks and benefits.

**Methods:**
Data are from baseline Herbal Heart Study visits of healthy 18-35-year-olds. Endothelial dysfunction was measured using flow-mediated dilation (FMD) via brachial artery ultrasound during reactive hyperemia; mean percentage change in arterial diameter calculated from two tests. Arterial stiffness was assessed using applanation tonometry with key central augmentation index (cAIx) as a key indicator. ANCOVA models assessed the association between CB+, FMD, cAIx, adjusting for demographics. Least squares means were estimated by CB+ status; multiple comparisons adjusted via Tukey-Kramer method to control for Type I error.

**Results:**
The sample [N=200, 25.2y (SD=4.8)] was 65.0% female and 54.5% Hispanic. CB+ were older than CB- (25.8y vs 24.1y, p=0.01). Mean FMD was higher in CB+ (8.61% ± 0.31) than CB- (7.65% ± 0.38, p=0.03). cAIx was higher in CB+ in CB- (10.6% ± 1.75) than CB- (6.34% ± 1.41, p=0.01). Central pulse pressure (cPP) was higher in CB+ (35.13 ± 0.80 mm Hg) compared to CB- (32.53 ± 0.96 mm Hg, p=0.02).

**Conclusion:**
Findings inform CB+ clinical care since it was associated with healthier endothelial function but more arterial stiffness. Research is needed to clarify details (i.e., cannabinoid consumption, route of cannabis consumption) to develop targeted interventions.

**Supported by:** NHLBI R01HL153467 (PI: Denise Vidot)

# COMPREHENSIVE POPULATION PHARMACOKINETIC ANALYSIS OF TETRAHYDROCANNABINOL AND ITS ACTIVE METABOLITE ACROSS INTRAVENOUS, ORAL, VAPED, AND SMOKED ROUTES

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**Introduction:** Studies describing the pharmacokinetics (PK) of Δ9-tetrahydrocannabinol (THC) and its metabolites are often limited by small sample sizes and lack of data on different inhalation methods and oral formulations. This analysis aims to develop a comprehensive population PK model for THC and its active metabolite, 11-OH-THC, across intravenous, oral, and inhalational routes.

**Methods:** Data from 16 controlled studies involving 368 subjects who received 1.25 – 50 mg THC via intravenous, oral (brownies, soft gel, or tablet), smoking, or vaping routes were pooled. Thirty subjects also received oral cannabidiol (CBD) with a CBD:THC ratio of 32-38:1. Structural and statistical models were tested to describe the time course of THC and 11-OH-THC. PK parameters and interindividual variabilities were estimated, with the effect of oral formulation and CBD co-administration also evaluated.

**Results:** Model parameters were reliably estimated. Oral absorption and bioavailability varied by formulation, with tablets absorbed in 1.5 hours, while brownies and soft gels took twice as long. Estimated bioavailability was 8% for brownies and 6% for tablets, and about three times higher for soft gels. Vaping had greater bioavailability than smoking (15 vs 26%). THC systemic clearance was 60 L/h, and metabolite clearance was slower (21 L/h). High CBD reduced THC clearance by 56% and metabolite clearance by 89%. Oral THC converted to 11-OH-THC was 48%, while other routes showed an up to 16-fold lower conversion.

**Conclusion:** This PK model provides a comprehensive understanding of THC and its active metabolite and will be the basis of future pharmacokinetic-pharmacodynamic relationship studies for THC across different routes and special populations.

## THE IMPACT OF PRODUCT FORMULATION ON THE PHARMCOKINETICS AND PHARMACODYNAMICS OF CANNABIS EDIBLES

Lakshmi Kumar[1], Austin Zamarripa[1], Elise Weerts[1], David Wolinsky[1], Jost Klawitter[2], Uwe Christians[2], Ryan Vandrey[1], Tory Spindle[1]

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**Introduction:** This ongoing human laboratory study compares the pharmacokinetic (PK) and pharmacodynamic (PD) effects of three different cannabis edible formulations: brownies (high fat vehicle), gummies (low fat vehicle) and beverages with "nanoemulsion" technology.

**Methods:** Healthy adults (n=4) completed nine, double-blind, outpatient sessions, separated by≥1 week, where they self-administered 1 of 3 cannabis edibles (i.e., gummy, drink, brownie) which contained either 0, 8.3, or 21.2 mg Δ9-tetrahydrocannabinol (THC). PD (i.e., subjective, cognitive, physiological effects) and PK (i.e., plasma concentrations of THC/metabolites) outcomes were assessed for 6 hours post-dosing.

**Results:** Each formulation produced dose-orderly PD effects. On average, the onset of subjective effects for drinks was 2-fold faster than brownies/gummies at both active doses. The time course and magnitude of effects were similar between brownies and gummies. At the high THC dose, cognitive impairment was greater for drinks versus brownies/gummies. Plasma THC levels showed that, at the high dose, drinks had a 60-min faster time to maximum concentration (Tmax) and a maximum concentration (Cmax) 1.65-3.86 times higher than brownies and gummies, respectively. Brownies had a Cmax 2.34 times greater than gummies (but similar Tmax).

**Conclusion:** When THC dose is held constant, the use of nanoemulsion technology or increasing dietary fat content in cannabis edibles may improve the bioavailability of THC. Further, nanoemulsion edibles showed an accelerated onset and magnitude of effects compared to conventional edible formulations (gummies and brownies). Formulation should be factored in the use of cannabis edibles in therapeutic, research, and other contexts, including cannabinoid drug development.

**Support:** National Institute on Drug Abuse (NIDA) Grants R01DA057201

# IMPACT OF ACUTE Δ9-TETRAHYDROCANNABINOL (THC) ON PHARMACOKINETICS, SUBJECTIVE EFFECTS AND COGNITIVE FUNCTION IN HEALTHY VOLUNTEERS

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**Introduction:** Cannabis produces a broad range of acute, dose-dependent psychotropic effects, such as feeling high, and is associated with acute small to moderate cognitive impairment, although this may depend on the respective cognitive domain. In the current randomized crossover study, we examined the impact of Δ9-tetrahydrocannabinol (THC), the main psychoactive constituent of cannabis, on pharmacokinetics, subjective effects and cognitive function in a large group of healthy volunteers.

**Methods:** 44 male occasional cannabis users received placebo and THC (6 mg) in two separate sessions using a Volcano vaporizer. Subjective drug effects were assessed using composite visual analogue scales (VAS) at several timepoints. Cognitive function was examined using eight neuropsychological tasks selected from the CANTAB® test battery. Blood samples were collected to measure THC plasma concentrations.

**Results:** THC significantly increased mean subjective ratings of **'**feeling high', 'altered perception' and 'dysphoria', and reduced 'relaxation' ($p<.015$). On the neuropsychological test battery, THC impaired outcome measures of reaction time, spatial working memory and one touch stockings of Cambridge, but none survived correction for multiple testing. Mean peak THC plasma concentration was 80.6±6.6 ng/ml. Higher concentrations were significantly associated with stronger subjective effects on all four VAS scales ($p<.05$).

**Conclusions:** THC plasma concentrations and reported subjective effects indicate that a moderate high dose of THC was administered. Nevertheless, we failed to demonstrate significant THC effects on any of the cognitive domains, including learning, memory and attention. The pharmacokinetics/pharmacodynamics (PK/PD) relationship suggests that peak THC plasma levels may be used to predict the individual subjective response.

# RESOURCE CENTER FOR CANNABIS & CANNABINOID RESEARCH (R3CR): A NEWLY FUNDED CENTER

Ikhlas Khan[1], Mahmoud ElSohly*[1], Mary F. Paine[2], Donald Stanford[3], Nandakumara Sarma[4], Robert Welch[5], Patrick C. Still[6], Inna Belfer[7], Heather L. Kimmel[8], Alexis Bakos[9], Kathleen Castro[10]

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**Introduction:** Despite widespread availability and established pharmaceutical potential of cannabis and derived cannabinoids, multiple barriers to conducting research remain, resulting in insufficient data on the basic mechanisms, safety, abuse potential, and efficacy for many of the cannabinoids and other constituents of cannabis. In 2022, NIH/NCCIH published a request for information (RFI, NOT-AT-22-026), which gathered information from investigators about scientific interests and barriers to conducting research in the field.

**Methods:** In response to the challenges raised in the RFI, a trans-NIH institute partnership (NCCIH, NIA, NIDA, and NCI) issued a funding opportunity (RFA-AT-24-006) to support the development and maintenance of a Resource Center for Cannabis and Cannabinoid Research (R3CR). Multiple institutions responded to the RFA, and the NCNPR and its partners (USP and WSU) received the award. The R3CR was established with three Cores: Regulatory Guidance Core (UM-NCNPR), Research Standards Core (USP) and Research Support Core (WSU). Seed grant funding will be made available through the R3CR.

**Results:** The R3CR is intended to reduce barriers to conducting research on cannabis and its constituents to enable researchers to generate more rigorous scientific evidence across a variety of research domains in both basic and clinical research.

**Conclusions:** The R3CR aims to facilitate research advances through synergistic interactions among experts in relevant commercial, basic science, clinical, and regulatory areas both within the R3CR and in collaboration with the extramural community.

## PARTIAL INVERSE AGONISM OF CB1 AS A DE-RISKED STRATEGY FOR OBESITY

Lucas Laudermilk[1], George Amato[1], Vineetha Vasukuttan[1], Andrew Harris[2], Sheryl Moy[3], Elaine Gay[1] and Rangan Maitra[1,4]

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**Introduction:** Full inverse agonism of CB1 is a clinically proven strategy for obesity and related diseases. However, full inverse agonists of CB1 like rimonabant produce psychiatric adverse effects in some patients, potentially due to an inhibition of basal signaling of the CB1 receptor, which is important for emotional welfare. While alternative strategies such as peripheralized CB1 antagonists, negative allosteric modulators and neutral antagonists are being considered, clinical success has been limited. Partial inverse agonism of CB1 has not been explored as a viable strategy that can produce efficacy for treating obesity while preserving basal CB1 activity. As such, the novel purine compound RTI-194 was developed and tested for safety and efficacy.

**Methods:** Various *in vitro*, absorption–distribution–metabolism–excretion–toxicity (ADMET) and pharmacokinetic (PK) studies were performed to characterize RTI-194. Positron Emission Tomography (PET) was used to establish receptor occupancy. In addition, behavioral profiling of RTI-194 was performed using intracranial electrical self-stimulation (ICSS) in rats, light-dark box and elevated plus maze in mice. This compound was tested in a mouse diet-induced model of obesity (DIO) for efficacy. Various disease related biomarkers were evaluated.

**Results:** The novel purine RTI-194 is a partial inverse agonist of CB1. Studies indicate that RTI-194 has excellent drug-like properties (e.g., oral bioavailability) and is highly specific for CB1. In behavioral studies, RTI-194 produced a benign profile compared to rimonabant, which produced robust aversive and anxiogenic effects. Oral administration of RTI-194 led to significant weight loss comparable to rimonabant. Various disease relevant biomarkers were improved upon treatment.

**Conclusions:** Partial inverse agonism is an exciting strategy for targeting the CB1 receptor. The novel purine RTI-194 administered therapeutically to DIO mice reversed obesity and biomarkers associated with disease progression and pathogenesis. Unlike rimonabant, RTI-194 did not produce adverse effects (e.g., anhedonia) in multiple behavioral assays in both mice and rats. These encouraging studies support further preclinical development of RTI-194 for obesity and other important indications.

**Acknowledgments:** We thank the NIDA drug supply program for providing various chemicals. We thank the histology, mouse behavior and rodent imaging cores at UNC for assorted services. We thank Rod Snyder and Yun Lan Yueh for providing expert bioanalytical support. This research was funded by grants AA031392, DA040460, DK130765, DA057168 and DK124615 from NIH.

**ENDOTOXEMIA ALTERS PLASMA ENDOCANNABINOID AND ETHANOLAMIDE PROFILES IN DAIRY COWS**

Madison N. Myers*[1], Miguel Chirivi[1], Jair Parales-Girón[2], Jose M. dos Santos Neto[2], Lynn C. Worden[2], Adam L. Lock[2], & G. Andres Contreras[1]

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**Introduction:** Endotoxemia induces systemic inflammation and metabolic disturbances than may lead to mortality. Endocannabinoids (eCB) are lipid mediators that regulate metabolism and immune responses, but their involvement in endotoxemia remains unestablished. Therefore, using a bovine model, we investigated how single and repeat endotoxin exposures during negative energy balance (NEB) alter circulating eCB.

**Methods:** This study took place over 28 d. Multiparous dairy cows (n=32) were assigned to one of four treatments (SAL-SAL, SAL-LPS, LPS-SAL, LPS-LPS; n=8/group) in a 2×2 factorial design. Two 4-d feed restriction periods (initiated on d8 and d20) induced a -15 Mcal energy deficit. Treatments (100mL saline, SAL; 1 ug/kg bodyweight lipopolysaccharide in SAL, LPS) were administered via jugular catheter on d10 and 22. Plasma eCB were quantified via LC-MS/MS at enrollment (BAS), prior to (0H) and 4h post-infusions (4H). Data were analyzed using linear mixed models (% change relative to BAS, $P<0.05$).

**Results:** NEB increased circulating 2-arachidonoylglycerol (2-AG, +55%) and anandamide (AEA, +15%). LPS elevated oleoylethanolamide (+22%; OEA) while suppressing 2-AG (-6%) at 4H. AEA (+26%) and linoleoylethanolamide (+7%; LEA) remained elevated for 12 d post-infusion 1. Following infusion 2, 2-AG increased (+26%) in SAL-LPS, and, in SAL-LPS and LPS-LPS, respectively, AEA (+15%, +8%), LEA (-30%, -15%), and OEA (-44%, -52%) declined.

**Conclusions:** Endotoxemia and NEB dynamically modulate eCB biosynthesis, with repeat exposures impairing eCB responses. Given the role of eCB in energy homeostasis and inflammation, our findings provide insight into eCB dysregulation in metabolic-inflammatory diseases, with potential implications for both animal and human health.

**ENDOCANNABINOIDS PRODUCED BY VISCERAL ADIPOSE TISSUE MAY LIMIT THE FORMATION OF NEW ADIPOCYTES**

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**Introduction:** A positive correlation exists between circulating endocannabinoids (ECs) and visceral fat indicating that adipose tissue (AT) is a significant producer of ECs which may act in an autocrine manner, activating cannabinoid 1 receptors (CB1R). CB1R activation is known to promote fat accumulation and contribute to the development of obesity-related metabolic diseases. However, the influence of ECs on the commitment of stem cells to the adipocyte lineage remains unclear.

**Methods:** ECs secreted by human and mouse visceral and subcutaneous AT (VAT / SAT) explants were quantified by LC-MS/MS. Tissues were collected from both healthy and obese donors. Commitment of stem cells from the vascular stromal fraction to adipocytes was assessed using spectral flow cytometry, RT-PCR and Oil Red O lipid staining.

**Results:** AT explants from both humans and mice produced significantly higher levels of 2-arachidonoylglycerol (2-AG) compared to arachidonoylethanolamide (AEA). The secretion rates of 2-AG and AEA by VAT explants from obese mice were higher than those from lean mice. A similar trend was observed with VAT explants from obese diabetic patients. To further investigate a potential role of ECs in AT remodeling, stem cells from VAT were exposed to the CB1R agonist arachidonyl-2-chloroethylamide. CB1R activation significantly delayed stem cell proliferation and commitment into pre-adipocytes. Consequently, the number of adipocytes capable of differentiation was reduced, as evidenced by the expression of adipocyte markers and lipid staining.

**Conclusion:** These findings suggest that excessive ECs production in VAT may restrict new adipocyte formation, favoring adipocyte hypertrophy and contributing to obesity-related metabolic disorders.

## CNS-SPARING CANNABINOID THERAPEUTICS: A DUAL CB1R/INOS BLOCKADE APPROACH TO TREAT METABOLIC SYNDROME DISORDERS

Pinaki Bhattacharjee,[1]* Szabolcs Dvorácskó, [1,2] Paul Volesky,[1] Nick Rutland,[1] Luca Maccioni,[3] Sergio A. Hassan,[4] Resat Cinar [2], Malliga R. Iyer[1]

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**Introduction:** Evolving research on endocannabinoids has transformed our understanding of human physiology and opened novel treatment approaches for many diseases. The anxiogenic effects have hindered the advancement of developing brain-penetrant orthosteric CB1 receptor (CB1R) antagonists, rendering this strategy impractical. Thus, allosteric modulators, neutral antagonists, and peripherally acting drugs are intriguing alternatives, but none are yet clinically available. The interaction between CB1R overactivation and iNOS overexpression is intriguing in obesity and diabetes.

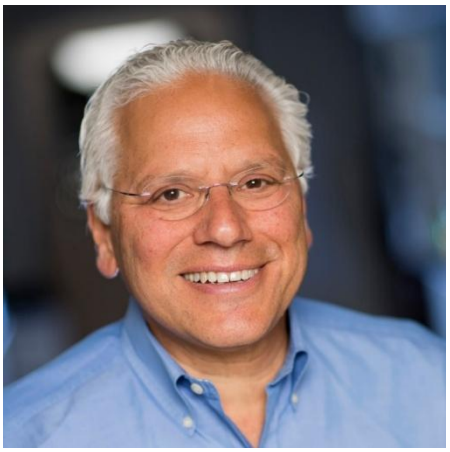
**Method:** Our extensive structure-activity analysis with *in vitro* radioligand binding, $GRAB_{eCB2.0}$ assay, GTPγS results, and GI motility test revealed that the tetrahydropyridazine-based analogues retained robust antagonistic activity at the CB1R.

**Result:** 1,4,5,6-tetrahydropyridazine based compounds demonstrated iNOS inhibition in RAW264.7 cells. Chirally separated active enantiomer of PB230 demonstrated limited brain penetration, in tissue distribution studies and showed promising outcomes in *in vivo* efficacy models for metabolic syndrome. In C57BL6/J mice with high-fat diet-induced obesity, administration of PB230 over a ten-day period led to decreased body weight and food intake, and improved glucose tolerance and insulin sensitivity with no observed anxiogenic effects (10 mg/kg) demonstrating its effective peripheral CB1R antagonism.

**Conclusion:** These findings have now culminated in the present work to identify a new class of molecules with improved pharmacological properties and promising activity against metabolic syndrome (MetS) disorders.

# ICRS 2025 Kang Tsou Memorial Lecture:
# 12:30-13:30, Wednesday July 9, 2025

## MEDICINAL EVOLUATION OF NATURAL HORMONES TO TRANSFORM DRUG TREATMENT OF OBESITY



Richard Di Marchi, Ph.D.
Distinguished Professor, Chemical Biology and
Gill Chair in Biomolecular Sciences
Indiana University, Bloomington Indiana

**Biography**

Richard DiMarchi is a Distinguished Professor of Chemistry and Gill Chair at Indiana University. He is a member of the National Academy of Medicine and the National Inventors Hall of Fame. He was Group Vice President at Eli Lilly and later at Novo, and recognized for discovery and development of Humalog®, rGlucagon®, and Forteo®. His academic research has broadened the understanding of glucagon physiology and the discovery of single molecule multimode agonists for the treatment of diabetes and obesity. Professor DiMarchi is co-inventor on more than one hundred U.S. patents and co-author to more than two hundred fifty peer-reviewed publications. He was identified as a top five translational researcher by Nature Biotechnology. He has co-founded eight biotech companies, notably Ambrx, Marcadia, Calibrium, and MBX. Professor DiMarchi has received the APS Merrifield career award in peptide sciences, the ACS Alfred Burger career award in medicinal chemistry, and the 2023 AAAS Bhaumik Breakthrough Award for GLP-1 Rx in obesity.

**Abstract**

The epidemic of obesity and its associated comorbidities represents a medicinal challenge that warrants broad molecular diversity. In concert with multiple collaborators, my IU laboratory has pioneered the recruitment of endogenous hormones and physiological mechanisms optimized for pharmacological purposes to address the heterogeneity constituted by the multiple diseases associated with the metabolic syndrome. From the earliest demonstration with lispro-insulin to the most recent discovery of single molecule, mixed incretin agonists we have pursued the discovery of chemically optimized macromolecules directed at the successful management of endocrine and related diseases. We have integrated classical small and large molecule-based pharmacology, while advancing the chemical methodology in synthesis of complex macromolecules. The integrated pharmacology of these peptides, proteins and nuclear hormones has provided a library of drug candidates replicated across multiple academic and commercial laboratories, clinical studies and inspired select registered drugs that deliver transformative therapeutic outcomes. The methodological approach to achieving these results may have merit in the search for medicines to treat other complex diseases correlative to advanced age.

## ICRS 2025 PRESIDENTIAL PLENARY: 8:30-9:30, Thursday July 10, 2025

1992-2025: FROM ENDOCANNABINOID-MEDIATED INTER-CELLULAR SIGNALING TO ENDOCANNABINOIDOME-MEDIATED INTER-KINGDOM CHEMICAL COMMUNICATION

Vincenzo di Marzo, Ph.D.
Professor, Faculty of Agricultural and Food Sciences &
Faculty of Medicine, Université Laval
Associate Research Director, Institute of Biomolecular Chemistry of the National Research Council (ICB-CNR)

**Biography**

Dr. Di Marzo is Canada Excellence Research Chair on the Microbiome-Endocannabinoidome Axis in Metabolic Health (CERC-MEND) at Laval University, Quebec, Canada (https://cerc-mend.chaire.ulaval.ca/en/home/), and Associate Research Director at the Institute of Biomolecular Chemistry of the National Research Council (ICB-CNR) in Pozzuoli, Italy (https://www4.na.icb.cnr.it/en/researchers-and-technologists/). He is also the coordinator of the Endocannabinoid Research Group (www.icb.cnr.it/erg) in the Naples region, and the director of the Joint International Research Unit between the Italian National Research Council and Université Laval, for Chemical and Biomolecular Research on the Microbiome and its impact on Metabolic Health and Nutrition (MicroMeNu, www.umilaval.cnr.it). Dr. Di Marzo has co-authored over 800 articles in peer-reviewed journals and has been listed as amoung the most Highly Cited Researchers (top 1% in the world) from 2014-2022. He is also a recipient of the ICRS Mechoulam award for his outstanding contributions to the cannabinoid research field.

## ASSOCIATIONS BETWEEN CANNABIS USE FREQUENCY, CIRCULATING CANNABINOID AND ENDOCANNABINOID LEVELS, AND PAIN SENSITIVITY

Elisa Pabon[*1,2], Adren Tran[3], Alexa Torrens[3], Anna Hilger[1], Conor H. Murray[1], Stephanie Lake[1], Timothy Fong[1], Daniele Piomelli,[3,4,5] and Ziva D. Cooper[1,6]

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**Introduction:** Cannabis containing delta-9-tetrahydrocannabinol can reduce pain. Frequent cannabis use may heighten pain sensitivity during abstinence, possibly due to altered circulating cannabinoid levels. This study examines the relationship between cannabis use frequency, acute pain sensitivity, and circulating cannabinoid levels in healthy adults who use cannabis.

**Methods:** Healthy adults (21-55 years, N = 38) who currently used cannabis were classified as "Light" (□4 days /week) or "Heavy" (□5 days /week). Cannabis abstinence > 12 hours was biochemically confirmed. Pain threshold (time to first pain sensation), tolerance (time until hand withdrawal), and subjective pain were assessed with the Cold Pressor Test (CPT). Blood samples were collected and analyzed for THC, 11-OH-THC, 11-COOH-THC, CBD, AEA, 2-AG, OEA, and PEA. Mixed effects models examined relationships between cannabis use frequency, circulating cannabinoids, and pain outcomes ($\alpha = 0.05$).
**Results:** Pain threshold, tolerance, and subjective pain ratings did not differ between "Heavy" and "Light" groups. However, THC, 11-OH-THC and 11-COOH-THC levels were significantly higher in the "Heavy" group (ps < 0.001. While most cannabinoid levels were unrelated to pain measures, THC ($p < 0.05$) and 11-OH-THC ($p < 0.001$) levels were positively related to subjective pain ratings.

**Conclusions:** Cannabis use frequency was not linked to pain sensitivity during short-term abstinence. However, more frequent use was associated with higher levels of THC and 11-OH-THC during abstinence, which were positively related to subjective pain ratings. Longer durations of abstinence may reveal increased pain sensitivity. These findings contribute to the discussion regarding the clinical use of cannabinoids as potential analgesics.

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# ENDOCANNABINOID SYSTEM ALTERATIONS IN PATIENTS WITH CHRONIC LOW BACK PAIN

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**Introduction:** Chronic low-back pain (CLBP) is a major unmet clinical need with significant socioeconomic impact. Negative affect and cognitive deficits are common comorbidities. The endocannabinoid (ECB) system modulates pain, negative affect, cognition, and their interaction. This study assessed pain, negative affect, cognition, quantitative sensory testing (QST) measures, and ECB system components in a CLBP cohort compared to healthy controls (HC).

**Methods:** 25 CLBP patients (52% female/48% male, 46±3.8 years) and 27 HCs (48% female/52% male, 40±2.1 years) were assessed for anxiety, depression, fatigue, pain-related fear, pain-catastrophising, and cognition. QST measures were taken on the forearm and lower back. Circulating ECBs and *N*-acylethanolamines (NAEs) were measured in serum via LC- MS/MS pre- and post-QST. Whole blood was analysed for a FAAH SNP (rs324420) and expression of genes encoding ECB system components. Data analysed with appropriate parametric or non-parametric statistical tests ($p<0.05$).

**Results:** CLBP participants exhibited higher anxiety, depression, fatigue, and pain catastrophising scores than HCs. CLBP participants were less sensitive to cold and warm detection on the arm/back, and to heat pain on the back. Prior to QST, CLBP patients had increased serum 2-AG and reduced AEA/NAE levels compared to HCs. Post-QST levels of all ECBs/NAEs, except OEA, decreased in both groups. No differences were found in ECB system gene expression or FAAH SNP prevalence.

**Conclusions:** CLBP patients exhibited increased negative affect and altered QST responses. Altered ECB/NAE levels in the CLBP participants were not attributable to the rs324420 SNP or expression of genes encoding components of the ECB system. Serum ECBs/NAEs were similarly reduced by QST in all participants. Further research is needed to elucidate the mechanisms underlying these ECB system alterations, and their functional implications for CLBP.

**Ethical Approval:** Participants were recruited and consented to procedures approved by the University of Galway and Galway University Hospital Research Ethics Committees.
**Acknowledgements:** Funding was provided by the Taighde Éireann – Research Ireland Government of Ireland Postgraduate Scholarship (GOIPG/2020/1496). The authors wish to thank the HRB Clinical Research Facility Galway.

# A RANDOMIZED PHASE II TRIAL OF MEDICAL CANNABIS TO REDUCE SYMPTOM BURDEN IN PATIENTS WITH ADVANCED PANCREATIC CANCER

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**Introduction:** Patients with pancreatic cancer experience burdensome symptoms, such as pain, nausea, appetite loss, insomnia, and anxiety. Cannabis has demonstrated efficacy in addressing cancer-related symptom burden and is available for eligible patients through state medical programs. The regulatory barriers to conducting interventional cannabis research require innovative trial designs that use standard-of-care medical cannabis.

**Methods:** Cannabis naïve patients with advanced pancreatic cancer experiencing pain, nausea, and/or cachexia and initiating systemic chemotherapy were randomized 1:1 to early cannabis (EC) versus delayed cannabis (DC) (NCT06605430). EC received 8 weeks of medical cannabis at no charge with education and dosing recommendations. DC received standard oncology care without cannabis for 8 weeks, and then received the cannabis intervention. Initial cannabis recommendations included a THC-dominant oral product at night (5mg), a THC/CBD balanced oral product (2.5mg/2.5mg) twice daily, and a THC-dominant vaporizer/oral spray (1-3mg) as needed. Patients self-titrated to achieve symptom control. Electronic surveys were completed weekly (NCI PRO-CTCAE) and monthly (PROMIS Global, cannabis/opioid logs).

**Results:** 26 patients were enrolled over 5 months (EC=14, DC=12) from one academic and one community oncology practice. Compared with the DC group, the EC group showed improved rates of change in the percentage of patients reporting moderate to very severe pain, appetite loss, insomnia, and anxiety over the first 4 weeks. By week 4, fewer EC patients used opioids and also reported a slightly lower mean morphine equivalent daily dose. Changes in PROMIS Global mental/physical scores were similar.

**Conclusions:** We demonstrate that conducting a pragmatic randomized interventional cannabis trial is feasible through an innovative trial design and collaboration between investigators and a state program. Early access to cannabis showed early signs of improved pain, appetite, insomnia and anxiety by week 4, and a trend towards lower opioid requirements a mong patients with pancreatic cancer initiating chemotherapy. These data highlight the potential for cannabis as a standard-of-care product to improve symptoms for patients with pancreatic cancer, while informing the need for and design of future larger trials.

| | **Early Cannabis (n = 14)[a]** | | **Delayed Cannabis (n = 12)[a]** | |
|---|---|---|---|---|
| Mean age (range) | 72 (64 -77) | | 68 (66 -70) | |
| % of patients with distant metastases (stage IV) | 43 | | 58 | |
| PROMIS mental/physical score, 0-100 scale [b] | BL 49/44 | 4W 45/41 | BL 47/47 | 4W 45/42 |
| % with moderate-very severe pain | BL 64 | 4W 67 | BL 80 | 4W 100 |
| % with moderate-very severe nausea | BL 36 | 4W 67 | BL 11 | 4W 50 |
| % with moderate-very severe appetite loss | BL 82 | 4W 56 | BL 40 | 4W 70 |
| % with moderate-very severe insomnia | BL 91 | 4W 33 | BL 80 | 4W 70 |
| % with moderate-very severe anxiety | BL 46 | 4W 25 | BL 50 | 4W 40 |
| % of patients using opioids | BL 29 | 4W 11 | BL 33 | 4W 46 |
| Mean morphine equivalent daily dose (MEDD), mg | BL 8 | 4W 3 | BL 5 | 4W 12 |

**Table 1.** Demographics and symptom measures among EC and DC patients at baseline (BL) and 4 weeks (4W).
[a]Patient totals may vary for certain measures, depending on available data. [b] Higher score = improved function.

# UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS OF MEDICAL CANNABIS FOR FIBROMYALGIA

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**Introduction:** Fibromyalgia is a common condition characterised by widespread chronic pain, associated with comorbid mental health disorders and reduced quality of life. Preclinical data suggests that major cannabinoids may have analgesic, anxiolytic and hypnotic effects, indicating potential benefits in fibromyalgia. However, high-quality clinical evidence remains limited. This study aims to assess the change in patient-reported outcome measures (PROMs) and incidence of adverse events (AEs) in patients prescribed cannabis-based medicinal products (CBMPs) for fibromyalgia.
**Methods:** This case series analysed data from the UK Medical Cannabis Registry (UKMCR). Patients were included if they enrolled ≥ 18 months prior to data extraction and had completed at least one baseline PROM questionnaire. The primary outcome was the change in PROMs [Fibromyalgia Symptom Severity, Fibromyalgia Widespread Pain Index, EQ-5D-5L, GAD-7, PGIC, SQS] from baseline to follow-up at 1, 3, 6, 12 and 18 months. Statistical significance was defined as $p < 0.05$. AEs were graded with CTCAE version 4.0.
**Results:** The analysis included 497 patients with a mean age of 44.66±12.02 years, with 341 (68.61%) female participants Most patients had previous cannabis experience (n=341, 68.61%). There was an improvement in all PROMs ($p<0.01$) from baseline to all follow-up periods. Higher CBD doses (>25.00 mg/day) and previous cannabis use were associated with increased odds of improvement on fibromyalgia-specific scales ($p<0.05$). A total of 227 patients (45.67%) reported 2,100 AEs (422.54%). The majority of AEs were mild-to-moderate (n=1,792, 85.33%), with fatigue being most common (n=153, 30.78%). Patients with baseline sleep impairment, prior cannabis use, or those using CBMPs as both oils and dry flower were less likely to report AEs ($p<0.05$).
**Conclusions:** This study demonstrates an association between treatment with CBMPs in fibromyalgia and improvements in pain, anxiety, sleep, and general quality of life. The high incidence of AEs in relation to other patient cohorts from the UKMCR may relate to the central sensitisation mechanism of fibromyalgia, which could increase susceptibility to adverse effects. These findings must be interpreted within the limitations of the study design. While randomised controlled trials are necessary to establish causality, this analysis provides valuable real-world data to inform their design.

# CANNABIS WITHDRAWAL SYNDROME AT ILLNESS ONSET IN FIRST- EPISODE PSYCHOSIS AND THE RISK OF SUBSEQUENT RELAPSE: A NESTED CASE-CONTROL STUDY

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**Introduction:** Cannabis withdrawal syndrome (CWS) has been identified as a possible trigger for the initial onset of first-episode psychosis (FEP). Here, we aimed to compare cases whose illness was immediately preceded by CWS with two groups: FEP heavy cannabis users and FEP non-users.

**Methods:** Cases and matched controls were identified from a clinical database of psychiatric healthcare records in London, UK. Cox proportional hazard models were used to investigate the effect of continued cannabis use after illness onset on the risk of psychosis relapse.

**Results:** 60 cases of FEP associated with cannabis withdrawal were matched with 60 FEP heavy cannabis users, and 60 FEP non-users. There were few differences in the demographic and clinical characteristics of the groups. Severe sleep impairment at illness onset was more common in the cannabis withdrawal group ($p<0.00001$).

Psychosis relapse within three years occurred in 41% of the cannabis withdrawal group, 51% of heavy users, 41% of non-users ($p=0.455$). Continued cannabis use was associated with an increased risk of psychosis relapse in the cannabis withdrawal group (HR=16.4 [95%CI: 2.2-121.9]; $p=0.006$), but not the heavy user group (HR=2.4 [95%CI: 0.85-7.0]; $p=0.10$). The interaction between case type and cannabis relapse produced a high but unstable hazard ratio (aHR=9.0 [95%CI: 0.9-94.3]; $p=0.067$; $aHR_{RidgeRegression}=3.0$ [95%CI: 1.6-8.9]).

**Conclusions:** People presenting with a first episode of psychosis in the context of cannabis withdrawal may be particularly sensitive to the effects of ongoing cannabis use. Clinicians should be aware that cannabis withdrawal may affect clinical outcomes in people with psychosis.

# PREDICTIVE ROLE OF INCREASED BLOOD ANANDAMIDE AT HOSPITAL ADMISSION IN ACUTE COVID PNEUMONIA RELATED RESPIRATORY FAILURE ON THE POST-COVID LUNG FIBROSIS

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**Introduction:** Subsets of COVID-pneumonia patients with acute respiratory failure experienced long- term respiratory dysfunction. Among survivors of severe COVID-19 pneumonia from the initial surge of COVID-19, over one half had impaired lung physiology at 6 months. However, mechanisms contributing to persistent pulmonary dysfunction following COVID-19 remain unclear. Elevated CB1R expression was noted in the lungs of patients who died from COVID-19 pneumonia. Our research indicated that CB1R activation exacerbates inflammation and tissue disrepair in mice. Additionally, higher levels of AEA in the lungs and serum of patients with pulmonary fibrosis correlated with poor lung function. These suggest overactivity of endocannabinoid/CB1R pathway may contribute lung disrepair in COVID-19 related respiratory dysfunction.

**Methods:** We measured endocannabinoid, inflammatory markers, and clinical parameters including pulmonary function at various time points during COVID-19 pneumonia respiratory failure. We aimed understanding pathogenic role of endocannabinoids in early and late respiratory complications across two independent cohorts including 125 subjects in different U.S. locations.

**Results:** We found that AEA and 2AG were significantly elevated in serum of acute COVID-19 patients. Both AEA and 2AG correlated with multiple inflammatory markers in COVID-19 pneumonia. Importantly, sustained elevation of AEA but not 2AG during hospitalization in days 1 and 9 in ventilated patients with acute hypoxic respiratory failure from COVID-19 pneumonia may hinder lung repair contributing to persistent pulmonary dysfunction at the 3-month follow-up.

**Conclusions:** Increased circulating endocannabinoids may be involved in the pathogenesis of COVID-19 pneumonia severity via CB1R that peripheral CB1R antagonism may be a promising therapeutic strategy for COVID-19 pneumonia.

# MODELING ADOLESCENT EDIBLE THC CONSUMPTION TO EXPLORE THE INFLUENCE OF SEX AND DOSE ON LONG-TERM NEURODEVELOPMENTAL OUTCOMES

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**Introduction**: Adolescent cannabis use can increase psychiatric risk later in life, yet usage rates remain high. Previous studies indicate that Δ9-tetrahydrocannabinol (THC) exposure during adolescence disrupts mesocorticolimbic circuits (i.e. prefrontal cortex [PFC], ventral tegmental area [VTA], and nucleus accumbens [NAc]), potentially causing long-lasting behavioural changes. Prior research has focused on systemic THC injections, which do not reflect typical human consumption methods. To address this, we investigated the long-term effects of adolescent edible THC consumption, a popular method of human cannabis use, in male and female rats, comparing the impacts of low and high-dose exposures.

**Methods**: Male and female rats received THC edibles (1-5 mg/kg, mixed with Nutella®) either once (low-dose) or twice daily (high-dose) during adolescence. Adult behaviour was evaluated through cognitive and affective tasks, and *in vivo* electrophysiology assessed glutamatergic PFC activity and dopaminergic VTA activity. Since significant changes were observed only in the twice-daily group, matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) and Western blotting were used to explore the metabolism of glutamate, GABA, and monoamine neurotransmitters in PFC-NAc-VTA pathways in this cohort.

**Results**: Low-dose THC consumption produced an anxiolytic effect in males; however, the high-dose THC edible caused sex-specific outcomes, resulting in anxiety behavioural in males and cognitive deficits in both sexes. The high-dose edible also produced PFC hyperactivity and altered VTA activity. Preliminary MALDI-IMS and Western Blot analysis revealed neurotransmitter metabolism disruptions in the PFC, NAc, and VTA following high-dose adolescent edible THC.

**Conclusions**: Adolescent consumption of edible THC leads to dose- and sex-dependent behavioural effects in adulthood, potentially mediated by excitatory/inhibitory imbalances and altered monoamine metabolism in mesocorticolimbic pathways produced by high-dose THC.

# INVESTIGATING THE RESPONSE OF THE ENDOCANNABINOID SYSTEM FOLLOWING REPETITIVE MILD TRAUMATIC BRAIN INJURY IN ADOLESCENT MALE AND FEMALE SPRAGUE-DAWLEY RATS

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**Introduction:** Mild traumatic brain injuries (mTBI) constitute 80% of all brain injury cases, with ~23% occurring in the adolescent populations. mTBIs are characterized by rotational movement of the brain due to biomechanical forces. Following the primary impact, blow or jolt, the secondary injury occurs, with heightened neuroinflammation due to proinflammatory cytokine production. While the primary injury cannot be prevented, the secondary injury may be mitigated with interventions focused on reducing neuroinflammatory hallmarks. The endocannabinoid (eCB) system, a highly expressed signalling system in the brain modulates various cognitive and neurobehavioral responses. eCBs like 2-Arachidonoylglycerol (2-AG) and anandamide (AEA) bind to cannabinoid receptors (CBR) located pre-synaptically on neurons and in glial cells to modulate neural activity and neuroinflammatory processes. The eCB system is primarily anti- inflammatory, with immune cells, including microglia, expressing CB1/2 receptors and secreting eCBs (2-AG specifically). Previous literature has demonstrated that pharmacological manipulation of this system has led to improvements in cognitive and inflammatory outcomes, thus providing a neural target for therapeutic intervention. The overall aim of this study is to characterize levels of 2-AG and AEA across four distinct brain regions at two different time points following RmTBI and examine changes occurring to inflammation and behavior.

**Methods:** Adolescent (P34) male and female Sprague-Dawley rats were administered 1 mTBI every 72-hours for a total of 5 hits. mTBIs were administered via the lateral impact device, a well validated model in literature. 3 primary endpoints were assessed: 1) eCB levels immediately post injury, 2) eCB levels one week post injury, 3) neurobehavioral outcomes and neuroinflammatory profiling. Brain tissue was harvested in both eCB cohorts to quantify endogenous 2-AG and AEA levels in 4 brain regions. Neurobehavioural assessments included the hanging bar task (motor), light-dark box task (LDB; approach-avoidance behavior) and novel object recognition task (NOR; cognitive). The hanging bar task was administered pre-RmTBI and 24 hours post-RmTBI. LDB was performed 1-week post-RmTBI and NOR task was performed 2 weeks post-RmTBI. Following all behavioural analyses, brains were perfusion-fixed and processed for immunohistochemistry to quantify microglia (density, morphology and phenotype).

**Results and Conclusions:** RmTBI significantly increased righting times (indicating loss of consciousness) in both males and females ($p>0.0001$) In males, RmTBI increased AEA in the hippocampus at the immediate timepoint and decreased 2-AG in the prefrontal cortex one-week post injury ($p=0.043$; $p=0.0104$). In females, RmTBI decreased 2-AG in the motor cortex a week following injury ($p=0.0101$). Inflammatory markers, including IBA1 are ongoing. This research demonstrated regional and temporal eCB changes following RmTBI. Further studies will investigate a therapeutic intervention to increase eCB levels post injury to assess inflammatory outcomes. **Acknowledgements:** This research was supported by the CIHR Foundation Grant and MIST program to MN Hill and Branch Out Neurological Foundation to L Javorcikova.

# EFFECTS OF ESTROUS CYCLE ON BEHAVIOR AND NEUROINFLAMMATION IN $\Delta^9$-THC-TREATED HIV-1 TAT TRANSGENIC FEMALE MICE

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**Introduction:** Fluctuations in ovarian hormones significantly impact endocannabinoid signaling and the dynamics of HIV-1 viral reservoir, specifically in women. This study aimed to (1) assess how the HIV-1 Tat protein affects behavior across estrous cycle and (2) determine whether acute $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) administration induces phase-specific behavioral effects in relation to the estrous cycle.

**Methods:** We used HIV-1 Tat transgenic female mice in two cohorts. One (7 mice/genotype) underwent 30-day estrous cycle monitoring via vaginal lavage. The second (8-11 mice/group) received vehicle or $\Delta^9$-THC (10 mg/kg) and underwent behavioral testing, and cytokine/chemokine analysis on brain samples.

**Results:** The results indicated that Tat expression significantly reduced proestrus frequency in Tat(+) females. Acute $\Delta^9$-THC decreased pain sensitivity in both groups during tail-flick and hot-plate assays. Hot-plate latency was cycle-dependent, with least sensitivity observed in estrus phase. $\Delta^9$-THC increased freezing time in Tat(+) mice, in proestrus and estrus phases. Anxiety-like behavior was heightened with □$^9$-THC, in both Tat(+) and Tat(–) mice, especially in proestrus phase. Proinflammatory cytokine/chemokine levels were most prominently affected by estrous cycle, with estrus phase increasing various proinflammatory markers in subcortical regions, whereas the opposite was seen in the spinal cord. Minimal effects were noted for acute $\Delta^9$-THC.

**Conclusion:** Our findings underscore the critical influence of estrous cycle on spontaneous nociception, anxiety-like behavior, and proinflammatory cytokine/chemokines, with behavior further being modulated by acute $\Delta^9$-THC treatment. These variations emphasize the need for more nuanced approaches, especially in research involving female subjects.

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## SEX DIFFERENCES IN STRESS RESPONDING IN A CLINICAL TRIAL OF PROGESTERONE IN CANNABIS USE DISORDER

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**Background:** Stress is a major contributor to relapse in cannabis use disorder (CUD), and stress-induced negative affect is a particular driver of use among women. The present study sought to examine if exogenous progesterone could block stress-induced craving and negative affect in men and women with CUD.

**Methods:** 148 non-treatment-seeking individuals with CUD (82 men, 66 women) received 200mg twice daily progesterone or placebo for one week while maintaining abstinence from cannabis. On study day 8, participants completed the Trier Social Stress Test. Stress measures (subjective stress, plasma cortisol, craving) were collected pre- and post-test. Effects of treatment, sex, and their interaction on stress measures were assessed using generalized linear mixed effects models and multinomial ordinal logistic regression models.

**Results:** There was no main effect of treatment on any stress measure ($p$'s>.05). However, a sex-by-treatment interaction was observed for craving ($p$<.05); progesterone reduced post-stress craving in women, but not men. A sex-by-subjective stress interaction was also observed on cortisol ($p$<.01), in which the association between stress and cortisol was positive in women, but insignificant in men, and this interaction was driven by participants randomized to progesterone. Conversely, a sex-by-stress interaction was observed for craving ($p$=.03), but the association between stress and craving was observed in men, not women.

**Conclusions:** While exogenous progesterone had no effect on stress-induced craving, cortisol, or negative affect overall, it did produce significant, differential effects by sex that primarily favored women. Future work examining the efficacy of progesterone in treatment-seeking women with CUD is of value.

## CANNABINOID REGULATION OF MURINE VAGINAL SECRETION

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**Introduction:** Tearing and salivation are wholly dependent on the activity of exocrine (lacrimal and salivary) glands whereas vaginal moisture and secretion rely on a combination of exudation and exocrine secretion. Exocrine gland disorders impact millions, and women with Sjögren's Syndrome often experience dry eye and mouth as well as vaginal dryness. Cannabis users' complaints of dry eye and 'cottonmouth' are well-known, but some female cannabis users also report vaginal dryness. The regulation of vaginal secretion by the cannabinoid signaling system is essentially unstudied. We recently reported that despite their small size and nocturnal nature, laboratory mice have measurable basal vaginal moisture and pheromone-stimulated secretory responses that are regulated by circadian and estrous factors.

**Methods:** We tested the regulation of vaginal moisture by cannabinoid CB1 receptors in this model.

**Results:** We now report that the cannabinoid receptor agonist CP55940 does not alter baseline vaginal moisture but prevents a stimulated secretory response due to a local perivaginal effect. Chronic intermittent CP55940 reduces basal vaginal moisture but also unmasks or induces a potentiating effect for CP55940, suggesting multiple sites of action. The acute and chronic effects likely occur via CB1 receptors. □[9]-tetrahydrocannabinol (THC), the chief psychoactive ingredient of cannabis, a partial agonist at CB1, has no acute or chronic effects.

**Conclusions:** In summary, strong acute activation of CB1 receptors in a murine model does not reduce vaginal moisture but does prevent a pheromone-stimulated vaginal secretory response. In contrast, chronic intermittent CB1 activation reduces baseline vaginal moisture. The extent to which these findings translate to humans remains to be determined.

# $\Delta^9$-TETRAHYDROCANNABINOL AND ITS EFFECTS IN A NEUROHIV MOUSE MODEL

Havilah P. Ravula*[1], Barkha J. Yadav-Samudrala[1], Laith E. Sawaqed[1], Sarah N. Arteaga[1], Essie Acquah[1], Sylvia Fitting[1]

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**Introduction:** $\Delta^9$-tetrahydrocannabinol (THC) has been studied for its neuroprotective benefits in disease and its ability to improve HIV-1-related symptoms in clinical and preclinical models. Chronic THC administration may cause tolerance to antinociceptive, hypothermic, and anxiolytic effects following acute THC administration; HIV status may further influence these effects. This study investigated the effects of an acute THC challenge after chronic THC exposure on behavioral and neuroinflammatory measures using a neuroHIV mouse model.

**Methods:** HIV-1 Tg26 transgenic mice (Tg26+/−, *n*=32(16f)) and their control littermates (Tg26−/−, *n*=31(16f)) received subcutaneous injections of vehicle solution or THC (3mg/kg), once/day, 5 days/week, for 90 days. After a 7-day drug-free period, all mice were given a high dose of THC (10mg/kg; intraperitoneally), and body temperature (BT), antinociception, locomotor activity (LA), and elevated plus maze (EPM) data were collected. To assess inflammation, we analyzed cytokine/chemokine levels via Bio-Plex and dystrophic microglia via immunohistochemistry.

**Results:** Chronic THC history resulted in significant downregulation of BT, reduced antinociception, increased anxiety-like behavior, and, for females, downregulation of proinflammatory/anti-inflammatory cytokines/chemokines. After acute THC challenge, vehicle history females showed significantly higher percent changes in BT, antinociception, and LA measures, along with less anxiety-like behavior. The Tg26(+/□) genotype resulted in increased antinociception, greater EPM distance traveled, and more cooccurrence with dystrophic microglia.

**Conclusions:** Female mice may develop tolerance to THC's hypothermic, antinociceptive, and anxiolytic effects; tolerance development may depend on HIV genotype. The sex and genotype effects seen in the behavioral assays may be elucidated by differential effects in the inflammatory measures.

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## ICRS 2025 MECHOULAM AWARDEE:
## 14:30-15:30, Thursday July 10, 2025

A NEW MECHANISM OF CANNABINOID TOLERANCE

Manuel Guzman, Ph.D.
Professor, Biochemistry and Molecular Biology
Complutense University of Madrid

**Biography**

Manuel Guzmán is Professor of Biochemistry and Molecular Biology at Complutense University of Madrid, and member of the Spanish Royal Academy of Pharmacy. He has served as President of the International Association for Cannabinoid Medicines (IACM) and the Spanish Society for Cannabinoid Research (SEIC). During the last 25+ years his research has focused mostly on the study of the mechanism of cannabinoid action. This work has allowed characterizing new effects and signaling pathways evoked by cannabinoid receptors, as well as suggesting new physiopathological implications derived from them, especially in the fields of oncology and neurology. He has published 220 scientific articles, as well as 6 patents on the potential therapeutic applications of cannabinoids as antitumoral and neuroprotective drugs. He has supervised 25 PhD theses.

**Abstract**

CB1R is the main molecular target of THC. Tolerance to THC develops upon repeated intake, which progressively decreases the efficacy and increases the risks of cannabis use. There is abundant evidence supporting the existence of pharmacodynamic adaptations at CB1R that are responsible for the process of cannabinoid tolerance. As for other GPCRs, CB1R desensitization typically involves short and longer-term molecular events. The mechanism of CB1R short-term desensitization (i.e., receptor uncoupling and internalization) is pretty well understood. However, the mechanism of CB1R longer-term desensitization (i.e., receptor downregulation by protein degradation) remains basically unknown. In this talk I will present work recently conducted by our group that unveils a molecular mechanism of CB1R degradation underlying cannabinoid tolerance.

# DEVELOPMENT OF A RAPID LC-MS/MS QUANTIFICATION METHOD IN MICE TO STUDY THE PHARMACOKINETICS AND ANXIOLYTIC EFFECTS OF CANNABIGEROL

Alex Mabou Tagne[1], Faizy Ahmed[1], Lana Debbaneh[1], Emma Raine Perranoski[1], Adren Tran[1], Aditi Das[2], and Daniele Piomelli[1,3,4,*]

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[4]Department of Pharmaceutical Sciences, University of California Irvine, Irvine, CA, USA.

**Introduction:** Despite increasing interest in cannabigerol (CBG), few animal studies have explored its pharmacological effects in relation to its pharmacokinetics. Understanding this relationship is crucial for comparing variables such as sex, age, and administration routes, which influence CBG absorption, metabolism, and distribution. To address this gap, we developed a rapid, sensitive LC-MS/MS method for quantifying CBG and its primary metabolite, cyclo-CBG, and applied it to rodent tissues. We also assessed CBG's effects on anxiety-related behavior in CD-1 mice.

**Materials and Methods:** CD-1 mice received a single intraperitoneal (IP) dose of CBG (10 mg/kg), and plasma/brain samples were analyzed over time using LC-MS/MS. Sample preparation involved protein precipitation with acetonitrile (ACN) and phospholipid removal via Enhanced Matrix Removal (EMR)-Lipid cartridges. Behavioral effects were assessed using elevated plus maze (EPM) and open field (OF) tests.

**Results:** The optimized method efficiently removed matrix interferences, including choline-containing phospholipids, and achieved excellent resolution (2.7) between CBG and cyclo-CBG. The 15-point calibration curves demonstrated strong linearity ($R^2 > 0.99$) over a broad concentration range (0.02 ng/mL–1.0 µg/mL). The limits of quantification were 0.1 ng/mL for CBG and 0.5 ng/mL for cyclo-CBG, with detection limits of 0.05 ng/mL and 0.2 ng/mL, respectively. Behavioral tests revealed anxiolytic effects of CBG.

**Conclusions:** We present a fast, reliable LC-MS/MS method for CBG quantification and demonstrate its applicability in pharmacokinetic and behavioral studies, facilitating future research on CBG's therapeutic potential.

## ANTINOCICEPTIVE EFFECTS OF CANNABIDIOL AND CANNABIGEROL IN AN INCISIONAL WOUND MODEL

Maria C. Redmond*[1,2,3,4], Catherine R. Healy[1,2,3,4], Rosmara Infantino[1,2,3], Mary Hopkins[1,2,3], Georgina Gethin[4,5,6], Abhay Pandit[4], David P. Finn[1,2,3,4]

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[4]CÚRAM, Research Ireland Centre for Medical Devices, University of Galway,
[5]School of Nursing and Midwifery, University of Galway,
[6]Alliance for Research and Innovation in Wounds, University of Galway, Ireland

**Introduction:** Wound-related pain is common yet poorly managed, and there is a need to identify new therapeutic approaches. Limited evidence suggests that cannabis and phytocannabinoids may reduce wound-related pain, but further study is warranted. We investigated the effects of systemic administration of cannabidiol (CBD) or cannabigerol (CBG) on nociceptive behaviour following back incision (BI) and on endocannabinoid levels.

**Methods:** Male Sprague-Dawley rats (150-200g on arrival, n=8-9/group) underwent BI or sham surgery. Back and paw mechanical withdrawal thresholds were assessed at baseline and post-surgical days (PSDs) 1, 4, 7, and 8 via manual and electronic von Frey tests, respectively. A single dose of CBD (3, 10 or 30 mg/kg i.p.), CBG (5, 10 or 20 mg/kg i.p.), or vehicle was administered one hour before the von Frey test on PSD 8. Rats were euthanised 90 minutes after CBD or 120 minutes after CBG administration.

**Results:** Following BI, rats developed primary and secondary mechanical hypersensitivity in the dorsum and hind paws, respectively. CBD (3 mg/kg) attenuated primary, but not secondary, hypersensitivity compared to vehicle-treated BI controls. CBG (10 and 20 mg/kg) attenuated primary hypersensitivity in the dorsum and secondary hypersensitivity in the ipsilateral paw. CBD (3 mg/kg) reduced AEA levels in the ipsilateral lumbar spinal cord compared to the contralateral side.

**Conclusions:** CBD and CBG can attenuate wound-related nociceptive behaviour in a dose-specific manner. Further research will investigate the mechanisms underlying CBD and CBG's antinociceptive effects and explore the potential of other cannabinoids and endocannabinoid system modulators in this incisional wound model.

**Acknowledgements:** Funding was provided by the Taighde Éireann – Research Ireland Government of Ireland Postgraduate Scholarship, Taighde Éireann – Research Ireland, and B. Braun Hospicare and is co-funded under the European Regional Development Fund under Grant Number 13/RC/2073-P2.

# EVALUATING THE THERAPEUTIC POTENTIAL OF CBD-CORTICOSTEROID COMBINATION THERAPY ON OSTEOARTHRITIS-RELATED CELL LINES IN VITRO

Tim Lefever*[1], Natalia Malek[1,2], Julia Jarco[2], Hunter Land[1]

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**Introduction**: Osteoarthritis (OA) disrupts the balance of joint cells, causing cartilage degradation, bone thickening, and inflammation driven by the synovium. Although corticosteroids (CSs) provide temporary symptom relief, their prolonged use can further damage the cartilage. This study explores whether combining cannabidiol (CBD) with corticosteroids can enhance joint health by protecting cartilage and reducing the necessary corticosteroid dose, thus limiting side effects. The research will assess the effects of CBD and combination on human chondrocytes (HC) osteoblasts (HOb) and synovial fibroblasts (HSF) by evaluating changes in cell viability, proliferation and gene expression.

**Methods**: Primary HC, HOb and HSF were treated with CBD and/or CSs across a range of concentrations up to 100 μM. Cell viability and proliferation were assessed in time using Incucyte S3 microscopic system. Gene expression of chondrogenic and osteogenic markers was performed with qRT-PCR technique.

**Results**: Dose-dependent toxicity was observed for both CBD and CSs across all tested cell lines. CBD exhibited cell-specific effects on the proliferation rate, while CSs consistently reduced proliferation at certain concentrations in all examined cell lines. Notably, CBD doses that enhanced cell proliferation in HC had no impact on the growth rates of HOb or HSF. In contrast, CSs demonstrated dose-dependent toxicity, consistent with previous studies, which was modified when CBD was added to the cell culture medium. Furthermore, changes in the expression profiles of the tested cells following CSs treatment were observed, with some of these alterations being reversed by CBD treatment.

**Conclusions**: Lack of increased toxicity and improvements in cellular health when combining CBD with CSs creates viable path for treating OA.

# EXPLORING THE PHARMACOKINETICS OF PHYTOCANNABINOIDS AND THEIR METABOLITES: AN ASSESSMENT OF ACUTE ORAL CANNABIGEROL (CBG) AND CANNABICHROMENE (CBC)

Ee Tsin Wong[1], Antasha Zainal[1], Eric Salazar[1], Jenny Ho[1], Blaine Phillips[1], Wenhao Xia[1], David Bovard[1], Elizabeth A. Cairns[2]*, Julia Hoeng[2]

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**Background**: Little is known about the pharmacokinetics of individual phytocannabinoids beyond delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Given the suggested therapeutic potential of other cannabinoids, it is essential to understand their oral bioavailability and kinetics. This study aimed to characterize the pharmacokinetics and bioavailability of cannabigerol (CBG) and cannabichromene (CBC), including for the first time some of their purported metabolites, increased plasma sampling to improve PK resolution, and IV administration for bioavailability determination.

**Methods**: Male Sprague Dawley rats (n=5-6) were administered an acute dose of oral (7.5-100 mg/kg in sesame oil; gavage) or intravenous (IV; 0.5 mg/kg) CBG or CBC under fasted conditions. Plasma was collected at 9 time points within 24 hours. UPLC-MS/MS was used to determine concentrations of CBG, 6',7'-epoxy-CBG, and 6'7'-dihydroxy-CBG (carmagerol), or CBC, and 8'-hydoxy-CBC. Pharmacokinetic profiles were evaluated by non-compartmental analysis (Phoenix WinNonLin, v8.3).

**Results**: Preliminary data show that following a 100 mg/kg oral dose Cmax was 1258.0 ± 344.7 ng/mL for CBG and 1379.1 ± 289.1 ng/mL for CBC, corresponding with Tmax at 220.0 ± 72.7 and 205.0 ± 97.5 minutes, respectively. Bioavailability was 5.1% for CBG and 29.4% for CBC. Only the 6'7'-expoxy-CBG and 8'-hydroxy-CBC metabolites reached sustained quantifiable levels post 100 mg/kg oral administration for assessment of PK profile.

**Conclusions**: These findings contribute to the understanding of the pharmacokinetic profiles of CBG and CBC, demonstrating differing bioavailability compared with other cannabinoids. These results provide important knowledge about the metabolic profiles of these two lesser studied phytocannabinoids and their metabolites in *in vivo* contexts.

# EFFECTS OF CANNABIDIOL (CBD) ON SPONTANEOUS OPIOID WITHDRAWAL IN MALE AND FEMALE RATS

Bryan W. Jenkins[1]*, Cerina Pang, Travis J. Wilberger, Robbie Y. Kuang, Won Park, Allie Hausker, Tylaah George, Tom Wang, Elise M. Weerts[1], Catherine F. Moore[1]

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**Introduction**: A key factor in continued opioid use is the emergence of a severe withdrawal syndrome after cessation that includes physical and psychological symptoms. Observational studies suggest cannabis may improve withdrawal symptoms. This study investigated the potential for chronic oral cannabidiol (CBD), a non-intoxicating cannabinoid, to alleviate symptoms in a multi-dimensional rat model of spontaneous opioid withdrawal.

**Methods**: Sprague Dawley rats (N = 100, equal sexes) were administered morphine (ascending doses: 10-50 mg/kg, s.c.) or vehicle (saline, s.c.) twice daily for 10 days. CBD isolate (10, 30 mg/kg, p.o.) or vehicle (sesame oil) was administered daily starting 12-hrs post-discontinuation (n = 8-9 per sex/group). Spontaneous withdrawal symptoms were evaluated across acute (38-hr) and protracted (up to 7-day) timepoints. Symptoms evaluated were physical (body weight, food intake, somatic signs, hyperalgesia) and psychological (irritability- and anxiety-like behaviors). Data were analyzed with ANOVAs and $p < 0.05$ was significant.

**Results**: Compared to non-dependent controls, morphine-dependent rats exhibited significant acute and protracted withdrawal symptoms, including reduced body weight and food intake, severe somatic signs, and persistent hyperalgesia ($p$'s $< 0.05$). CBD did not alleviate physical or psychological withdrawal symptoms at the doses tested.
**Conclusions**: Oral CBD isolate may not be effective in treating opioid withdrawal symptoms. Other cannabis constituents in CBD products may contribute to improved symptoms reported by users. These findings necessitate further research on the potential therapeutic benefits of cannabinoids for alleviating withdrawal symptoms in patients with OUD.

# $\Delta^9$-TETRAHYDROCANNABINOL EFFECTS ON PHYSIOLOGY AND BEHAVIOR ARE ALTERED IN MICE LACKING REGULATOR OF G PROTEIN SIGNALING 12

A. Matt Reck[1], David P. Siderovski[2], & Steven G. Kinsey[1]

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[2]Department of Pharmacology & Neuroscience, University of North Texas Health Science Center, Fort Worth, TX, USA

**Introduction:** Agonist-stimulated G protein-coupled receptors (GPCRs), like the $CB_1$ and $CB_2$ cannabinoid receptors, activate intracellular signaling cascades that are normally opposed by a superfamily of "Regulators of G protein Signaling" (RGS proteins). RGS12 is one of the largest members of this regulatory protein family and is known to oppose kappa opioid receptor signaling; the analgesic effects of kappa agonists are seen to be potentiated in mice lacking *Rgs12* gene expression. The RGS domain of RGS12 associates with $G_{i/o}$ alpha subunits, an integral part of the signaling cascade following cannabinoid receptor activation. Moreover, RGS12 is expressed in many central and peripheral tissues that likely also express $CB_1$ and $CB_2$ receptors. Thus, it is plausible that RGS12 might also regulate the effects of cannabinoid drugs through its intracellular activity opposing GPCR-activated signal transduction. In the present study, we worked to determine whether RGS12 deletion affects various behavioral and physiological effects of $\Delta^9$-THC.

**Methods:** Adult male and female RGS12 constitutive knockout or wildtype cagemate mice (C57BL/6J background strain) were administered $\Delta^9$-tetrahydrocannabinol (up to 100 mg/kg, s.c. in 1:1:18 parts kolliphor:ethanol:saline) cumulatively, 50 min prior to testing for body temperature, catalepsy (bar test), and spinal antinociception (tail immersion in 56°C water).

**Results:** $\Delta^9$-THC dose-dependently induced hypothermia (≥10 mg/kg), catalepsy (≥30 mg/kg), and antinociception (≥1 mg/kg) in both knockout and wildtype mouse cohorts. RGS12 deletion potentiated THC-induced spinal antinociception and hypothermia, where greater outcomes were observed in the RGS12 knockout mice.

**Conclusions:** RGS12 *knockout* mice showed both greater antinociception and hypothermia caused by THC. These data suggest that RGS12 modulates the effects of cannabinoid receptor agonism, likely through its intrinsic, negative regulatory activity at $G_{i/o}$-coupled GPCRs. Future work will determine the extent to which RGS12 deletion may also potentiate THC tolerance and withdrawal. Given that humans may express different RGS12 polymorphisms, these data suggest a potential physiological mechanism through which individual differences in THC effects occur.
Acknowledgments: This work was supported financially by the National Institute on Drug Abuse [NIH R01 DA048153] and the UConn Center for Advancement in Managing Pain.

## ICRS 2025 EARLY CAREER AWARDEE: 17:15-17:45, July 10, 2025

# PAMTASTIC VOYAGE: EXPLORING CB1 PHARMACOLOGY WITH POSITIVE ALLOSTERIC MODULATOR (PAM)-ANTAGONISTS

Thomas Gamage, Ph.D.
Assistant Professor, Neuroscience & Physiology
SUNY Upstate Medical University, Syracuse, NY

Dr. Gamage is an assistant professor in the Department of Neuroscience and Physiology at SUNY Upstate Medical University in Syracuse, NY. He first began studying the stimulus properties of endocannabinoids using the drug discrimination paradigm as an undergraduate in Dr. Jenny Wiley's laboratory at Virginia Commonwealth University. He earned his PhD in Pharmacology and Toxicology at VCU under the mentorship of Dr. Aron Lichtman. Dr. Gamage's dissertation work examined the endocannabinoid system as a target for the development of pharmacotherapeutics for the treatment of opioid dependence, investigating the effects of endocannabinoid catabolic inhibitors on somatic and affective components of morphine withdrawal. During this time, he also developed a deep interest in CB1 allosteric modulators, conducting the first systematic investigation into the in vivo effects of the CB1 allosteric modulator Org27569.

Dr. Gamage completed a postdoctoral fellowship Dr. Mary Abood's lab, building on his work on CB1 allosteric modulators, using molecular pharmacology techniques to investigate the effects of Org27569 on ERK signaling. In 2016, Dr. Gamage established a molecular pharmacology laboratory at RTI International, working closely with Drs. Brian Thomas and Jenny Wiley. At RTI, Dr. Gamage investigated the molecular pharmacology of synthetic cannabinoid receptor agonists and minor phytocannabinoids. There, he began a long-running collaboration with Drs. Yanan Zhang and Thuy Nguyen studying novel CB1 allosteric modulators, serving as principal investigator on a K01 awarded by the National Institute on Drug Abuse. This collaborative research demonstrated that these compounds, known as positive allosteric modulator (PAM)-antagonists, exhibit a unique pharmacological profile that provides a powerful means for targeting highly active CB1 receptor populations.
In 2023, Dr. Gamage established a new lab at SUNY Upstate Medical University where he continues his work investigating CB1 allosteric modulators, minor phytocannabinoids, and the spatiotemporal nature of cannabinoid receptor signaling. This work is supported by the National Center on Complementary and Integrative Health.

ICRS 2025

35th ANNUAL SYMPOSIUM

JULY 6-10 • BLOOMINGTON, IN

# DATA BLITZ & POSTER PRESENTATION ABSTRACTS



Back to posters

# MORPHINE-INDUCED ALLODYNIA IS ATTENUATED BY MAGL INHIBITION OR CB1 POSITIVE ALLOSTERIC MODULATION

Maria Jaakson[1], A. Matt Reck[1,2], Carl Rodriguez[1,2] & Steven G. Kinsey[1]

[1]School of Nursing, University of Connecticut, Storrs, CT, USA
[2]Department of Psychological Sciences, University of Connecticut, Storrs, CT, USA

**Introduction:** Opioid-induced hyperalgesia (OIH) is a condition where prolonged use of opioids causes heightened sensitivity to various painful and nonpainful stimuli, including touch and temperature. OIH occurs via mu opioid receptor-dependent and -independent mechanisms, coinciding with drug tolerance. The endocannabinoid system, which interacts with the opioid system in pain regulation, may be a target for counteracting OIH and restoring normal pain processing. The goal of the present study was to assess the ability of the monoacylglycerol lipase (MAGL) inhibitor, JZL184, or the CB1 selective positive allosteric modulator, ZCZ011, to reduce mechanical and cold allodynia induced by repeated morphine treatment.

**Methods:** Under general anesthesia, female and male C57BL/6J mice (n=10; 5f/5m) were implanted with an osmotic minipump that delivered 60 mg/mL/day of morphine sulfate or sterile saline vehicle. Mechanical allodynia and acetone-induced cold allodynia were quantified over 7 days post-surgery. On day 7, JZL184 (40 mg/kg, i.p.), or ZCZ011 (40 mg/kg, i.p.) was administered 120 or 75 min before allodynia testing, respectively.

**Results:** Morphine induced both mechanical and cold allodynia, which became statistically significant, as compared to control, starting on day 4 post surgery ($p < 0.01$). The MAGL inhibitor JZL184 attenuated mechanical allodynia ($p = 0.168$). In a separate cohort of mice, the CB1 positive allosteric modulator ZCZ011 attenuated morphine-induced mechanical and cold allodynia ($p > 0.999$)

**Conclusions:** These data suggest that cannabinoid modulating drugs are a potential strategy for attenuating morphine-induced allodynia. Future studies will evaluate a wider dose range of JZL184 and ZCZ011 while investigating potential cannabinoid receptor mechanisms.

Acknowledgments: This work was supported financially by the National Institute on Drug Abuse [R01 DA048153, R21 DA052690].

# ASSESSMENT OF ACEA ANTINOCICEPTIVE SEX DIFFERENCES AND TOLERANCE DEVELOPMENT IN MURINE MODELS OF INFLAMMATORY AND NEUROPATHIC PAIN

Robert C Barnes*[1], Dakota Robison[1], America Alanis[1], Mikaela Aleman[1], Satish Banjara[1], Josee Guindon[1]

[1]Department of Pharmacology and Neuroscience, Texas Tech University Health Sciences Center, Lubbock, TX, USA

**Introduction:** Chronic pain causes a significant reduction in patient quality of life and is commonly caused by rheumatic disease, characterized by inflammatory pain, and chemotherapy-induced peripheral neuropathy (CIPN). ACEA is a selective agonist of cannabinoid receptor 1 (CB1), a receptor that is frequently implicated in nociceptive regulation. In this study, we evaluated ACEA for antinociceptive efficacy, tolerance development, and sex differences in the formalin model of inflammatory pain and our CIPN model.

**Methods:** This study was performed in adult male and female C57BL/6j and S426A/S430A CB1 transgenic mice. In the formalin model, mice received daily ACEA injections, with the last such injection followed by a subcutaneous injection of 2.5% formalin into their left hind paw and pain quantification. In the CIPN model, neuropathic pain was established through weekly injections of cisplatin and assessed through the Von Frey and acetone tests. After neuropathic pain establishment, mice received daily injections of ACEA or vehicle.

**Results:** ACEA provided significant antinociceptive benefit without significant sex differences in peak antinociception in both models. In both models, tolerance developed more rapidly in female mice but was observed in all mice during the testing period. S426A/S430A transgenic mice performed similarly to C57 mice, with the notable exception of delayed tolerance development.

**Conclusions:** This study demonstrates the antinociceptive efficacy of ACEA in two murine models relevant to chronic pain, while also establishing the limiting factor of tolerance development. Further research is needed to better understand the mechanism of CB1 tolerance and to develop strategies to limit its development.

Acknowledgements: funding via NIH R01 NIDA 044999-01A1S1, NIH R01 NIDA 044999-01A1, and Grant 121035 Texas Tech University.

# NOVEL CANNABICHROMENE DERIVATIVE LACKING ABUSE LIABILITY EFFECTIVELY REDUCES PAIN IN MICE

Miguel A. De Leon[1]*, Rebecca E. Ozborn[1], Hannah M. Harris[2], Iram Shahzadi[3], Waseem Gul[3], Mahmoud ElSohly[3], Nicole M. Ashpole[1]

[1]Department of Biomolecular Sciences, University of Mississippi, Oxford, MS
[2]Department of Psychiatry, Columbia University, New York, NY
[3]ElSohly Laboratories Incorporated, Oxford, MS

**Introduction**: Pain is the primary cause of disability in the United States, and there has been a growing interest in developing cannabinoid-based therapies for treating pain. While many studies have identified beneficial effects of Δ9-tetrahydrocannabinol ($\Delta^9$-THC) against pain, the therapeutic potential of $\Delta^9$-THC is limited due to its psychoactive effects and potential abuse liability. Therefore, a cannabinoid devoid of these limitations would be a better therapeutic candidate. Cannabichromene (CBC) has non-psychoactive and anti-inflammatory effects, leading our team to develop novel derivatives of CBC that could enhance bioavailability and therapeutic potential. The current study evaluates the effectiveness of a novel CBC derivative, CBC-Val-HS.

**Methods**: To assess the bioavailability of these two compounds, a pharmacokinetic study was conducted (10mg/kg, i.v., po), and blood concentrations were measured at varying timepoints (e.g., 30 min, 60 min, 2 hrs). To assess the efficacy of CBC-Val-HS compared to CBC against inflammatory pain, an abdominal writhing assay was conducted. Male and female mice were administered increasing doses of CBC or CBC-Val-HS (0.1mg/kg- 50mg/kg, i.p.) 30 minutes prior to administration of 0.7% acetic acid. The number of abdominal writhes associated with inflammatory pain was measured for 30 minutes. The efficacy of CBC-Val-HS against cisplatin-induced neuropathic pain was also assessed using the electronic Von Frey test. The psychoactive potential of CBC-Val-HS, and potential abuse liability, was evaluated in the classic tetrad assay and conditioned place preference assay.

**Results**: CBC-Val-HS showed greater bioavailability than CBC with both IV and oral administration. Administration of CBC and CBC-Val-HS decreased the number of abdominal writhes in a dose-dependent manner with 10mg/kg or higher fully blocking inflammatory writhing. Similar to CBC, 10mg/kg CBC-Val-HS alleviated cisplatin-induced neuropathic pain, and reduced tail flick and hot plate latencies in the tetrad assay, without reducing locomotion or dropping core body temperature. No abuse liability was detected in the place preference assay.

**Conclusions**: Together, these data indicate that CBC-Val-HS can attenuate inflammatory, chemotherapy-induced, and thermal pain as effectively as CBC. Importantly, CBC-Val-HS has greater bioavailability than CBC and lacks psychoactive effects, suggesting greater therapeutic and commercialization potential.

# ALLOSTERIC LIGANDS TARGETING THE CB1 CANNABINOID RECEPTOR SUPPRESS INFLAMMATORY PAIN WITHOUT PRODUCING RESPIRATORY DEPRESSION OR MOTOR IMPAIRMENT

Ifeoluwa D. Solomon*[1,2], Sumanta Garai[3], Ganesh Thakur[3] and Andrea G. Hohmann[1,2,4]

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## Introduction

The CB1 cannabinoid receptor is a therapeutic target for treatment of pathological pain, but orthosteric CB1 activation produces undesirable on-target side effects. Medicinal chemistry efforts have, consequently, shifted their focus to allosteric modulators that engage sites distinct from the classical CB1 binding site to boost CB1-dependent signaling. GAT211, initially identified as a CB1 PAM, is a racemic molecule containing GAT228 (*R*) and GAT229 (S) enantiomers. Whether differences in allosteric activation or positive allosteric modulation translate into differences in therapeutic efficacy or unwanted cannabimimetic effects remains unknown.

## Methods

We evaluated the therapeutic efficacy of GAT228 and GAT229 enantiomers, in a preclinical model of inflammatory pain induced by intraplantar injection of complete Freund's adjuvant and assessed whether they would lack adverse side effects (respiratory depression, motor impairment) associated with the orthosteric CB1 agonist CP55,940.

## Results

Both the GAT228 and GAT229 attenuated mechanical hypersensitivity induced by intraplantar injection of complete Freund's adjuvant (CFA). Whereas CP55,940 produced robust respiratory depression and motor impairment, the CB1 allosteric agonist GAT228 and the CB1 PAM GAT229 failed to do so.

## Conclusion

GAT228 and GAT229 show preclinical efficacy in an inflammatory pain model without eliciting cannabimimetic side effects including motor impairment or respiratory depression unlike the CB1 othosteric agonist (CP55,940) under identical conditions. These findings identify CB1 allosteric agonists and PAMs, as a promising, safer therapeutic approach for pain management compared to direct orthosteric agonists such as CP55,940. Overall, this work contributes to offering a novel pathway for pain management that minimizes the risk of adverse effects.

**Acknowledgments** Supported by DA009158 and DA047858 (to AGH). IS was supported by a Sharon Brehem Fellowship.

# ANTINOCICEPTIVE EFFECTS OF FAAH OR MGL INHIBITION IN A PRECLINICAL MODEL OF LOW BACK PAIN

Mary A. Hopkins[1,2,3], Maria C. Redmond[1,2,3,4], Catherine R. Healy[1,2,3,4], David P. Finn*[1,2,3,4]

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**Introduction:** Chronic low back pain (CLBP) is an unmet clinical need with significant socioeconomic impact. Endocannabinoid system (ECS) alterations have been reported in patients with CLBP. Here, we investigated the effects of inhibitors of the endocannabinoid- catabolising enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL) on nociceptive behaviour in a novel rat model of CLBP following intervertebral-disc-injury (IVDI).

**Methods:** Forty Male Sprague-Dawley rats (7-8 weeks, 245-335g on arrival, n=10/group) underwent IVDI or sham surgery under isoflurane anaesthesia. Base-of-the-tail mechanical withdrawal thresholds were assessed at baseline, post-surgical days (PSD) 2, 7, 14, 20, and 21 via electronic von Frey (VF). One hour before VF on PSD21, a single dose of URB597 (1 mg/kg i.p.), MJN110 (5 mg/kg i.p.) or vehicle (ethanol:Kolliphor:saline (1:1:18)) was administered. Rats were euthanised 90min post-drug, and levels of anandamide (AEA), 2- arachidonoyl glycerol (2-AG), *N*-palmitoylethanolamide (PEA) and *N*-oleoylethanolamide (OEA) were measured in discrete brain regions, spinal cord and plasma using LC-MS/MS. Data were analysed with ANOVA followed by Tukey's *post-hoc* test ($p<0.05$).

**Results:** Rats developed IVDI-induced mechanical hypersensitivity at the base-of-the-tail from PSD2 onwards. URB597 and MJN110 attenuated mechanical hypersensitivity compared with vehicle-IVDI controls. URB597 increased levels of AEA, OEA and PEA in all brain regions assayed, and in the spinal cord and plasma. MJN110 increased levels of 2-AG in all brain regions, but not in spinal cord or plasma.

**Conclusions:** These results provide the first preclinical evidence that modulation of the ECS (via pharmacological inhibition of FAAH or MGL) may represent a viable analgesic approach for CLBP associated with IVDI.

**Ethical Approval:** Experimental procedures were approved by the Animal Care and Research Ethics committee, University of Galway. The experiments were completed under licence from the Health Products Regulatory Authority in the Republic of Ireland, in accordance with the EU Directive 2010/63. The study was designed in accordance with the ARRIVE guidelines.

**Acknowledgements:** Funding was provided by the Taighde Éireann – Research Ireland Government of Ireland Postgraduate Scholarship (GOIPG/2020/1496).

# LOW-DOSE CP55,940 CHANGES BODY COORDINATION PATTERNS DURING TREADMILL RUNNING IN MARKER-BASED 3D MOTION CAPTURE OF MICE

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**Introduction:** Our previous studies revealed impairment in mouse running speed and changes in step kinematics induced by CP55,940, recorded with marker-based motion capture. Here, we aimed to test whether principal component analysis (PCA) unravels distinct dominant whole-body coordination patterns (i.e. body deformations from mean) following CP55,940 compared to vehicle control.

**Methods:** We used adult male C57BL/6 mice in a within-subject, randomized design (n=6- 10). We recorded voluntary running on a treadmill after treatment with vehicle (1:1:18 EtOH, Kolliphor, saline) or a low dose of CP55,940 (0.3 mg/kg) for 30 s at increasing speeds (15, 20, 30 and 40 m/min) until failure. Mice were implanted with permanent markers located on the hips, back, hindlimb knees, and ankles. A high-speed, high-resolution 3D motion capture system (Qualisys) was used to track 3D trajectories and velocity of markers during the task. PCA was performed on mean subtracted pooled marker trajectories.

**Results:** In mice running at 20 m/min under baseline conditions, 87% of variance in the whole-body deformations around a mean body pose was represented by a 6-dimensional principal component (PC) space. Dominant PCs were conserved between individual animals, and the number of PCs decreased at higher speeds, signifying reduced locomotory strategies (“gaits”). Following treatment with CP55,940, PCs representing the body configurations changed compared to vehicle control, indicating that low-dose CP55,940 modified the whole-body deformation dynamics during treadmill running.

**Conclusions:** PCA reveals distinct coordination patterns and their alternations induced by CP55,940. The results call for application of whole-body analytical approaches to naturalistic movements such as locomotion.

**Acknowledgements:** Research was supported by the Japan Society for Promotion of Science (JSPS).

# THE ENDOCANNABINOID SYSTEM IN NEURAL STEM CELLS AS A BRIDGE AMONG ADULT HIPPOCAMPAL NEUROGENESIS, STRESS RESILIENCE AND ENVIRONMENTAL CHALLEGES

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**Introduction:** Adult hippocampal neurogenesis (AHN) facilitates memory and emotional regulation, profoundly affected by stress, which can impact resilience to depression and anxiety. This study evaluates how anandamide (AEA) and cortisol can influence neural stem cells (NSCs) dynamics, exploring their roles within the endocannabinoid and glucocorticoid pathways.

**Methods:** Murine hippocampal neurospheres were treated with 1 μM AEA and 5 μM cortisol, individually and in combination, for 24 hours and one week. We assessed NSCs proliferation and differentiation by quantifying Ki-67 and Doublecortin (DCX) expressions through flow cytometry.

**Results:** Immediate treatment (24 hours) with AEA or cortisol showed no significant changes in Ki-67 or DCX levels, indicating negligible short-term effects on NSCs dynamics. Chronic exposure (7 days), however, demonstrated that AEA significantly enhanced both Ki-67 and DCX expressions, suggesting improved NSCs proliferation and differentiation. Conversely, cortisol alone did not significantly alter these markers but did show enhanced effects when combined with AEA.

**Conclusion:** Our study underscores the significant role of AEA in enhancing the *in vitro* proliferation and differentiation of NSCs. Notably, while AEA exhibits a clear promotive effect on neurogenesis, cortisol did not demonstrate any significant impact on NSCs behavior within the studied time- frames. This finding suggests that AEA may act independently of the glucocorticoid pathway in short-term settings. Future studies will extend these experiments to include longer treatment durations, in order to ascertain whether prolonged exposure to cortisol may elicit delayed effects on NSCs dynamics.

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# DEVELOPMENT OF A SUITABLE MOUSE MODEL TO STUDY STRESS RESPONSE MECHANISMS ASSOCIATED TO CB1 RECEPTOR

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**Introduction:** Adult hippocampal neurogenesis (AHN) is a fine-tuned process that generates new functional hippocampal neurons. Bioactive lipids of the endocannabinoid system (ECS) influence AHN, stress responses and mood, particularly *via* cannabinoid 1 ($CB_1$) receptor [1, 2]. Studies show sex-dependent impact of stress on AHN and ECS [3]. Here, we aimed to develop a mouse model to study stress response mechanisms and unravel the role of $CB_1$ in stress resilience.

**Methods:** Male and female $CB_1^{-/-}$ and wild type (WT) C57BL/6J mice were assigned to control (standard housing) or Repeated Restraint Stress (RRS) groups (n=3/5). RRS mice underwent 1-hour daily restraint for 3 days per week. After 4 weeks, behavioural tests were performed *via* Open Field (OF) and Novel Object Recognition test (NORT).

**Results:** No changes were observed for the OF parameters (time spent in the periphery and number of entries in the center) in both male and female WT mice. Instead, the NORT expressed as "novel object preference (%)" was impaired in RRS male WT mice compared to controls, but remained unchanged in female WT mice. To assess $CB_1$ involvement in stress resilience, RRS is running in $CB_1^{-/-}$ mice, and the same behavioural tests will follow. These data will be presented at the congress.

**Conclusions:** We developed a mouse model of physical restraint stress, which is characterized by cognitive functional impairment in male but not in female mice, suggesting a sex-dependent effect of our experimental paradigm. Our model has now been translated to $CB1^{-/-}$ mice, in order to investigate the role of $CB_1$ in stress resilience.

**Acknowledgements:** The authors are grateful for financial support to the European Union - Next Generation EU funds (Progetto PRIN2022PNRR - *The endocannabinoid system in the neural stem cells as a bridging among adult hippocampal neurogenesis, stress resilience and environmental challenges* - Avviso MUR - D.D. n. 1363 of 01/09/2023 - PNRR - Missione 4 Istruzione e Ricerca - Componente 2 - Investimento 1.1).

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# ART12.11, A NOVEL CANNABIDIOL:TETRAMETHYLPYRAZINE COCRYSTAL, ALLEVIATES STRESS-INDUCED DEPRESSIVE SYMPTOMS

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**Introduction:** Clinical and pre-clinical studies show promising outcomes for cannabidiol (CBD) in treating mood disorders, though certain pharmacokinetic limitations hinder its therapeutic applications. We have recently shown promising anti-depressant behavioural effects following the acute administration of ART12.11, a novel cannabidiol:tetramethylpyrazine (CBD:TMP) cocrystal. The ART12.11 cocrystal aims to improve the pharmacotherapeutic potential of CBD by combining it with the co-former tetramethylpyrazine (TMP). Here, we explore the behavioural effects of chronic ART12.11 administration and compare it with an established anti-depressant.

**Methods:** Using translational behavioural pharmacology, we are characterizing the anti-depressant and cognitive effects of chronic oral ART12.11 treatment in male Sprague Dawley rats following exposure to chronic stress. Additionally, we are using Sertraline, a well- established Selective Serotonin Reuptake Inhibitor (SSRI), as a positive control. We are also investigating the potential of ART12.11 as an adjunct therapy by co-administering it with Sertraline.

**Results:** We speculate that chronic administration of ART12.11 will alleviate depressive-like symptoms and minimally impact cognition. We expect Sertraline treatment to also improve depressive-like symptoms, though it may negatively impact cognition. Finally, we anticipate the ART12.11 + Sertraline co-administration to enhance anti-depressant efficacy. A full panel of results, including multiple depression and cognition behavioural tests, will be available by June 2025 and presented at ICRS.

**Conclusions:** Given previous findings regarding ART12.11, we speculate that chronic administration of ART12.11 will reverse stress-induced deficits and produce anti-depressant behavioural effects, comparable to Sertraline administration. This will further enhance the notion that ART12.11 is a promising pharmacotherapeutic development for the treatment of mood disorders.

# ROLE OF 2-AG/CB1 RECEPTOR SIGNALING IN STRESS POTENTIATED RELAPSE TO COCAINE SEEKING IN RATS

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**Introduction:** Glucocorticoids activate endocannabinoid signaling at the GABA interneuron-pyramidal cell synapse in the medial prefrontal cortex (mPFC), likely through increased CB1 receptor signaling by 2-archidonoylglycerol (2-AG) to promote cocaine seeking. The mechanism by which glucocorticoids, such as corticosterone (CORT), enhance 2-AG-mediated signaling remains unknown.

**Methods:** We utilized electrophysiology, a self-administration/cocaine seeking protocol in rats, and eCB GRAB approaches within tissue culture.

**Results:** We have shown that CORT rapidly decreases mini-inhibitory postsynaptic currents (mIPSCs) via a presynaptic cannabinoid 1 receptor-mediated mechanism. This effect was blocked by inhibition of the 2-AG synthetic enzyme, diacylglycerol lipase. Since G protein coupled receptor-mediated activation of Gq signaling cascades can lead into 2-AG synthesis, we delivered a Gq palmitoylated palpeptide and additional Gq inhibitors into the prelimbic PFC. We find that Gq blockade reduces CORT-potentiated reinstatement and the effect of CORT to reduce mIPSCs in PFC slices.

**Conclusions:** Our data suggest that Gq signaling, likely leading to the production of diacylglycerol, is required for CORT to inhibit GABA release at the pyramidal cell synapse in the mPFC. The resulting disinhibition of excitatory input into the nucleus accumbens results in drug seeking behavior in previously drug exposed rats.

# SYNTHETIC CANNABINOID WIN-55 INCREASES MITOCHONDRIAL OXYGEN CONSUMPTION IN HEALTHY OPTIC NERVE HEAD ASTROCYTES WHILE DECREASING LACTATE RELEASE IN STRESSED ASTROCYTES

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**Introduction**: Optic nerve head astrocytes (ONHAs) undergo metabolic changes in glaucoma. Cannabinoid signaling has been shown to reduce intraocular pressure, but the effects on cellular metabolism have not been explored. We hypothesize that the CB1 receptor modulates bioenergetic profiles of ONHAs by increasing the metabolic capacities of mitochondria in addition to increasing lactate production.

**Methods**: Primary rat ONH astrocytes were isolated and seeded into Seahorse 24-well culture plates or 6-well BioFlex® membrane plates. To measure oxygen consumption, cells were treated for 24 hours with WIN-55 (1, 10, 100, 1000nM) before performing a Mitochondrial Stress Test. Membrane plates were similarly treated with WIN (100 or 1000nM) before 24 hours stretch-stress. Conditioned media was used to determine extracellular lactate concentration.

**Results**: We observed a concentration-dependent increase in basal respiration in cells treated with WIN-55, but only 1000nM had significantly higher basal respiration than the control ($p=0.0115$) and 1nM ($p=0.0463$). ATP-linked respiration only significantly increased between 1000nM and the control ($p=0.0218$) with an increasing concentration-dependent trend overall. ANOVA showed no differences in lactate concentration among healthy cells. Analysis showed significance among stretched groups ($p=0.0145$) but post-hoc analysis found no significance. However, 100nM decreased lactate 1.8-fold during stretch-stress conditions.

**Conclusions**: Our preliminary results demonstrate a possible effect of ONH astrocyte CB1 receptor signaling in modulating mitochondrial metabolism. However, further biological replicates are needed to clarify observed trends and significant increases in oxygen consumption. Confirming these findings could offer novel targets for modulating metabolic changes observed at the ONH during glaucomatous pathology.

# DIFFERENTIAL EFFECTS OF CB1 RECEPTOR ANTAGONISM ON HIPPOCAMPAL LONG-TERM DEPRESSION DURING AGING

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**Introduction:** The temporally and spatially restricted release of endogenous cannabinoids in the brain can mediate or modulate synaptic plasticity, including long-term potentiation and long-term depression (LTD), in a synapse-specific manner. This is especially relevant in the hippocampus, a key structure involved in learning and memory. Brain aging is associated with a decline in cognitive function that parallels changes in synaptic plasticity. Endocannabinoid signaling also undergoes marked changes over the lifespan, with decreased activity in middle-aged animals and variable outcomes reported in aged animals. The goal of the present studies was to explore the role of altered endocannabinoid signaling in age-dependent changes in hippocampal LTD.

**Methods:** Experiments were performed on CD-1 and C57/Bl6 mice using protocols approved by the University of Connecticut Institutional Animal Care and Use Committee. Sagittal slices containing the hippocampus were cut with a vibratome and transferred to a recording chamber continuously perfused with carboxygenated artificial cerebrospinal fluid at room temperature. Field excitatory postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum of the hippocampus in response to Schaffer collateral stimulation. LTD was induced by a 10-minute exposure to the metabotropic glutamate receptor agonist (S)-3,5-dihydroxyphenylglycine (DHPG).

**Results:** We examined the role of endocannabinoid signaling in DHPG-induced LTD by recording fEPSPs in the CA1 stratum radiatum in hippocampal slices from adolescent (1 - 2 months), mature (3 - 6 months), and aged (22 - 24 months) mice. In adolescent and mature animals, significant LTD (30 - 40% decrease from baseline) could be induced by 10 min of exposure to DHPG and lasted at least sixty minutes. This form of LTD was dependent on mGluR activation because it was completely blocked by combined exposure to the mGluR1 antagonists MPEP and CPCCOEt. In adolescent animals, the magnitude of LTD was significantly reduced by blocking cannabinoid receptor activation with the CB1 receptor antagonist NESS-0327. In contrast, the magnitude of LTD in mature animals was generally unaffected by blocking cannabinoid receptor activation. In aged mice, LTD was not consistently evoked, and the magnitude of LTD when present was significantly lower than in juvenile and mature animals. Interestingly, however, the magnitude of LTD in aged animals was increased by blocking cannabinoid receptor activation with NESS-0327.

**Conclusions:** These results indicate that the magnitude and prevalence of DHPG-induced LTD are age-dependent, as are the effects of CB1 receptor blockade. This suggests that endocannabinoid signaling plays differential roles in synaptic plasticity across the lifespan. Understanding the age-dependent roles of endocannabinoid signaling will be critical in unraveling their contributions to synaptic plasticity, learning, and memory during early development and aging.

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# TACKLING CCK/CB1R EXPRESSING GABAERGIC INTERNEURONS IN BLA FOR THEIR ROLE IN FEAR EXTINCTION

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**Introduction:** Previously, we demonstrated the importance of cortical inputs into the basolateral amygdala (BLA) for extinction learning and showed that augmented endocannabinoids signal through their presynaptic receptors to modulate synaptic plasticity during extinction learning (Gunduz-Cinar et al 2023). However, the precise contribution of increased endocannabinoids on the CB1Rs expressing BLA cholecystokinin (CCK) interneurons are still not known.

**Methods:** Our previous attempt to manipulate these GABAergic interneurons using the CCK-Cre-Dlx-FLpo double transgenic mice appeared to be not specific to the CB1Rs that are abundant on just the CCK interneurons, but also a variety of other cells (Rovira-Estaban et al 2019). In this study, using the intrsect viral strategy we explored the role of calcium binding protein of CCK/CB1Rs (NECAB 2) (Miczan et al 2021) and gamma synuclein (Sncg) (Dudok et al 2021) that showed selectivity in hippocampus. Using both viral promoters and mouse line we characterized their molecular expression in BLA and their behavioral roles with optogenetic and/or chemogenetic manipulations during fear extinction.

**Results:** Profiling of Necab2 as a promoter for BLA CCK/CB1R-interneurons showed expression majorly on the GABAergic neurons but with lack of specificity evidenced by co-expression on NPY, VIP and PV INs. Moreover, CB1Rs were not found abundant on Necab2 expressing interneurons. While Sncg profiling is still ongoing, our preliminary data showed co-expression on both Vglut1 and GAD 65 expressing neurons in BLA.

**Conclusion:** Understanding the precise role of the CB1R expressing interneurons will provide us information on input neuron modulation during fear and extinction learning in BLA. The results of this study will guide us in the development of better therapeutic strategies for anxiety and stress related disorders.

# OVERABUNDANT ENDOCANNABINOIDS IN NEURONS ARE DETRIMENTAL TO COGNITIVE FUNCTION

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**Introduction:** 2-Arachidonoylglycerol (2-AG) is the most prevalent endocannabinoid involved in maintaining brain homeostasis. Previous studies have demonstrated that inactivating monoacylglycerol lipase (MAGL), the primary enzyme responsible for degrading 2-AG in the brain, alleviates neuropathology and prevents synaptic and cognitive decline in animal models of neurodegenerative diseases. However, the cellular mechanism is still unclear.

**Method:** We assessed spatial learning and memory using the Morris water maze testing, while synaptic functional and structural alterations in the hippocampal dentate gyrus (DG) were evaluated through field potential recordings, whole-cell recordings, electron microscopy and Golgi-staining-based analysis. Single-cell RNA sequencing was subsequently employed to profile activity-dependent changes in synaptic gene expression across hippocampal subregions.

**Results:** We show that selectively inhibiting 2-AG metabolism in neurons induces CB1R-dependent impairments in learning and memory in mice. This cognitive impairment appears to result from decreased expression of synaptic proteins and synapse numbers, impaired long-term synaptic plasticity and cortical circuit functional connectivity, and diminished neurogenesis. Interestingly, the synaptic and cognitive deficits induced by neuronal MAGL inactivation can be counterbalanced by inhibiting astrocytic 2-AG metabolism. Transcriptomic analyses reveal that inhibiting neuronal 2-AG degradation leads to widespread changes in expression of genes associated with synaptic function.

**Conclusions:** These findings suggest that crosstalk in 2-AG signaling between astrocytes and neurons is crucial for maintaining synaptic and cognitive functions and that excessive 2-AG in neurons alone is detrimental to cognitive function.

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# DOES CB2 CANNABINOID RECEPTOR INVOLVE IN THE REGULATION OF NEUROTROPHIC FACTORS AND NEUROGENESIS IN ADULT BRAIN?

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**Introduction:** The full spectrum of the role of $CB_2$ cannabinoid receptor (CB2R) is still not clearly understood. Recently several studies have reported the presence of functional CB2R in neuronal cells that regulate several important brain functions including rewarding effect of abuse drugs and adult brain neurogenesis. In our laboratory, we have shown CB1R plays a key regulatory role in EtOH-mediated effects on neuroprogenitor cell fate related to adult hippocampal neurogenesis. However, the role of CB2R in the regulation of adult neurogenesis is poorly understood. In the current study using chronic binge-like alcohol drinking model we have studied a) the effect of exogenous or endogenous activation of CB2R on adult rat hippocampal neurogenesis and b) the role of CB2R on ethanol effect on hippocampal neurogenesis.

**Method:** Adult male Wistar rats (body wt. 280-320 gm) were treated with vehicle or CB2 receptor agonist HU308 (15 mg/kg/day, ip) or with ethanol (5 g/kg/day, ig) alone or in combination for 10 days. Animals were sacrificed 24 hr following the last treatment. The effects of the drug treatments on neurogenesis, cell proliferation, apoptosis and CREB activation in dentate gyrus and prefrontal cortex were assessed immunohistochemically by DCX+IR (doublecortin positive), Ki67+IR and cleaved caspase-3 and pCREB staining respectively in brain slices.

**Results:** We found that CB2R agonist HU-308 treatment did not produce any significant change in basal DCX+IR and Ki67+IR compared to vehicle treated animals following treatment. We also found that CB2R agonist did not alter ethanol- mediated inhibition of neurogenesis and augmentation of apoptosis. Interestingly, we observed that CB2R activation and ethanol treatment individually produced a significant increase on pCREB positive cells number in the dentate gyrus.

**Conclusion & Future Direction:** Together, these findings suggest that CB2R activation does not directly play a role in the modulation of neurogenesis and/or EtOH-mediated effects on neuroprogenitor cell death and neurogenesis in the adult brain. However, CB2R activation significantly produced activation of CREB in the neurogenic niche area of the brain. Currently, using CB2R knock-out mice and CB2R expressing microglia cells, we are investigating the role of CB2R in the regulation of CREB, c-SRC and BDNF signaling with or without ethanol treatment.

# INACTIVATION OF MONOACYLGLYCEROL LIPASE IN ASTROCYTES ATTENUATES SEIZURE SEVERITY AND POST-SEIZURE INJURY

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**Introduction:** Monoacylglycerol lipase (MAGL) is a key enzyme responsible for degrading the endocannabinoid 2-arachidonoylglycerol (2-AG). Its inactivation has been shown to produce anti-inflammatory and neuroprotective effects in neurodegenerative and inflammatory disease models. However, the impact of cell-specific MAGL inactivation on epilepsy remains unclear. This study aimed to investigate whether MAGL disruption attenuates seizure severity and post-seizure neuronal damage and whether these effects are cell-type specific.

**Method**: Kainic acid (KA) was administered to induce seizures in wild-type (WT) and genetically modified MAGL knockout mice, including astrocyte-specific knockout (AKO), neuron-specific knockout (NKO), and total knockout (TKO) models. CB1 knockout (KO) mice were also included. JZL-184, a pharmacological MAGL inhibitor, was used to evaluate its effects on seizure induction, progression, survival, and behavioral deficits. Immunohistochemical analyses of GFAP and Iba1 were performed to assess astrocyte and microglial reactivity.

**Results**: TKO and AKO mice exhibited greater resilience to KA-induced seizures, showing longer latency and lower Racine scores. Both models also demonstrated reduced KA-induced neuroinflammation, as indicated by decreased GFAP and Iba1 expression. In NKO mice, JZL-184 administration significantly prolonged seizure latency. However, in CB1 KO mice, JZL-184 did not affect seizure latency but reduced mortality.

**Conclusion**: Astrocytic MAGL inactivation provides neuroprotection by reducing seizure severity and mitigating neuroinflammation. These findings highlight the therapeutic potential of targeting astrocytic endocannabinoid signaling in epilepsy.

# TEMPORAL AND SPATIAL CHARACTERIZATION OF THE VACUOLIZATION PHENOTYPE OF MICE LACKING GDE1

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**Introduction**: Glycerophosphodiester phosphodiesterase 1 (GDE1) was identified as a key enzyme in the conversion of glycerophospho-N-acyl ethanolamines (GP-NAEs) to NAEs including the endocannabinoid anandamide. GDE1 is an intracellular membrane protein with unknown physiological functions and pathological significance in vertebrates. Here, we describe a comprehensive anatomical characterization of the progressive histopathological phenotype of the brain of mice lacking GDE1.

**Methods**: Fluorescent immunolabeling was performed on every third 50 □m-thick section prepared from 4% PFA-fixed brains. *Gde1*+/+ and *Gde1*-/- mouse brain sections were compared at *postpartum* days p30, p80 and p360. Tyrosine-hydroxylase antibody was used to delineate monoaminergic nuclei and F-actin was labelled with fluorescent phalloidin. Samples were imaged by a Nikon A1R confocal microscope.

**Results**: Low intensity vacuolization in brain parenchyma could already be detected at the age of p30. The two most prominent regions affected at this early stage were the pyramidal layer of hippocampal CA1 region and the locus coeruleus. By the age of p80 there was a substantial increase in the size and the density of vacuoles. Several monoaminergic regions were heavily affected including the locus coeruleus, the substantia nigra pars compacta, the ventral tegmental area and the ala cinerea nucleus (A2/C2) all contained high density of large vacuoles. Several non-monoaminergic regions were also strongly or moderately affected by vacuolization: the hippocampal CA1 region, central amygdala, cerebellum, and the pontine nucleus reticularis. Interestingly, the severity of the phenotype was comparable between age p80 and age p360.

**Conclusions**: Most neurodegenerative diseases that are associated with extended vacuolization cause premature death. While we observed a remarkable histopathological phenotype in the brains of *Gde1*-/- mice, it is important to note that these mice do not die prematurely. Thus, these observations raise the intriguing possibility that a cellular adaptive mechanism protects neurons from the abnormal accumulation of GDE1 substrates such as glycerophospho-N-acyl ethanolamines by sequestering these lipid species in vacuoles.

# DNA BREAKDOWN IN SPERM CELLS LEADS TO MALE INFERTILITY IN THE ABSENCE OF THE GDE1 ENZYME

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**Introduction:** Male infertility is an emerging and significant problem in Western societies. Various factors contribute to sterility, including genetic predispositions, medical conditions, injuries and environmental factors including lifestyle choices. Thus, a better understanding of the cell biology of reproductive organs and sperm cells is required to identify the specific molecular pathological mechanisms that underlie infertility. Here, we present evidence that the glycerophosphodiester phosphodiesterase enzyme GDE1 is present in sperm cells and its loss causes male infertility.

**Methods:** Molecular, anatomical and behavioral approaches were used in this study. Female mice were placed in male cages to study sexual behavior, recording mounting and intromission for 30 minutes. Tissues were cryosectioned after PFA fixation. Epididymal sperm samples were analyzed using HTF-saline smears, and morphological assessments were performed with various immunohistochemical and immunofluorescent labeling techniques.

**Results:** By using multicolor fluorescent immunolabeling with a novel antibody for GDE1 and confocal microscopy, we found that GDE1 protein levels are abundant in the head and neck of sperm cells. In the absence of GDE1 in sperm cells obtained from *Gde1-/-* knockout mice, we noticed globozoospermia, an abnormal sperm morphology condition that is linked to male infertility. Notably, two independent approaches to visualize DNA in sperm cells failed to detect genetic materials in the vast majority (>98%) of *Gde1-/-* sperm cells. Interestingly, the number and motility of sperm cells remained intact in the absence of GDE1. Additionally, we also found that GDE1 protein is present in specific epididymal cell types and the morphology of sperm cells is abnormal already in the epididymis of *Gde1-/-* knockout mice. We observed no changes in the sexual and breeding behavior of mice in the absence of GDE1.

**Conclusions:** These findings show GDE1 is essential for sperm maturation in the epididymis. Importantly, loss of GDE1 causes male infertility due to robust DNA breakdown and abnormal sperm morphology (globozoospermia) without affecting breeding behavior. Considering the conserved protein sequence of GDE1 in mammals, we propose that loss-of-function genetic variants of the *GDE1* gene may also contribute to male infertility in humans.

# NEURONAL DISTRIBUTION OF GDE1, AN N-ACYLETHANOLAMINE-SYNTHESIZING ENZYME IN THE MOUSE CNS

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**Introduction:** The glycerophosphodiester phosphodiesterase 1 (GDE1) is a membrane-associated enzyme that hydrolyzes glycerophospho-N-acylethanolamines to N-acylethanolamines in the brain and participates in bioactive N-acylethanolamine biosynthesis such as anandamide, N-palmitoylethanolamine, and N-oleoylethanolamine. While its metabolic function has been extensively studied in vitro by biochemical and cell biology approaches, its in vivo function remains poorly understood. To address this question, we performed comparative analysis of the regional and cellular distribution of GDE1, validated our data in *Gde1-/-* knockout mice and extended our observations to postmortem human brain tissue. Notably, *Gde1-/-* brain showed the morphological hallmarks of spongiform encephalopathy. Therefore, we also tested the hypothesis that the absence of GDE1 in the brain is associated with a specific reactive change of glial cells in response to the damage.

**Methods:** Mice were anesthetized then transcardially perfused with 4% paraformaldehyde and postfixed overnight at 4°C. Human brain samples were fixed with 4% paraformaldehyde immersion fixation. Immunolabeling was performed on 40-µm-thick free-floating brain slices. Multiplex immunofluorescence labeling was performed using a novel primary antibody for GDE1 and with neurochemical markers for various cell types. Sections were imaged using a NIKON A1R confocal laser scanning microscope.

**Results:** High levels of GDE1-positive immunolabeling were observed in the principal excitatory neurons throughout the forebrain in wild-type mice but not in *Gde1-/-* knockout mice. Similar high intensity GDE1-immunolabeling was found in principal cells such as pyramidal neurons and granule cells in the human hippocampal samples. Colocalization analysis demonstrated that GDE1 is also present in GABAergic interneurons in the hippocampus. In contrast, GDE1 protein levels in astrocytes, oligodendrocytes and microglial cells are under the detection threshold for immunolabeling. Brain samples from *Gde1-/-* mice exhibit extreme vacuolization in neurons that are associated with strong reactive astrogliosis.

**Conclusions:** The cell-type-specific distribution of GDE1 in mice and humans indicate that GDE1 plays a broad neuron-specific role in the metabolism of bioactive N-acylethanolamines. A striking vacuolization develops in the absence of GDE1, but we could only detect astrogliosis and no microglial response that is typically seen in such neurodegenerative conditions.

# CB1R MEDIATED REGULATION OF CHRONIC ETHANOL-INDUCED NEUROIMMUNE GENE EXPRESSION IN HIPPOCAMPUS AND PREFRONTAL CORTEX OF ADULT RAT BRAIN

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**Introduction:** Recently neuroimmune gene RAGE the (Receptor for Advanced Glycation End products) ) and Toll like receptor4 (TLR4) as well as their endogenous agonist, HMGB1 (high-mobility group box 1) has been implicated in alcohol addiction. Studies from different laboratories have found that chronic ethanol treatment persistently upregulated RAGE, TLR4, HMGB1, and that alcohol dependence is associated with increased expression of these neuroimmune genes. However, inspite of increased incidence of alcohol and cannabinoid exposure during adolescent and adulthood the effects of combined ethanol and cannabinoid treatment on these neuroimmune genes is not studies. In the current study using acute binge-like alcohol drinking model, we have investigated a) the effect of ethanol and CB1 cannabinoid receptor(CB1R) agonist (ACEA) on the expression of RAGE and HMGB1 in adult rat hippocampus and prefrontal cortex and b) the role of CB1R antagonist SR141716A (SR1) on ethanol and ACEA induced effects on the expression of RAGE and HMGB1.

**Method:** Adult male Wistar rats (body wt. 350 -380 gms) were treated with vehicle or binge level ethanol (5 g/kg, ig/day) or CB1R agonist ACEA (3 mg/kg, i.p./day ) alone or in combination with CB1R antagonist SR141716 (SR1; 3 mg/kg, ip) for 10 consecutive days. For antagonist treatment, SR1 was administered 30 min prior to ethanol or ACEA treatment and animals were sacrificed 3hr. following the last treatment. The effects of the ethanol and ACEA alone or in combination in presence of SR1 on HMGB1, RAGE, Il-1β, TNFα, Il-6, IL-8 expression as well as Toll-like receptor2/4 (TLR2 and TLR 4) activity was measured in dentate gurus and prefrontal cortex were assessed.

**Results & Conclusion:** We found that chronic treatment with ethanol or CB1R agonist ACEA alone and in combination significantly increased HMGB1, RAGE, Il-1β, TNFα, Il-6, expression in both the dentate gyrus of hippocampus and the prefrontal cortex region of the brain in a SR1-dependent manner. TL2 and TLR 4 expressions and activity were significantly augmented by ethanol and ACEA in both the brain regions and SR1 pre-treatment attenuated those response. Together, these findings clearly suggest that CB1R plays a key regulatory role on EtOH - mediated neuroimmune gene expression in the brain. Thus targeting CB1R for regulation of ethanol-mediated effects on these neuroimmune factors may have therapeutic potential for alcohol addiction.

# CHRONIC MICRODOSING OF LSD IN MICE CAUSES INCREASES IN ENDOGENOUS LIPID SIGNALING MOLECULES IN THE CORTEX WITH LITTLE EFFECT IN THE PLASMA AND HIPPOCAMPUS 24 HOURS AFTER FINAL TREATMENT

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**Introduction:** Serotonergic psychedelics like psilocybin and LSD have recently been garnering increased attention for a potential role in treating psychiatric disorders due, in part, to their potential to enhance neuronal plasticity. Clinically microdosing these psychedelics to psychiatric patients has become a topic of interest in hopes of harnessing the functional effects of the substances without producing the recreational 'high'. In that endocannabinoid and related lipids are associated have neuromodulatory effects and some studies suggest that psychedelics influence eCB levels, here we test the hypothesis that chronic mircrodosing of LSD will drive differential changes in a broad range of plasma and CNS signaling lipids.

**Methods:** 12 adult male C57/BL6J mice 3 months old were randomly divided into two experimental groups; 30 ug/kg LSD per day or vehicle daily for 7 days. All treatments were administered via intraperitoneal injections. Injections took place between 10 AM and 12 PM every day. The brain tissue and plasma were collected 24 hours after the final injection and kept in –80 until brain area dissections and lipid extractions. Tissues underwent methanolic extraction and partial purification via C18 columns and analysis through high-performance liquid chromatography-tandem quadrupole mass spectrometry (API 7500 Sciex).

**Results:** Overall, endogenous lipids in plasma remained largely unchanged 24 hours after the final injection with only 5% of lipids being modulated. Similarly, only 3% of hippocampal lipids were changed 24 hours after the last injection. By contrast, 12% of cortical lipids showed significant increases. Among those modulated in the cortex were the free fatty acids, members of the *N*-acyl GABA, and *N*-acyl serine families. Interestingly, *N*-oleoyl alanine was the only endolipid that changed in both the hippocampus and the cortex, increasing significantly in both tissues.

**Conclusions:** Overall, we show that chronic microdosing LSD in a mice model have little effect on systemic plasma lipids 24 hours after the last treatment; however, significant difference in cortex endolipids are observed.

# BEYOND THE DIAGNOSTIC BINARY: USING THE RESEARCH DOMAIN CRITERIA TO UNDERSTAND THE RELATIONSHIP BETWEEN CANNABIS CONSUMPTION AND ANXIETY

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**Introduction:** Evidence regarding whether cannabis use creates anxiolytic or anxiogenic effects is inconclusive. We hypothesize that classification of anxiety in the binary is a major contributing factor to inconclusive findings. Anxiety encompasses multiple symptoms that need to be studied from a translational framework such as the Research Domain Criteria to provide relevant scientific data.

**Methods:** This study employed a mixed-method design using quantitative measures (Socio-demographics, STAI, & CUDIT-R) to describe the population and deductive content analysis of semi-structured interviews to test the hypothesis.

**Results:** The study sample (N=30) comprised of both medical (n=19) and recreational cannabis consumers (n=11). In the overall sample, 63.33% of the participants identified as male and the sample's age range was 18–69 years, with an average of 35.33 years (SD 14.18). Average age at first recreational cannabis use was 16.27 (SD 3.59) years, and the participants reported 14.17 (SD 14.95) years of regular cannabis use. The study sample average STAI State score was 34.43 (SD 10.12) and STAI Trait score was 39.43 (SD 10.42). Thirty-nine anxiety codes as defined by the DSM-5-TR were identified. Qualitative analysis showed of the 39 codes identified 13 were negative valance system, 2 were positive valance system, 6 were cognitive system, 8 were social processes, and 10 were arousal and regulatory systems, none were sensorimotor.

**Conclusions:** The construct of anxiety is more nuanced than that captured by the diagnostic label. Future studies should replicate and expand this approach to quantify under what conditions cannabis use has anxiolytic or anxiogenic effects.

## SENSORY PROCESSING DYNAMICALLY MODULATES ENDOCANNABINOID LEVELS IN THE PRIMARY SOMATOSENSORY CORTEX

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The endocannabinoid system (ECS) plays a pivotal role as a neuromodulator, finely tuning various physiological functions. Endocannabinoids (eCBs), released from postsynaptic neurons, exert tonic and phasic influences on synaptic transmission. Despite the established impact of eCB signaling on synaptic function, direct, real-time visualization of eCB levels has remained a significant experimental hurdle. In this study, we utilized in vivo two-photon imaging with the genetically encoded eCB sensor GRAB-eCB2.1, enabling us to dynamically monitor eCB fluctuations in awake, head-fixed mice. We observed that eCB levels predominantly oscillated at low frequencies (0–0.5 Hz, 75%), with fewer oscillations detected in the mid (0.5–1.5 Hz, 12.5%) and high-frequency ranges (1.5–2.5 Hz, 12.5%). Notably, whisker stimulation, which naturally elevates neuronal activity in the somatosensory cortex, shifted eCB oscillations towards the mid and high-frequency range. Conversely, enhancing overall neuronal activity by suppressing GABAergic inhibition increased eCB fluctuations but, paradoxically, shifted high-frequency oscillations towards lower frequencies. Similarly, pharmacological inhibition of monoacylglycerol lipase (MAGL) with JZL184, to increase eCB (2-AG) levels, reduced mid- and high-frequency oscillations, driving eCB dynamics towards lower frequencies. These findings collectively demonstrate that high-frequency eCB oscillations are dependent on MAGL activity and that eCB fluctuations are dynamically coupled to neuronal activity patterns within the somatosensory cortex. Ultimately, our study offers insights into the nuanced regulation of eCB signaling and its intricate relationship with network activity.

# SEX-SPECIFIC EFFECTS OF PRENATAL THC AND EARLY LIFE STRESS ON ANXIETY AND DEPRESSIVE-LIKE BEHAVIOUR: THE IMPACT ON ELECTROPHYSIOLOGICAL PROPERTIES IN THE VENTRAL TEGMENTAL AREA AND BASOLATERAL AMYGDALA

Enzo Perez-Valenzuela, Taygun Uzuneser, Marieka DeVuono, Daniel Hardy, Walter Rushlow, Steven R. Laviolette

**Introduction:** The prevalence of cannabis use during pregnancy among young women (18-24 years) has risen by 19% between 2009-2016. Notably, maternal cannabis use has been linked to increased maternal rejection and withdrawal from offspring. Prenatal exposure to cannabis heightens susceptibility to co-occurring environmental stressors during or after prenatal life, potentially neuropsychiatric risk. We hypothesized that prenatal THC exposure (PCE) combined with early life stress (ELS) would exacerbate anxiety and depressive-like behaviours through dysregulation of neuronal activity states in the Ventral Tegmental Area (VTA) and Basolateral Amygdala (BLA).

**Methods:** In this study, pregnant Wistar rats were exposed to edible THC (5 mg/kg) or vehicle from gestational day 7 to 21. Subsequently, litters were separated into a maternal separation group (daily 3-hour maternal separation from postnatal day 2-15) or a control group without separation. Mood-related behavioural phenotypes of male/female offspring were assessed during adulthood using social interaction, elevated plus maze, light/dark box, sucrose preference, food novelty seeking and forced swimming test. Following behavioural testing, single-unit electrophysiological and local field potential (LFP) recordings were performed in the VTA and BLA.

**Results:** Our finding revealed a sex-depend effect of PCE and ELS in anxiety and depressive symptoms. While male rats showed an anxiogenic effect only with ELS, female rats increased their anxiety-like behaviour only with the combination of PCE and ELS, suggesting a sex-specific resilience against early insults. On the other hand, the depressive-like symptoms were unaltered with PCE or ELS in male offspring rats; meanwhile, in female offspring rats, ELS induced a depressive phenotype. Single-unit electrophysiology recording results only revealed decreased pyramidal neuron activity in PCE male offspring. LFP showed that ELS increased the theta power in BLA of male offspring, PCE increased alpha and beta power, and the combination reduced delta power. Interestingly, in the VTA the combination of PCE and ELS increased the oscillation power of theta, alpha, beta, and gamma. In female offspring, ELS reduced gamma power in BLA and increased delta power in the VTA. PCE reduced alpha and theta power, and the combination decreased beta power in BLA.

**Conclusions:** The present study demonstrates that PCE and ELS exert sex-dependent effects on anxiety- and depressive-like behaviours, highlighting potential sex-specific resilience mechanisms where males were more susceptible to anxiety and females to depression. Electrophysiological findings revealed heterogeneous modulation of PCE and ELS in the oscillatory power in both the BLA and VTA. These findings underscore the complex interaction between prenatal cannabis exposure and early-life adversity in shaping neuropsychiatric risk and highlight the importance of considering sex differences in the long-term consequences of perinatal cannabinoid exposure.

# DAILY CANNABIS USE LINKED TO DAILY ANXIETY REDUCTIONS IN INDIVIDUALS USING CANNABIS FOR ANXIETY COPING

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**Introduction:** Previous research has shown that delta-9-tetrahydrocannabinol (THC) may be anxiogenic, while cannabidiol (CBD) may be anxiolytic. Cannabis use is often driven by coping with anxiety, though its repeated, daily impact on anxiety remains unclear.

**Methods:** Participants (N=345; 62% female, 33±13 years of age) intending to use cannabis to reduce their anxiety were randomly assigned to a cannabis product that was CBD-dominant (CBD; n=119), equal parts THC and CBD (THC+CBD; n=112), or THC-dominant (THC; n=114) to use *ad libitum* for 30 days. Daily surveys were completed indicating if participants had used their product (yes/no) and their current anxiety (0-10 scale). To assess the impact of last 24-hour use, group, time and all possible interactions on daily anxiety, a linear mixed-effects model was run controlling for cannabis anxiety expectancies.

**Results:** The use of cannabis within the past 24 hours was marginally associated with lower daily anxiety ($F(1,9160)=3.55$, $p=.0597$). A marginal group-by-time interaction ($F(2,9160)=2.29$, $p=.101$) indicated that the rate of decrease in anxiety differed by group (THC+CBD: $b=-0.032$, 95%CI[-0.041,-0.024]; THC: $b=-0.018$, 95%CI[-0.026,-0.010]; CBD: $b=-0.033$, 95%CI[-0.041,-0.024]). Post hoc testing indicated that the rate of decrease in anxiety from day 1 to day 30 was not as large in the THC group compared to the CBD ($b= 0.014$, $p=.053$) or THC+CBD ($b= 0.015$, $p=.038$) groups.

**Conclusions:** The *ad libitum* use of cannabis products was associated with marginal reductions in daily anxiety. Consistent with previous literature, anxiety reductions were stronger in products containing moderate or high amounts of CBD when compared to THC-dominant products.
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# CANNABIDIOL IS A PATHWAY-SELECTIVE NEGATIVE ALLOSTERIC MODULATOR OF THE μ OPIOID RECEPTOR

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**Introduction** Synthetic μ-opioid receptor (MOR) agonists like fentanyl are potent analgesics but their use is limited by tolerance, dependence, and respiratory depression. Negative allosteric modulators (NAMs) of MOR offer a potential strategy to enhance opioid safety if they selectively modulate specific MOR signaling pathways. While cannabidiol (CBD) was shown to attenuate fentanyl-induced cAMP suppression, its effects on other MOR signaling pathways remain unexplored, leaving its role in opioid pharmacology insufficiently defined. Here, we use a suite of signaling assays to systematically characterize the effects of CBD on MOR signaling cascades.

**Methods** Fentanyl-induced signaling was quantified with fluorescent MAP kinase activity reporters, $BRET^2$ assays for β-arrestin1/2 and Gi protein recruitment, and in-cell western blotting for receptor internalization, using HEK-293 and 293A cells transiently or stably expressing MOR.

**Results** CBD acted as a pathway-selective MOR NAM, inhibiting fentanyl-induced β-arrestin1/2, Gi protein, and MAPK recruitment with low micromolar potency, while not affecting receptor internalization.

**Conclusion** CBD, and potentially other phytocannabinoids, may serve as scaffolds for developing more potent and pathway-selective MOR NAMs, offering a strategy for improving opioid pharmacology and safety.

**WITHDRAWN**



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# A SPOONFUL OF SUGAR HELPS THE MEDICINE GO DOWN: ORAL ADMINISTRATION OF CANNABINOIDS ALLEVIATES UC SYMPTOMS AND IMPROVES DISEASE CONDITION

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**Introduction:** Ulcerative colitis (UC) is a type of chronic inflammatory bowel disease affecting the distal colon. Due to the unknown etiology and the debilitating pathogenesis of the disease, developing treatment options remains a challenge. Patients report using cannabis to self-medicate and at the recommendation of clinicians. While the impact of cannabis on IBD remains unclear, patients continue to report improved quality of life with use, importantly cannabinoids are known to have an anti-inflammatory effect that may impact UC.

**Methods:** The well-studied DSS model will be used to evaluate the impact of hemp extracts on UC in mice. Hemp extract will be administered to mice at the time of the DSS treatment either parenterally or through diet (mixed in Nutella). Disease activity index (DAI) scoring will be performed daily, and colons will be collected following treatment and assessed using histological and immunohistochemical staining.

**Results:** Previously, we have shown that intraperitoneal administration of hemp extract reduces symptoms of colitis, normalizes the metabolome, and changes the microbial composition of the gastrointestinal tract. Our preliminary findings show that oral administration of hemp extract reduces colonic damage and inflammatory cytokine markers.

**Conclusions:** To date, only extracts containing the psychoactive cannabinoid tetrahydrocannabinol have been clinically tested for treating IBD, and these studies have had mixed results. Our animal studies indicate that hemp extracts may offer an alternative treatment option, and future studies will use the chronic model of colitis, ideally leading to a clinical trial in UC patients.

# PERINATAL CANNABINOIDS EXPOSURE DIFFERENTIALLY IMPACTS ASTROCYTE CYTOARCHITECTURE IN THE OFFSPRING DEVELOPING PREFRONTAL CORTEX

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**Introduction:** Approximately 250,000 fetuses are born each year in the US following perinatal cannabis exposure (PCE). Phytocannabinoids (pCB) consumed by mothers, such as Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), can cross the placental barrier and reach the fetal brain. Exogenous pCB exposure is likely to alter the endocannabinoid system (ECS), which is critical for brain development, increasing the risks of impaired executive function, psychosis, and depression in offspring. Preclinical studies modeling human PCE with THC have identified lasting changes in the prefrontal cortex. However, we have limited information on the lasting impacts of PCE with CBD or the combination of THC and CBD. Astrocytes play a crucial role in neurovascular coupling, with their extensive processes connecting to synapses and blood vessels. During postnatal brain development, neurons significantly influence the morphology of astrocytes. Accumulating literature highlights the importance of the ECS in modulating astrocyte functions. Here, we aim to determine the effects of PCE on astrocyte morphology.

**Methods and results:** To achieve this, we first established dosing paradigms to model human PCE involving THC, CBD, or a 1:1 THC and CBD combination throughout pregnancy using CD-1 mice, an outbred wild-type mouse strain. Next, we quantitatively examined the impacts of PCE on astrocyte distributions and morphologies using Aldh1l1-eGFP astrocyte reporter mice and by sparsely labeling astrocytes in CD-1 mice with AAV5.GfaABC1D.Lck-GFP. Specifically, we assessed the branching complexity, total volume, surface area, and neuropil infiltration volume of astrocytes in the prefrontal cortex of control versus PCE progenies. In addition to astrocyte morphology, we also analyzed the densities and distributions of neurons and astrocytes. Lastly, we quantified the overall densities of both excitatory and inhibitory synapses in the prefrontal cortex.

**Conclusion:** We will share the data generated with our newly developed preclinical PCE model, helping to address PCE's impact on astrocyte morphology and neuronal synapses in the prefrontal cortex. In summary, our dataset suggests that astrocyte in male versus female are display different sensitivity window to cannabinoids during development, in addition with CBD being the main player contributing to general cellular complexity and increased subcortical excitatory synaptic input to astrocyte territory.

# EFFECTS OF PRENATAL THC EXPOSURE ON THE MESOCORTICAL DOPAMINERGIC PATHWAYS IN MICE: AN IMMUNOLABELING ANALYSIS

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**Introduction:** Marijuana use during pregnancy is increasing in the United States and is frequently reported to be used for management of nausea associated with morning sickness, as well as mood disorders. The main psychoactive component of marijuana, delta-9-tetrahydrocannabinol (THC), is lipid-soluble and can cross the placenta during gestation. Previous research has indicated the presence of cannabinoid receptors in developing fetal tissue, and additional work is required to determine how prenatal exposure may affect the developing postnatal brain, particularly during adolescence as reward pathways undergo maturation. Previous work from our laboratory has shown reduced expression of dopaminergic markers in the striatum during early adolescence of mice prenatally exposed to THC, particularly in the hilar region of granule cells forming the Islands of Calleja in the ventral striatum. In addition to the observed effects on mesolimbic dopaminergic pathways, we aimed to further investigate the effects of prenatal THC exposure on monoaminergic systems within the mesocortical dopaminergic pathways projecting to the medial prefrontal cortex (mPFC).

**Methods:** Pregnant dams were injected subcutaneously with 3mg/kg THC or saline vehicle daily from gestational day 5 to delivery. Monoaminergic innervation of the mPFC was analysed from offspring at postnatal day 35, 65, or 130 using immunofluorescent labelling for dopamine transporter (DAT), tyrosine hydroxylase (TH), and noradrenaline transporter (NET). Immunofluorescent signal was quantified from 60x confocal microscope images by training pixel classifiers to recognize axons and boutons.

**Results:** Dopaminergic innervation in the infralimbic (IL) region of the mPFC is sparse in early adolescence compared to striatal regions. Yet we found a significant reduction after prenatal THC exposure which normalizes from early adolescence into adulthood, with the greatest reduction in DAT signal being observed at P35. However, total noradrenergic innervation of the mPFC is not significantly affected by prenatal THC exposure at P35, suggesting that dopaminergic, but not noradrenergic, inputs to the mPFC show susceptibility to *in utero* THC exposure.

**Conclusions:** Our anatomical data suggests that *in utero* exposure to THC affects mesocortical dopaminergic innervation in adolescence. Given the role of dopamine in modulating the activity of excitatory pyramidal efferents from the mPFC to regions involved in fear and reward, such as the amygdala, it is likely that the alteration in dopaminergic innervation could have behavioural effects during early adolescence. Considering the reported hyperdopaminergic responsiveness in the ventral striatum in the same preclinical model, it remains to be seen whether the observed changes represent an adaptive or a maladaptive process in the prefrontal cortex.

# RISKS OF CANNABINOID EXPOSURE ON BIRTH OUTCOMES: A SYSTEMATIC REVIEW

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**Introduction:** With the changing legal landscape, the acceptance and availability of cannabis products have increased. Cannabis products are generally considered "natural" and relatively safe by consumers. However, growing empirical evidence indicates cannabis negatively affects human health. In contrast to the well-known teratogenic effects of alcohol and nicotine products, safety of cannabis product use during pregnancy has not been established. The goal of this systematic review was to determine patterns that exist in human and rodent literature on the effects of prenatal exposure to cannabis products and delta-9-tetrahydrocannabinol (THC) on birth outcomes.

**Methods:** A systematic review of rodent and human studies was conducted using PRISMA guidelines. Rodent and human literature searches were conducted in PubMed, Scopus and CINAHL. Twenty-one rodent and 36 human studies were selected for review.

**Results:** In both human and rodent studies, prenatal exposure to cannabis was significantly associated with lower birth weights, but not with gestational age in rodents or humans. In most rodent studies, prenatal exposure to cannabis did not affect mortality or litter size. In human studies, there is a tendency for infants exposed to cannabis during pregnancy to have worse health at delivery.

**Conclusion:** Findings indicate that cannabis exposure in utero may be associated with worse birth outcomes; however, the results are mixed and vary by species and outcome. Methodological differences and scant existing research may have contributed to this inconsistency. Given the legalization of cannabis product use for recreational and medicinal purposes is growing, additional research is necessary to determine its influence on fetal and infant health outcomes.

# SEX DIFFERENCE IN ENDOGENOUS LIPIDS IN SUBMANDIBULAR AND EXTRAORBITAL LACRIMAL GLANDS

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**Introduction:** This study seeks to examine the influence of sex on Submandibular gland (SMG) and extraorbital lacrimal glands (ELG) within lipid signaling systems. Salivary and lacrimal glands play critical roles in oral and ocular health. The SMG is a major salivary gland responsible for salivation, which is essential for maintaining both gastrointestinal and oral health. Meanwhile, the ELG secretes tears that protect and maintain ocular surfaces. Despite their vital functions, little scientific attention has been paid to how lipid signaling molecules in the SMG and ELG regulate salivation and tearing. Recent work in our laboratory shows that the endocannabinoid (eCB) system, including its small-molecule lipid mediators, modulates parasympathetic inputs to both glands in a sex-dependent manner. Identifying how these pathways differ between males and females may deepen our understanding of sex-specific disease susceptibility, such as in autoimmune conditions (e.g., Sjögren's syndrome).

**Methods:** SMG and ELG from female and male mice were dissected within 2 hours of either lights on or off, lipids extracted, partially purified, and analyzed using High-Performance Liquid Chromatography coupled with tandem Mass Spectrometry (HPLC/MS/MS; API 7500).

**Results:** In the SMG, females had higher levels overall in most of the lipids screened compared to males regardless of the time of day, including 2-arachidonoyl glycerol (2AG) and N-acyl ethanolamines (NAEs). Additional lipids, including members of the N-acyl-alanine, N-acyl-leucine, N-methionine, N-acyl-phenylalanine, N-acyl-tyrosine, N-acyl-serine, and free fatty acids were higher in females. Importantly, N-glycine and N-acyl-valine species were only higher in female under day light cycle  One lipid species, N-acyl-taurines, were significantly lower in females. Similarly, levels of ELG lipids were higher overall in females regardless of the time of day. Unlike the SMG, *N*-acyl-taurine levels were also higher in females.

**Conclusion:** Comparisons between day and night conditions indicated that lipid levels were generally consistent, though there were some notable exceptions. This suggests that the observed sex differences are primarily driven by sex-specific factors rather than circadian influences, providing new insights into the molecular mechanisms underlying salivation and tearing. Our findings demonstrate distinct differences in lipid signaling profiles between males and females, with females exhibiting higher levels of several key lipids, including 2AGs, NAEs, and their congeners, with a key difference being N-acyl-taurine levels lower in the SMG but higher in the ELG. These results align with previous research indicating that the eCB system plays a crucial role in regulating these glands in a sex-specific manner. These findings highlight sex-based differences in lipid signaling, which may help explain diseases like Sjögren's syndrome. Since salivary and lacrimal glands are essential for oral and eye health, understanding their lipid pathways could lead to better treatments.

# FAAH INHIBITION OFFERS SEX-SPECIFIC PROTECTION IN A NEUROHIV TAT TRANSGENIC MOUSE MODEL OF NEUROINFLAMMATION

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**Introduction:** Human immunodeficiency virus-1 (HIV-1) is a chronic disease that invades the brain, causing neurological injury by releasing neurotoxic factors such as the HIV-1 viral protein transactivator of transcription (Tat). Tat disrupts calcium equilibrium, contributing to excitotoxicity, neuronal injury, and neuroinflammation. While combination antiretroviral therapy (cART) has improved the quality of life for individuals with HIV-1, prolonged viral presence in the brain often leads to HIV-associated neurocognitive disorders (HAND), which is associated with impaired prefrontal cortex (PFC) function. Drugs targeting the endocannabinoid (eCB) system elicit neuroprotective and anti-inflammatory effects, making them promising therapeutic strategies for neurodegenerative disorders. One such target is fatty acid amid hydrolyze (FAAH), the primary catabolic enzyme of the eCB anandamide/AEA.

**Methods:** This study tests whether the selective FAAH inhibitor, PF-3845, ameliorates neuroinflammation in an HIV-1 Tat transgenic mouse model. Tat(+) and Tat(−) (mixed sex) mice received chronic administration of PF-3845 or vehicle (i.p., 10mg/kg, 12d) and were sacrificed 2hr post final injection. A Bio-Plex assay and mass spectrometry quantified respective cytokine/chemokine levels and eCB levels in the PFC and hippocampus.

**Results:** Vehicle-treated Tat(+) mice showed a significant upregulation of proinflammatory cytokines and chemokines in the PFC, but not hippocampus, compared to Tat(−) mice. PF-3845 significantly reduced levels of IL-1β, IL-3, IL-5, IL12p40, CXCL-1, CCL4/MIP-1β in Tat(+) females, but not in males. Assessment of eCB levels (AEA, 2-AG) in the PFC and hippocampus are ongoing.

**Conclusions:** Inhibiting FAAH, the major catabolic enzyme of anandamide, promotes a sex-dependent protection in the HIV-1 Tat-induced model of neuroinflammation.

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**DUAL FAAH INHIBITING/HYDROGEN SULFIDE GENERATING COMPOUNDS**

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**Introduction:** FAAH has been linked to improved wound healing and decreased nociception. Hydrogen Sulfide ($H_2S$) is a gasotransmitter known to help regulate ROS and RNS. A compound that inhibits FAAH and produces $H_2S$ has potential to effectively treat inflammation and two such compounds are 19-113 and 19-125.

**Methods:** Compounds 19-113 and 19-125 were synthesized using known carbamoylimidazolium salts chemistry. FAAH activity and MAGL selectivity was determined using commercially available kits from Cayman Chemical. Hydrogen sulfide generation was measured in aqueous solution using DMPD in 7.2 M HCl plus $FeCl_3$ in 1.2 M HCl to generate methylene blue; methylene blue levels were measured by absorbance at 655 nM. Methylene blue was also used to measure $H_2S$ in Saccharomyces cerevisiae after lysis. Finally, $H_2S$ generation was measured in Saccharomyces cerevisiae live cells that have not been lysed using WSP-1.

**Results:** Both 19-113 and 19-125 were potent FAAH inhibitors with IC50s of 58 nM and 25 nM respectively, and they were found to be highly selective for FAAH over MAGL with both having IC50s of greater than 30 μM for MAGL. After these compounds react with FAAH, they will generate the leaving group 4-hydroxythiobenzamide (4-HBT) which generated $H_2S$ in a dose dependent manner in aqueous solutions and Saccharomyces cerevisiae cells. Hydrogen sulfide levels of over 100 nM were measured in aqueous and cell lysates for both 4-HBT and 19-113.

**Conclusions:** Two promising compounds, 19-113 and 19-125, that are potent inhibitors of FAAH and that generate $H_2S$ have been identified.

# MAPPING AND TRANSCRIPTIONAL REGULATION OF THE HUMAN CANNABINOID RECEPTOR 1 (CNR1) PROMOTER

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**Introduction:** Within the field of medical cannabis, biological compounds like cannabinoids and endocannabinoids, and their endogenous receptors have been the major focus of research. Two main cannabinoid receptors make up the ECS, cannabinoid receptor type 1 (CB1R) and cannabinoid receptor type 2 (CB2R). One aspect of the ECS that has been neglected is the transcriptional regulation of these receptors via their promoter and enhancer regions and associated transcription factors.

**Methods:** To map the promoter regions of the human *CNR1* gene (the gene encoding CB1R), a 997 base pair fragment from the sequence upstream of *CNR1* was cloned into a secreted luciferase reporter vector. The activity of this full-length construct was tested for activity in human cell lines from three tissues: nervous tissue (SHSY-5Y), kidney tissue (HEK293T), and colonic epithelium (HCT116). To begin to map key regulatory regions within this promoter, a series of deletions were constructed, and the activity of these constructs were accessed in the three cell lines.

**Results:** Through this mapping, we have identified 2 key regulatory regions within the promoter. Additionally, the data indicate that increases in cAMP levels suppresses gene expression in a cell type specific manner.

**Conclusion:** *In silico* modeling predicts a number of transcription factors that may play a role in the regulation of CNR1 at the regions identified in the promoter. This work begins to address the transcriptional regulation of *CNR1*, and future studies will focus on the implication of this regulation in disease.

# SIGNIFICANT CIRCULATING AND LOCAL SKIN CHANGES IN LEVELS OF ENDOCANNABINOIDS AND RELATED LIPIDS IN IMQ-INDUCED PSORIATIC-LIKE MOUSE MODEL

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**Introduction**: Psoriasis is a common chronic inflammatory skin disease characterized by inflammation of the epidermis. The pathogenesis is still widely unknown, and specifically the role of lipid biomarkers in both skin and plasma. Due to the involvement of endocannabinoids in inflammatory processes, this study employed a targeted lipidomic approach to examine the concentration of 101 endogenous lipids, including endocannabinoids, in the plasma and skin using a mouse model of psoriasis.

**Methods:** 20 male C57B16/J mice, all 2.5 months of age, divided into two experimental groups. A psoriatic-like skin irritation was induced in the treatment group with 62.5 mg of topical imiquimod (IMQ) cream administration for six days on the shaved backs and right ears. Lipidomics was performed on plasma and skin samples from the control and treatment groups through lipid extraction, partial purification via C18 solid phase extraction columns, and analysis through high-performance liquid chromatography-tandem quadrupole mass spectrometry. In addition, skin punches were analyzed using qPCR to determine changes in eCB-related proteins.

**Results:** qPCR data on skin punches showed that levels of DAGLa and b, NAPE-PLD, and CB1 significantly decreased, whereas levels of CB2 and MAGL significantly increased. Lipidomics analysis revealed an overall pattern of eCB congeners significantly increasing in the skin and decreasing in the plasma of the psoriasis model including the eCBs Anandamide and 2-AG. A key exception in this phenomenon was nine stearic acid amino acid conjugates (and not SEA) increased in both the plasma and skin in the psoriasis model. Bile acids were also evaluated in these tissues and demonstrated dramatic significant differences, but in a third phenotype. Cholic acid significantly decreased in both the plasma and skin, while deoxycholic acid, taurocholic acid, tauroursodeoxycholic acid, taurodeoxycholic acid, and taurochenodeoxycholic acid increased in both plasma and plasma.

**Conclusions:** This study demonstrates profound systemic and localized changes in eCBs and related endogenous signaling lipids as well as eCB-related RNA in the IMQ-induced psoriasis mouse model. These results provide novel insight into the relationship between endocannabinoids and related endogenous lipids and psoriasis that have the potential to be exploited for novel therapeutics.

# INVESTIGATING THE CELLULAR MECHANISMS OF CANNABIDIOL'S ANTI-NEUROINFLAMMATORY EFFECTS IN SONG CONTROL NUCLEI FOLLOWING DAMAGE TO VOCAL PRE-MOTOR CORTEX

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**Background:** Cannabidiol (CBD) is FDA-approved for childhood seizure syndromes associated with developmental delays, including language. We found that it preserves learned song patterns in zebra finches following partial electrolytic ablation of the vocal motor cortex (HVC). To investigate its anti-neuroinflammatory effects, we examined astrocyte activity, autophagy, and apoptosis using immunohistochemistry (IHC).

**Methods:** While optimizing antibody conditions, we observed region-specific autofluorescence (AF) changes in song-related brain areas. The emission spectra and subcellular localization suggest AF is lysosomal lipofuscin, a marker of oxidative stress. AF levels differed between VEH- and CBD-treated groups, prompting further analysis. We assessed CBD's effects on gliosis (GFAP), autophagy (LC3), apoptosis (cleaved caspase-3), and AF in song nuclei using IHC. AF changes were examined alongside cellular markers to evaluate CBD-mediated modulation of lysosomal processes and neuroinflammation.

**Results:** CBD reduced LC3 and cleaved caspase-3 in HVC, RA, and Area X, suggesting lower autophagic demand, reduced apoptosis, and neuroprotection. CBD increased lysosomal-associated AF in HVC and RA, indicating enhanced lysosomal activity, while AF decreased in Area X, reflecting distinct injury responses between motor and learning circuits. CBD bilaterally increased gliosis (GFAP) in HVC, suggesting it primed the system in a way that promotes recovery.

**Conclusions:** CBD modulates neuroinflammation, autophagy, and cell survival, promoting recovery in song-related regions. Ongoing PCR experiments are assessing IL1B expression changes to determine the minimal pre-treatment duration for anti-inflammatory effects. These findings provide mechanistic insight into CBD's therapeutic potential for conditions involving inflammation and cellular stress.

# IMPACT OF CANNABIS AND NICOTINE CO-USE STATUS ON THE SUBJECTIVE DRUG EFFECTS OF INHALED DELTA-9-TETRAHYDROCANNABINOL

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**Introduction:** People who use cannabis are more likely to use nicotine, and concurrent use ("co-use") may influence abuse related effects and craving of either substance. This study examined differences in subjective effects of inhaled delta-9-tetrahydrocannabinol (THC) in people that co-use cannabis and nicotine compared to those who only use cannabis (controls).

**Methods:** Data from two placebo-controlled, within-subjects studies with low and high doses of inhaled THC were analyzed. Subjective drug effects related to abuse liability (i.e., "High", "Good Effect") and drug craving were compared between co-use and control participants. A mixed ANOVA examined group differences for subjective effects across drug condition and time.

**Results:** Co-use (N=7, 5 female, 35.8 ±8 years) and control (N=7, 6 female, 29.1 ±6 years) participants did not differ in cannabis use frequency (co-use, 4.5 +/-2 days/wk; controls, 4.2 ±2 days/wk). Cigarette craving was significantly greater in the co-use compared to control group ($p$=.024) in all conditions. In the co-use group only, placebo administration tended to increase cannabis craving, and THC tended to reduce cannabis craving, though these results did not reach statistical significance ($p$=.083). Ratings of "High" and "Good Effect" were greater in THC conditions compared to placebo ($p$<0.05); no differences were observed between groups.

**Conclusions:** Typical abuse-related subjective effects did not differ between groups. However, people who co-use cannabis and nicotine exhibit elevated cannabis and nicotine craving and may be more sensitive to THC-induced suppression of cannabis craving than people who only use cannabis. These findings point to a potential mechanism that may contribute to continued patterns of cannabis use among people who use cannabis and nicotine.

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**WITHDRAWN**



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# CHARACTERIZING VAPORIZED AND SMOKED PUFF TOPOGRAPHY AMONG ADULT CANNABIS USERS

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**Introduction:** Rapid and accurate surveillance of cannabis use is necessary for tracking public health implications of novel cannabis products, formulations, and routes of administration (ROA). Subject-rated surveys frequently quantify inhaled cannabis use via estimated weight of raw material or number of puffs consumed. Alone, neither conveys an accurate quantification of THC exposure, but analysis of puffing behavior in controlled studies could be used to increase the validity of puff count as a viable metric.

**Methods:** This secondary analysis of three cannabis self-administration studies compared puff topography across ROA (vaporized, smoked), formulation (THC concentrate, plant material), and THC dose (placebo, active THC range 10-30mg) in healthy adults. Cannabis administration was similar across studies; participants inhaled cannabis ad libitum in a 15-minute dosing interval until all product was consumed. Analyses included differences by ROA, formulation, dose and participant characteristics on key topography outcomes.

**Results:** Participants (n=53; 49% female) averaged 31 years (SD=9) and reported cannabis use on 23 (SD=33) days in the past 90. Puff topography differed by ROA and gender (ps<.05): avg puff volume was larger for vaporized (M=279 $\pm$ 121 mL) vs. smoked cannabis (M=223 $\pm$121 mL), and for males (M=279 $\pm$ 169 mL) vs. females (M=196 $\pm$ 103 mL). Puffs were larger at the beginning vs. end of a given bout and decreased in volume by THC concentration.

**Conclusions:** Puff topography varies by ROA, product type, puff number within a bout, and participant characteristics. These data can inform algorithms estimating THC doses using self-reported puff counts in survey research.

# PREVALENCE AND PATTERNS OF CANNABIS PRODUCT ADULTERATION: AN ANALYSIS OF WEDINOS DATA

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**Introduction:** The increasing global consumption of cannabis, particularly within unregulated markets, raises concerns about potential health risks associated with contaminants and adulterants. This study investigates the presence of active compounds and adulterants in illicit cannabis products within the UK using data from the Welsh Emerging Drugs & Identification of Novel Substances (WEDINOS) service.

**Methods:** This case series analysed 1,635 samples of cannabis products submitted to WEDINOS between September 2013 and July 2024. Sample characteristics and active compound analysis were performed by the WEDINOS service. Geographical distribution was determined using postcode data mapped to Government Office Regions. Statistical analysis was entirely descriptive.

**Results:** Of the samples analysed, 695 (42.51%) did not contain any naturally occurring cannabinoids, and in 162 (9.91%) samples, no active compound was identified. Over one-third (n=624; 38.17%) of tested samples contained a psychoactive adulterant, with synthetic cannabinoid receptor agonists being the most common (n=435; 26.61%). Vape products showed the highest contamination rate, with 35.69% (n=227) containing naturally occurring cannabinoids and 57.23% (n=364) containing psychoactive adulterants. Regional variation was observed, with samples from the South East showing the highest proportion containing cannabis or cannabis-derived compounds (n=190; 67.14%).

**Conclusions:** This study highlights the challenges of adulteration of illicit cannabis products in the UK, with a concerning prevalence of synthetic cannabinoid receptor agonists, particularly in vape products. While this study may not be fully representative of the UK's illicit cannabis market, its findings underscore the significant risks consumers face due to a lack of regulation and quality control. This emphasises the need for greater consumer awareness, harm reduction strategies, and policy reform to mitigate the risks associated with adulterated cannabis within the illicit market.

# MATERNAL OBESITY ALTERS CANNABINOID RECEPTOR CONTENT IN PANCREATIC ISLETS OF RAT OFFSPRING ASSOCIATED WITH CHANGES IN GLYCEMIC HOMEOSTASIS

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**Introduction:** Maternal obesity is associated with increased risk of type 2 diabetes in the offspring. The endocannabinoid system (ECS) plays an important role in regulating pancreas development and function, with CB1 decreasing insulin secretion. Obesity is related to the ECS hyperactivation, however, the effect of maternal obesity on ECS of the pancreatic islets of rat offspring is unknown.

**Methods:** Female rats were fed a control diet (C; 9% lipids) or obesogenic diet (OD; 40% lipids) for 9 weeks before mating and during gestation and lactation. Pancreas and blood samples of male and female offspring were collected at birth and weaning for immunohistochemical analysis (CB1 and CB2) and hormonal profile. Data were analyzed by two-way ANOVA (*$p<0.05$).

**Results:** Maternal OD increased CB1 content in the islets of male neonate rats (+26.45*), mainly in the β cells. This profile was associated with decreased insulinemia (-44%*) and glycemia (-11%*). At weaning, maternal OD increased islet diameter in female offspring (+5.6%*), without CB1 changes in both sexes. There was a sex and an interaction effect on pancreatic CB2 content, with lower CB2 in the islets of OD females compared with OD males (-22%*). This profile was associated with increased glycemia (+6.2%*) and insulinemia (+40.9%*) and decreased glucagon (-37.3%*) and GLP-1 (-33%*) serum levels, with males more affected.

**Conclusion:** These results suggest that sex-specific alterations of the cannabinoid receptors in the developing pancreatic islets may be involved in the developmental origins of metabolic syndrome programmed by maternal obesity.

# 2-AG MEDIATES THE BEHAVIORAL TRANSITION FROM SEX TO FEEDING BEHAVIOR IN A WILD SNAKE

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**Introduction:** The mechanisms regulating seasonal changes in behavior are not well understood. Endocannabinoids modulate neuronal signaling in reproductive, metabolic and locomotive control centers and are widely distributed throughout the vertebrate nervous system, making them a strong candidate for facilitating seasonal transitions. Red-sided garter snakes (*Thamnophis sirtalis parietalis*) are a powerful comparative study organism where the relationship between endocannabinoid signaling and seasonal sex and feeding behavior can be isolated, readily interrogated, and interpreted within the context of the animal's ecology.

**Methods:** First, we asked if brain endocannabinoid concentrations vary with migratory status or sex. In Manitoba, Cananda, non-migratory snakes (n = 16 males, 15 females) were captured at their breeding grounds and migratory snakes (n = 16 males, 12 females) were intercepted at the beginning of their migration to feeding grounds. Brain tissue was collected for LC-MS/MS analysis of 2-arachidonoyl-*sn*-glycerol (2-AG) and anandamide. Second, we asked if manipulating endocannabinoid signaling altered seasonal reproductive or food-motivated behavior. Male snakes (n = 126) were captured during their mating season and treated with (n = 18 in each): vehicle control, cannabinoid-1 receptor inverse agonist (AM251, 0.5 or 5 mg/kg), cannabinoid receptor agonist (CP-55940, 0.1 or 1 mg/kg), or an inhibitor of the 2-AG degradative enzyme MGL (JZL184, 1.6 or 16 mg/kg). Snakes were housed in outdoor arenas and received daily intraperitoneal treatment injections for 15 days. Throughout the experiment, courtship behavior and preference for reproductive vs feeding opportunities were measured.

**Results:** We found that 2-AG levels in the reptilian homologue of the mammalian hippocampus decreased significantly as male and female snakes began their migration to feeding grounds ($P$ = 0.038). Furthermore, blocking cannabinoid-1 receptor with AM251 (0.5 mg/kg) significantly reduced male courtship behavior ($P$ = 0.032), but not locomotor activity ($P$ = 0.835), after 7 days of treatment. Remarkably, despite 8 months of aphagia during hibernation, increasing endogenous levels of 2-AG with JZL184 (1.6 mg/kg) delayed the onset of feeding behavior and extended reproductive behavior in males ($P$ = 0.046).

**Conclusions:** Our results support a role for 2-AG in the seasonal maintenance and termination of reproductive behavior. Because the endocannabinoid system is highly conserved across vertebrates, these data are broadly applicable to understanding the neuroendocrine mechanisms that mediate trade-offs between reproduction and self-maintenance.

**Acknowledgements**: We thank the Manitoba Department of Natural Resources and Indigenous Futures for permission to conduct these studies and for logistical support in the field.

# CHRONIC CO- EXPOSURE TO ETHANOL AND CANNABINOIDS CAUSE SIGNIFICANT DECREASE IN ADULT BRAIN NEUROGENESIS AND HIPPOCAMPAL RELATED LEARNING AND MEMORY FUNCTIONS IN ADULT RATS

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**Introduction:** Several studies from different labs including our lab has established that acute as well as chronic exposure to ethanol or cannabinoids, cause a decrease in adult brain neurogenesis and severe impairment in hippocampal related cognitive functions. However, very little is known about the effects of the combined exposure on adult brain neurogenesis as well on the cognitive functions. Furthermore, very few studies have attempted to establish the correlation between the reduction in adult neurogenesis and impairment of the hippocampal related cognitive functions. In this present study, we wanted to study **a)** Effects of co-exposure of ethanol and cannabinoids (exogenous & endogenous) on adult brain neurogenesis and hippocampal related learning and memory functions **b)** If there is any correlation between reduction of adult brain neurogenesis and hippocampal related functions and **c)** if CB1 receptor has any regulatory role in any of these processes.
**Method:** Adult male Wistar rats at post-natal day (PND) 80 (body wt. 350-500 gm) were treated with vehicle or ethanol (5 mg/kg/day, ig) or CB1R agonist ACEA (3 mg/kg/day, ip,) or JZL195 (3 mg/kg/day, ip; dual inhibitor of endocannabinoid degrading enzymes FAAH& MAGL), for 10 days(till PND 90) in the presence and absence of CB1R antagonist SR141716 (SR1;3 mg/kg/day, i.p).For combination treatment ethanol and cannabinoid drug doses were reduced to half and SR1 was administered 20 min prior to ethanol, ACEA or JZL195 treatment. Body weight was taken every alternate day and 24 hr. following the last treatment, animals were subjected to Y-maze or NOR (Novel Object Recognition) tasks respectively using standard protocols. After 24 hours of the last trial for both the tests, animals were sacrificed and brains collected. Immunohistochemical analysis was carried out to assess for changes in neurogenesis, ((DCX+IR), cell proliferation (Ki67) & apoptosis (cleaved caspase-3).
**Result:** We found that chronic co-exposure to ethanol,CB1R agonist ACEA or endocannabinoid inactivation enzyme inhibitor(JZL195) alone or in combination significantly impaired both spatial memory (as indicated by the arm entry and dwell time in the novel arm in a Y-maze test) and novel object recognition ability ( total exploration time).Similarly, we also found that, these treatments significantly reduced adult neurogenesis (DCX+IR) which was accompanied by increased cell death (i.e.,cleavedcaspase-3 + IR cells).Interestingly, pretreatment with CB1R antagonists SR141716 significantly ameliorated the ethanol/cannabinoid-induced impairment of spatial memory function and novel object recognition ability and reversed ethanol-induced inhibition of adult neurogenesis and cell death. Furthermore, we also found a positive correlation between the reduction in adult brain neurogenesis and the impairment of hippocampal related brain functions.
**Conclusion:** Chronic treatment with ethanol, cannabinoids alone or in combination significantly impaired hippocampal-dependent spatial recognition and novel object recognition memory performance and as well caused a significant reduction in neurogenesis accompanied with an increased cell death in adulthood in a CB1 receptor-dependent manner. These findings suggest that eCB/CB1R system can be a potential target for therapeutic intervention in these processes.

# EFFECTS OF SEX AND CB2R ON SYSTEMIC CANDIDA ALBICANS INFECTION IN MICE

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**Introduction:** Males are generally more susceptible to microbial infections than females. The impact of sex on *Candida albicans* (*C. albicans*) infections remains understudied. *C. albicans* is an opportunistic fungal pathogen that mostly affects the immune compromised. CB1R and CB2R are the most widely studied cannabinoid receptors of the endocannabinoid system. CB2R has been shown to play a role in immune cell function. Neutrophils, which are affected by CB2R activation, are known to be essential in the fight against *C. albicans* infections.

**Methods:** To investigate the role of sex and CB2R in systemic *C. albicans* infection, we infected CB2R+/+ and CB2R-/- male and female mice with $5x10^5$ *C. albicans* cells/20g mouse. Some mice were then monitored for survival and morbidity for up to 12 days. Other mice were used to assess tissue fungal load, serum cytokine levels and neutrophil levels in bone marrow (BM) and blood.

**Results**: CB2R+/+ and CB2R-/- male mice had lower survival rates and greater weight loss than CB2R+/+ and CB2R-/-females. Tissue fungal loads were comparable between the sexes and genotypes. Interleukin-6 (IL-6) levels were higher in males compared to females regardless of genotype. IL-6 plays a role on neutrophil egression from BM, so we looked at neutrophil levels in BM and blood. CB2R-/- mice had lower levels of blood neutrophils compared to CB2R+/+, suggesting that CB2R may regulate neutrophil egression from the BM in this type of infection in mice.

**Conclusions:** Sex and CB2R may modulate the immune response to systemic *C. albicans* infection in mice.

## EFFECT OF CB2R ON VULVOVAGINAL CANDIDIASIS IN MICE

Lauren Wong, Virginia Jacinto-Torres, Nancy E. Buckley

**Introduction:** Vulvovaginal candidiasis (VVC) or vaginal yeast infection is a fungal infection primarily caused by Candida albicans (C. albicans). It is estimated that about 75% of women will experience VVC at least once in their lifetime. Susceptibility to VVC greatly depends on factors such as sexual activity, immune status, and substance use. Neutrophils are the primary innate immune cells in the fight against VVC. Neutrophils are produced in the bone marrow (BM). During VVC, neutrophils exit (egress) the BM and enter the vagina to combat the infection. Neutrophils are known to express CB2R and to be affected by CB2R activation.

**Methods:** To investigate whether CB2R plays a role in neutrophil egression from the BM during VVC, CB2R+/+ and CB2R-/- female mice were injected subcutaneously with 17β-estradiol (E2) to synchronize the estrous cycle. Three days later, the mice were infected by placing 5 x 106 C. albicans yeast cells into the vagina. Mice were then monitored daily for any signs of disease (e.g. weight loss, activity levels, hair coat appearance). The vagina, vaginal lavage (VL), uterus, and BM were collected 4 days after the infection. Vagina and uterus fungal loads were determined by counting C. albicans colony forming units (CFU). VL and BM cells were analyzed via flow cytometry to assess neutrophil egression.

**Results:** Thus far, it seems VL neutrophil levels are reduced in CB2R-/- compared to CB2R+/+ mice.

**Conclusions:** CB2R may modulate neutrophil egression.

# DAILY REDUCTIONS IN PAIN ARE ASSOCIATED WITH THE USE OF EDIBLE CANNABIS PRODUCTS CONTAINING THC IN THOSE WITH CHRONIC LOW BACK PAIN

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**Introduction:** Previous research has explored the analgesic effects of delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). It is still unclear if repeated, daily use of cannabis products containing a spectrum of THC and CBD are associated with daily decreases in pain intensity over an extended period.

**Methods:** Participants (N=243: 56% female, 46±12 years of age) intending to initiate cannabis use to mitigate their chronic low-back pain self-selected an edible cannabis product to use *ad libitum* for 14 days. Ratios of THC to CBD present in the product were used to determine product chemovar group: CBD (n=97), THC+CBD (n=112), and THC (n=34). Participants completed daily surveys indicating if they had used their product in the past 24 hours (yes/no) and rated their current pain intensity (0-10 scale). A linear mixed-effects model controlling for pain expectancy assessed if use in past 24 hours, product group, and time impacted daily pain intensity.

**Results:** There were significant use-by-group ($F(2,2953)=3.39$, $p=0.03$) and group-by-time ($F(2,2953)=3.45$, $p=0.03$) interactions. Compared to non-use, the use of THC+CBD (difference=-0.42, SE=0.12, $p<.01$) and THC (difference=-0.64, SE=0.20, $p=.02$) products within the past 24 hours were associated with lower pain intensity. Decreases in pain intensity from day 1 to day 14 were only observed in the THC+CBD group ($b=-0.049$, 95%CI[-0.07, -0.03].

**Conclusions:** *Ad libitum* use of edible cannabis products within the past 24 hours containing THC were associated with decreased daily pain intensity. Further, products containing relatively equal amounts of THC and CBD were associated with decreases in pain over the course of 14 days.

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# DIFFERENTIAL EFFECTS OF $\Delta^8$-THC PAIN-DEPRESSED CLIMBING

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**Introduction:** Visceral pain has a large global impact, decreasing quality of life and productivity. Cannabis products are used to treat various types of pain, and preclinical studies support the use of various cannabinoids to decrease visceral pain. However, these reports tend to rely on pain-induced behaviors, in particular abdominal stretching behaviors induced by intraperitoneal injection of acetic or lactic acid. These results, while promising, are also subject to locomotor confound. In contrast, pain-depressed behaviors, such as spontaneous climbing, are restored by some analgesics and may offer stronger external validity than pain-induced tests alone. The purpose of this project was to determine the analgesic effects of the minor cannabinoid delta-8-tetrahydrocannabinol (i.e., $\Delta^8$-THC) using a mouse acetic acid-induced visceral pain, as assessed using both pain-induced and pain-depressed assays.

**Methods:** Adult female and male mice were administered acetic acid (0.6%) or distilled water and tested either for abdominal stretching/belly pressing (i.e., pain-induced behavior), or climbing on a vertical 0.635 $cm^2$ wire mesh surface (i.e., pain-depressed behavior). Mice were pretreated with either the minor cannabinoid $\Delta^8$-THC (5, 10, 25, or 50 mg/kg, ip) or vehicle 60 min prior to acetic acid administration. Abdominal stretching/pressing behaviors were hand scored from videos, and climbing was quantified manually and by ANY-maze tracking software.

**Results:** Acetic acid induced stretching behaviors and were reduced by $\Delta^8$-THC treatment (5, 50 mg/kg; $p<0.001$), consistent with analgesia. Acetic acid depressed wire climbing. However, $\Delta^8$-THC depressed climbing behavior regardless of acetic acid treatment ($\geq$5 mg/kg; $p<0.01$), consistent with a sedative or somnolent effect of $\Delta^8$-THC.

**Conclusion:** These data suggest that $\Delta^8$-THC may reduce visceral pain, although the somnolent effects present an experimental confound, underscoring the importance of using of both pain-induced and -depressed behaviors in preclinical research.

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# CHARACTERISATION OF COGNITION-, ANXIETY- AND DEPRESSION- RELATED BEHAVIOUR AND THE ENDOCANNABINOID SYSTEM IN A RAT INCISIONAL WOUND MODEL

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**Introduction:** Anxiety and depression are common comorbidities in individuals with chronic wounds. The endocannabinoid system (ECS) has a role in the modulation of cognition and negative affect. This study characterised cognition-, anxiety- and depression-related behaviour in the hairy skin back incision (BI) wound model in rats of both sexes and investigated alterations in the ECS.

**Methods:** Male and female Sprague-Dawley rats (150-200g on arrival, n=9/group) underwent BI or sham surgery. Anxiety-related behaviour was assessed using the light-dark box, elevated plus maze (EPM) and open field tests between post-surgery days (PSDs) 5 and 30. The sucrose preference test assessed depression-related behaviour on PSDs 17-18. Cognition-related behaviour was assessed using the novel object recognition test on PSD 31. Rats were euthanised on PSD 35. Quantification of endocannabinoids (2-AG and AEA) and *N*-acylethanolamines (PEA and OEA) was carried out by LC-MS/MS. RT-qPCR assessed endocannabinoid-related gene expression in discrete brain regions.

**Results:** There was no effect of BI on cognition-, anxiety- or depression-related behaviour. Female rats spent more time in the open arms of the EPM and had higher locomotor activity than their male counterparts. Post-mortem analysis revealed higher striatal levels of 2-AG in females compared to males, which positively correlated with locomotor activity. Female BI rats had increased levels of mRNA encoding *mgll* in the striatum vs female shams.

**Conclusions:** These results indicate sex differences in anxiety-related behaviour, locomotor activity, and the ECS. Further work is required to determine the implications of increased striatal expression of mRNA encoding *mgll* post-incision in female rats.

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# PILOT STUDY OF TOPICAL CANNABINOIDS FOR MUSCULOSKELETAL PAIN

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**Introduction:** Chronic musculoskeletal pain is a highly prevalent and disabling condition. In recent years, topical cannabinoid formulations (creams, salves, etc.) have become widely popular for treating joint pain. However, there is a paucity of research on these products. The goal of this pilot observational study was to investigate the use of topical cannabinoids for chronic musculoskeletal pain.

**Methods:** Eligible participants with chronic musculoskeletal pain who planned to use topical cannabis products completed baseline assessments (including pain, function, cognition measures) prior to initiating use of a topical cannabis product of their choice, with follow-up assessments at 24 hours, 2 weeks, and 2 months.

**Results**: 15 participants were included (3 male, 12 female; mean age 40.2 years, range 23-64). PROMIS Pain Interference scores decreased from 59.2 at baseline to 56.7 at two months, meeting the minimal clinically important difference (MCID) of >2 T-score point change. Patient Global Impression of Change scores were 3.1 at 24 hours and 4.5 at two months. There was no significant change in PROMIS Cognitive Function 6A scores. At two months, 78% of participants rated topical cannabis “as good as or better” than their usual pain medications. No adverse effects were reported.

**Conclusions**: This pilot study suggests topical cannabinoids may provide clinically meaningful pain relief for individuals with chronic musculoskeletal pain. Cognitive function remained stable, indicating that topical cannabinoid products likely act on localized tissues without significant systemic effects. Further research with a larger sample size is warranted to explore long-term efficacy and optimal formulations of topical cannabinoids.

# THE FATTY ACID BINDING PROTEIN 5 INHIBITOR ART26.12 ALLEVIATES OSTEOARTHRITIS PAIN

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**Introduction:** Knee osteoarthritis (OA) is a highly prevalent progressive degenerative joint disease that manifests as pain in the affected joint. Current treatment options rely largely on prescribing non-steroidal anti-inflammatory drugs (NSAIDs); however, NSAIDs suffer from poor long-term efficacy and their chronic use leads to adverse effects including elevated blood pressure and gastrointestinal ulceration. Fatty acid binding protein 5 (FABP5) is a cytosolic chaperone that mediates endocannabinoid transport and facilitates endocannabinoid inactivation. In parallel, FABP5 potentiates the release of pro-inflammatory cytokines and chemokines in inflamed tissue. ART26.12 is a novel selective FABP5 inhibitor that exhibits broad antinociceptive activity in preclinical models. The goal of this study was to evaluate the efficacy of ART26.12 in a model of OA pain.

**Methods:** The experiments were approved by the Stony Brook University Institutional Animal Care and Use Committee (#277150). OA was induced in female Sprague Dawley rats using the destabilization of the medial meniscus (DMM) model. X-ray imaging tracked the progression of OA throughout the twelve-week time course of the experiment. After the eighth week, rats were treated acutely with vehicle, naproxen (8 mg/kg), or ART26.12 (10, 25, or 50 mg/kg) via the oral route. Static weight bearing/incapacitance was evaluated prior to as well as 1h and 4h post-administration. The rats continued receiving each treatment BID for 4 weeks and incapacitance readings were collected weekly during this phase.

**Results:** The progression of OA was confirmed by narrowing of the knee joint space, subchondral bone damage, and the emergence of weight bearing asymmetry. Acute administration of vehicle had no effect on incapacitance while naproxen increased weight bearing on the ipsilateral (arthritic) limb 4h after dosing. Administration of 10 mg/kg ART26.12 elicited an increase in weight bearing 1h after administration while the 25 mg/kg and 50 mg/kg doses elevated weight bearing at both 1h and 4h time points. Chronic dosing with ART26.12 resulted in a dose dependent increase in weight bearing that persisted throughout the four-week time window. Similar effects were observed with naproxen. The treatments did not affect weight in the experimental subjects.

**Conclusions:** Acute and chronic oral dosing with ART26.12 (10-50 mg/kg) reduces OA pain with comparable efficacy to naproxen. ART26.12 constitutes a promising non-opioid, non-steroidal treatment for OA pain.
Acknowledgements: This work was funded by Artelo Biosciences

# ASSESSMENT OF ANTINOCICEPTION AND TOLERANCE TO INHIBITORS OF FATTY ACID AMIDE HYDROLASE AND MONOGLYCERIDE LIPASE IN A MURINE MODEL OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

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**Introduction:** Chemotherapy-induced peripheral neuropathy (CIPN) is a common and limiting side effect of cancer treatment. Despite this, there is currently a paucity of adequate treatment options for CIPN. Fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MGL) are the two enzymes primarily responsible for terminating endocannabinoid ligand signaling through ligand degradation. In this study, we evaluated the antinociceptive efficacy, risk of tolerance development, and potential for estrous cycle changes due to FAAH inhibition (via centrally mediated URB597 and peripherally restricted URB937) and MGL inhibition (via JZL184) in our CIPN model.

**Methods:** Neuropathic pain was established in 10-week old male and female C57BL/6j mice via weekly injections of cisplatin and assessed daily via the Von Frey and acetone tests. The estrous cycle was tracked through daily vaginal lavage, slide staining and subsequent microscopy. After the establishment of neuropathic pain, mice received daily injections of either vehicle, JZL184, URB597, or URB937.

**Results:** All three endocannabinoid inhibitors produced significant antinociception. In female mice only, complete tolerance developed to the antinociceptive effects of all three treatments. All compounds produced significant estrous cycle alterations that persisted until the development of tolerance, which occurred simultaneously with antinociceptive tolerance.

**Conclusion:** This study demonstrates the significant antinociceptive potential of endocannabinoid degradation enzyme inhibitors while also identifying a key limiting factor in their use – the swift development of tolerance in female mice. A need for further research to identify and mitigate the development of tolerance, and to better understand the mechanism of estrous cycle changes, has also been identified.

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# EXPANDING ACCESS TO NON-PSYCHOTROPIC CANNABINOIDS (NPCS) IN CLINICAL PRACTICE

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**Introduction:** The use of NPCs has not reached widespread acceptance in clinical practice, despite mounting pre-clinical and clinical evidence of efficacy and safety. Significant barriers to chronic NPC use include an unreliable supply chain of effective doses, and lack of physician education. We have developed a cost-effective, web-based "real world clinical trial" platform which accelerates patient data collection on NPCs.

**Methods:** We created a proprietary, secure, HIPAA compliant web-based platform (Lumina**TM**), which tracks drug to drug interactions and patient entered data through a patient portal: symptoms using either rating scales or integers, adverse events, side effects, medical history, weight, and current pharmaceuticals. Providers were able to e- prescribe any of 5 medical grade pure molecules in vegan gel caps: CBD 75 mg and 150 mg; CBN 30 mg, CBG 100 mg, and CBC 50 mg, which were then shipped directly to the patient.

**Results:** Data collection began in August of 2024. To date, 4 providers in 3 states have adopted Lumina, with 84 patients enrolled, and 212 patient visits, over 8 months. Patient acceptance and engagement with Lumina was high, with a 75% compliance rate for patient populated data. Physician engagement has overall been very positive with a growing provider base.

**Conclusions:** Lumina has proven to be a powerful and effective tool, and is able to accumulate reliable efficacy and safety data quickly. It can also identify effective combinations of molecules. This platform is invaluable for expanding access to therapy, and designing future clinical trials.

# UK MEDICAL CANNABIS REGISTRY: AN UPDATED CLINICAL ANALYSIS OF CHRONIC PAIN OUTCOMES

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**Introduction:** Chronic pain is the most common reason why cannabis-based medicinal products (CBMPs) are prescribed in the UK. There is also rising evidence on the effects of phytocannabinoids and terpenes in altering nociceptive signalling and the emotional and cognitive manifestations of pain. Despite this, there is a paucity of high-quality randomised controlled trial evidence of efficacy. The case series aims to provide an updated analysis of changes in patient-reported outcome measures (PROMs) and reported adverse events in patients prescribed CBMPs for chronic pain.

**Methods:** Cases enrolled in the UK Medical Cannabis Registry ≥ 2 years prior to 6th January 2025 and had completed a minimum of 1 baseline PROM were extracted. The primary outcomes for this study were changes in the Brief Pain Inventory-Short Form (BPI-SF), Short Form – McGill Pain Questionnaire 2 (SF-MPQ-2) and Pain visual analogue scale (Pain VAS). Secondary outcomes were changes in the EQ-5D-5L Index value and prescribed opioids, in addition to reported adverse event prevalence. $P<0.050$ was considered statistically significant.

**Results:** After application of inclusion criteria, 3,744 patients with chronic pain were included in the analysis. The mean age of participants was 46.36 ± 13.92 years. The majority of patients were already current consumers of cannabis (n=2,152; 57.48%). The mean BPI Severity score reduced from 5.97 ± 1.71 at baseline to 1 (5.05 ± 1.82; $p<0.001$), 3 (4.85 ± 1.79; $p<0.001$), 6 (4.74 ± 1.80; $p<0.001$), 12 (4.75 ± 1.74; $p<0.001$), 18 (4.74 ± 1.76; $p<0.001$), and 24 months (4.81 ± 1.71; $p<0.001$). There was also a reduction in BPI Interference subscale, SF-MPQ-2, and Pain VAS at each follow up compared to baseline ($p<0.001$). Prescribed opioids, measure in oral morphine equivalents reduced from 33.09 ± 68.32 mg/day at baseline to 26.77 ± 50.55 mg/day at 24 months. There were 538 (14.37%) participants who reported a minimum of one adverse event.

**Conclusions:** This study demonstrated an associated improvement in pain-specific outcomes from 1 month after initiating CBMPs up to 2 years of enrolment in the UK Medial Cannabis Registry. There was also an associated reduction in opioid prescribing over the same period. Fewer than 1 in 5 individuals reported an adverse event during follow up. Whilst randomised controlled trials are necessary to understand the true efficacy and safety of CBMPs, observational evidence such as this may help in guiding current clinical practice and future research.

# UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS FOR COMPLEX REGIONAL PAIN SYNDROME

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**Introduction:** Complex regional pain syndrome is characterised by severe, persistent pain and significant impacts on health-related quality of life. Cannabis-based medicinal products (CBMPs) represent a potential novel therapeutic option, with increasing research on their effect on neuropathic pain. However, no clinical trials to date have evaluated the effects of CBMPs in individuals with complex regional pain syndrome. This study aims to assess changes in patient-reported outcome measures (PROMs) and the prevalence of adverse events associated with CBMPs prescribed for complex regional pain syndrome.

**Methods:** This case series assessed changes in PROMs over 6 months in complex regional pain syndrome patients enrolled in the UK Medical Cannabis Registry. Adverse events were recorded and graded using the Common Terminology Criteria for Adverse Events version 4.0. Statistical significance was defined as $p<0.050$.

**Results:** Sixty-four patients were included in the analysis. The mean age of patients was 41.91 ± 11.25 years. Forty-two (65.63%) patients were current or ex-users of cannabis. Pairwise comparisons demonstrated significant improvements ($p<0.050$) in Brief Pain Inventory (Pain Severity and Interference), Short-Form McGill Pain Questionnaire-2 (Neuropathic Pain, Affective Descriptors, Overall Score), Pain Visual Analogue Scale, European Quality of Life-5 Dimension-5 Level (Index Value, Pain and Discomfort, Anxiety and Depression), Generalised Anxiety Disorder-7 and Single-Item Sleep Quality Scale at 1-, 3-, and 6-month follow-up compared to baseline. Clinically important differences in Pain Visual Analogue Scale were associated with previous cannabis use ($p=0.007$). Five patients (7.81%) reported a total of 50 adverse events (78.13%). Of these, 15 (30.00%) were classified as mild, 15 (30.00%) as moderate, 20 (40.00%) as severe.

**Conclusions:** This study represents the first analysis of outcomes in individuals with complex regional pain syndrome prescribed CBMPs. The findings suggest that initiation of CBMPs is associated with improvements across multiple validated PROMs. While these results are consistent with existing literature, they must be interpreted cautiously given the study's limitations. CBMPs were associated with improvements in both pain severity and interference. Participants also reported enhancements in key metrics of health-related quality of life. These findings support the need for high-quality randomised controlled trials to determine the efficacy of CBMPs in improving complex regional pain syndrome symptoms.

# UK MEDICAL CANNABIS REGISTRY:AN UPDATED CLINICAL ANALYSIS OF ENDOMETRIOSIS-ASSOCIATED CHRONIC PAIN

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**Introduction:** Endometriosis is a chronic inflammatory condition affecting up to 10% of biological females of reproductive age. Common symptoms include cyclical and continuous chronic pelvic pain. Cannabis-based medicinal products (CBMPs) have been increasingly evaluated for their efficacy in chronic pain, however, there is limited high-quality randomised controlled trial data on chronic pain secondary to endometriosis. This study aims to assess the changes in pain-specific and general patient-reported outcome measures in patients treated with CBMPs for endometriosis-associated chronic pain.

**Methods:** This cases series analysed data from the UK Medical Cannabis Registry. Inclusion criteria were patients treated for a primary indication of endometriosis, enrolled 2 years or more prior to data extraction, and completed one or more baseline patient-reported outcome measures (PROMs). Primary outcome measures were changes in the Brief Pain Inventory- Short Form (BPI), Short Form McGill Pain Questionnaire 2 (SF-MPQ-2) and pain visual analogue scale (Pain VAS) from baseline up to 2 years. Statistical significance was defined as $p<0.050$.

**Results:** One hundred and one patients met inclusion criteria with a mean age of 35.53 ± 7.59 years. Thirty-one (30.69%) of participants were unemployed. Forty-two (41.48%) patients were already current users of cannabis at baseline. At 2 years the median CBD and THC doses were 26.03 mg/day [IQR:19.00-60.50 mg/day] and 123.75 mg/day [IQR:99.00-236.00 mg/day] respectively. There was a reduction in BPI Pain Severity, BPI Pain Interference, SF- MPQ-2 total score, and Pain VAS at 2 years compared to baseline ($p<0.010$). There was a reduction in co-prescribed opioids from baseline (19.86 ± 17.21 oral morphine equivalents/day) to 2 years (14.82 ± 15.92 oral morphine equivalents/day; $p=0.015$). Eighteen (17.82%) participants reported one or more adverse events. The most common were fatigue (n=16; 15.84%), lethargy (n=15; 14.85%), and headache (n=13; 12.87%).

**Conclusions:** There was an improvement in pain severity and interreference of pain on activities of daily living among individuals with endometriosis prescribed CBMPs. There was also a reduction in prescribed opioids in this cohort. Most participants tolerated treatment well, with no life-threatening or disabling adverse events.

# UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS FOR EPILEPSY

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**Introduction:** Approximately one-third of epilepsy patients fail to achieve seizure remission despite optimal therapeutic management. Cannabis-based medicinal products (CBMPs) have shown promise as a potential therapy. However, the limited high-quality evidence regarding their efficacy and safety necessitates further investigation. This study aimed to examine changes in epilepsy-specific and general health-related quality of life (HRQoL) using validated patient-reported outcome measures (PROMs) in individuals with treatment-resistant epilepsy.

**Methods:** This case series analysed patients with epilepsy from the UK Medical Cannabis Registry. Changes in Quality of Life in Epilepsy-31 (QOLIE-31), Single-item Sleep Quality Score (SQS), EQ-5D-5L, Generalised Anxiety Disorder-7 (GAD-7) and Patient Global Impression of Change (PGIC) were evaluated between baseline and follow-up at 1, 3, and 6 months. Adverse events (AEs) were documented and classified by severity. Statistical significance was set at $p<0.050$.

**Results:** The analysis included 134 patients. The mean age of patients was 36.87 (± 12.00) years. Thirty (22.39%) participants were cannabis naïve. Median cannabis grams for current and ex users (n=104; 77.61%) were 5.50 (2.00-20.00) grams. There was an improvement in QOLIE-31 total mean score from baseline to 1, 3, and 6 months ($p < 0.001$) and across all 7 domains of the QOILE-31. An improvement from baseline in overall HRQoL was noted following 1, 3, and 6 months (all $p<0.001$) as shown by the change in EQ-5D-5L index. Forty patients (29.85%) achieved a minimal clinically important difference in QOLIE-31 at 6 months. Multivariate analysis showed that those aged 55+ years old had increased odds for a meaningful positive change in QOLIE score (OR = 8.59, 95% CI = 1.17-62.87; $p = 0.034$). Eighteen AEs (13.43%) were reported by 5 patients (3.73%), predominantly of mild to moderate severity.

**Conclusions:** The proportion of patients achieving clinically significant improvement aligns with existing literature on CBMPs for epilepsy. The incidence of AEs was lower than in comparable studies, potentially attributable to the high proportion (67.16%) of non-cannabis- naïve individuals in the cohort. CBMP initiation was associated with improvements across all PROMs, and the treatments were well-tolerated. Nevertheless, randomised controlled trials are essential to establish causality.

# UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS FOR AUTISM SPECTRUM DISORDER

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**Introduction:** Autism spectrum disorder is a neurodevelopmental disorder associated with significant behavioural and psychological challenges, often persisting into adulthood. Current interventions for challenging behaviours and comorbid psychiatric disorders have limited efficacy and tolerability. This study aims to evaluate changes in health-related quality of life (HRQoL), anxiety, and sleep quality in individuals with autism spectrum disorder treated with cannabis-based medicinal products (CBMPs).

**Methods:** This observational case series analysed data from the UK Medical Cannabis Registry (UKMCR) on patients with autism spectrum disorder treated with CBMPs. Demographic and clinical data were collected at baseline, with patient-reported outcome measures assessed at 1, 3, 6, 12, and 18 months. Primary outcomes included changes in anxiety (GAD-7), sleep quality (SQS), and HRQoL (EQ-5D-5L). Secondary outcomes included the incidence and severity of adverse events. Statistical significance was defined as $p<0.050$.

**Results:** One hundred and thirty individuals met the inclusion criteria, with a mean age of 34.07±11.49 years. Significant improvements were observed in GAD-7 ($p<0.001$) and SQS ($p<0.001$) scores from baseline to 18 months. EQ-5D-5L index values improved from baseline (0.43±0.30) to 18 months (0.51±0.32, $p<0.001$), and PGIC scores increased from 1 month (5.43±1.49) to 18 months (5.65±1.32, $p=0.013$). Thirty-four participants (26.15%) reported a total of 232 (178.46%) adverse events, with most being mild (n=88; 67.69%) or moderate (n=99; 76.15%). Forty-two adverse events (32.31%) were classified as severe and three (2.31%) as life-threatening/disabling.

**Conclusions:** Treatment with CBMPs was associated with improvements in HRQoL, anxiety, and sleep outcomes in patients with autism spectrum disorder over an 18-month period. There was a favourable safety profile, with 73.85% of patients not reporting any adverse events. However, these findings must be interpreted cautiously within the context of the study's limitations, including the absence of a control group. Further high-quality randomised controlled trials are needed to confirm the long-term efficacy and safety of CBMPs in autism spectrum disorder.

# UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS FOR MIGRAINE

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**Introduction:** Migraine is a common primary headache disorder and a leading cause of disability among working age adults. Cannabis-based medicinal products (CBMPs) have gained rising attention as novel therapies for acute treatment of migraine attacks. However, there is limited evidence of their effectiveness as prophylaxis for episodic or chronic migraine. This case series aims to assess the change at 2 years in headache-specific and general patient- reported outcome measures (PROMs) in patients with migraine enrolled in the UK Medical Cannabis Registry.

**Methods:** Patients with episodic and chronic migraine that has not responded to licensed therapies were included in this analysis from the UK Medical Cannabis Registry. Primary outcomes were changes in the Headache Impact Test-6 (HIT-6), Migraine Disability Assessment (MIDAS), and EQ-5D-5L Index Value at 1, 3, 6, 12, and 24 months from baseline. Adverse events were graded using the Common Terminology Criteria for Adverse Events 4.0. P-values $<0.050$ were considered statistically significant.

**Results:** There were 203 participants who met the inclusion criteria. There were 114 (56.15%) males in the cohort and the mean age was 39.49 ± 11.31 years. The median lifetime cannabis exposure between current and former users at baseline was 5.13 [2.00-18.00] gram years. At 24 months, the most common product combinations were dried flower and oil (n=90; 44.33%), dried flower only (n=64; 31.53%), and oil only (n=32; 15.76%). HIT-6 improved from baseline to all follow up periods ($p<0.001$). MIDAS and EQ-5D-5L Index Value remained improved to 18 months ($p<0.010$), however there was no difference from baseline at 24 months ($p>0.050$). Thirty-one (15.27%) participants reported an adverse event. The most common adverse event was headache (n=22; 10.84%).

**Conclusions:** Participants in this analysis demonstrated an associated improvement in headache-specific and general outcome measures. However, the effects appeared to be diminished at 24 months. Moreover, the most common adverse event was headache. Further assessment is necessary to identify whether pharmacological tolerance to the effects of CBMPs occurs at 24 months or whether this outcome is due to other factors and whether the adverse events were secondary to treatment-related effects or were headaches related to the participant's underlying migraine diagnoses.

# UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS FOR POST-TRAUMATIC STRESS DISORDER

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**Introduction:** Post-traumatic stress disorder (PTSD) is a mental health condition characterised by persistent symptoms including flashbacks, nightmares, avoidance of reminders, and hyperarousal following exposure to trauma. For recommended talking and pharmacological therapies, the non-response rate approaches 50% and 40%, respectively. Cannabis-based medicinal products (CBMPs) have emerged as novel treatments for PTSD. This study's primary aim was to assess the changes in PTSD-specific and general patient-reported outcomes measures (PROMs) over 24 months for PTSD patients treated with CBMPs.

**Methods:** This observational case series included PTSD patients enrolled on the UK Medical Cannabis Registry for 24 months or longer. Changes in PTSD-specific symptoms (IES-R), anxiety (GAD-7), sleep quality (SQS), and general HRQoL (EQ-5D-5L) were assessed at 1, 3, 6, 12, 18, and 24 months.

**Results:** There were 446 patients who met the inclusion criteria for the present analysis. Three-quarters (n=335; 75.11%) of individuals were current consumers of cannabis at baseline. The median lifetime cannabis exposure of those individuals was 10.00 [3.00-24.00] gram years. At 24 months the median CBD dose was 22.00 mg/day [11.00-66.00 mg/day]. The THC dose was 214.50 [115.50-279.56 mg/day]. There was an improvement in IES-R, SQS, GAD-7, and EQ- 5D-5L Index Value at 1, 3, 6, 12, 18, and 24 months compared to baseline ($p<0.001$). Seventy- five (16.82%) participants reported adverse events. The most common were fatigue (n=45; 10.09%), fatigue (n=42; 9.42%), and headache (n=38; 8.52%).

**Conclusions:** Treatment with CBMPs was associated with improvements in PTSD symptoms, anxiety, sleep, and health-related quality of life at up to 24 months. Although the study's observational nature limits causal conclusions, these findings support further assessment of CBMPs. There were a high proportion of current cannabis consumers at baseline who transitioned to legal CBMPs. Future studies should look to assess the outcomes in cannabis naïve individuals.

# UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS FOR DEPRESSION

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**Introduction:** Depression is a common mental health condition characterised by persistent low mood, anhedonia, changes in sleep and appetite, and impaired cognitive function. Cannabis- based medicinal products (CBMPs) may be considered in patients in the UK who have not responded to licensed therapies. However, in comparison to other common conditions where CBMPs are prescribed there is a paucity of high-quality evidence for their efficacy and safety. The primary aim of this study is to assess changes in mood up to 2 years in patients with depression.

**Methods:** This case series used data from individuals enrolled in the UK Medical Cannabis Registry, where depression was the primary indication for therapy. Primary outcomes were mean change in depression severity and the proportion of responders (≥ 50% reduction in severity) measured using the Patient Health Questionnaire-9 (PHQ-9) at 24 months. P- values<0.050 were statistically significant.

**Results:** In total, 698 patients with depression were included in the analysis, of which 498 (71.35%) were male. The majority of participants were either current users (n=517; 74.07%) or previous users (n=125; 17.91%) of cannabis at baseline. The most common combinations of product at 24 months were dried flower only (n=363; 52.01%), dried flower and medium-chain triglyceride oils (n=227; 32.52%) and medium-chain triglyceride oils only (n=58; 8.31%). The mean PHQ-9 score at baseline was 17.06 ± 6.22. This was lower at 1 (10.41± 6.57; p<0.001), 3 (9.48 ± 6.06; p<0.001), 6 (9.26 ± 6.09; p<0.001), 12 (8.93 ± 6.06; p<0.001), 18 (8.40 ± 5.91; p<0.001), and 24 months (8.39 ± 5.89; p<0.001). There were 397 (56.88%) participants who reported a reduction in PHQ-9 value ≥50% at 24 months. Sixty-three (9.03%) patients reported adverse events.

**Conclusions:** Treatment with CBMPs was associated with a reduction in depression severity in this cohort of patients. More than 1 in 2 individuals at 2 years reported a clinically significant response to treatment. Considering this cohort of patients had treatment-resistant depression, which was unresponsive to 2 or more licensed therapies, this is an interesting finding. However, the limitations of this analysis must be considered, including almost three-quarters of participants already using cannabis at baseline. This selection bias may have contributed to the observed outcomes.

# UK MEDICAL CANNABIS REGISTRY: AN UPDATED CLINICAL OUTCOMES ANALYSIS ACROSS ALL CONDITIONS

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**Introduction:** Cannabis-based medicinal products (CBMPs) were rescheduled in the UK on 1st November 2018, allowing them to be prescribed for patients with unmet clinical need. However, despite this legal change there is a paucity of high-quality clinical evidence to guide clinical practice. Consequently, there are barriers to access and limited resources to guide present practice. This case series aims to assess the changes in patient-reported outcome measures and prevalence of adverse events in a population prescribed CBMPs in the UK.

**Methods:** Data was extracted from the UK Medical Cannabis Registry on 6th January 2025. Participants were restricted to those who had enrolled a minimum of 2 years prior to analysis and completed a minimum of one baseline patient-reported outcome measure. The primary outcomes were changes in EQ-5D-5L, generalised anxiety disorder-7 (GAD-7) and single- item sleep quality scale (SQS) from baseline to 1, 3, 6,12, 18, and 24 months. Adverse events were recorded in accordance with the common terminology criteria for adverse events version 4.0.

**Results:** In total, 8,945 participants were included in the study of which 5,358 (59.90%) were males. The mean age was 42.26 ± 13.35 years. Nearly two-thirds (n=5550; 62.05%) were already current consumers of cannabis at baseline. The most common diagnoses were chronic non-cancer pain (n=2276; 25.44%), anxiety (n=1355, 15.15%) and fibromyalgia (n=789; 8.82%). There were improvements in ED-5D-5L, GAD-7, and SQS at 1 month following initiation of medical cannabis and these were sustained at 2 years ($p<0.001$). On multivariable logistic regression, female sex was associated with an increased likelihood of reporting an improvement in EQ-5D-5L (odds ratio: 1.28; 95% confidence interval:1.15-1.43; $p<0.001$). Mean oral morphine equivalent at baseline was 29.01 ± 59.54 mg/day. At 2 years, 455 (22.88%) of individuals who were prescribed opioids reports a clinically significant reduction in dose. There were 1,221 (13.65%) participants who reported an adverse event.

**Conclusions:** This latest analysis provides an updated analysis of outcomes for medical cannabis patients in the UK up to 2 years. Whilst these results must be interpreted in the context of the study's notable limitations, these show the medical cannabis was largely well- tolerated. Moreover, there was an improvement in health-related quality of life, anxiety and sleep. These findings can help inform future focused assessment through randomised controlled trials.

# MEDICINAL CANNABIS ON PRESCRIPTION IN THE NETHERLANDS: PATIENT CHARACTERISTICS AND PATTERNS OF USE

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**Introduction:** General practitioners and specialists in the Netherlands can prescribe medicinal cannabis, which pharmacies dispense upon prescription. Annually, approximately 6.000 patients receive medicinal cannabis. Despite the frequent prescription of medicinal cannabis, little is known about patient characteristics or patterns of medicinal cannabis use in the Netherlands.

**Methods:** Patient records from two Dutch pharmacies specialized in the processing and dispense of medicinal cannabis were analysed. Data were collected from patients who received their first prescription of medicinal cannabis in 2024, including demographic, clinical information and characteristics of medicinal cannabis use (e.g. medical indication, cannabis variety, administration method, dosage, frequency, duration, and number of dispenses).

**Results:** Preliminary data indicate that in 2024, 1.411 patients were prescribed medicinal cannabis through Dutch medicinal cannabis pharmacies. Patients had a mean age of 61±17 years, with 41.5% being male and 58.5% female. The primary indications for prescribing medicinal cannabis were chronic pain (64%), insomnia (21%), terminal-stage cancer (12%), and muscle spasms (3%). The vast majority of patients (97%) received medicinal cannabis in oil form. Data analysis is ongoing to further characterize medicinal cannabis patients and usage patterns.

**Conclusions:** In the Netherlands, medicinal cannabis is regularly prescribed for treatment of a broad range of medical indications (e.g. chronic pain, insomnia and cancer). This includes mainly older patients, and cannabis oil is the most frequently dispensed cannabis formulation. Annual monitoring of characteristics of medicinal cannabis patients and patterns of use is invaluable in informing both scientific research and policy making in the field of medicinal cannabis.

# DEMOGRAPHIC AND CLINICAL FACTORS ASSOCIATED WITH RECREATIONAL CANNABIS USE IN FEMALE PSYCHIATRIC OUTPATIENTS WITH SUICIDAL IDEATION

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**Background:** Given the widespread legalization of recreational cannabis across the United States, it is crucial to examine sociodemographic, affective, and behavioral factors associated with cannabis use to identify at-risk populations. Female individuals exhibit a faster transition from first use to problem use than their male counterparts. Further, suicide is a leading cause of death in reproductive-aged female individuals, with rates in this group showing the steepest increases in recent years. Considering these co-occuring trends, there exists an urgent public health need to identify characteristics of female recreational cannabis users with suicidality to inform prevention and intervention efforts.

**Methods:** Demographic, clinical diagnostic, and daily psychiatric symptom and substance use data from baseline and washout phases across three randomized controlled trials were analyzed. Data was primarily collected after the legalization of recreational cannabis in Illinois. Female individuals were recruited for past-month suicidal ideation (SI) or as healthy controls with no history of SI.

**Results:** Of 257 participants, 140 (54%) reported cannabis use. Participants with SI only and SI with a history of suicide attempt used cannabis more frequently than healthy controls (OR=5.23, $p=0.005$; OR=3.20, $p<0.001$, respectively). Sexual minority status also positively predicted cannabis use (OR=2.69, $p=0.001$).

**Conclusion:** In a sample of reproductive-aged female participants, those with past-month suicidal thoughts were more likely to use cannabis than controls, though cannabis use was not significantly associated with suicide attempt. As cannabis use continues to rise, clinicians and researchers must be informed of risk factors for use, and how use impacts psychiatric outcomes and suicidality.

# 19 PATIENTS REPORT SEIZURE FREEDOM WITH MEDICAL CANNABIS TREATMENT FOR DRUG RESISTANT EPILEPSY: A CASE SERIES

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**Introduction**: Seizure freedom is the primary goal of epilepsy treatment. More treatments that produce SF in drug-resistant epilepsy (DRE) are needed. Cannabis-based products for medicinal use (CBPMs) containing cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) have been shown to induce SF in DRE. However, there remains a paucity of published real-world evidence in both pediatrics and adults on SF resulting from CBPM therapy.

**Methods**: This is a retrospective case series at an outpatient neurology clinic in Toronto, Canada, on patients with DRE who experienced significant SF during CBPM treatment. All patients were treated via the clinic's stepwise treatment protocol. The study describes clinical features of patients and their CBPM-related SF.

**Results**: We had 19 DRE cases that experienced seizure freedom—15 pediatric, 4 adults (10.9% of the total DRE cohort). The median cumulative SF duration was 245 days, split between continuous SF periods lasting at least 90 days. Five patients had continuous SF periods lasting ≥1 year. Most patients used CBD+THC regimens. Three patients weaned all concomitant ASMs. Adverse events (AEs) were reported by half of the patients.

**Conclusions**: The results of the study support prioritizing CBPMs in cases of DRE. It also supports research into identifying clinical and biological biomarkers for DRE cases that may achieve seizure freedom under CBPM treatment. Lastly, the study supports improving the accessibility of CBPMs, examining seizure freedom in future CBPM epilepsy trials, and assessing the role of THC in reducing seizures.

# CANCER PATIENT-REPORTED RISKS AND BENEFITS OF CANNABIS: DIFFERENCES BASED ON CONSUMPTION BEFORE AND AFTER DIAGNOSIS

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**Purpose:** Evidence suggests that cannabis may alleviate cancer-related symptoms, yet its long-term effects remain unclear. This study examines perceptions of the risks and benefits of cannabis consumption (CB+).

**Methods:** Data are from the Cannabis and Cancer Study. Socio-demographics, cancer stage, and CB+ were collected via REDCap. The NCI-Cannabis Core Questionnaire was used to assess perceptions of cannabis risks and benefits. Chi-squared/Fisher's exact tests examined differences in perceived risks and benefits; statistical significance was $p < 0.05$.

**Results:** In the sample (N=437), 45.8% reported CB+ in the past 30-days. The most frequently reported perceived risk was impaired memory (31.5%); the most frequently reported perceived benefit (62.0%) was the relief of stress, anxiety, or depression. The perceived risk of increased use of illicit substances other than cannabis was significantly higher in those who started using cannabis after diagnosis (15.4%) versus before diagnosis (5.9%, $p = 0.03$). Among perceived benefits, improved nausea (58.1% vs 32.3%, $p = 0.03$), decreased use of illicit substances other than cannabis (23.0% vs 6.2%, $p < 0.01$), enjoyment or recreation (42.2% vs 18.5%, $p < 0.001$). was significantly different between participants who initiated cannabis use before their cancer diagnosis and those who initiated use after their cancer diagnosis.

**Conclusion:** Respondents with cancer perceive cannabis as beneficial for symptom relief; concerns about memory impairment and substance use persist. Understanding these perceptions can help inform clinical guidance and tailored patient education on cannabis use in cancer care.

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# RECRUITMENT CHALLENGES IN CLINICAL STUDIES OF CANNABIS

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**Introduction:**
Studies of cannabis as alternative treatments for health conditions has increased in recent years, particularly after legalization of medical/recreational use in many states. While recruitment into clinical research can be challenging across disciplines, enrollment into cannabis studies can be particularly difficult and is associated with unique challenges.

**Methods:**
Screen fail data from multiple clinical studies on the health effects of cannabinoids were reviewed. A matrix of challenges to enrollment was constructed and potential ways to overcome the challenges were discussed and are presented here.

**Results:**
Primary challenges identified were: current regular use of cannabis, unwillingness to reduce/discontinue cannabis use, concerns with federal legal status of cannabis, consequences of testing positive for THC, and questions about the source/safety of investigational cannabis products. Potential strategies include stakeholder engagement in the community to better educate on current safeguards of clinical research, such as FDA oversight of clinical trials and the Certificate of Confidentiality that is available from NIH.

**Conclusions:**
Familiarity with cannabis in the community has proven to both help and hinder recruitment into clinical studies. Federal restrictions and historical campaigns condemning cannabis have discouraged some potential participants against enrolling in research studies. In contrast, many potential participants are already regularly using cannabis and are unwilling to reduce/discontinue personal use as required for most clinical studies. Despite state legality, many employer policies prohibit THC use; thus, participants could risk employment by enrolling in cannabis clinical trials. Community educational engagement activities may help to offset these challenges.

# CANNABIS USE IN ENDOMETRIOSIS: A SCOPING SYSTEMATIC REVIEW

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**Introduction:** Endometriosis affects 6-10% of reproductive-age females, causing chronic pelvic pain and significantly impacting quality of life. Current treatment options remain limited, prompting increasing interest in cannabis as a potential therapeutic intervention. This scoping systematic review aimed to comprehensively evaluate the clinical literature on cannabis use among individuals with endometriosis, examining efficacy, safety, patient experiences, and potential therapeutic implications.

**Methods:** A systematic search was conducted across MEDLINE, PubMed, and EMBASE databases on 16 January 2024. The review included in-human and clinical studies evaluating cannabis effects in non-pregnant adults with endometriosis. The quality of each study which met inclusion criteria was assessed using the Newcastle-Ottawa Scale.

**Results:** Nine completed studies encompassing 1,787 participants and four ongoing clinical trials were analysed. All nine (69.0%) completed studies were cross-sectional. Of nine completed studies, seven (77.8%) utilised cross-sectional online surveys, one (11.1%) used a combination of surveys and focus groups, and one (11.1%) analysed data from a cannabis usage tracking app. The median Newcastle-Ottawa score was 4 (range: 3-6). Five (38.5%) studies explored patients' reasons for using cannabis. The most common indications were pain (57.3% to 95.5%), sleep improvement (95.5% of users in one study), and gastrointestinal distress (15.2% to 78.5%). Four (30.8%) studies compared the effect of cannabis use on conventional prescribed medications. However, the different methods used precluded pooling of results. Of the four (30.8%) studies that reported on the impact of cannabis use on use of conventional medications, all found that most patients were able to reduce their conventional medications. Four (30.8%) studies reported on the adverse effects of cannabis use, with ranges of 10.2% to 52.0% of respondents reporting at least one side effect.

**Conclusions:** The review highlighted substantial methodological constraints inherent in the existing research. These limitations include the exclusive reliance on observational studies, potential recall and selection biases, significant heterogeneity in cannabis formulations and dosages, and a fundamental lack of rigorous randomised controlled trials. While observational evidence suggests there may be potential benefits of cannabis in managing endometriosis-associated symptoms, there remains a critical and urgent need for high-quality prospective longitudinal research and randomised controlled trials to definitively establish the safety and efficacy of cannabis as a therapeutic intervention for endometriosis.

**WITHDRAWN**



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# SUBJECTIVE RESPONSES TO CANNABIS RELATED TO RECENT MOOD

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**Introduction:** Cannabis effects vary among individuals. Studies outside of the cannabis field suggest that recent mood might influence subjective drug effects and contribute to continued drug use. We examined whether recent mood influences responses to cannabis in controlled laboratory settings.

**Methods:** In this double-blind, placebo-controlled, within-subjects study, male and female volunteers (n = 68; 23 females) participated in 3 laboratory sessions. Smoked cannabis (0%, 4%, and 10% THC) was administered in a randomized order (one dose per session). We analyzed self-reported feelings of being "High," "Anxious," or "Depressed" from visual analog scales scored as peak change from baseline. We also analyzed behavior from a cannabis self-administration task, wherein participants purchased up to three puffs ($1/puff) of the same blinded dose (0%, 4%, or 10%) consumed 3 hours earlier. All dependent measures were examined as a function of Beck Depression Inventory (BDI) or Beck Anxiety Inventory (BAI) scores obtained during screening. Two-way repeated measures analysis of variance (RM-ANOVA) compared groups with "minimal" depression (BDI scores < 10) or anxiety (BAI scores < 8) to those above these thresholds.

**Results:** Participants primarily expressed "minimal" depression (n = 62/68) and anxiety (n = 59/68) symptoms. There were dose-dependent effects of cannabis on "High" and number of puffs (ps < 0.001), but not on "Anxious" or "Depressed." Moreover, there were no significant interactions with depression or anxiety symptoms.

**Conclusions:** Future analyses may incorporate more individuals with depression and anxiety symptoms, the current analysis shows little indication that recent mood significantly affects responses to cannabis.

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# SEX DIFFERENCES IN THE ACUTE EFFECTS OF CANNABIS (SEDICAN): STUDY DESIGN AND OBJECTIVES

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**Introduction:** Cannabis is one of the most commonly used psychoactive substances worldwide and an increasing amount of people use cannabis for medicinal purposes. Although epidemiological studies indicate differences between men and women in cannabis use patterns and characteristics, sex differences in the acute effects of cannabis are unclear. Here, we present study design and objectives of a randomised clinical trial entitled 'Sex differences in the acute effects of cannabis (SEDICAN)'.

**Methods:** 48 healthy occasional cannabis users (24 males and 24 females) are allocated to either of two randomised controlled study arms. Each arm consists of three study visits that involve cannabis administration using a Volcano vaporizer. Participants will receive placebo cannabis and a low and high dose of either Bedrocan (22% THC; <1.0% CBD) or Bediol cannabis (6.3% THC; 8.0% CBD).

**Results:** The main study parameters are subjective, behavioural, cognitive, cardiovascular and pharmacokinetic outcomes. Subjective drug effects are assessed using visual analogue scales. Behavioural assessments include questionnaires and interviews measuring psychotic symptoms, anxiety and mood. Cognitive function is examined using the WAIS-IV test battery. Heart rate and blood pressure are frequently measured. Blood samples are collected to measure cannabinoid plasma concentrations.

**Conclusions:** Anticipated findings will provide important information on sex differences in the acute effects of cannabis as well as on potential risk factors associated with cannabis use. Moreover, the impact of THC dose and CBD/THC ratio on acute cannabis effects will be examined. This is relevant in the context of rising numbers of patients using cannabis for medicinal purposes.

# COMPREHENSIVE IN VITRO AND IN VIVO EVALUTION OF INDAZOLE-BASED SYNTHETIC CANNABINOID RECEPTOR AGONISTS

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**Introduction**: Since their first appearance as designer drugs in the early 2000s, synthetic cannabinoid receptor agonists (SCRAs) have been continuously structurally modified by clandestine labs to bypass regulations. This rapid evolution has resulted in increasingly potent and efficacious variants with potentially significant health risks, among which indazole-based SCRAs are one of the most potent and prevalent series. Our group conducted a comprehensive evaluation of a library of indazoles by assessing their in vitro and in vivo activities, aiming to establish trends in potency/efficacy, pharmacokinetic properties and pharmacological effects to assist the legal classification and detection of future SCRAs in forensic and clinical settings.

**Methods**: A library of indazole-based SCRAs were synthesized incorporating various head and tail groups. They were characterized by $^{1}H$ and $^{13}C$ NMR, LCMS, and HPLC. Their activities were assessed in CB1 and CB2 calcium mobilization, [$^{3}H$]CP55,940 radioligand binding, and β-arrestin assays. Their metabolic stability was evaluated against mouse (MLM) and human liver microsomes (HLM). One such compound CUMYL-BUTINACA was evaluated for hypothermic and discriminative stimulus effects in mice and compared to the effects of $\Delta^{9}$-tetrahydrocannabinol.

**Results**: Compounds with a five-carbon tail group exhibited single-digit nanomolar potency at both CB1 and CB2 receptors in calcium mobilization and radioligand binding assays along with moderate CB1 selectivity, whereas shorter tail groups, such as propyl, tend to favor CB2 agonism. Head groups including CUMYL, AMB, AB, MDMB, and ADB conferred high potency and some CB1 selectivity in the calcium assays, but similar or greater binding affinity for CB2 compared to CB1 in the binding assay. Most compounds functioned as full agonists at CB1, with the majority fully activating recruitment of β-arrestin-2, though a few exhibited only partial recruitment at 10 μM suggesting potential for bias. The PICANA series displayed short half-lives in MLM and HLM, with the exception of AB-PINACA, MDMB-PINACA, and ADB-PINACA (clearance rates <50 mL/min/kg). Both CUMYL-BUTINACA and $\Delta^{9}$-tetrahydrocannabinol produced dose-dependent hypothermia and CUMYL-BUTINACA produced $\Delta^{9}$-tetrahydrocannabinol-like discriminative stimulus effects. CUMYL-BUTINACA was more potent than $\Delta^{9}$-tetrahydrocannabinol in vivo.

**Conclusions**: A comprehensive evaluation was conducted on indazole-based SCRAs for their potency, selectivity, PK properties, and in vivo pharmacological effects. This effort establishes the structure, property, and activity trend of this rapidly expanding class of psychoactive substances.

**Acknowledgement:** We thank the NIDA drug supply program for providing various chemicals. We thank Rod Snyder and Yun Lan Yueh for providing expert bioanalytical support. This work was funded by the NIDA grant DA040460.

# EXPLORING CB2 AS A THERAPEUTIC TARGET IN PSORIASIS

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**Introduction:** The skin endocannabinoid system (ECS) regulates lipid synthesis and keratinocyte proliferation, and its dysregulation is implicated in psoriasis. Cannabinoids, through their anti-inflammatory and antiproliferative effects, offer a promising therapeutic avenue in this condition. However, understanding the precise mechanisms through which cannabinoids exert their effects is crucial for optimizing their therapeutic potential. To address this, we focused on the role of CB2 in psoriasis pathogenesis.

**Methods:** Psoriasis was induced in mice via topical imiquimod (IMQ) for six days. Back and ear thicknesses and the Psoriasis Area and Severity Index (PASI) were recorded daily and at the endpoint, plasma and skin samples were collected for immunohistochemistry, RT-qPCR, and lipidomic analysis. The CB2 agonist AM1710 was administered from two days before IMQ treatment throughout the experiment. For knockout studies, both global and keratinocyte-specific CB2 knockout mice were analyzed.

**Results:** IMQ treatment induced psoriatic-like skin inflammation, with elevated AEA and 2- AG levels in affected skin and reduced levels in plasma. AM1710 treatment reduced back skin thickness, clinical scores (especially scaling), and the level of inflammatory cytokines in IMQ-treated mice. Global CB2 deletion had minimal effects on disease severity, while keratinocyte-specific CB2 deletion significantly exacerbated inflammation and scaling.

**Conclusions:** We characterized the endocannabinoid changes in the imiquimod-induced mouse model of psoriasis and examined CB2's role in the disease. Our findings indicate that CB2 activation alleviates psoriasis symptoms, likely through keratinocyte modulation, making it a possible novel therapeutic target for psoriasis.

**WITHDRAWN**

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# INITIAL STUDIES REGARDING THE ACUTE EFFECTS OF WIN55,212-2 ON URINARY WATER AND ELECTROLYTE EXCRETION IN ALKALI-LOADED MICE

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**Introduction:** The kidney endocannabinoid system has multiple physiologic and pathophysiologic functions. Cannabinoid receptor type-1 (CB1R) agonists acutely act as diuretics with variable effects on urine $Na^+$, $K^+$, and $HCO3^-$ excretion. The kidney collecting duct (CD) regulates total body electrolyte, acid/base, and water balance through specific functions of principal cells (PCs) and intercalated cells (ICs). We previously identified IC-specific CB1R localization in the CD and that WIN55,212-2 (WIN) induced central diabetes insipidus, a disorder characterized by hypotonic polyuria from vasopressin deficiency, in acid-loaded and non-acid-loaded mice (Rein JL, et al. Am J Physiol Renal, 2024). Since ICs are critical to maintaining acid/base and electrolyte homeostasis, we sought to extend our findings and characterize the acute effects of WIN on alkali-loaded mice.

**Methods:** Metabolic studies were performed in male and female C57BL/6 mice aged 8-12 wks that were alkali-loaded for 7 d with 280 mM $NaHCO3^-$ and 2% sucrose in the drinking water. Mice were then injected IP once with either 1.5 mg/kg WIN or vehicle (VEH; 10% Tween-80 and 10% DMSO in PBS) and urine was collected over 2 hrs followed by terminal blood collection. Results are presented as mean ± SD with significance by unpaired 2-tailed Student's *t*-test if $P \leq 0.05$.

**Results:** Alkali-loaded mice were similarly hypokalemic regardless of WIN (2.8 ± 0.5 mmol/L; n=12) vs. VEH (2.7 ± 0.6 mmol/L; n=10) (P=0.6). Alkali-loaded mice treated with WIN had a lower urine osmolality (646 ± 110 vs. 1587 ± 250 mmol/kg H2O; P<0.00001) and less urine $K^+$ excretion (UKV) (0.09 ± 0.04 vs. 0.18 ± 0.08 µmol/gBW/2hrs; P=0.011) but had similar urine $Na^+$ (UNaV) and $Cl^-$ excretion (n=8) compared to VEH (n=11). In a separate set of experiments, WIN-treated non-alkali loaded male mice (n=6) also had a lower UKV (0.44 ± 0.17 vs. 0.85 ± 0.26 µmol/gBW/2hrs; P=0.006) but similar UNaV compared to VEH (n=8).

**Conclusion:** WIN-treatment reduced urine $K^+$ excretion in non-alkali-loaded mice and further attenuated urinary $K^+$ losses in hypokalemic alkali-loaded mice. WIN-treated alkali-loaded mice also produced relatively dilute urine, consistent with past experiments. This research integrates existing knowledge regarding the diuretic effects of cannabinoids and the influence of CB1R on kidney function, while adding new insights about total body water and electrolyte homeostasis.

**Acknowledgements:** Funded by Veterans Affairs BLR&D CDA-2 IK2BX006144 (JLR). The views expressed in this abstract are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs or the United States government.

# TARGETING ENDOCANNABINOID SYSTEM DEFICITS AS A THERAPEUTIC STRATEGY FOR GULF WAR ILLNESS IN A MOUSE MODEL

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**Introduction:** Gulf War Illness (GWI) is a chronic condition marked by a diverse array of symptoms, including pain, fatigue, anxiety, and cognitive impairments. These symptoms are believed to result from damage caused by exposure to toxic chemicals during the Gulf War (GW), such as pesticides, nerve agents, and prophylactic drugs, under unpredictable stress. We hypothesized that the pathogenesis of GWI might be rooted in long-lasting disruption of the endocannabinoid (ECB) system, a signaling complex that serves important protective functions in the brain.
**Methods:** Using a well-established mouse model of GWI, we assessed the levels of ECB molecules and ECB-related genes in the brain, and examined whether pharmacological treatment targeting ECB deficits could alter the behavioral phenotype of GWI mice.
**Results:** we found that levels of the ECB messenger, anandamide, were significantly reduced in the brain of diseased mice, compared to healthy controls. Moreover, the transcription of the Faah gene, which encodes the enzyme fatty acid amide hydrolase (FAAH) that breaks down anandamide, was notably elevated in the prefrontal cortex and brain microglia of GWI mice. Furthermore, treatment with the FAAH inhibitor URB597, administered immediately after GW chemical exposure, normalized brain anandamide levels and corrected behavioral deficits in these animals, including heightened anxiety-like and depression-like behaviors, and defective extinction of fearful memories.
**Conclusions:** Together, these results suggest that exposure to GW chemicals produce a deficit in brain ECB signaling. Pharmacological recovery of anandamide-mediated ECB signaling through the FAAH inhibition may serve as an effective therapeutic approach for GWI.

# CANNABINOID 2 RECEPTOR INCREASES EXPRESSION IN THE PERIPHERY AND BRAIN OF AGED MICE

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**Introduction:** The cannabinoid 2 receptor (CB2) is involved in immune modulation and expressed on peripheral immune cells and activated microglia. Quantifying CB2 protein expression has been challenging due to poor antibody specificity. Although increased CB2 levels have been described in neurodegeneration, expression in healthy aging and age-related immune responses remains unclear. Therefore, we characterized CB2 expression in splenocytes and the brains of young and aged CB2-GFP reporter mice.

**Methods**: Mice aged 2-4 months (n=37, 18M and 19F) and 18-23 months (n=46, 24M and 22F) were injected peripherally with LPS ($1.5x10^6$ EU/kg; IP) or saline and brains and spleens were collected 24 hours after injection. Splenocytes were evaluated for GFP expression using flow cytometry and for cnr2 and inflammatory transcripts using qPCR. Brains were evaluated for GFP expression and microglial activation using flow cytometry and immunohistochemistry and for distribution of cnr2 transcript using RNAscope.

**Results:** Splenocyte qPCR results demonstrated significant inflammatory responses to LPS suggesting a peripheral response can be detected 24hrs following an acute mild dose. Aged mice exhibited significantly less IL-1b when stimulated with LPS ($p<0.0001$) and more IL-10 ($p<0.0001$) than young mice. Evaluation of cnr2 transcript in splenocytes showed that young mice treated with LPS have a significant reduction in cnr2 ($p=0.0297$) that was not mimicked in aged mice. Whereas GFP expression (reporter for CB2) of splenocytes as detected by flow cytometry showed significant increases in monocytes from aged compared to young mice regardless of Ly6C expression. In the brain, as expected IBA1+ area increased with LPS treatment in the hippocampus of young mice ($p<0.05$). This effect was not found in brains of aged mice. GFP expression significantly increased on microglia of aged mice compared to young. Similarly, preliminary evaluation of cnr2 in the substantia nigra using RNAscope demonstrated increased cnr2 transcript.

**Conclusions:** Our results suggest that in splenocytes of aged mice, CB2 increases in monocytes compared to young animals yet reaches a ceiling effect and does not alter expression following LPS treatment. Furthermore, CB2 expression increases in the brain were identified in microglia and neutrophil subsets of CNS immune cells from aged mice. Overall, our results suggest that CB2 increases with age, however differential expression have been found across cell type and sex. Further analysis needs to evaluate CB2 expression across different brain regions. Functional assays will be needed to determine whether these changes in CB2 are beneficial to the aged immune system in both the periphery and the brain.

# EFFICIENT TERPENYLATION OF POLYKETIDE ESTERS WITH ISOPIPERITENOL. SYNTHETIC METHODS TO TRANSFORM PRODUCTS INTO CANNABINOID ACIDS

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**Introduction:** Access to endogenous cannabinoid acids (*i.e.*, CBGA, CBDA, THCVA) through the processing of plant extracts can be complicated by unwanted decarboxylation in the presence of sunlight or heat upon storage. Stereo- and regiocontrolled organic synthesis pathways present unrivaled solutions to obtaining high purity APIs in support of clinical research across the ECS.

**Methods:** CitraChem's MA-based R&D laboratory has developed a chemistry platform in which 5-*n*-alkyl-4-(methoxycarbonyl)resorcinols are key intermediates for regioselective Friedel-Crafts terpenylation reactions with geraniol or (+)-isopiperitenol. The resulting methyl cannabigerolate and methyl cannabidiolate products form cleanly without the need for blocking groups due to the *meta*-directing effect of the ester moiety. Organic acids (PTSA/CSA) and Lewis acids ($BF_3 \cdot Et_2O$) function as catalysts in these convergent reactions, with toluene as solvent and $MgSO_4$ to absorb the water of ionization.

**Results:** Methyl cannabigerolate and methyl cannabidiolate were prepared in >60% yield and >98% purity following methods above. The methyl ester of CBDVA was also synthesized from the requisite *n*-propyl polyketide ester, the latter available by procedures we reported previously. Attempts to apply traditional basic hydrolysis conditions (aq. NaOH or KOH, LiOH in THF, etc.) to each ester led only to return of unreacted starting materials: Phenolic deprotonation renders the conjugated esters completely inert to acyl substitution even under conditions of high temperature. This challenge has been addressed by a strategic shift toward SN2-based demethylation reactions in which sulfide nucleophiles proved superior.

**Conclusions:** Cannabinoid acids (CBGA/CBDA) were synthesized by ester dealkylation. These carboxylic acids set a stage for conversion to novel aniline derivatives by Curtius rearrangement.

# EXPLORING BEHAVIORAL AND MORPHOLOGICAL EFFECTS OF MINOR PHYTOCANNABINOIDS IN ZEBRAFISH DEVELOPMENT

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**Introduction**: Cannabis and cannabinoid accessibility has steadily risen over the past decade due to the relaxation of laws that had previously restricted their possession and use. Studies have shown that early-life exposure to $\Delta^9$-tetrahydrocannabidiol (THC), causes sex-dependent brain development deficits and persistent behavioral disorders in humans. Our lab has found similar behavioral endpoints in zebrafish exposed to THC developmentally. Specifically, $\Delta^9$-THC exposure resulted in dose-dependent hyperactivity and anxiety-like behavior (i.e., thigmotaxis) that persist well into adulthood. Unfortunately, despite increased consumer interest, little is known on the developmental effects of cannabidiol (CBD), cannabinol (CBN), cannbigerol (CBG) and other minor cannabinoids. We hypothesize that exposure to minor cannabinoids will also impact behavioral and morphological development.

**Methods**: Developing zebrafish (6-96 hours post fertilization) were exposed to increasing concentrations (0.03-2 μM) of 8 different cannabinoids- $\Delta^9$-THC, CBD, CBN, CBG, hexahydrocannabinol (HHC), tetrahydrocannabivarin (THCV), $\Delta^{10}$-THC, and $\Delta^{8}$-THC. Photolocomotor responses and developmental morphology were assessed at 120 hours post fertilization using the Zebrabox. To examine the contribution of canonical cannabinoid receptors to changes in behavior, zebrafish lacking cannabinoid receptor 1 (*cnr1-/-*) and cannabinoid receptor 2 (*cnr2-/-*) were also exposed to the cannabinoids.

**Results**: Our results indicate $\Delta^8$-THC, CBD, CBG, HHC, and THCV all significantly reduce photolocomotor response in a dose-dependent fashion. THCV and $\Delta^{8}$-THC showed significant reduction in dark phase activity at doses as low as 0.12 μM, while doses of 2 μM were required to see impaired locomotor response with $\Delta^{9}$-THC, CBG, and HHC. CBN and $\Delta^{9}$-THC elicited no behavioral modifications even at the highest dose. Morphological changes largely correlated with the behavioral impairments. Our initial studies in *cnr2-/-* fish also reveal significant toxicity with CBD. Further evaluations of the minor cannabinoids within the transgenic fish are ongoing.

**Conclusion**: Similar to $\Delta^{9}$-THC, several minor cannabinoids alter morphology and behavior of developing zebrafish, particularly in the very early stages of embrolarval development. The impact of cannabinoid receptors on these changes is still being evaluated. We will continue this study to observe the persistence of morphological, hyperactive, or anxiogenic behaviors into adulthood.

# INVESTIGATING THE ANALGESIC, ABUSE-RELATED, AND THC SPARING EFFECTS OF MYRCENE, A CANNABIS-BASED TERPENE, IN A WITHIN-SUBJECTS PLACEBO-CONTROLLED STUDY

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**Introduction:** Cannabis is frequently used for pain, but adverse effects limit clinical utility. Preclinical studies show that myrcene, a cannabis-based terpene, has potential analgesic effects, does not possess abuse-related characteristic of delta-9-tetrahydrocannabinol (THC), and may spare adverse consequences of THC. This study assesses the analgesic, abuse-related, and THC-sparing effects of myrcene alone, and in combination with THC, in healthy volunteers.

**Methods:** In this ongoing placebo-controlled, within-subject study, participants inhale vaporized myrcene (0.0, 0.5, 12.0 mg) and THC (0.0, 5.0, 15.0 mg), alone and in combination, across nine sessions. Subjective drug effects are measured with visual analog scales; pain threshold and tolerance using the Cold Pressor Test (CPT); and subjective pain ratings using McGill Pain Questionnaire (MPQ) / 'Painfulness' & 'Bothersomeness' scales.

**Results:** Preliminary data (N = 8 female, 4 male) shows that relative to placebo myrcene does not produce abuse-related subjective drug effects, whereas THC produces higher ratings ($p < 0.001$). When combined, this THC effect is not altered. Myrcene does not elicit analgesic effects, but may decrease subjective pain ratings ($p < 0.10$), whereas THC elicits analgesic effects ($p < 0.05$) and likely reduces subjective pain ratings ($p < 0.10$). When combined, myrcene may reduce THC- induced analgesia ($p < 0.10$) and subjective pain ratings ($p < 0.05$).

**Conclusions:** This preliminary dataset shows that myrcene does not produce abuse-related or consistent analgesic effects, whereas THC elicits both effects. In combination, myrcene may reduce THC's analgesic effects, which has potential clinical implications in the use of cannabis for pain relief.

**Funding:** US NIH (R01AT010762, UL1TR00188), IRACDA at UCLA (K12GM106996), CIHR Postdoctoral Fellowship to SLBaglot, CIHR Banting Fellowship to SLake, and the Semel Charitable Foundation.

# EXPLORING THE THERAPEUTIC EFFECTS OF CBD AND THC ON PRE-CLINICAL RODENT MODELS OF MIGRAINE

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**Introduction:** Migraine is a complex neurological disorder affecting approximately 15% of the global population. The neuropeptide calcitonin gene-related peptide (CGRP) is implicated in migraine pathophysiology, as elevated CGRP levels correlate with migraine attacks, and its administration induces migraine-like symptoms in both humans and animal models. Current migraine treatments, including triptans, ditans, beta-blockers, and CGRP-targeting therapies, provide relief for only about 50% of patients, highlighting the need for novel therapeutic approaches.The endocannabinoid system (ECS) has been implicated in migraine pathophysiology, with evidence suggesting its dysregulation in migraine patients. This has led to growing interest in targeting the ECS for novel migraine therapeutics. Specifically, CB1 receptor activation has been shown to inhibit CGRP release from trigeminal neurons. Emerging clinical and preclinical studies suggest that cannabis-based treatments may provide therapeutic benefits. Our previous research demonstrated that pretreatment with a 100:1 CBD:THC ratio effectively reduced light aversion and spontaneous pain induced by i.p. administered CGRP in mice, without adverse effects.

**Methods:** To investigate whether cannabinoids could alleviate migraine like symptoms of light aversion and pain, we pretreated mice with THC and CBD followed by central (i.c.v.) administration of CGRP. Light aversion behavior was assessed using a light-dark box assay and spontaneous pain was assessed using the automated squint assay.

**Results:** Peripheral cannabinoid injection of 100:1 CBD:THC rescues central CGRP-induced light aversion (Figure 1) and spontaneous pain (Figure 2) without notable adverse effects.

**Conclusions:** Our findings indicate that cannabinoids may mitigate central CGRP-mediated migraine symptoms, supporting their potential as alternative migraine therapies. Further studies are needed to optimize dosing and elucidate mechanisms.

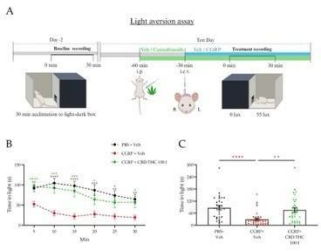


Figure 1:

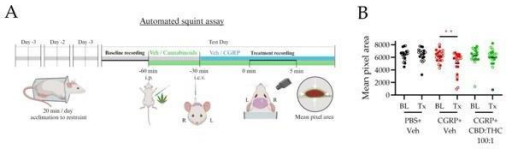


Figure 2:

# A HIGH-FAT MEAL SIGNIFICANTLY IMPACTS THE BIOAVAILABILITY AND BIPHASIC ABSORPTION OF CANNABIDIOL (CBD) FROM A CBD-RICH EXTRACT IN MEN AND WOMEN

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**Introduction:** Cannabidiol (CBD), one of the major components of *Cannabis sativa*, is attracting increasing attention for its alleged health-promoting properties. Low-dose CBD preparations, unofficially classified as food supplements in many countries, are widely available and popular.
Their widespread use highlights the need for a better understanding of the pharmacokinetics of CBD, its primary metabolite 7-hydroxy-cannabidiol (7-OH-CBD), and factors affecting this.

**Methods:** Pharmacokinetics of CBD and 7-OH-CBD were investigated over a 48-hour period following administration of a capsule containing a CBD-rich cannabis extract equivalent to 70 mg CBD, in healthy male (n=5) and female (n=6) participants. To study the impact of a standardized high-fat meal consumed 30 minutes before compared to fasting, a randomized crossover design was used.

**Results:** Both the geometric mean ratio (GMR) for Cmax (17.4; 90% CI 12.4–24.2) and AUC (9.7; 90% CI 7.7–12.3) of CBD were significantly higher in the fed condition compared to fasting, indicating a substantial food effect on CBD bioavailability. A notable double peak phenomenon was observed after meal consumption, with a less pronounced effect in the fasted state.

**Conclusions:** Healthcare professionals and CBD users should be aware that consuming CBD with a meal significantly increases its bioavailability. Standardizing CBD intake to meals is important for achieving consistent and optimal effects. The observed second peak phenomenon in both CBD and its metabolite 7-OH-CBD is an interesting finding, contributing to sustained high plasma concentrations. This may be (partially) attributed to lymphatic transport, enterohepatic recirculation, and/or a secondary meal effect.

# ASSOCIATION BETWEEN LOW ORAL DOSES OF SYNTHETICALLY PRODUCED CANNABIDIOL (CBD) AND PLASMA CONCENTRATION OF ANANDAMIDE (AEA), PALMITOYLETHANOLAMINE (PEA) AND OLEOYLETHANOLAMINE (OEA) IN HEALTHY OCCASIONAL CANNABIS USERS

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**Introduction**: CBD, an exogenous ligand of the endocannabinoid system, has unclear effects on N-acylethanolamines such as AEA, PEA and OEA. This study investigates the acute impact of marketed CBD doses on the plasmatic levels of these mediators in occasional cannabis users, addressing a significant gap in the understanding of low-dose CBD in non-therapeutic contexts.

**Methods**: In a triple-blind, placebo-controlled, randomized, crossover trial, 70 volunteers were randomized to ten sequences of four oral doses of CBD oil (20mg, 50mg, 100mg and 200mg) and placebo (0mg). Blood samples were collected at baseline (pre-dosing) and at 60-, 120-, 210-, 300-, and 360-minutes post-ingestion. Plasma concentrations of AEA, PEA and OEA were measured using LC-MS/MS.

**Results**: Administration of 50mg and 200mg of CBD significantly decreased plasma concentrations of PEA (50mg: Cohen's d= 0.513 [CI: 0.171-0.854]; 200mg: Cohen's d= 0.472 [CI: 0.131-0.812]) and OEA (50mg: Cohen's d= 0.586 [CI: 0.244-0.927]; 200mg: Cohen's d= 0.430 [CI: 0.090-0.771]) compared to placebo ($p<0.05$), but had no significant effect on AEA. Doses 20mg and 100mg of CBD had no effect on plasma levels of AEA, PEA and OEA. All analytes decreased consistently across timepoints at all doses, including placebo.

**Conclusions**: Doses of 50mg and 200mg of ingested CBD reduced plasma levels of PEA and OEA, but not AEA. These findings improves our understanding of the biological effects of cannabinoid-based products, while highlighting the complex, dose-dependent effects of CBD on endocannabinoid signaling.

# DOSE VARIABILITY AND MANUFACTURING QUALITY: ANALYSIS OF CBD GUMMY PRODUCTS

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**Introduction:** Gummy-based CBD products are widely available, but this product type has inherent manufacturing challenges (e.g., incompatibility of a hydrophilic matrix and hydrophobic compounds) which impacts product homogeneity. The goal of this study was to determine the variability of cannabinoid concentrations within a bottle of CBD gummies (i.e., gummy-to-gummy dose variability) across 10 unique products.

**Methods:** 10 CBD gummy products were obtained and cannabidiol (CBD) and Δ9-tetrahydrocannabinol (Δ9-THC) concentrations were analyzed. For each product, the mass of 12 individual gummies was determined, each gummy was separately cryomilled and 6 sample replicates were aliquoted for extraction and analysis (total of 72 samples per product). LC-MS/MS with separation was carried out using a Phenomenex Kinetex® C8 column and 12-minute gradient program.

**Results:** Primary outcomes were variability in 1) gummy mass and 2) CBD and Δ9-THC concentrations. Across the products tested, masses ranged from 3.1074 to 5.3345 grams, with a coefficient of variation (CV) range of 0.7% to 7.8%. The mean CBD concentration range detected: 5.73 to 26.93 mg per gummy (CV range: 2.1% to 27.1%) and mean Δ9-THC concentration range: 0.079 to 0.840 mg per gummy (CV range: 3.1% to 23.5%).

**Conclusion:** This study, while limited to examining 10 products, allowed for analysis of 720 samples. The results demonstrate an appreciable variability in the cannabinoid doses available within each bottle. This variability subjects consumers to inconsistent dosing, even when taking a product per label instructions. None of the analyzed products disclosed the presence of Δ9-THC on the label, which is highly problematic.

# CLINICAL STUDY DESIGN FOR A RANDOMIZED, OPEN-LABEL, 4-WAY CROSSOVER, PHARMACOKINETIC STUDY COMPARING A SOLID TO AN OILY CANNABIDIOL FORMULATION UNDER FASTED AND FED CONDITIONS

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**Introduction**: Cannabidiol (CBD) is an effective API for the treatment of epilepsy in children. The current marketed drug is an oily solution. For clinical practice, a tablet is considered more patient-friendly. Therefore, > 160 different solid formulations of CBD have been produced and tested *in vitro*. A spray-dried nano-emulsified prototype was selected for the planned pharmacokinetic (PK) study. The aim was to generate a robust clinical study design to compare an oily to a solid CBD formulation.

**Methods**: ICH GCP guidelines with integrated Addendum E6 (R2) were applied.

**Results**: Assessing the PK properties of the solid CBD formulation in healthy adults compared to an oily formulation using the $AUC_{0\text{-}24h}$ of the CBD plasma concentration in fed and fasted state after single administration of CBD was defined as primary objective. From previous clinical CBD studies, it is known that oral CBD bioavailability is very low and plasma CBD clearance is very high. Therefore, the PK properties of the plasma metabolites of CBD, 7-hydroxycannabidiol (7-OH-CBD) and cannabidiol-7-oic acid (7-COOH-CBD) were included. Furthermore, the $AUC_{0\text{-}24h}$ sums of all measured plasma levels of CBD, 7-OH-CBD and 7-COOH-CBD were covered in the objectives. All PK parameters: $C_{max}$, $T_{max}$, $T_{1/2}$, $AUC_{0\text{-}24h}$ and $AUC_{inf}$ will be determined. The power calculation allowed for n=32 subjects. The 16 males and 16 females will be randomly assigned into 4 groups that will be equally distributed into 4 treatment regiments in open-label, 4-way crossover design.

**Conclusion**: The PK study design was defined according to good clinical practice standards.

# QUILLAJA EMULSION ALTERING THC HUMAN PK PROFILE COMPARED TO DISTILLATE INFUSED GUMMY

Harold Han

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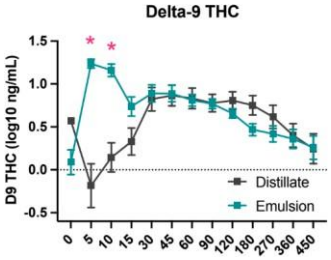
**Introduction:** Quillaja based cannabis emulsion has big potential when applied in medicinal cannabis gummies. This study demonstrates the drastically different human PK profile comparing THC quillaja emulsion infused gummy with THC distillate infused gummy.

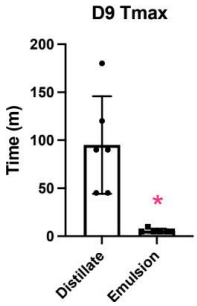
**Methods:** IRB approved human PK study was conducted among 6 testers to test 15mg THC in two types of pectin gummies. This was a double-blinded, randomized trial where the gummies were either infused with distillate or quillaja emulsion. Blood samples were taken over a 7.5 hour span and THC, 11-OH-THC and THC-COOH were analyzed.

**Results:**

Peak blood absorption by time Δ9

| | DISTILLATE - THC | | | | | | EMULSION - THC | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tester 1 | Tester 2 | Tester 3 | Tester 4 | Tester 5 | Tester 6 | Tester 1 | Tester 2 | Tester 3 | Tester 4 | Tester 5 | Tester 6 |
| 0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.7 | 0.0 | 0.0 | 0.0 | 0.7 | 1.2 | 2.3 | 0.0 |
| 5 | 0.4 | 0.4 | 0.0 | 0.0 | 7.8 | 0.7 | 29.8 | 17.0 | 14.7 | 18.2 | 12.9 | 15.0 |
| 10 | 1.4 | 1.4 | 0.8 | 0.9 | 8.7 | 0.8 | 33.1 | 13.0 | 12.3 | 12.4 | 13.3 | 11.2 |
| 15 | 2.5 | 2.1 | 2.6 | 1.3 | 8.9 | 0.8 | 14.1 | 7.1 | 6.5 | 4.7 | 4.2 | 2.1 |
| 30 | 12.8 | 9.1 | 7.9 | 5.8 | 10.0 | 1.6 | 8.0 | 9.2 | 10.9 | 10.6 | 8.6 | 2.8 |
| 45 | 14.2 | 10.8 | 4.7 | 5.3 | 14.7 | 2.7 | 5.8 | 8.7 | 13.3 | 15.2 | 5.6 | 3.9 |
| 60 | 11.4 | 10.5 | 3.8 | 5.5 | 14.8 | 3.8 | 4.4 | 5.5 | 10.4 | 10.4 | 5.4 | 5.1 |
| 90 | 5.7 | 7.9 | 5.2 | 4.2 | 16.5 | 4.2 | 3.5 | 6.2 | 8.0 | 7.0 | 4.9 | 6.0 |
| 120 | 4.0 | 6.9 | 9.7 | 6.4 | 14.0 | 3.8 | 2.9 | 3.9 | 6.2 | 5.6 | 3.8 | 5.4 |
| 180 | 3.7 | 4.7 | 5.1 | 15.0 | 9.4 | 2.6 | 1.4 | 3.0 | 4.2 | 3.9 | 2.9 | 3.3 |
| 270 | 2.6 | 2.5 | 3.3 | 10.6 | 10.7 | 1.8 | 0.0 | 1.2 | 4.3 | 3.1 | 3.1 | 2.5 |
| 360 | 1.6 | 0.8 | 3.2 | 8.7 | 8.8 | 2.4 | 0.0 | 1.4 | 3.8 | 3.4 | 3.1 | 1.1 |
| 450 | 1.4 | 0.4 | 2.2 | 7.3 | 8.5 | 1.2 | 0.0 | 0.8 | 3.4 | 3.6 | 2.8 | 0.8 |



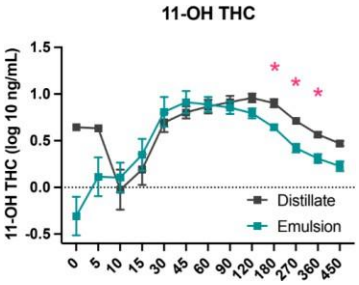




Raw data on THC concentration marking Cmax Significant faster onset time observed

Averaged THC results with 6 testers

Peak blood absorption by time 11-OH-THC

| | DISTILLATE - 11-OH-THC | | | | | | EMULSION - 11-OH-THC | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tester 1 | Tester 2 | Tester 3 | Tester 4 | Tester 5 | Tester 6 | Tester 1 | Tester 2 | Tester 3 | Tester 4 | Tester 5 | Tester 6 |
| 0 | 0.0 | 0.0 | 0.0 | 0.0 | 4.4 | 0.0 | 0.6 | 0.2 | 0.0 | 1.0 | 0.0 | 0.0 |
| 5 | 0.0 | 0.0 | 0.0 | 0.0 | 4.3 | 0.0 | 2.2 | 0.5 | 0.0 | 2.0 | 0.0 | 0.0 |
| 10 | 0.5 | 0.0 | 0.6 | 0.5 | 4.0 | 0.0 | 3.0 | 0.6 | 0.0 | 2.5 | 0.5 | 1.5 |
| 15 | 2.0 | 0.6 | 2.4 | 0.7 | 4.5 | 0.0 | 6.1 | 1.5 | 0.4 | 3.0 | 3.2 | 3.6 |
| 30 | 6.7 | 4.3 | 10.9 | 3.2 | 6.6 | 2.2 | 22.5 | 7.4 | 1.6 | 8.1 | 9.0 | 3.7 |
| 45 | 10.1 | 7.4 | 6.0 | 4.2 | 8.4 | 4.2 | 17.6 | 14.2 | 3.1 | 8.2 | 10.6 | 4.5 |
| 60 | 12.1 | 10.4 | 5.2 | 5.8 | 9.2 | 4.5 | 13.0 | 9.5 | 4.5 | 8.7 | 10.1 | 4.3 |
| 90 | 11.4 | 11.1 | 5.4 | 5.4 | 11.7 | 7.1 | 11.8 | 6.4 | 6.1 | 8.7 | 8.6 | 3.9 |
| 120 | 10.1 | 10.0 | 13.0 | 7.5 | 9.2 | 6.2 | 9.6 | 6.5 | 6.4 | 5.6 | 6.8 | 3.8 |
| 180 | 8.6 | 10.3 | 7.3 | 10.6 | 6.6 | 5.7 | 5.3 | 3.7 | 4.7 | 3.6 | 5.1 | 4.0 |
| 270 | 5.0 | 4.9 | 4.2 | 7.4 | 5.3 | 4.6 | 4.1 | 2.3 | 2.9 | 2.7 | 2.4 | 1.9 |
| 360 | 3.6 | 3.0 | 3.3 | 4.4 | 4.3 | 3.7 | 3.0 | 1.8 | 1.7 | 2.6 | 2.0 | 1.5 |
| 450 | 3.8 | 2.6 | 2.6 | 3.1 | 3.1 | 2.7 | 2.4 | 1.5 | 1.3 | 2.4 | 1.7 | 1.2 |




Raw data on 11-OH-THC marking Cmax testers

Averaged 11-OH-THC results with 6

**Conclusions:** This study demonstrated the fast onset profile of medicinal gummy infused with quillaja based emulsion. Cmax was reached for all testers within 10 minutes of ingestion with quillaja emulsion infused gummy. We observed a similar concentration level of 11-OH-THC between distillate gummy and emulsion gummy. This fast acting feature could bring huge benefits to patients who need instant gratification when consuming medicinal gummies.

# CANNABIS AND OLDER PERSONS: IDENTIFYING STATE POLICIES AND LOCAL CONDITIONS MOST RELEVANT TO AGE-RELATED MEDICAL USE

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**Introduction**:

While previous research has associated state cannabis regulation with individual access and use, little is known about state regulations and local conditions most relevant to older persons who use cannabis for medical purposes.

**Method:**

We observed cannabis legalization across the United States, focusing on regulations most critical to older persons including access, program eligibility and qualifying conditions and providers, potency limits, care giver autonomy, delivery and home growth options. We then used individual item measures to construct aggregated scores of state policies and charted changes within and among state policies from program inception through 2024. We linked these state policies with local conditions considered most salient to older adults who use cannabis for medical purposes including access to medical dispensaries and local opioid prescribing rates.

**Results:**

We created five geographically-based "cannabis clusters" in which vectors we aligned a set of quantified features reflecting state and local conditions. One cluster reflects a local environment considered to be "most permissive" with state regulations facilitating access for persons with age-related qualifying conditions and an ample supply of medical dispensaries. In contrast, we created a cluster reflecting "most restrictive" local contexts with state no regulations facilitating access for persons with age-related qualifying conditions, no access to medical dispensaries, and an above average opioid prescribing rate.

**Conclusions:**

Identification of geographic clusters that facilitate or hinder cannabis use among older persons can be used to advance scientific understanding about variation in cannabis use among older persons across the United States.

# BUILDING THE CANNABIS DATA REPOSITORY: DISSEMINATION OF CANNABIS USE MEASUREMENT TOOLS TO QUANTIFY MEDICINAL CANANBIS USE BEHAVIOR AND ITS IMPACT ON HEALTH

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**Introduction:** With more than 5.5 million registered patients in state-regulated medicinal cannabis programs, the United States has seen a significant increase in the complexity of cannabis products. There is an urgent need for validated tools that capture cannabis product type, dosage, and quantity; and a need for a unified framework to accurately its health effects. The objective of this study was to validate the new Cannabis Use Questionnaire (CUQ) and to construct a Core Assessment Battery for Cannabis (CABC) that will evaluate the efficacy of therapeutic cannabis use.

**Methods:** For the validation of the CUQ, a test-retest reliability study and a psychometric analysis is currently in progress. For the CABC, a literature review was conducted to compile validated assessments for key health-related endpoints relevant to medicinal cannabis use. The list of assessments was evaluated based on accessibility, validation, prevalence, adaptability and brevity.

**Results:** The CUQ employs 12 base questions across 9 product category domains (e.g., flower, e-liquid, concentrates, oils, edibles, topicals) and captures the predominant chemical composition, intended route of administration, and dose. Test-retest reliability findings and psychometric properties of the instrument will be presented at the meeting for the first time. The CABC comprises of 10 health domains that can be adopted for cross-study comparison across observational cannabis studies (including anxiety, depression, sleep, quality of life, and pain).

**Conclusions:** For the first ever in cannabis research history, the CUQ and CABC will provide a consistent framework for evaluating the therapeutic potential of cannabinoids, ensuring comparability and reliability across studies.

# ASSESSMENT OF RESPONSE PATTERNS TO CANNABIS-BASED MEDICINAL PRODUCTS: A LONGITUDINAL CLUSTERING ANALYSIS USING EQ-5D-5L IN THE UK MEDICAL CANNABIS REGISTRY

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**Introduction:** Cannabis-based medicinal products (CBMPs) have potential therapeutic benefits, yet their effects, particularly from unlicensed products, remain underexplored. Patient-reported outcome measures (PROMs) are useful for evaluating CBMP efficacy. Longitudinal clustering can analyse patterns of change in PROMs over time, linking these patterns to CBMP and patient-related variables. This approach aims to enhance understanding of how different CBMPs impact patient outcomes and identify factors influencing these responses. This study aims to identify distinct patterns of patient response to CBMPs using longitudinal clustering techniques and to explore associations between these patterns and various demographic and treatment-related factors.

**Methods:** Data was extracted from the UK Medical Cannabis Registry on 6th January 2025. Trajectory k-means clustering to EQ-5D-5L index values collected at baseline, 1 month, 3 months, 6, 12, 18 and 24 months. Univariate logistic regression and multivariate penalised logistic regression with least absolute shrinkage and selection operator (LASSO) combined with stability selection were employed to characterise the clusters and examine their associations with CBMP and patient-related variables.

**Results:** Ten distinct trajectories of patient responses were identified. Six trajectories showed improvement from baseline but plateaued after one month. One trajectory was largely static from baseline (Cluster A, mean difference -0.02). Cluster C and Cluster H demonstrated a benefit at 1 month, but continued improvement at following dates. Cluster I meanwhile demonstrates a reduction from baseline until 24 months (mean change -0.28). Regression analyses revealed significant associations between demographic, lifestyle, and CBMP-related factors and cluster membership ($p<0.050$).

**Conclusions:** This is the first study applying longitudinal clustering to PROM data for CBMPs. Findings are exploratory, and further research is needed, including using other clustering methods, a broader range of PROMs, and external validation, before drawing definitive conclusions. However, this approach may be useful in helping to guide clinical practice as to who are suitable candidates for medical cannabis in the absence of much high-quality data to inform clinical guidelines.

# CANNABIS AS THERAPY FOR ADULT AUTISM SPECTRUM DISORDER (ASD): IMPROVEMENT OF THE ADAPTIVE AND EMOTIONAL BEHAVIOR

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**Introduction:** Autism Spectrum Disorder (ASD) encompasses neurodevelopmental disorders marked by deficits in social interaction, communication, and repetitive behaviors. Genetic and environmental factors influence its etiology, making early diagnosis and intervention crucial. However, many adults remain undiagnosed in childhood due to lower support needs or misdiagnosis. These individuals often develop comorbidities in adolescence, such as anxiety, depression, and eating disorders, which impair social interaction and quality of life. Pharmacological treatment is non-specific, with psychotropic drugs mainly used to mitigate symptoms. Clinical studies indicate a link between endocannabinoid system changes and behavioral modifications in ASD, with cannabis treatment showing promise in children. However, research on its effects in autistic adults remains scarce, and clinical trials typically use fixed doses of pure cannabidiol (CBD) without titration adjustment. This study aims to evaluate the social, adaptive, and emotional behavior and pharmacological effects of phytocannabinoids in adults diagnosed with ASD.
**Methods:** Fourteen participants aged 18-55 years received Whole CBD Revivid® (100 mg/ml or 3,000 mg) cannabis oil, starting at 20 mg/day and increasing to 200 mg/day, administered twice daily after meals. ssessment tools included the Vineland-3 behavioral questionnaire before treatment (T0) and at T15, T30, T60, T90, and T120 days. A follow-up was conducted at T180 days. Clinical valuation, global status monitoring, and adverse event tracking were performed using the Dose, Adverse Effects, Improvements with Cannabis (DEMC®) protocol. Results: Initial findings from the DEMC protocol within 15 days indicated notable improvements. Among 52 records, 34.6% reported better sleep, 13.5% improved mood, and 9.6% reduced anxiety and irritability. Additional improvements included reduced sadness (7.7%), decreased agitation (5.8%), enhanced communication (5.8%), improved attention and focus (5.8%), and reduced repetitive movements (3.8%). On the Vineland-3 Scale, substantial changes were observed between T0 and T15. Internalizing behavior, assessing anxiety, depression, and social withdrawal, improved, with a 10.3% reduction in symptoms (17.28 to 15.5 points). Communication skills, including receptive, expressive, and written abilities, increased from 72.14 to 78.10 points (8.26% improvement). Adverse reactions were minimal, with 57.1% of participants reporting no side effects. The most common adverse effect was gastrointestinal discomfort (42.9%). No cases of sleep disorders, sexual dysfunction, weight gain, or motor coordination issues were reported.
**Conclusions:** Preliminary data from the DEMC protocol and Vineland-3 scale suggest that cannabis treatment can improve adaptive behavior, communication, and emotional regulation in autistic adults with good tolerability and minimal adverse effects. These findings indicate that cannabinoid therapy could be a viable first-line treatment for ASD in adults, addressing core symptoms and associated comorbidities effectively.

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# IMPACT OF CANNABIS EXPOSURE ON GUT BARRIER FUNCTION IN METABOLIC HEALTH AND DISEASE

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**Introduction:** The endocannabinoid (eCB) system is a lipid-derived signaling pathway that controls food intake, energy homeostasis, and reward (Argueta, et al., 2019). This system is expressed throughout the body, including in the gastrointestinal tract, where it becomes dysregulated in diet-induced obesity (DIO) and may participate in gut barrier function; however, it is unclear if over activating the eCB system with cannabis is protective or detrimental for gut function. Indeed, studies from our lab suggest that the eCB system in intestinal epithelial cells controls gut barrier function and associated inflammation, and its activity exerts a protective influence during diet-induced obesity in mice (Wiley & DiPatrizio, 2022). Moreover, studies suggest that activating the eCB system with phytocannabinoids prevents colitis and increases colonic barrier integrity (Becker 2021). Conversely, other studies show that pharmacological activation of cannabinoid receptors in lean mice leads to an increase in plasma levels of lipopolysaccharide, which suggests a compromised gut barrier function (Alhouayek, et al., 2011). Given these discrepancies as to the protective or detrimental roles for the eCB system in gut health and function, we examined the impact of THC (i.e., the primary intoxicating chemical in cannabis), as well as whole cannabis extracts, on gut barrier function in a diet-induced obese mouse model that displays a mild phenotype of disrupted barrier function. The knowledge gained from these investigations will provide critical insights into the therapeutic potential of cannabis as a treatment for diseases that affect gastrointestinal barrier function.

**Methods:** Male and female conditional intestinal epithelium-specific CB1R-deficient (intCB1R-/-), and control mice with functional CB1Rs in the intestinal epithelium (intCB1+/+) were given ad libitum access to water and either a low-fat/no-sucrose diet or a Western diet high in fat and sucrose for 60 days. Intestinal permeability was assessed utilizing 4kDa FITC-dextran 4 hours after oral gavage. After 8 weeks, mice were weight-matched into separate groups and administered 5mg/kg of pure $\Delta^9$ THC or whole cannabis extracts matched for $\Delta^9$ THC content for 14 days. Small and large intestine mucosa were scraped and flash-frozen for subsequent qPCR and lipid extractions.

**Results:** DIO mice displayed increases in intestinal permeability. Notably, pure $\Delta^9$ THC and whole cannabis extracts significantly reduced this effect in an intestinal CB1R- and sex-dependent manner, which suggests a protective effect for activating the eCB system with cannabis. Moreover, $\Delta^9$THC and whole cannabis extracts reversed DIO-induced downregulation in expression of the tight junction protein, occludin, and improved dysregulated expression of inflammatory genes in the small intestine in male mice.

**Conclusion:** These findings suggest that CB1Rs in the intestinal epithelium play a critical role in diet-induced gut barrier dysfunction, which can be reversed with cannabis exposure. Future studies will aim to further elucidate specific mechanisms underlying interactions between cannabis exposure, CB1Rs, and sex-dependent effects on gut barrier integrity.

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