ICRS 2026

36TH ANNUAL SYMPOSIUM ON THE CANNABINOIDS

CONFERENCE PROGRAM

JUNE 28–JULY 2, 2026 | DIJON, FRANCE

36TH ANNUAL SYMPOSIUM OF THE

INTERNATIONAL CANNABINOID RESEARCH SOCIETY

DIJON, FRANCE

JUNE 28 – JULY 2, 2026

Exhibition and Trade Center of Dijon
3 Bd de Champagne, 21000,
Dijon, France

International Cannabinoid Research Society
Winston Salem, NC, USA

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36th ANNUAL SYMPOSIUM ON THE CANNABINOIDS PRELIMINARY PROGRAM

Exhibition and Trade Center of Dijon,
3 Bd de Champagne, 21000, Dijon, France

Sunday, June 28, 2026	
16:00	**Registration** Exhibition and Trade Center of Dijon 3 Bd de Champagne, 21000, Dijon, France
18:00 - 20:00	**Welcome Reception** Cellier de Clairvaux 27 Bd de la Trémouille, 21000 Dijon, France

Day 1. Monday, June 29, 2026			
8:30-8:45	Welcome		
8:45-10:30	**Session 1. The Endocannabinoid System: Medicinal Chemistry, Chemical Biology, AI & Drug Discovery** **Chairs: Uwe Grether & Malliga Iyer**		
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19:00	**Gala Dinner Banquet** Le Stade Gaston-Gérard 17 Rue du Stade, 21000 Dijon, France		

Poster Session 1 - June 29, 2026			
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ORAL PRESENTATION ABSTRACTS

Session 1 Keynote Presentation

ORAL DECODING SUCCESS: THE NEW INGREDIENTS OF DRUG DISCOVERY ACCELERATING CLINICAL IMPACT THROUGH AI, HTE, CHEMICAL BIOLOGY, AND THE FIVE-STAR MATRIX

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The pervasive challenge of high attrition rates in drug development, particularly within neuroscience, necessitates a paradigm shift toward more integrated, predictive, and patient-centric research frameworks. This keynote lecture explores the synergistic integration of medicinal chemistry with four "new ingredients" designed to bridge the gap between bench and bedside: Artificial Intelligence (AI), High-Throughput Experimentation (HTE), Chemical Biology, and the "Five-Star Matrix" translational framework[1]. At the core of this holistic approach is the **Five-Star Matrix**, a comprehensive translational framework that sequentially links drug biodistribution, target binding, proximal functional effects, and distal biological outcomes to ultimate clinical disease effects. By identifying translatable biomarkers across diverse systems—ranging from biochemical assays to clinical patient populations—this matrix fosters cross-disciplinary collaboration and a "quick win/fail fast" mindset, ensuring that only the most robust hypotheses proceed to clinical trials. Modern medicinal chemistry is further empowered by the convergence of **AI and Machine Learning (ML)** with miniaturized **HTE**. This synergy enables the rapid exploration of vast chemical spaces through deep graph neural networks trained on high-quality experimental data sets. By incorporating synthetic feasibility and multi-dimensional optimization (MDO) from the outset, researchers can significantly reduce cycle times in the hit-to-lead phase while simultaneously optimizing potency and ADMET properties[2]. Furthermore, **Chemical Biology** provides the essential toolset for validating these transitions. The development of high-quality, labeled chemical probes and advanced technologies like nanoBRET and activity-based protein profiling (ABPP) allows for the direct measurement of target engagement and selectivity in physiological settings, providing vital confidence before advancing candidates. The utility of these integrated strategies will be illustrated through representative examples from **endocannabinoid system drug discovery programs**, including the development of selective monoacylglycerol lipase (MAGL) inhibitors. These case studies demonstrate how the combination of reaction prediction, structural insights, and a solid translational foundation can accelerate the delivery of innovative medicines with high clinical impact.

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BIOORTHOGONAL PROBES TO VISUALIZE ENDOCANNABINOIDS AND THEIR METABOLISM

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Introduction: Although the enzymatic machinery and mode-of-action of the endocannabinoids AEA and 2-AG have been relatively well explored, less is known about the transport of the lipids themselves. More specifically: how are endocannabinoids exported, traverse the synaptic cleft and eventually taken up for degradation? Traditional tools such as isotope labels lack sufficient spatiotemporal resolution to answer these questions.
Methods: We have synthesized analogues of AEA and 2-AG that bear a cyclopropene click handle (cAEA & c2-AG). The probes were visualized with confocal microscopy through reaction with a fluorogenic tetrazine. The metabolism of the probes was tracked with MS following reaction with a bespoke tetrazine. Model systems studied included (live) cell culture, *ex vivo* murine acute brain sections and *in vivo* administered mice.
Results: cAEA and c2-AG retained affinity for CB1/2 and activated the endocannabinoid sensor $GRAB_{eCB2.0}$. In addition, c2-AG was hydrolyzed by MAGL as efficiently as endogenous 2-AG. Live cell imaging in U87-MG cells revealed a higher cellular accumulation of c2-AG compared to cAEA. Furthermore, uptake of cAEA in Neuro2a cells could be abolished by pharmacological FAAH inhibition. Finally, the distribution of cAEA was visualized in *ex vivo* murine acute brain sections and post-mortem analysis of *in vivo* administered mice.
Conclusions: We show that cAEA and c2-AG retain characteristics of their endogenous counterparts. Since the methodology for visualization and metabolism is compatible with various model systems, cAEA and c2-AG will enhance the spatiotemporal resolution of future studies on endocannabinoid transport.

CHEMICAL PROBES FOR VISUALIZING AND UNLOCKING THE MULTIFACETED ROLE OF MONOACYLGLYCEROL LIPASE

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Introduction: Monoacylglycerol lipase (MAGL) is a pivotal catabolic serine hydrolase within the endocannabinoid system (ECS) and plays a crucial role in regulating the brain concentrations of 2-arachidonoylglycerol (2-AG). Inhibiting MAGL leads to an increase in 2-AG levels while simultaneously reducing the levels of arachidonic acid and pro-inflammatory eicosanoids in the central nervous system. This reduction in neuroinflammation suggests that MAGL inhibition holds significant therapeutic potential for the treatment of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Multiple Sclerosis (MS), and Amyotrophic Lateral Sclerosis (ALS), all of which are characterized by chronic neuroinflammatory processes. However, the distribution and role of MAGL in physiological and pathological processes remain poorly understood. **Methods and Results**: We report the design, synthesis, and validation of a versatile fluorescent probe platform targeting MAGL. Structure-based and reverse-design approaches enabled the development of reversible and irreversible probes that maintain high affinity and selectivity independent of the reporter unit. Incorporation of a BODIPY moiety into the ligand structure yielded miniaturized, drug-like probes with high solubility, permeability, and picomolar potency across species. The probes were extensively validated using biochemical assays, flow cytometry, and confocal imaging in live cancer cells, primary neurons, and human iPSC-derived brain organoids, and further demonstrated versatility through red-shifted analogs, 18F-labeling, and PROTAC design. **Conclusions:** The fluorescent probes enabled effective visualization of MAGL activity in ECS cellular signaling and inflammatory processes, determination of target occupancy in patient-derived cells, and monitoring of disease progression. These highly selective and potent probes offer powerful tools for studying and validating MAGL and its therapeutic potential in ECS-related disorders.

A NOVEL CB2R-BASED APPROACH TO SUPPRESS LEUKOCYTE GUT-HOMING AND ATTENUATE INFLAMMATION IN CROHN'S DISEASE WITH STABILISED PEPTIDE ANTAGONISTS

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Introduction: Endocannabinoid signaling via CB_2R on leukocytes represents a poorly understood regulator of immune function. We have previously demonstrated that $CD4^+$ T cell-specific CB_2R deficiency suppresses T cell trafficking to the murine small intestine. Conversely pharmacological CB_2R activation increases gut integrin expression, promoting adherence to the endothelium in.vitro. Our goal was to develop a novel CB_2R-based approach to suppress gut-homing and attenuate inflammation in Crohn's disease, an incurable chronic intestinal disease.

Methods: We are investigating the antagonism of this receptor with small, stable constrained peptides (Selektides) that we have discovered with selection campaigns against endogenously expressed receptor on human immortalized Jurkat T cells. A panel of 10 candidate antagonists have been examined for functional modulation of the receptor using glucose influx assays, β-arrestin recruitment, phospho-ERK signalling, and cell surface expression of the integrin α4β7.

Results: A lead candidate Selektide with an IC_{50} value of 5.4 nM was prepared for in.vivo study in a chronic model of Crohn's, the $TNF^{\Delta ARE/+}$ mouse. Subcutaneous administration in 8–12-week-old mice with fully developed chronic ileitis showed a marked reduction in infiltrating $CD4^+$ and $CD8^+$ T cells and in $CD19^+$ B cells. This effect was restricted to intestinal tissue and lymphoid tissue without impacting systemic immune phenotype. Detailed examination of the histology of tissue samples from the gut of these mice will establish the full therapeutic potential of CB_2R antagonism with these peptides.

Conclusion: We have identified a highly selective biologic inhibitor of CB_2R with favourable pharmacokinetics and demonstrable anti-inflammatory potential in.vivo.

UNBIASED IDENTIFICATION OF PUTATIVE CLINICAL BIOMARKERS OF TARGET ENGAGEMENT WITH THE SELECTIVE FATTY ACID-BINDING PROTEIN 5 INHIBITOR ART26.12

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Introduction: ART26.12 is a first-in-class reversible fatty acid-binding protein (FABP) 5 inhibitor that recently successfully completed a Single Ascending Dose study (ART26.12-100) in healthy volunteers. Due to its novel nature, clinically relevant biomarkers of target engagement are unknown. Discovery of target engagement biomarkers are important as early proof-of-mechanism and may help optimise dosing strategies and patient stratification in future studies. We set out to determine potential proteomic and lipidomic plasma biomarkers in an unbiased manner, assessing time-course data from healthy volunteers dosed with ART26.12 (300mg). **Methods:** Plasma from four patients dosed with ART26.12 (300 mg) was taken pre-dose and at 1h, 2h, 4h, 8h, 12h, 24h, and 48h, aliquoted and flash frozen. Untargeted proteomics was carried out by Creative Proteomics, using tandem mass tag mass spectroscopy (MS). Untargeted lipidomics was carried out by Creative Proteomics, using ultra performance liquid chromatography MS. Proteomic analysis was undertaken using Science Machine, an agentic AI bioinformatics platform that ran the specific requested algorithms and tools in the back end. Further proteomic and lipidomic analysis were undertaken in R. **Results:** Proteomic analysis identified widespread proteome changes, peaking at 4h. Proteins were grouped into nine clusters based on time-course/patient profiles, with enrichment seen in extracellular matrix (cluster 1-3), adaptive immunity (cluster 5), skin development (cluster 4) and protein translation (cluster 9). A network of proteins associated with FABP5 with high confidence was constructed (using the STRING database). Of 80 proteins in the network, 31 proteins were detected in the proteomics dataset, with 21 showing upregulation by ART26.12. Separately, statistical analysis of early timepoints and comparison with in-house proteomic preclinical datasets arrived at a concordant set of differentially expressed proteins, with consistent changes in alarmins (S100 family), apoliproteins (APO family), FABPs (FABP1, FABP2, FABP4) and MMPs (MMP9, MMP14). Lipidomic analysis showed that the number of lipids significantly different to baseline increased with each hour, peaking at 12 hours and falling at later timepoints. This was primarily driven by upregulated lipid species. Early changes surrounded phosphatidylcholine remodelling, enrichment of oxidised membrane phospholipids, and general downregulation of triglyceride species. Specific candidate biomarkers beginning at 1–2h and persisting through at least one timepoint include PCs (e.g, 12:0_12:0, 16:0_16:0, 24:1_12:1), oxidised membrane phospholipids (e.g., PG 17:4CHO) and oleic acid-containing triglycerides (e.g., 16:1_18:1_18:1). **Conclusions:** Unbiased proteomic and lipidomic analysis identified putative plasma biomarkers of FABP5 target engagement in healthy humans. These included proteins associated with lipid metabolism, inflammation, the extracellular matrix, membrane phospholipids and triglycerides. Further work is underway to confirm these results and link functionally with FABP5.

INSIGHTS INTO THE TETRAHYDROPYRIDAZINE MOLECULAR SCAFFOLD CLASS OF PERIPHERAL CB1 ANTAGONISTS FOR THE TREATMENT OF METABOLIC DISORDERS

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Introduction: The development of metabolic syndrome (MetS) often stems from obesity and contributes to comorbid conditions such as dyslipidemia, organ fibrosis, and cardiovascular or renal dysfunction. Multiple signaling pathways influence these chronic metabolic diseases, and the endocannabinoid (EC) system plays a well-recognized role in regulating MetS pathophysiology. Inhibiting cannabinoid-1 receptors (CB_1R) has been shown to improve metabolic abnormalities, including excess adiposity, insulin resistance, and hepatic steatosis. With the withdrawal of rimonabant—the first brain-penetrant CB_1R antagonist—research has shifted toward strategies that localize CB_1R blockade to peripheral tissues to avoid central effects. Continued efforts in this direction have shown promise in both preclinical and clinical studies, underscoring the need for novel therapeutic options targeting peripheral CB_1R antagonism.

Methods and Results: Building on our ongoing efforts, we have developed novel CB1 antagonists based on a tetrahydropyridazine molecular scaffold and amino acid pendants designed to minimize brain penetration. Using rational design, multiparameter optimization (MPO) scoring, and molecular dynamics (MD) simulations, we identified and prioritized compounds for detailed investigation. The most promising candidates were subsequently evaluated for their CB_1R inhibitory activity through comprehensive in vitro and in vivo studies.

Conclusion: We identified compounds having nanomolar affinity and selectivity for CB_1R and acting as functional antagonists. Based on our in vitro ADME results and in vivo PK studies, we advanced a lead compound (e.g PB75B). Results from this study and evaluation of this series of compounds as cannabinoid receptor antagonists along with their therapeutic potential in treating MetS will be presented.

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INVESTIGATING THE ROLE OF THE ENDOCANNABINOID SYSTEM IN THE PATHOPHYSIOLOGY OF RETINOPATHY OF PREMATURITY

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Introduction: Retinopathy of prematurity (ROP) is the leading cause of childhood blindness. Over the past decades, polyunsaturated fatty acids (PUFAs) have emerged as contributors to ROP pathophysiology and/or potential management. Interestingly, studies suggest that the PUFA-derivatives endocannabinoids (EC) regulate many cellular features such as inflammation and energy metabolism. Given that the retina, which is particularly rich in plasmalogens, is the most PUFA-enriched tissue in the body, we investigated whether the EC system (ECS) is involved in ROP pathophysiology.

Methods: Using the oxygen-induced retinopathy (OIR) mouse model, we combined lipidomics with transcriptomic and proteomic approaches to comprehensively characterize OIR-induced adaptations of the retinal ECS. A plasmalogen-deficient mouse model ($DAPAT^{-/-}$) was used to get complementary insight into the potential role of plasmalogens in the retinal ECS regulation.

Results: OIR was associated with pronounced lipidomic alterations in the mouse retina, characterized by significant modifications of phospholipids, especially PUFA-containing, and EC concentrations, notably anandamide (AEA), palmitoylethanolamide (PEA), and docosahexaenoyl ethanolamide (DHEA). Transcriptomic and proteomic analyses further revealed marked dysregulation of EC-related genes and proteins, including biosynthetic enzymes (NAPEPLD, DAGLA) and degrading enzymes (FAAH, MAGL), which were differentially up- or down-regulated. Our findings on $DAPAT^{-/-}$ mice also suggest that plasmalogens are involved in the regulation of the ECS in the retina.

Conclusions: This study provides the first evidence of ECS dysregulation in the retina in a mouse model of ROP. Identification of key molecular and cellular contributors advances our understanding of ROP pathophysiology and highlights EC signaling as a potential mechanistic axis in disease progression.

IMPACTS OF CO-ADMINISTERED ALCOHOL AND VAPORIZED CANNABIS ON SIMULATED DRIVING ABILITY AND ABUSE LIABILITY

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Introduction: Increasingly, alcohol/cannabis co-use is a world health concern. Controlled research on the interactive effects of vaporized cannabis and alcohol on driving and abuse liability, however, remains limited.

Methods: Eleven healthy adults (mean age=26 yrs, 27% female) completed 7 sessions in an ongoing, double-blind, placebo-controlled laboratory study. Participants self-administered drinks with placebo (PL; 0.0% target breath alcohol concentration [BAC]) or alcohol, in combination with vaporized cannabis (delta-9-tetrahydrocannabinol [D9-THC]) or PL (0mg D9-THC). Dose conditions were: PL+PL; PL+5mg D9-THC; PL+25mg D9-THC; 0.05% BAC+PL;
0.05% BAC+5mg D9-THC; 0.05% BAC+25mg D9-THC; and 0.08% BAC+PL. Simulated driving and subjective effects were measured before and repeatedly after dosing (up to 6.5 hours). Puff topography was recorded.

Results: Alcohol with 25mg D9-THC produced greater driving impairment than the same alcohol or cannabis dose alone (p=.002). Co-use driving impairment persisted up to 4.5 hours post-dosing, despite recovery of self-reported driving confidence. Driving impairment from 25mg D9-THC was similar in magnitude to 0.08% BAC; impairment from 0.05% BAC+25mg D9-THC exceeded 0.08% BAC. Cannabis/alcohol co-use increased drug liking (p<.001) versus alcohol alone. Alcohol increased cannabis puffing intensity (e.g., greater puff volumes; p=.043).

Conclusions: Alcohol and vaporized cannabis co-use exacerbated simulated driving impairment and increased abuse liability relative to cannabis or alcohol alone. Alcohol also intensified puffing behavior, potentially elevating D9-THC exposure. Thus, alcohol/cannabis co-use may increase the likelihood of negative outcomes such as automobile accidents, even at relatively modest doses. These findings highlight heightened alcohol/cannabis co-use risks and warrant consideration in global policy, treatment, and prevention efforts.

INHIBITION OF DIAGLYCEROL LIPASE DISRUPTS THE HEAD-TWITCH RESPONSE INDUCED BY PSILOCYBIN

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Introduction: Serotonergic psychedelics are emerging as promising therapeutics for psychiatric disorders due to their robust effects on mood, perception, and cognition. In rodents, agonism of the serotonin 2A receptor (5-HT2AR) induces the head-twitch response (HTR), a behavioral proxy for psychedelic activity which predicts hallucinogenic potency in humans. 5-HT2AR activation engages phospholipase C signaling to produce diacylglycerol (DAG), a key precursor for the endocannabinoid 2-arachidonoylglycerol (2-AG) via diacylglycerol lipase (DAGL). Although antagonism of cannabinoid type 1 receptors enhances the HTR, the specific contribution of DAG signaling and 2-AG synthesis to psychedelic-induced behavioral outcomes remains unclear.

Methods: Mice were pretreated with the DAGL inhibitor DO34 (30 mg/kg) two hours before psilocybin injection (0.5, 1, 2, or 3 mg/kg). Animals were then placed in a behavioral chamber and video recorded for 30 minutes. HTRs and other behaviors, including rearing and grooming, were quantified. To relate behavioral dynamics to eCB signaling, cortical 2-AG signal was recorded using fiber photometry over the course of psilocybin exposure, and advanced video tracking software for animal pose estimation (SLEAP) was used to extract time-locked behavioral motifs.

Results: Pretreatment with DO34 significantly reduced the number and altered the temporal distribution of HTRs following administration of psilocybin. DAGL inhibition also produced dose-dependent changes in cortical 2-AG signaling.

Conclusions: These findings implicate DAGL-dependent 2-AG synthesis as a downstream modulator of psychedelic-induced behavior. Ongoing studies combining targeted mass spectrometry and genetically encoded biosensors will quantify how psilocybin alters endocannabinoid dynamics, providing a deeper understanding of how 2-AG shapes behavior during psychedelic states.

HOW DOES CANNABIS USE AFFECT THE BRAIN'S REWARD SYSTEM? A 12-MONTH LONGITUDINAL FMRI STUDY OF ADULTS AND ADOLESCENTS WHO USE CANNABIS AND AGE-MATCHED CONTROLS

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Introduction: Substance use has been associated with blunted brain responses to non-drug rewards but findings in people who use cannabis are mixed. Adolescents may be uniquely vulnerable to cannabis-related disruption to reward processing due to ongoing neuromaturation, but longitudinal research is lacking.

Methods: In this longitudinal fMRI study, we compared brain measures of reward anticipation in 46 adolescents (16-17 years) and adults (26-29 years) who used cannabis (1-7 days/week) and 50 age-matched controls with the Monetary Incentive Delay task at baseline and 12-month follow-up. We looked at activity at the whole-brain level and in region of interest (ROI) analyses adjusted for cigarette/roll-up use, depression, and risk-taking.

Results: ROI analyses showed that reward anticipation activity decreased in the right (p=.05, η_p^2=.04) and left (p=.02, η_p^2=.05) ventral striatum from baseline to follow-up in participants who used cannabis compared with control participants. These effects remained in unadjusted models and when including only participants who consistently used or abstained from cannabis during the study period. There were no significant interactions between cannabis user-group and age-group, or between user-group, age-group, and time. There were also no cannabis user-group main or interaction effects in full sample ROI analyses for the thalamus, insula, or supplementary motor area, or in exploratory whole-brain analyses.

Conclusions: Cannabis use may be associated with reductions in non-drug reward anticipation activity in the ventral striatum, a key part of the brain's reward system. However, there was no evidence of adolescent resilience or vulnerability to cannabis-

related changes in brain reward anticipation activity.

ICRS 2026 Presidential Plenary Lecture 1: 12:00-13:00, Monday June 29, 2026

A DRUGGABLE ACSL4 ISOFORM MOONLIGHTING FUNCTION FACILITATES ENDOCANNABINOID PLASMA-MEMBRANE DIFFUSION AND TUNES CB1 RECEPTOR SIGNALING

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Biography: Jürg Gertsch studied cultural anthropology at the Universidad Central de Venezuela, and neurosciences and biochemistry at the University of Sussex and the Biozentrum Basel. He received his M.Sc. in Biochemistry (1997) and his doctorate in pharmacognosy, ethnobotany, and natural product chemistry (2002) from the Swiss Federal Institute of Technology Zurich, under the supervision of Drs. Otto Sticher and Sir Ghillean Prance. From 2003-2009, he was a postdoctoral researcher and group leader at the Institute of Pharmaceutical Sciences in the laboratory of Dr. Karl-Heinz Altmann. From 2009-2013, he was an assistant professor of membrane biochemistry, and a full professor of Biochemistry and Pharmaceutical Biology at the Medical Faculty of the University of Bern since 2014. Dr. Gertsch's research group focuses on the molecular pharmacology of the endocannabinoid system, general drug discovery, cannabinoid pharmacology, and translational research. Since 2014, he has served as deputy and co-director of the Institute of Biochemistry and Molecular Medicine at the University of Bern. He has co-organized IMCCB-25 in Bern and is a co-chair of the 2027 GRC on Cannabinoid Function in the CNS. He is a co-founder of Synendos Therapeutics and Tasteomics.

Abstract: The endocannabinoid system is a lipid signaling network involved in neuromodulation, metabolism, and immune response. Although the enzymatic control of endocannabinoid (eCB) synthesis and degradation is well understood, the mechanisms underlying eCB reuptake across the plasma membrane remain unclear. Using the WOBE437-derived clickable probe RX-237 we identified ACSL4 isoform 2 (ACSL4-iso2) as a bifunctional protein facilitating eCB diffusion across the plasma membrane while the enzymatic function activates arachidonic acid (AA) and recycles 2-arachidonoylglycerol. We describe a druggable moonlighting function that transports eCBs and drives arachidonate incorporation into the plasma membrane. Using tailored genetic and pharmacological tools, we selectively inhibited both functions. CB_1R signaling was modulated by ACSL4-iso2 through high-affinity binding of eCBs and by remodeling plasma membrane lipid composition, impacting passive diffusion of bioactive lipids, and supporting retrograde eCB signaling at synapses. We propose a model in which eCB diffusion at plasma membrane and AA metabolism jointly sustain the autocrine eCB tone. Our study uncovers a druggable mechanism for facilitated eCB plasma membrane diffusion that mediates the pharmacological effects of selective endocannabinoid reuptake inhibitors including SYT-510, which is in clinical development.

THE CANNABIDIOL FOR ACUTE PSYCHOSOCIAL STRESS AND NAUSEA (CAPSTAN) CLINICAL TRIAL

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Introduction: Clinical trials suggest anxiolytic effects of cannabidiol (CBD) at doses ≥ 300 mg while preclinical research has described anti-nausea effects of CBD in animal models. Few studies have investigated the efficacy of lower oral doses (≤ 150 mg) of CBD, typical of over-the-counter products. Here, we used a series of virtual reality (VR) tasks to investigate the acute effects of 150 mg CBD on anxiety and nausea.

Methods: A randomised, double-blind, placebo-controlled, parallel-group clinical trial involved healthy volunteers ≥ 18 years of age. Participants were administered a single dose of 150 mg CBD or placebo 90 minutes before completing three custom-programmed VR challenges. These were the 'Public Speaking', 'Walk the Plank' and 'Rollercoaster Ride' tasks, designed to elicit social anxiety, fear of heights and motion sickness respectively. The primary outcomes were subjective anxiety and nausea measured on 100 mm visual analogue scales (VASs) throughout the test session. Secondary outcomes included salivary cortisol, heart rate, skin conductance and other subjective feelings (i.e., calm, bored, tense and excited).

Results: Sixty-nine participants were randomised and all were included in the final sample. The Public Speaking and Rollercoaster Ride tasks effectively increased subjective ratings of anxiety and nausea, respectively. CBD significantly reduced self-reported anxiety immediately after the Public Speaking task ($p=0.0485$, Dunn-Šidák corrected) but had no significant effects on nausea or other secondary outcomes.

Conclusion: CBD exhibited modest anxiolytic effects on psychosocial stress but no effects on nausea. This study confirms the feasibility and effectiveness of VR approaches in pharmacological studies of anxiety and nausea in healthy participants.

ACUTE AND LONGER- TERM EFFECTS OF EDIBLE CANNABIS USE OF DIFFERING CANNABINOID RATIOS FOR OLDER ADULTS USING FOR PAIN, SLEEP, AND MOOD PROBLEMS

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Introduction: Many older adults use cannabis for pain, sleep problems, and mood, but few studies rigorously assess the products, dosages, cannabinoid profile, or effects of cannabis in older adults.

Methods: We recruited 326 older adults (Mage=69.8) who either did (n=267) or did not (n=69) want to use edible cannabis for pain, sleep disturbance, or negative mood. Participants chose and used either CBD dominant, THC dominant, or CBD+THC edible products ad libitum for 4 weeks and during an acute use session in our mobile pharmacology laboratory.

Results: Cannabis use was associated with acute improvement in pain (p=.001), and there was a significant CBD x THC x time interaction $F(1,306) = 5.40$, $p<.05$): at 0 mg of THC, there was no association between CBD mg and pain relief, while at 15 and 30 mg of THC, more mg of CBD produced more pain relief. Higher THC in blood was associated with greater intoxication ($p<.001$) while higher CBD in blood was associated with lower intoxication ($p<.05$). Sleep disturbance significantly decreased over the four weeks of use for the CBD+THC and THC groups (p's<.001) and marginally decreased for the CBD group (p=.076), with no change for the non-use group (p=.796). There were few changes in pain or in mood in any group over four weeks.

Conclusions: This study adds to the growing literature examining recreational market cannabis use among older adults and identifies significant outcomes experienced by those who use varying cannabinoid ratios of edible cannabis products for common complaints of aging.

PRIMARY RESULTS OF A RANDOMIZED CLINICAL TRIAL OF VAPORIZED CANNABIS VERSUS ORAL OXYCODONE FOR CHRONIC SPINE PAIN

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Introduction: Chronic spine pain is a highly prevalent and disabling musculoskeletal condition for which opioids are commonly prescribed, but are often ineffective. Cannabinoids may be beneficial for pain management in this population, but high quality studies are lacking. The purpose of this double-blind crossover trial was to compare the acute analgesic effects of cannabis to a common opioid for chronic spine pain.

Methods: Participants attended 3 separate 4-hour visits where they received one of the following drugs: active vaporized cannabis (5.4% delta-9 tetrahydrocannabinol, THC), active oxycodone (5-10mg), or placebo. The primary outcome was change in numerical rating scale (NRS) pain scores (0-10 points) after drug administration; secondary outcomes included Patient Reported Global Impression of Change (PGIC) scores.

Results: 35 participants with chronic spine pain (17 males, 16 females, 2 unspecified; mean age 46.6 years) were randomized in this trial. Cannabis led to significantly greater reductions in NRS scores as compared to oxycodone ($p<0.001$ at 10 minutes and 1-hour post-administration) as well as placebo ($p<0.001$ at all timepoints). These reductions in NRS pain scores further met the criteria for Substantial Clinical Benefit. No significant differences in NRS scores were seen between oxycodone and placebo at any timepoint. PGIC scores were also significantly higher in the cannabis administration condition ($p<0.001$).

Conclusions: Vaporized cannabis produced a rapid onset in chronic spine pain relief that was superior to a commonly prescribed opioid. We are currently conducting a follow-up trial to study the analgesic effects of long-term daily use of cannabis for chronic spine pain.

IMPACTS OF MINOR CANNABINOIDS AND TERPENES ON MENOPAUSE AND PERIMENOPAUSE SYMPTOMS

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Introduction: The menopausal transition is associated with adverse symptoms, and existing treatments often involve significant side effects and risks. Many midlife women report using cannabis to manage menopause- and perimenopause-related symptoms, yet the efficacy of cannabinoids for these symptoms remains largely unexplored. However, evidence from other populations suggests that cannabinoids like cannabidiol (CBD) and cannabigerol (CBG) may reduce common menopause symptoms (e.g., anxiety, pain), highlighting a critical public health gap.

Methods: Using a longitudinal, double-blind, placebo-controlled clinical trial, we assessed the effects of a hemp-derived minor cannabinoid (e.g., CBD, CBG) and terpene (e.g., linalool) formulation on psychological and cognitive symptoms of perimenopause and menopause. 104 healthy midlife women were randomized to cannabinoid or placebo conditions. Participants completed symptom questionnaires, cognitive assessments, and twice-daily ecological momentary assessments (EMAs) of symptom severity over three months. The first month served as a no-treatment baseline, followed by two months of twice-daily oral administration of the product or placebo.

Results: Preliminary mixed factorial ANOVAs revealed several main effects of time, no main effects of product, and one significant interaction. Specifically, the placebo group reported higher EMA-rated depression severity during baseline compared to subsequent months (*p*s
< .05). Side effects were minimal, did not differ by condition, and no participants reported intoxication or impairment.

Conclusions: Null product effects may reflect insufficient tetrahydrocannabinol (THC) content and/or analytic limitations. Multilevel modeling is underway to more sensitively assess longitudinal change and potential moderators (e.g., hormonal therapy use). Continued research on cannabinoid-based interventions must remain a priority in women's health.

EXAMINING THE PSYCHOLOGICAL AND PHYSIOLOGICAL EFFECTS OF CANNABIGEROL (CBG) IN A DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSSOVER CLINICAL TRIAL

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Introduction: Cannabigerol (CBG) is a minor cannabinoid that may have therapeutic properties, but human clinical trials are needed to examine these effects and potential side effects. The current study was designed to examine the acute effects of CBG on psychological (anxiety, stress, mood, and memory) and physiological (pain tolerance, blood pressure, pulse, brain activity) outcomes.

Methods: A double-blind, placebo-controlled, crossover trial was designed to investigate the effects of 50 mg of CBG relative to placebo. Participants (N = 100) are randomly assigned to ingest either 50 mg of CBG or placebo in one session and the other product in a second session (separated by one week). Participants provide assessments of blood pressure, pain tolerance, brain activity, anxiety, stress, mood, intoxication, impairment, and side effects prior to ingesting each product (T0) and at three timepoints after ingesting each product.

Results: Preliminary results (*n* = 66) revealed an effect of CBG on changes in anxiety such that anxiety was reduced significantly more in the CBG condition than placebo condition. Further, short-term memory test performance was superior in the CBG condition relative to the placebo condition. There were no effects of CBG, relative to placebo on the other outcomes and there was no evidence of impairment, intoxication, or side effects.

Conclusions: This study provides additional support for CBG as an anxiolytic in humans and indicates that CBG may have a subtle positive impact on memory without intoxication or impairment. This work should help inform future clinical trials and consumers of CBG.

Mary E. Abood ICRS Women in Cannabinoid Research Award Lecture: 15:45-16:15, Monday June 29, 2026

EMBRACING COMPLEXITY IN CANNABIS CLINICAL SCIENCE: USING MACHINE LEARNING TO IDENTIFY BIOMARKRS OF CANNABIS USE DISORDER

Cinnamon Bidwell, PhD
Associate Professor, Institute of Cognitive Science
University of Colorado Boulder

Introduction: Cannabis use is increasing globally, with expanding product diversity and potency outpacing the scientific understanding of its health effects. My research program has sought to characterize the behavioral, clinical, and biological effects of cannabis across product forms, focusing on THC exposure, endocannabinoid signaling, and their links to risk, abuse liability, and health outcomes. This work has evolved toward an integrative framework that situates exogenous cannabinoids within a broader lipid signaling system, incorporating endocannabinoid tone and downstream lipid mediators to better capture the complexity of cannabis-related biology. **Methods:** Across a series of studies, we combine controlled laboratory, naturalistic, and clinical approaches with multimodal biomarker assessment. In the current work, machine learning was applied to high-dimensional biobehavioral data spanning endocannabinoids, exogenous cannabinoids, and lipid mediators to identify multivariate signatures of cannabis use disorder (CUD). Harmonized data from cannabis-focused cohorts (Discovery: N = 504; Validation: N = 181) were analyzed using elastic net regression with nested cross-validation (10 outer, 5 inner folds). Three biomarker panels, cannabinoids (16), endocannabinoids (17), and lipid mediators (55), were modeled individually and in combination to evaluate associations with CUD. **Results:** Models integrating all three biomarker panels demonstrated the strongest performance in the discovery sample (RMSE = 1.34, R^2 = 0.35) and were significantly associated with CUD in an independent validation sample (p = .03). Lipid mediator models alone were also significant (p = .01), whereas models excluding lipid mediators or relying solely on cannabinoids or endocannabinoids showed minimal associations. Across studies, findings highlight that THC exposure and endocannabinoid tone are important but insufficient in isolation, and that broader lipid signaling networks provide more robust and consistent associations with risk-related outcomes.**Conclusions:** This body of work underscores the need to move beyond reductionist approaches in cannabis science toward multilevel, systems-based models that integrate biological, behavioral, and real-world data. By combining multimodal datasets with machine learning approaches, this research advances the identification of biologically grounded, generalizable signatures of cannabis-related risk and supports the development of more precise, translational frameworks for understanding cannabis use and disorder in the context of modern, high-potency exposures.

ICRS Mid-Career Award Lecture: 8:45-9:15, Tuesday, June 30, 2026

INTEGRATING ENDOCANNABINOID AND PHYTOCANNABINOID SCIENCE TO ADDRESS PAIN AND INFLAMMATION

Steven G. Kinsey, PhD
Professor, Elisabeth Deluca School of Nursing Director,
Center for the Advancement in Managing Pain
University of Connecticut, Storrs, CT USA

Biography: Dr. Steven G. Kinsey is a Professor of Nursing and Director of the Center for Advancement in Managing Pain at the University of Connecticut. His research program investigates how endocannabinoid and phytocannabinoid signaling regulate pain, inflammation, stress responsivity, and cannabinoid dependence. He has published extensively on the pharmacology of monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) inhibitors, demonstrating their efficacy in reducing neuropathic pain, inflammatory arthritis, and opioid-related hyperalgesia. His work has also defined behavioral and neurobiological mechanisms of cannabinoid withdrawal, including studies of Δ^9-THC, synthetic cannabinoids, and CB_1 positive allosteric modulators. More recently, his laboratory has expanded to examine the analgesic and anti-inflammatory potential of minor phytocannabinoids and their interactions with established pain pathways. Dr. Kinsey's research has been continuously supported by the National Institutes of Health for more than a decade. He is committed to advancing rigorous cannabinoid science and has served on the ICRS Board of Directors since 2013, as Secretary, Treasurer, and currently as Executive Director.

Abstract: Cannabinoid research continues to reveal powerful opportunities for treating chronic pain, inflammation, and substance use disorders. My work has focused on defining how endocannabinoid signaling regulates neuropathic and inflammatory pain, with particular emphasis on targeting metabolic enzymes such as monoacylglycerol lipase (MAGL). In preclinical models, MAGL inhibition produces robust reductions in mechanical and cold allodynia, attenuates inflammatory arthritis-induced swelling and functional deficits, and interacts synergistically with established analgesics. These findings highlight the therapeutic potential of enzyme targeted approaches that enhance endogenous cannabinoid tone while minimizing the cognitive and abuse related liabilities associated with CB1 orthosteric agonists. Our recent work has expanded to examine minor phytocannabinoids, including CBN, CBC, CBL, and Δ8 THC, which display distinct pharmacological profiles. Early evidence suggests that these lesser studied constituents of Cannabis sativa may modulate pain and inflammation through partially overlapping but mechanistically unique pathways, opening new avenues for therapeutic development. As part of receiving the ICRS Mid-Career Award, this lecture will also reflect on the essential role the International Cannabinoid Research Society has played in shaping my scientific trajectory from early trainee presentations to collaborations that have defined major directions in my program of research.

NEUROMETABOLIC ASPECTS OF CANNABIDIOL MECHANISMS IN A MITOCHONDRIAL DISEASE MODEL

Gunter van der Walt[*1], Armin Kouchaeknejad[1], Jianshen Yu[1], Inés Reynoso[3], Albert Quintana[1,2,] Jürg Gertsch[3], Emma Puighermanal[1]

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Introduction: Mice with constitutive knockout of the mitochondrial respiratory chain protein NDUFS4 (Ndufs4KO) develop a fatal encephalomyopathy, effectively recapitulating the phenotype of human Leigh Syndrome, the most prevalent primary mitochondrial disease (MD). Daily Cannabidiol (CBD) treatment extends lifespan and broadly rescues the phenotype of Ndufs4KO mice. Although metabolic alterations represent a key intersection of MD neuropathology and CBD pharmacology, their interactive effect on brain metabolism remains unknown. This study aims to elucidate the neurometabolic aspects of CBD therapeutic mechanisms in MD.

Methods: Ndufs4KO and control mice were chronically treated with CBD (100 mg/kg, i.p.) or vehicle from weaning until endpoint. Brains were extracted for LC-MS metabolite quantification against panels of glycerophospholipid, amino acid and neurotransmitter standards. After data normalization, metabolites were selected by statistical significance (Student's t-test $p<0.05$) and effect size (Cohen's $D>1.0$) for pathway analysis.

Results: Of the 67 metabolites measured, 34 were significantly altered in Ndufs4KO brains, implicating arachidonic acid, phospholipid, glutamate and sulfonic amino acid metabolism. CBD treatment ameliorated KO-driven depletions of key homeostatic metabolites □aspartate, oxylipins and ethanolamine endocannabinoids□ while bolstering protective glutathione, taurine, GABA and glycine levels. These metabolic shifts align with pallidal endocannabinoid receptor transcript changes and GABAergic dysregulation underlying epilepsy in NDUFS4 models.

Conclusion: These data provide the first targeted metabolomics evidence supporting CBD's multimodal therapeutic mechanisms in MD, including antioxidant activity, lipid-remodelling and neuroprotection. By implicating brain-region specific responses to alterations in global endocannabinoid tone, this work highlights the often-overlooked metabolic aspects of CBD neuropharmacology, supporting a systems biology view of cannabinoid biology in neurological disorders.

CHRONIC CANNABIDIOL RESTRUCTURES THE ENDOCANNABINOID LANDSCAPE IN AGED HIV-1 TAT TRANSGENIC MICE

Barkha J Yadav-Samudrala*[1], Morgan L Johnson[1], Ashima Ale[1], Katherine Jiang[1], William Lee[1], Amy Nguyen[1], Dorian Ho[1], Essie Acquah[1], Isabella C Orsucci[1], Laith E Sawaqed[1], Gabriella Boyer[1], Justin L Poklis[2], Wei Jiang[3,4], Sylvia Fitting[1]

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Introduction: As the population of older individuals living with HIV expands, identifying non-euphoric therapeutic interventions for HIV-associated complications is vital. While cannabidiol (CBD) shows neuroprotective potential, its effects following prolonged exposure in an aging population remain poorly understood. This study evaluates the impact of chronic CBD on behavioral outcomes and endocannabinoid (eCB) signaling in aged male and female HIV-1 Tat transgenic mice.

Methods: Aged (15–18 months) male and female Tat transgenic mice received chronic CBD (3 mg/kg, s.c.) for 12 weeks. Behavioral assays assessed object recognition memory, anxiety, nociception (tail flick/hot plate), and locomotion. Post-mortem, liquid chromatography-tandem mass spectrometry and western blot mapped eCB ligands (AEA, 2-AG, AA), metabolic enzymes (FAAH, MAGL), and receptors (CB_1R, CB_2R, GPR55) across the prefrontal cortex, amygdala, brainstem, and spinal cord.

Results: Chronic CBD improved recognition memory specifically in female mice. While CBD did not affect spinal tail-flick sensitivity, it universally increased supraspinal hot plate sensitivity. Treatment also elevated baseline body temperature and modulated body mass without impairing locomotion. Critically, CBD restructured the eCB landscape across all CNS regions in a highly sex- and genotype-dependent manner. Cannabinoid receptor and GPR55 expression exhibited distinct regulatory shifts governed by significant three-way interactions (sex x genotype x treatment).

Conclusion: These findings demonstrate that chronic CBD interacts with the eCB system of aged mice across all CNS regions in a sex- and genotype specific manner. The results underscore the necessity of considering age, sex, and treatment duration when evaluating cannabinoid-based therapies for managing neuroHIV.

CHRONIC NEUROINFLAMMATION IS REQUIRED TO ALLOW CB_2-MEDIATED CONTROL OF IN.VIVO MICROGLIAL FUNCTION

Julián Romero*[1], Isabel Bravo Pérez-Plá[1], Samuel Ruíz de Martín Esteban[1], Laura Álvarez Gallardo[1], Almudena López Escobar[1], Laura Martín Pérez[1], Ana M. Martínez-Relimpio[1], Iván Rodríguez-Martín[1], Ricardo Mostany[2], Cecilia J. Hillard[3], M. Teresa Grande[1]

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Introduction: Cannabinoid CB_2 receptors are expressed by microglial cells. Their expression in the healthy brain is low and is dramatically increased under chronic neuroinflammatory conditions. Methodological aspects of currently available methods have limited our understanding of CB_2 pathophysiological relevance.
Methods: We used in.vivo two-photon microscopy to study the role that CB_2 receptors may play in microglial cells. This approach allows the study of microglia in its own biological context with minimal disruption. We generated several strains of transgenic mice and performed laser-induced micro-lesions to the brain cortex. We compared microglial responses in normal, physiological conditions ($Cx3cr1^{GFP}$ and $Cx3cr1^{GFP}/CB_2^{-/-}$ mice), as well as in a model of chronic neuroinflammation ($5xFAD/Cx3cr1^{GFP}$ and $5xFAD/Cx3cr1^{GFP}/CB_2^{-/-}$mice), in which amyloid deposition triggers an intense inflammatory response. These mice express green fluorescent protein (GFP) in microglial cells only, thus allowing the measurement of morphological changes (soma and processes) after laser-induced injury.
Results: The acute injury to the brain parenchyma induced a profound morphological rearrangement of nearby microglia, as previously described, in both physiological as well as pathological conditions. In the absence of chronic neuroinflammation, CB_2 deletion did not modify microglial morphological features. In the context of chronic neuroinflammation, however, the absence of CB_2 led to significant changes in microglial soma volume and sphericity as well as in the volume and length of cell processes.
Conclusions: Our results confirm that CB_2 receptors play a significant role in microglial function only in the context of chronic neuroinflammation, being negligible when the damage is induced in the healthy brain.

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EXPLORING BIASED SIGNALING OF CLINICAL AGONISTS FOR THE CANNABINOID CB2 RECEPTOR WITH A NOVEL FUNCTIONAL MULTIPLEX ASSAY

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Introduction: Activation of cannabinoid receptors holds immense therapeutic potential in various pathological conditions due to its wide involvement in cellular signalling. However, most CBR agonists fail in clinical trials due to lack of efficacy, low receptor selectivity or adverse effects. Studying biased signalling, where activation of one pathway is preferred over another, can contribute to more successful CBR clinical outcomes. Hence, a more representative method to distinguish biased signalling might help combat obstacles in successful CBR drug design.

Methods: In this project, we developed a multiplex assay that simultaneously detects CB_2R agonist-mediated cAMP inhibition via GloSensor technology and β-arrestin-2 recruitment via Nanobit system in one cellular system. The set-up was validated using reference agonist CP55,940. With radioligand binding studies, binding affinity profiles were determined for CBR benchmark agonists, endocannabinoids and clinical agonists. Next, we explored functional responses of these CB_1 and CB_2 receptor clinical agonists and calculated bias factors to show the applicability of the assay.

Results: Foremost, we present a robust multiplex assay for reliable detection of functional bias between cAMP inhibition and β-arrestin-2 recruitment for which two luminescent signals of separate technologies can be accurately distinguished. For CB_1R clinical agonist JWH145, we were able to show modality bias as β-arrestin-2 recruitment is not activated at all. Furthermore, testing clinical CB_2R agonists in the multiplex assay resulted in observation of trends for biased signalling, but not in significant bias.

Conclusions: In conclusion, we have developed a multiplex assay for CBreceptors that can detect biased agonism. This provides new advances for drug design and discovery for cannabinoid receptors. Ultimately, this can be utilized to relate diverging agonist- or disease-induced functional responses to desired clinical outcomes.

NOVEL, POTENT CB1 ALLOSTERIC MODULATORS

Ruth A. Ross[1], Claudia Lutelmowski[1], Kim S. Sugamori[1], Catharine A. Mielnik[1], Iain R. Greig[2]

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Introduction: The CB_1 receptor allosteric modulator, ABM300, ameliorates psychosis-type behaviours in the dopamine transporter knockout (DATKO) genetic mouse model of hyperdopaminergia (Mielnik *et al.*, 2021). Here we identify novel CB_1 allosteric modulators that have improved potency, metabolically stability and brain penetration.

Methods: Molecules were screened *in vitro* against CP55, 940-induced β-arrestin recruitment (Eurofins PathHunter® assay), inhibition of cAMP and pERK1/2 phosphorylation in PathHunter® CHO-K1 CNR1 cells (Eurofins DiscoverX). Molecules were tested for *in vivo* efficacy on psychosis-like behaviours in DATKO mice.

Results: We identified potent oxadiazole-indole (ABM480) and indole sulfonamides (ABD1085). They displayed similar *in vitro* effects on CB1 agonist signalling to ABM300 as inhibitors of CP-55,940 mediated β-arrestin recruitment (IC_{50} values 5-30 nM) and cAMP inhibition (IC_{50} values 300-600 nM). However, ABD1085 was significant less potent as an inhibitor of CB1-agonist induced pERK. Unlike ABM300 and ABM480, both of which significantly increased [^{3}H]CP binding, ABM1085 had no effect on CB1 agonist binding. Pharmacokinetic analysis revealed that ABM480 and ABD1085 achieved significantly higher and more prolonged concentrations in the brain compared to ABM300. *In vivo*, ABM480 was highly potent, decreasing hyperactive exploratory phenotypes in the DATKO genetic model at 1mg/kg while ABD1085 (10 mg/kg) had no effect.

Conclusions: The unexpected lack of efficacy of ABD1085 in hyperdopaminergic models as compared to the PAM antagonists (ABM300 and ABM480) may be related to the fact that it is less potent as an inhibitor of CB1 agonist induced pERK signalling and / or, that it is a β NAM.

Acknowledgements: This project was funded by CIHR grant (PJT-173364).
Mielnik *et al.*, Neuropsychopharmacology. 2021 46(2):413-422.

INVESTIGATING THE LINK BETWEEN EARLY LIFE STRESS, SOCIAL STRESS RESPONSE, AND IN-VIVO BRAIN FATTY ACID AMIDE HYDROLASE LEVELS IN INDIVIDUALS WITH SOCIAL ANXIETY DISORDER

Christina F. Pereira[1,5*], Rheina Firdiawati[1,3], Pablo M. Rusjan[6], Rachel F. Tyndale[1,3,4], M. Ishrat Husain[1,4], Jerry Warsh[1,4,5], Bernard Le Foll[1-5,7], Gwyneth Zai[1,4,5], Isabelle Boileau[1,3-5], Stefan Kloiber[1,3-5]

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BACKGROUND: Early life stress (ELS) increases the risk of Social Anxiety Disorder (SAD). Alterations in the endocannabinoid system (ECS) enzyme Fatty Acid Amide Hydrolase (FAAH) after stress have been reported in preclinical research, but clinical research is limited. This analysis examined the relationship between ELS, brain FAAH levels, and response to social stress using the Montreal Imaging Stress Task (MIST).

METHODS: Participants with SAD (n=19, M/F:9/10; age:25.16±5.80) completed the Childhood Trauma Questionnaire (CTQ), positron emission tomography (PET) scanning with the FAAH probe ([^{11}C]CURB), *FAAH* C385A genotyping, and visual analogue scales (VAS) of 10 emotional domains during the MIST. Partial correlations with FAAH genotype assessed the relationships between brain FAAH, CTQ, and VAS scores. Pearson's correlations assessed the relationship between CTQ and VAS scores.

RESULTS: CTQ Emotional Abuse scores were significantly positively correlated with FAAH levels in whole brain (r=0.584,p=0.014); insula; hippocampus; thalamus; and prefrontal, temporal, and parietal cortices ($p<0.05$). Whole brain FAAH levels were significantly positively correlated with MIST-induced increases in feeling overwhelmed (r=0.589, p=0.034). Significant negative correlations emerged between Emotional Abuse scores and MIST-induced changes in happiness (r=-0.633,p=0.011), confidence (-0.752,p=0.001), and insecurity (r=-0.640,p=0.010).

CONCLUSIONS: Our preliminary findings address a critical gap in knowledge of the neurobiological mechanisms linking childhood trauma and SAD; suggesting a potential link between early negative experiences, altered social stress response, and in-vivo FAAH levels. Future work replicating these findings and investigating how ELS may impact the ECS and social stress response is needed to understand this complex relationship and potentially support development of ECS-targeting treatments.

SYT-510, A CLINICAL CANDIDATE FOR A FIRST-IN-CLASS SELECTIVE ENDOCANNABINOID REUPTAKE INHIBITOR, DEMONSTRATES EFFICACY IN MULTIPLE PRELCLINICAL CNS DISEASE MODELS AND A FAVORABLE PHASE 1 SAFETY PROFILE

Andrea Chicca*[1,2], Inés Reynoso-Moreno[2], Celine Maeder[1], Txomin Lalanne[3], Luis Fernando Quiñones Olvera[2], Sandra Glasmacher[1], Juerg Gertsch[2]

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Introduction: The endocannabinoid system (ECS) exerts a key neuromodulatory role in the brain. Its dysfunction has been implicated in a variety of CNS conditions such as anxiety, movement disorders, seizures and neuroinflammation. While ECS modulators have been tested in humans with some therapeutic benefits, more effective modulators with better specificity and efficacy are needed.

Methods*: In vitro* assays were employed to characterize SYT-510's mode of action and specificity. *Ex vivo* electrophysiological recordings in brain slices were used to gain insight on the neuromodulatory function of SERIs. *In vivo* animal models were utilized to study the efficacy of SYT-510 on symptoms relevant to CNS disorders.

Results: SYT-510 inhibits AEA and 2-AG cell reuptake by targeting a newly identified endocannabinoid membrane transporter. In brain slices, SYT-510 effectively modulated CB1R-mediated short-term synaptic plasticity in regions relevant to movement, learning and memory, seizure and anxiety. The effect is lost by knocking down the eCB membrane transporter. SYT-510 showed pharmacological effects in multiple animal models of CNS disorders which correlated with moderate increases in eCB levels. SYT-510 does not elicit sedation, abuse potential and psychotropic effects.

Conclusions: SYT-510 is the first candidate of SERIs, a novel class of ECS modulators that potently and selectively inhibit endocannabinoid reuptake, thereby rebalancing and gently enhancing the ECS to ultimately restore normal brain function under disease conditions. SERIs show broad therapeutic potential across multiple CNS disorders. SYT-510 is currently in Phase 2 clinical development.

A NOVEL CANNABINOID RECEPTOR 1 (CB1) PAM-ANTAGONIST DAMPENS AMPHETAMINE-INDUCED DOPAMINE LEVELS; EVIDENCE OF CB1-MEDIATED INDIRECT MODULATION OF HIGH DA STATES

Catharine A. Mielnik*[1], Brandon L. Oliver[2], Alexandra Arcenas[2], Natalie E. Zlebnik[2], Ruth A. Ross[1]

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Background: Several psychiatric conditions are linked to dopamine (DA) dysregulation (DA pathologies). D2-targeting compounds the gold standard for "antipsychotics". We focus on indirect modulation of DA via endocannabinoid (EC) signaling. Cannabinoid Receptor 1 (CB1) is a potential target in DA pathologies; EC system integrates DA signaling. Based on targeting DA directly pitfalls, indirect targeting may be advantageous. CB1 offers indirect modulation. We show proof-of-principle via novel class of CB1 allosteric modulators; CB1 positive allosteric modulator (PAM)-antagonists. CB1 PAM-antagonists increase agonist affinity while blocking signalling. HyperDA is likely exacerbated by positive feedback loop between DA and 2-arachidonoylglycerol (2-AG). High levels of 2-AG/CB1 signalling appear to drive the pathology. We will measure DA levels *in vivo* to establish a mechanism of action for our novel CB1 PAM-antagonists.

Methods: Using amphetamine-induced hyperDA model, we measure changes in DA release following administration of CB1 PAM-antagonist (ABM300, 10 mg/kg i.p. 30-min pre-treatment) in C57Bl/6J mice. Using fluorescent $GRAB_{DA}$ sensor to record DA, we simultaneously measure DLS/NAc DA release during open-field exploration.

Results: Pre-treatment with ABM300 dampens amphetamine-induced DA release, decreasing area under the curve (AUC) in DA signal; DLS (AUC: $p=0.016$), NAc (AUC: $p=0.0676$). DA transient peak width/peak prominence were unchanged in DLS/NAc. DA dynamics mirror behavioural effects; ABM300 dampens amphetamine-induced psychomotor activity.

Conclusion: We provide mechanistic insight for indirect modulation of DA occurring in the striatum via CB1 using our novel CB1 PAM-antagonist. PAM-antagonists could be a "seek and destroy" mechanism that targets agonist-activated GPCRs, offering a novel approach to generate "antipsychotic"-like molecules.

THE ROLE OF THE ENDOCANNABINOID SYSTEM IN ALCOHOL USE DISORDER (AUD): NOVEL INSIGHTS FROM A PROSPECTIVE CLINICAL STUDY

Sara L. Kroll*[1,2], Jürg Gertsch[3], Ines Reynoso[3], Vinzenz C. Schmid[1], Kathrin Stilinovic[4], Olena Zhabenko[4], and Boris B. Quednow[2,4]

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Background: Alcohol use disorder (AUD) remains a major global health burden, and effective treatments for abstinence are still lacking. Emerging preclinical evidence indicates a link between the endocannabinoid system (ECS) - with its main endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) - and AUD. Here, we investigated the ECS involvement in AUD in a larger human sample at treatment entry and over the time course of abstinence.

Methods: Plasma endocannabinoid levels were assessed in 42 individuals with AUD at treatment entrance (T0) and 40 healthy controls. Additional data were collected 2-3 weeks (N=38), 3 months (N=27), and 6 months (N=28) after treatment onset within AUD. Moreover, the motive for drinking alcohol was investigated.

Results: We found significantly elevated plasma levels of 2-AG ($F(1,74)=9.4$, $p=.003$, $d=.65$) and AEA ($F(1,77)=12.0$, $p<.001$, $d=.78$) in individuals with AUD compared to controls at T0. Linear mixed models showed a significant TIME effect ($t(1,20.5)=-3.3$, $p=.003$) and at trend level TIME*Abstinence interaction ($t(1,21.0)= 1.84$, $p=.080$) for 2-AG. The motive to drink for reward was a significant predictor of elevated basal 2-AG levels ($B=.09$, $p=.046$), whereas the motive to drink due to relief was a predictor at trend level of higher basal AEA levels ($B=.014$, $p=.075$).

Conclusion: Our findings provide novel translational evidence for ECS involvement in AUD suggesting that higher 2-AG levels may facilitate abstinence. Results support preclinical findings of distinct ECS involvement in stress and reward processes, suggesting personalized treatment approaches based on the individual's preferred drinking motives.

Kang Tsou Memorial Lecture: 12:00-13:00, Tuesday, June 30, 2026

PARKINSON'S DISEASE: FROM GENES TO PERSONALIZED TREATMENTS

Thomas Gasser, MD, PhD

Professor & Director,
Hertie-Institute for Clinical Brain Research,
University of Tübingen, Germany

Dr. Gasser is a professor of neurology and has been director of the Department of Neurodegenerative Diseases at the Hertie-Institute for Clinical Brain Research since 2002 and chairman of the Board of Directors at the Center of Neurology, University of Tübingen, Germany since 2008. In 2010 he became Dean of research of the Medical Faculty at the University of Tübingen and since 2013 he has also been coordinator for clinical studies at the Tübingen partner of the German Center for Neurodegenerative Diseases (DZNE).

His main areas of research are the genetic and molecular basis of Parkinson's disease, dystonias and other movement disorders, including the mapping, cloning and functional characterization of Mendelian genes causing Parkinson's disease and other movement disorders, as well as their diagnosis and treatment but also the identification of common genetic variability which contributes to the sporadic forms of the disease.

In 2024 Dr. Gasser received the Breakthrough Prize in Life Sciences, together with Dr. Andrew Singleton and Dr. Ellen Sidransky, which honors transformative advances towards understanding living systems and extending human life, for his groundbreaking research into genetic risk factors for Parkinson's disease.

ENDOCANNABINOID/CB1R OVERACTIVATION: FANNING THE FLAMES OF LUNG INFLAMMATION IN ALCOHOL USE DISORODER

Lenny Pommerolle[1], Seray B. Karagoz[1], Abhishek Basu[1], Muhammad Arif[1], Ilhem Messaoudi[2], Kathleen Grant[3], Ellen L. Burnham[4], Resat Cinar*[1]
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Introduction: Chronic alcohol consumption predisposes the lungs to acute tissue injury, pneumonia, and alcoholic lung disease by compromising immune function, altering alveolar macrophages (AMs), which contributes to increased susceptibility to inflammatory and fibroproliferative disorders. The endocannabinoid system, particularly cannabinoid receptor 1 (CB1R), is upregulated in pulmonary fibrosis. However, the impact of chronic alcohol consumption on the pulmonary endocannabinoid/CB1R system remains unexplored.

Methods: To investigate this with translational human relevance, experiments were performed using mouse models (NIAAA alcohol diet), non-human primate rhesus macaque models (voluntary drinking alcohol self-administration), and biospecimens from humans with Alcohol Use Disorder (AUD) (n=96, 87% male Age=45) and controls (n=40, 65% male, Age =36).

Results: In the non-human primate model, the endocannabinoid anandamide (AEA) levels in bronchoalveolar lavage fluid (BALF) doubled following ethanol induction, while endocannabinoid 2-arachidonoylglycerol (2-AG) remained unchanged. However, after six months of voluntary ethanol consumption, both AEA and 2-AG levels increased dramatically (~22-fold) compared to baseline. In the mouse model, AEA levels, but not 2-AG, were significantly elevated in BALF and lung tissue. *Cnr1* (CB_1R) gene expression increased, while the AEA-degrading enzyme *Faah* was downregulated by ~60%. The synthetic and degrading enzymes of 2-AG, respectively, *Daglb1* and *Mgll* were also reduced following ethanol exposure. Flow cytometry revealed CB_1R upregulation in lung leukocytes, particularly in macrophages 9hr-post binge of ethanol. In agreement with the animal models, AEA also significantly increased in the BALF of participants with AUD compared to controls.

Conclusion: Across different species and alcohol drinking models as well as in humans with AUD, chronic alcohol consumption overactivated endocannabinoid/CB_1R system, significantly increasing AEA and 2-AG levels while upregulating CB_1R in lung macrophages. Together, these observations suggest that CB_1R may represent a therapeutic target with potential to mitigate alcohol-related tissue injury in the lung warranting investigation of endocannabinoid/CB_1R-directed interventions in alcohol-associated pulmonary vulnerability.

MAGL INHIBITION SUPPRESSES PSORIASIFORM INFLAMMATION VIA CB2

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Introduction: Psoriasis is a chronic inflammatory skin disease characterized by epidermal hyperproliferation and cutaneous inflammation. The skin endocannabinoid system regulates its homeostasis, and growing evidence supports a protective role for cannabinoid receptor type 2 (CB2) signaling in psoriasiform inflammation. Monoacylglycerol lipase (MAGL), the primary enzyme degrading 2-arachidonoylglycerol (2-AG), represents an attractive therapeutic target by enhancing endogenous cannabinoid signaling while limiting inflammatory lipid mediator production. Here, we tested whether LEI-515, a novel, reversible, peripherally restricted MAGL inhibitor, attenuates psoriasiform pathology and whether these effects depend on CB2 signaling.

Methods: Psoriasis was induced in WT and global CB2 knockout mice by daily topical application of 62.5 mg of 5% imiquimod cream for six days. LEI-515 (10 mg/kg p.o.) was administered beginning two days prior to imiquimod treatment and continued throughout disease induction. Disease severity was assessed by skin thickness and clinical scoring. Epidermal proliferation and signaling were evaluated by Ki67 and pSTAT3 immunohistochemistry, inflammatory cytokine expression by RT-qPCR, and scratching behavior by video-based quantification.

Results: In WT mice, LEI-515 significantly reduced imiquimod-induced disease severity, including decreased skin thickening, lower clinical scores, and reduced epidermal hyperproliferation. LEI-515 treatment was also associated with reduced scratching behavior and attenuated inflammatory cytokine expression. In contrast, LEI-515 failed to improve clinical, histologic, or behavioral outcomes in global CB2 knockout mice, indicating that CB2 signaling is required for the protective effects of MAGL inhibition.

Conclusions: These findings identify MAGL inhibition as a CB2-dependent strategy to suppress psoriasiform inflammation and support further investigation of skin-targeted MAGL-based therapies for psoriasis.

INSULIN RESISTANCE AS A KEY DETERMINANT OF ENDOCANNABINOID OVERPRODUCTION IN OBESE VISCERAL ADIPOSE TISSUE

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Introduction: Circulating endocannabinoids (ECs) correlate positively with visceral adipose tissue (VAT), implicating VAT as a key source of ECs that may contribute to adipocyte dysregulation and obesity-related metabolic complications. Although adipocytes harbor the enzymatic machinery required for EC biosynthesis, how this pathway is regulated in metabolic disease remains unclear.

Methods: Using an explant-based approach, we quantified EC production in visceral and subcutaneous adipose tissue (VAT and SAT) from diet-induced obese (DIO) mice and from human controls (CON), obese non-diabetic (OND) or obese diabetic (OD) subjects. Regulatory mechanisms were investigated combining data from CB1R-deficient mice, pharmacological modulation and transcriptomic profiling of human VAT.

Results: In lean mice, ECs release followed the pattern OEA > PEA = 2-AG >> AEA, without depot specificity. In insulin-resistant DIO mice VAT selectively exhibited hypersecretion of 2-AG and AEA. In humans, 2-AG predominated and was higher in VAT than SAT in controls.
Strikingly, VAT explants from OD patients secreted significantly higher levels of 2-AG, while N-acylethanolamines showed only a modest increase compared with OND and CON. SAT secretion rates remained unchanged. β-adrenergic stimulation selectively increased 2-AG production in control VAT, an effect lost in DIO mice suggesting a state of catecholamine resistance. RNA-seq analysis of OD human VAT revealed a pro-inflammatory, lipid mediator transcriptional signature coupled to suppressed insulin-responsive adipocyte program.

Conclusion: Together, mouse and human data identify insulin resistance as the major determinant of VAT-derived 2-AG overproduction establishing this EC as a key mediator of metabolic dysregulation in type 2 diabetes.

MITOCHONDRIA-ASSOCIATED CB1 RECEPTORS ARE NEW PLAYERS IN THE CONTROL OF ENERGY AND GLUCOSE METABOLISM

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Introduction: Cannabinoid receptor type 1 (CB1R) is highly expressed in the brain, with lower levels observed in the pancreas, two vital organs that contribute to maintaining physiological glycemia through inter-organ humoral and neural communications. CB1R have been found to localize not only at the plasma membrane (pmCB1), but also associated with mitochondrial membrane (mtCB1) in neurons and astrocytes. Available evidence on the role of CB1R in the modulation of insulin secretion has been found to be conflicting. Hence, the goal of our work was to understand the relative contribution of pmCB1 and mtCB1 to glucose homeostasis.
Methods: We employed an animal model expressing a mutant form of CB1 (designated DN22-CB1), which lacks *in vivo* mitochondrial localization and functions, yet maintains pmCB1-related signaling. A full metabolic profile of the DN22-CB1 mice was realized. Pancreatic islets were isolated to further dissect the islet-autonomous responses under different metabolic stimuli. **Results:** Our observations revealed that DN22-CB1 mice were diabetic due to reduced insulin secretion, while maintaining insulin sensitivity. Vagal signaling was not altered as indicated by neural recordings following glucose injection. By electron microscopy and respirometry, we demonstrated functional mtCB1R expression in pancreatic beta-cells. Glucose-stimulated insulin secretion data obtained from isolated islets of chow-fed DN22-CB1, full CB1-KO and their control littermates suggest that both pmCB1 and mtCB1 participate to the regulation of insulin secretion in complex ways. We observed profound differences in protein content, endocannabinoids content, and cells' kinome activity in response to glucose in pancreatic DN22-CB1 islets. Finally, we observed that mtCB1R expression is needed for the well-known incretin-induced potentializing of insulin secretion, which is currently the gold-standard treatment for diabetes. **Conclusion:** This study identified novel molecular mechanisms regulating glucose-dependent insulin secretion, which may lead to targeted pharmacological interventions.

SPATIALLY RESOLVED PROTEOMIC ANALYSIS OF ENDOCANNABINOID SYSTEM MARKERS IN SALIVARY GLANDS OF PATIENTS WITH SJÖGREN'S SYNDROME

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Introduction: The organization and expression of endocannabinoid system components in Sjögren's disease salivary glands remain poorly understood, even though these molecules may influence glandular function and immunity. With current treatments largely limited to symptom relief, better molecular insights are needed. Cannabinoids may offer therapeutic benefits; therefore, this study uses spatial proteomics to map the endocannabinoid system and identify new treatment targets.

Methods: We utilized imaging mass cytometry (IMC) alongside single-cell spatial transcriptomics to analyze endocannabinoid system profiles in core biopsies from Sjögren's patients (10 males, 10 females) and healthy controls. This methodology enabled precise, high-resolution measurement of ECS components, characterization of immune cell populations, and detailed mapping of their spatial relationships.

Results: We observed a pronounced increase in the colocalization of the CB2 receptor with the GPR55 receptor in the salivary glands of Sjögren's disease patients. This enhanced receptor colocalization was closely associated with increased immune cell infiltration within the glandular tissue of affected individuals. Notably, our spatial proteomic analyses revealed significant sex-dependent differences in the composition of immune cell populations infiltrating the salivary glands. Additionally, we detected elevated expression levels of cannabidiol (CBD) targets, including TRPV1 and PPARγ, in samples from patients with Sjögren's disease compared to healthy controls.

Conclusions: These findings reveal that Sjögren's disease alters the spatial organization of endocannabinoid system components in the salivary glands and increases the presence of cannabinoid-responsive targets alongside immune cell infiltration. The results highlight the therapeutic potential of targeting the endocannabinoid system in addressing glandular inflammation in Sjögren's disease.

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ICRS Lifetime Achievement Award Lecture: 8:45-9:15, Wednesday, July 1, 2026

CANNABINOID CHEMISTRY: FORM, FORMULATION, AND FUNCTION

Brian Thomas, PhD

Empirical Pharmaceutical Services, LLC

Brian F. Thomas is a pharmacologist, toxicologist, and analytical scientist with more than four decades of experience spanning cannabinoid pharmacology, analytical chemistry, drug discovery, pharmaceutical development, and regulatory science. He received his Ph.D. in Pharmacology and Toxicology from Virginia Commonwealth University in 1992, where his doctoral research in the laboratory of Dr. Billy R. Martin focused on cannabinoid physicochemical properties, structure-activity relationships, receptor binding, and efficacy. He was also the first recipient of the Lauren A. Woods Award for outstanding graduate research in the Department of Pharmacology and Toxicology. Dr. Thomas spent more than 28 years at RTI International, where he served in senior scientific leadership roles, including Senior Director and Principal Scientist in the Center for Analytical Chemistry and Pharmaceutics. During that time, he led major NIH, FDA, and contract research programs involving the preparation, analysis, stability assessment, storage, and distribution of research drugs, cannabinoids, and tobacco research products, while also contributing to Drug Master Files, Tobacco Product Master Files, and Chemistry, Manufacturing, and Controls documentation to support clinical research. He later served as Senior Director of Analytical Chemistry, Discovery Sciences, and Pharmaceutics at Canopy Growth Corporation and as Director of Analytical Sciences at The Cronos Group, with responsibility for analytical characterization, quality systems, stability testing, formulation support, and regulatory documentation across research and production environments. He is currently Principal of Empirical Pharmaceutical Services, LLC, where he provides evidence-based consulting in analytical chemistry, pharmacology, toxicology, pharmaceutical sciences, and regulatory strategy. Dr. Thomas also holds adjunct faculty appointments at Wake Forest University School of Medicine and North Carolina Central University, serves on the Board of Directors of the College on Problems of Drug Dependence, and holds scientific advisory roles with several biotechnology organizations. He has authored or co-authored more than 120 peer-reviewed publications and numerous book chapters, co-authored two books, and has been widely recognized for his leadership and scientific contributions, including election as a Fellow of the College on Problems of Drug Dependence in 2019, the 2025 International Cannabinoid Research Society Lifetime Achievement Award, the 2025 Mahmoud ElSohly Award from the American Chemical Society, and Board of Directors of the College on Problems of Drug Dependence in 2026.

DISSECTING THE ROLE OF CB1R EXPRESSING GABAERGIC INTERNEURONS IN THE BASOLATERAL AMYGDALA DURING FEAR EXTINCTION

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Introduction: There is a large body of work investigating the role the endocannabinoid system in anxiety-related disorders. We have shown that endocannabinoid signaling from the basolateral amygdala (BLA) on cortical inputs into the BLA mediates extinction learning through the modulation of glutamate release (Gunduz-Cinar et al 2023 *Neuron*, *111*(19), 3053-3067). However, it remains unclear whether local-inhibitory BLA microcircuits are also regulated by endocannabinoids though the modulation of GABA.
Methods: Our previous attempts to manipulate GABAergic BLA interneurons using the CCK-Cre-Dlx-FLpo double transgenic mice (Rovira-Estaban et al 2019 *eNeuro*, *6*(6) 0220-19.2019) or utilizing the intersct viral strategy with a NECAB2 -Cre promoter, a calcium binding protein unique to CCK/CB1R neurons (Miczan et al 2021 *Cerebral Cortex*31(3):1786-1806.), lacked the specificity we desired. This has led us to search for an alternative marker for CB1R-expressing BLA interneurons, namely gamma synuclein (sncg), which is highly co-expressed with CNR1 in interneuron populations in hippocampus (Dudok et al 2021 *Neuron*109(6)997-1012). In the present study, using a combination of immunohistochemistry and RNAscope, we characterized the selective co-expression of sncg for CB1R-expressing BLA interneurons and validate potential viral strategies to be leveraged for bidirectional manipulations in fear extinction using optogenetics, and fiber photometry recordings in these interneurons in mice.
Results: In amygdala, sncg gene expression mostly overlaps with GAD2 expressing cells and only a small portion overlaps with the Slc17a7 expressing cells. Targeting these cells with the intersect viral approach confirmed these results and showed that CB1Rs are co-expressed on the GABAergic Sncg+ interneurons. Optogenetic inhibition of these cells impaired extinction learning and CRISPR mutation of the CB1Rs on all GABAergic interneurons impaired retrieval of extinction memory. Currently, we are doing experiments to understand the dynamic control mechanism of the endocannabinoids at the basolateral amygdala outputs using fiber photometry.
Conclusion: Identifying an effective targeting strategy and mechanism of action for controlling the output of CB1R-expressing interneurons in the BLA enables future studies of their role in anxiety, fear and extinction learning, and informs the development of improved therapeutic approaches for anxiety-related disorders.

ANALYZING NATURAL MOUSE BEHAVIOR USING MACHINE LEARNING AND POSE ESTIMATION: IMPACT OF THC AND ROLE OF ENDOCANNABINOID SIGNALING

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Introduction: How THC impairs natural behaviors in mice and to what extent changes in endocannabinoid (**eCB**) signaling are involved remain unknown. Computer vision tools, such as DeepLabCut and SLEAP, have begun to transform current behavioral research by allowing for high-resolution tracking of individual points on animals during the expression of behavioral phenotypes. Genetically encoded fluorescent sensors allow for real-time detection of changes in the levels of select signaling molecules produced endogenously and include $GRAB_{eCB2.0}$ that detects sub-second changes in eCB levels with high resolution in mouse brain.

Methods: We developed a video-monitored behavioral platform combined with machine learning classifiers to unravel discrete changes in mouse spontaneous natural behaviors studied in a linear track. Changes in neuronal activity (GCaMP) and eCB signaling ($GRAB_{eCB2.0}$) were monitored in the medial prefrontal cortex (**mPFC**) using fiber photometry.

Results: THC infusion into the mPFC disrupted key natural behaviors, including walking kinematic features characteristic of catatonia-like responses. THC increased mPFC GABAergic activity preceding walk initiation events. Pose-defined closed loop optogenetic stimulation of mPFC GABAergic neurons demonstrated that THC exacerbates selected parameters of motor impairment. Surprisingly, THC induced a time-locked, movement-induced, transient potentiation of mPFC eCB release and ensuing CB_1R-mediated synaptic inhibition. Our video-monitored behavioral platform also reliably detected kinematic features occurring following sciatic nerve ligation as a model of neuropathic pain, and the approach and disengagement behavior of mice to a reward, sucrose delivery system.

Conclusions: THC-modifies the mPFC E/I balance and output via rapid dynamic eCB release that control natural behaviors in mice.

GLUTAMATERGIC SIGNALING ALTERATIONS IN CA1 PYRAMIDAL NEURONS MEDIATE THC-INDUCED MEMORY IMPAIRMENT

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Introduction: The hippocampus plays a pivotal role in memory formation, and cannabinoids, including Δ^9-tetrahydrocannabinol (THC), modulate hippocampus-dependent memory, alter multiple forms of synaptic and structural plasticity, and disrupt the excitatory/inhibitory (E/I) balance. The CA1 region has been identified as a critical locus for these effects, where repeated THC exposure has been associated with impaired long-term potentiation and reduced dendritic spine density. Here, we sought to identify the cell type–specific molecular mechanisms underlying these alterations following chronic THC exposure, with the goal of developing pharmacological strategies that preserve the therapeutic potential of THC while preventing memory impairment.

Methods: To decipher the THC-induced translatome, we used Wfs1-CreERT2:RiboTag mice expressing HA-tagged ribosomes selectively in CA1 pyramidal neurons. Mice received daily intraperitoneal injections of THC (10 mg/kg) or vehicle for six days, after which HA-associated mRNAs were isolated and analyzed by RNAseq. On the basis of these transcriptomic signatures, we tested whether pharmacological modulation of the glutamatergic system could normalize circuit function and rescue memory consolidation.

Results: Analyses of differentially translated transcripts revealed significant enrichment of Gene Ontology and KEGG categories related to memory, synaptic organization, structural plasticity, and, notably, glutamatergic transmission, including altered expression of glutamate receptor subunits in THC-treated mice. Restoring E/I signaling recovered performance in hippocampus-dependent memory tasks after repeated THC exposure, while preserving THC-induced analgesia.

Conclusions: Collectively, our data identify CA1 pyramidal neuron–specific pathways dysregulated by chronic THC, link these changes to glutamatergic dysfunction and memory deficits, and provide proof-of-concept that targeting synaptic balance can dissociate the therapeutic and adverse cognitive effects of cannabinoids.

KLS-13019: A SYNTHETIC CBD ANALOGUE THAT REVERSES SYMPTOMS AND PRESERVES CHEMOTHERAPEUTIC EFFICACY IN NOVEL TRANSLATIONAL MODELS OF CIPN

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Introduction. Chemotherapy-induced peripheral neuropathy (CIPN) is a common, often persistent complication of chemotherapy, characterized by pain, numbness, tingling, and cold sensitivity in the hands and feet, affecting roughly one third of patients. With no FDA-approved treatments and the vast majority of patients relying on prescription opioids, there is a critical need for non-opioid therapies. We developed KLS-13019, a synthetic cannabidiol analogue and GPR55 antagonist with improved efficacy, safety, and bioavailability, which, when given orally, prevents and durably reverses mechanical hypersensitivity in paclitaxel and oxaliplatin CIPN models in mice and rats. However, most preclinical CIPN studies are conducted in tumor-free animals and rarely pair neuropathy outcomes with measures of tumor burden or employ high-speed videography to capture nuanced pain-like behaviors, limiting translational relevance. Because our data indicate that the optimal therapeutic window is at or shortly after chemotherapy initiation, we hypothesized that KLS-13019 would alleviate paclitaxel-induced pain-like behaviors without compromising chemotherapeutic efficacy or altering tumor progression in a cancer-bearing model using advanced behavioral assessments. **Methods.** In the first set of experiments, we evaluated the direct impact of KLS-13019 on cancer cell viability using colorectal (RKO, MC38) and liver (Hep3B, HepG2) tumor cell lines in vitro and an in vivo tumor model using mice injected with MC38 murine colon cancer cells. In the second set of experiments, we evaluated the effect of KLS-13019 in a combined model of CIPN and tumor response. Rats first received an injection of the carcinogen N-methyl-N-nitrosourea (MNU) to induce mammary tumors. Once tumors became palpable, animals were treated with vehicle, paclitaxel, or paclitaxel plus KLS-13019. We then assessed neuropathic pain–like behaviors and monitored tumor growth over time to determine whether KLS-13019 could prevent or reverse paclitaxel-induced CIPN in tumor-bearing rats without compromising chemotherapeutic control of the cancer. Finally, in a pilot study, we implemented high-speed videography to capture whole-body responses to a wider range of tactile stimuli, deriving a multidimensional behavioral signature of touch and pain-like responses across conditions. Using supervised and unsupervised machine learning, unique behavioral signatures were defined and their ability to predict pain and/or recovery was tested across groups and conditions. Together, these findings show that KLS-13019 can be evaluated concurrently for its ability to preserve chemotherapeutic efficacy, alleviate neuropathic pain in tumor-bearing animals, and normalize complex pain-related behaviors using advanced quantitative videography. **Results.** In the first set of experiments, we found that KLS-13019 inhibited proliferation of liver and colorectal cancer cells in vitro and reduced primary tumor growth in the orthotopic mouse model of colorectal cancer. In the second set of experiments, we found that KLS-13019 co-administered with paclitaxel attenuated paclitaxel-induced mechanical and cold allodynia while concurrently decreasing mammary tumor size, indicating that CIPN relief was achieved without compromising, and potentially improving, chemotherapeutic tumor control. Lastly, in our pilot high-speed videography rodent CIPN study, KLS-13019 treatment normalized multidimensional, whole-body, tactile-evoked pain-like responses compared with paclitaxel alone, supporting the feasibility of using advanced kinematic signatures as sensitive endpoints for neuropathic pain modulation. **Conclusions.** These data suggest that KLS-13019 is a promising dual-action candidate that can both reverse CIPN and maintain or enhance the antitumor effects of standard chemotherapy. By integrating tumor-bearing models with sophisticated behavioral quantification, this work establishes a more clinically relevant preclinical platform for developing non-opioid treatments for cancer therapy–related neuropathic pain.

CANNABIDIOL FOR SPINAL CORD INJURY CHRONIC NEUROPATHIC PAIN: A RANDOMIZED CLINICAL TRIAL

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Introduction: Chronic neuropathic pain affects more than two-thirds of patients with spinal cord injury (SCI). Current treatments have limited efficacy and significant side effects. Cannabidiol (CBD) has demonstrated efficacy in preclinical neuropathic pain models and is often used by patients with chronic pain, albeit at low doses (< 300 mg/day) that may be ineffective. Here we investigated the effect of a relatively high (800 mg/day) daily CBD dose on chronic neuropathic pain related to SCI.

Methods: This was a randomized, double-blinded, placebo-controlled, crossover clinical trial conducted at the Neuroscience Research Australia Research Institute. Forty participants were randomized and thirty-eight included in the primary analysis (n=6/38 female; age (mean±SD): 55±14 years). All participants consumed oral CBD and placebo over two six-week periods separated by a four-week washout. The CBD was titrated upwards to 800 mg/day over 2-weeks. The primary outcome was change in self-reported pain intensity on a Visual Analogue Scale from 0 (no pain) to 10 (worst pain imaginable). Secondary outcomes included ratings of depression, stress, anxiety, and sleep. Plasma and urinary CBD and metabolite concentrations were assessed during both arms.

Results: A significant treatment by phase (p<0.001) interaction effect was observed on pain intensity. During the active phase, pain scores were lower with CBD (mean±SEM: 3.82±0.23) compared to placebo (mean difference=−0.54, SEM=0.15, p<0.001), with a moderate effect size favouring CBD (Hedges' g=-0.38). Fourteen participants (≈37%) demonstrated clinically significant (>30%) pain intensity reductions during CBD treatment compared to 4 participants (≈11%) during placebo. Adverse events, almost all of a minor nature, were reported by 26 participants with CBD (n=67 events), and by 20 participants (n=51 events) with placebo.

Conclusions: Six-weeks of daily treatment with high-dose oral CBD significantly reduced the self-reported intensity of neuropathic pain in individuals with SCI, with a modest effect size. CBD was generally well-tolerated. These results support further research into the use of high-dose CBD for treating chronic neuropathic pain.

Trial Registration: anzctr.org.au Identifier: ACTRN12622000634774.

INHIBITION OF FATTY ACID BINDING PROTEIN 5 ALLEVIATES NEUROPATHIC PAIN FOLLOWING SPINAL CORD INJURY IN MICE

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Introduction: Up to 60-70% of spinal cord injury (SCI) patients suffer from lifelong neuropathic pain (NP) that is poorly responsive to available analgesics. Hyperexcitability and spontaneous activity of nociceptors has been suggested to play a causal role in the development of NP following SCI. Increased activity of CaV3 channels enhances nociceptor hyperexcitability and promotes the development and persistence of NP. Fatty acid binding protein 5 (FABP5) is a cytosolic chaperone that regulates endocannabinoid metabolism and inflammatory signaling pathways. FABP5 is expressed in nociceptors and may modulate CaV3 channel activity and ensuing pain. The goal of this study was to determine whether FABP5 inhibition using the novel selective FABP5 inhibitor ART26.12 or genetic FABP5 deletion inhibits CaV3 channel activity and reduces NP.

Methods: The experiments were approved by the Stony Brook University Institutional Animal Care and Use Committee (#891445). WT and FABP5 KO mice were used in this study. SCI was induced by a midline spinal cord contusion at T10. Mechanical allodynia was measured with von Frey filaments utilizing the up-down method. Spontaneous pain was measured via conditioned place preference (CPP) paradigm. For *in vitro* electrophysiology, the action potential clamp technique was used in dissociated dorsal root ganglia neurons isolated from SCI and sham mice to measure the interspike CaV3 and total calcium currents as well as spontaneous activity.

Results: SCI induced mechanical allodynia in WT mice, which was fully reversed following administration of 40 mg/kg ART26.12. In the CPP paradigm, WT SCI mice treated with ART26.12 spent a greater amount of time in the ART26.12 paired chamber and decreased amount in the vehicle-paired chamber, which was not observed in the sham controls, suggesting that ART26.12 relieves ongoing pain. ART26.12 (5 μM) reduced the total interspike calcium charge and the interspike CaV3 charge in SCI nociceptors, effects that were also observed in SCI nociceptors from FABP5 KO mice. Lastly, SCI increased the incidence of spontaneously active nociceptors in WT mice, which was reduced in ART26.12-treated and FABP5 KO nociceptors to levels comparable to sham controls.

Conclusions: These results indicate that FABP5 inhibition in nociceptors reduces CaV3 current, mechanical hypersensitivity, and spontaneous pain in mice subjected to SCI. ART26.12 represents a promising non-opioid analgesic with a unique mechanism of action for the management of SCI pain.

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Δ⁹-TETRAHYDROCANNABINOL AND CANNABIDIOL SYNERGISTICALLY ALLEVIATE HYPERALGESIS IN A HUMANIZED MOUSE MODEL OF SICKLE CELL DISEASE

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Introduction: Sickle cell disease (SCD) affects circa 7.7 million people worldwide and is characterized by chronic and episodic pain that substantially impairs quality of life. Current opioid-based therapies have limited efficacy and serious adverse effects. Cannabis has emerged as a potential alternative, yet the analgesic efficacy of its primary components, Δ⁹-tetrahydrocannabinol (THC) and cannabidiol (CBD), remains unclear. Here, we evaluated the antinociceptive effects of THC and CBD, alone and in combination, in a humanized mouse model of SCD.

Methods: Male HbSS-BERK and HbAA-BERK mice received intraperitoneal injections of THC, CBD, or THC-CBD combinations. Mechanical (automated Von Frey) and cold (dry ice) hyperalgesia were assessed alongside motor coordination (rotarod), anxiety-like behavior (EPM), and cognition (NOR). Isobolographic analyses determined drug interactions. Pharmacokinetic profiling of cannabinoids and endocannabinoids was performed by LC–MS/MS. Oxidative stress (malondialdehyde, MDA) and inflammatory markers (Il-1β, Il-10 mRNA) were quantified in spinal cord and spleen.

Results: THC and CBD produced dose-dependent antinociception in HbSS-BERK mice. Isobolographic analysis revealed significant synergy at ED_{50} ratios, yielding robust and sustained analgesia lasting ≥14 days with chronic dosing. THC-CBD co-administration increased systemic exposure to THC, CBD, and 2-arachidonoylglycerol. SCD mice exhibited elevated MDA and Il-1β mRNA expression. CBD reduced MDA and Il-1β, THC reduced spinal MDA and increased Il-10, while the combination suppressed Il-1β and elevated Il-10 in both tissues.

Conclusions: THC and CBD synergistically produce sustained analgesia in SCD mice through complementary antioxidant and anti-inflammatory mechanisms. These findings provide a mechanistic rationale for optimizing THC:CBD ratios in future clinical trials targeting SCD pain.

CANNABIDIOL ATTENUATES CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHIC PAIN THROUGH A MECHANISM THAT REQUIRES THE ENZYME N-ACYL PHOSPHATIDYLETHANOLAMINE-SPECIFIC PHOSPHOLIPASE D (NAPE-PLD)

Carlos Henrique Alves Jesus[1,2], Ailing Li[1,2], Luana Caroline de Assis Ferreira[1,2], Ken Mackie[1,2,3], Andrea G. Hohmann[1,2,3]

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Introduction: Cannabidiol (CBD) is a non-psychoactive component of cannabis that has been studied as a potential therapy for chronic pain. CBD attenuates behavioral hypersensitivities in preclinical models of neuropathic pain. However, a lack of understanding of the mechanisms underlying the therapeutic effects of CBD has hindered development and application of CBD to mechanism-based therapies for pain. We asked whether the analgesics effects of CBD were dependent upon the enzyme NAPE-PLD.

Methods: We used a mouse model of chemotherapy-induced peripheral neuropathy (CIPN) to evaluate the acute and chronic antinociceptive effects of CBD and its mechanisms of action. Pharmacological specificity was assessed using antagonists targeting cannabinoid type 1 and type 2 receptors (CB1 and CB2) and peroxisome proliferator–activated receptors gamma and alpha (PPARγ and PPARα). Mechanistic pathways were further examined using GPR55 and NAPE-PLD knockout (KO) mice given repeated CBD dosing during both the development and maintenance phases of paclitaxel-induced CIPN.

Results: CBD suppressed paclitaxel-induced behavioral hypersensitivities; these effects were attenuated by a PPARα antagonist, but not by PPARγ, CB1 or CB2 antagonists. CBD reduced both the development and maintenance of neuropathic nociception in a CIPN model in wild-type mice but these effects were absent in NAPE-PLD KO mice. By contrast, anti-allodynic efficacy of CBD was fully preserved in GPR55 KO mice.

Conclusions: Pharmacological blockade of the PPARα receptor and genetic deletion of NAPE-PLD abolished the antinociceptive effects of CBD in a model of CIPN, suggesting a pivotal role for NAPE-PLD and PPAR receptors in CBD-mediated analgesia in chemotherapy-induced neuropathic pain.

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THC-INDUCED BEHAVIOR, PHYSIOLOGY, AND WITHDRAWAL EFFECTS ARE EXACERBATED BY RGS12 DELETION

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

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Introduction: Regulator of G-protein Signaling (RGS) proteins are considered negative regulators of the intracellular signal cascade initiated by G-protein Coupled Receptor (GPCR) activation. Cannabinoid receptors are GPCRs that selectively signal through $G\alpha_{i/o}$ subunits. RGS12 is the largest member of the RGS superfamily and preferentially associates with $G\alpha_{i/o}$ subtypes, suggesting a plausible interaction between RGS12 and cannabinoid receptors. This study determined if RGS12 deletion alters the *in vivo* effects of Δ^9-tetrahydrocannabinol (THC). To this end, we compared THC-induced acute tetrad effects, drug tolerance, and rimonabant-precipitated withdrawal behaviors in RGS12 knockout vs. wildtype mice.

Methods: Adult male and female RGS12 (C57BL/6J background) knockout or wildtype littermate mice were administered THC (up to 100 mg/kg, s.c. in 1:1:18 parts kolliphor:ethanol:saline) cumulatively, 50 min before tetrad testing (i.e., bar test, tail immersion, hypothermia, and spontaneous locomotion). A separate group of RGS12 (-/-) or RGS12 (+/+) littermates received THC (30 mg/kg, s.c. BID x6 days) with tetrad testing 50 min after each AM injection (~0800) to assess tolerance. To precipitate withdrawal, all mice were injected with rimonabant (3 mg/kg, i.p.) 30 min after the morning THC injection on day 6 and scored for somatic signs of withdrawal during a 1 hr observation.

Results: THC dose-dependently induced hypothermia (≥10 mg/kg), catalepsy (≥30 mg/kg), and antinociception (≥1 mg/kg) in both genotypes. RGS12 (-/-) mice exhibited potentiated THC effects for antinociception (30 mg/kg) and hypothermia (30 mg/kg). Genotype did not affect the rate of tolerance development. However, RGS12 deletion increased precipitated THC (30 mg/kg) withdrawal-induced paw tremors and head twitches.

Conclusions: RGS12 negatively modulates acute THC-induced antinociception and hypothermia. Additionally, RGS12 deletion potentiates the frequency of somatic withdrawal behavior following repeated THC administration. Thus, RGS12 may function to modulate both the acute effects and abuse liability of cannabinoids through its regulatory role on receptor functionality.

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.

Mechoulam Awardee Lecture: 12:00-13:00, Wednesday, July 1, 2026

HARNESSING THE ENDOCANNABINOID SYSTEM TO COMBAT PAIN AND ADDICTION

Andrea G. Hohmann, PhD
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Department of Psychological & Brain Sciences, Indiana University, Bloomington, IN

Biography: Andrea G. Hohmann is a Linda and Jack Gill Chair of Neuroscience and Distinguished Professor of Psychological and Brain Sciences at Indiana University Bloomington. Dr. Hohmann received her Ph.D. from Brown University in the laboratory of J. Michael Walker. She completed postdoctoral training as a Pharmacology Research Associate Fellow in functional neuroanatomy in the laboratory of Miles Herkenham at the National Institute of Mental Health (NIMH). Dr. Hohmann served as a Staff Research Fellow at the National Institute of Dental and Craniofacial Research (NIDCR) in pain and neurosensory mechanisms in the laboratory of M.A. Ruda. Dr. Hohmann served as an Assistant, Associate and Full Professor at the University of Georgia before she was recruited to Indiana University. Dr. Hohmann's research exploits novel mechanisms for suppressing pain with an emphasis on targeting the endocannabinoid signaling system for therapeutic benefit. Her lab uses behavioral, pharmacological, neurophysiological, genetic models, biochemical assays, as well as neuroanatomical and molecular approaches to validate therapeutic targets and her contributions span over three decades. Her work was the first to show that cannabinoids suppress activity in nociceptive neurons. She documented that dorsal root ganglion cells synthesize CB1 receptors and are transported to the periphery and provided evidence for peripheral cannabinoid analgesic mechanisms. Her laboratory showed that endocannabinoids are mobilized on demand to produce stress-induced analgesia and, together with the Piomelli lab, validated MAGL as a target for analgesic drug development. Her work elucidated the molecular architecture of 2-AG signaling in the periaqueductal gray and spinal cord. Her lab elucidated CB2 cannabinoid analgesic mechanisms and used conditional knockout mouse lines to identify cell types necessary and dispensable for CB2 analgesic mechanisms. The Hohmann lab also documented therapeutic potential of CB1 positive allosteric modulators as an analgesic strategy and CB1 negative allosteric modulators as an anti-addiction strategy. Dr. Hohmann was a former recipient of the Young Investigator Award from the International Cannabinoid Research Society as well as the Ester Fride Award from the International Association for Cannabinoid Medicines (IACM) for contributions to cannabinoid basic research. She was awarded a Lilly Presidential Life Science Professorship at Indiana University. Dr. Hohmann is an elected fellow of the American Association for the Advancement of Science (AAAS) in recognition of outstanding contributions to the progress of science.

Abstract: The opioid epidemic has led to a resurgence of interest in medical marijuana as an analgesic strategy. Here, I review therapeutic strategies that target the endocannabinoid signaling system that offer considerable promise for suppressing pathological pain without the unwanted effects of THC in cannabis. I highlight foundational studies documenting that cannabinoids act at a neural level to suppress pain, and evidence that endocannabinoids are mobilized on demand to produce adaptive changes in pain responsiveness. These contributions provided a foundation for development of brain permeant and peripherally restricted inhibitors of endocannabinoid deactivation. I address the therapeutic potential of peripheral cannabinoid analgesic mechanisms and CB2 cannabinoid receptor signaling for suppressing neuropathic and inflammatory pain. Finally, I address the therapeutic promise of positive allosteric modulation of CB1 cannabinoid receptor signaling as an analgesic strategy and the potential of negative allosteric modulation of CB1 cannabinoid receptor signaling as an anti-addiction strategy that offers a more circumscribed and potentially desirable therapeutic profile compared to orthosteric CB1 agonists and antagonists.

ICRS Early Career Award Lecture: 8:45-9:15, Thursday, July 2, 2026

USING HUMAN LABORATORY STUDIES TO ADVANCE CANNABIS REGULATORY SCIENCE

Tory Spindle, PhD

Associate Professor, Behavioral Pharmacology Research Unit
Department of Psychiatry and Behavioral Sciences
Johns Hopkins University School of Medicine

Dr. Spindle conducts human laboratory studies to characterize the behavioral pharmacology of cannabis and cannabis constituents. His research seeks to understand how factors such as route of administration, dose, product formulation/chemical composition profile, and user factors impacts the pharmacokinetic and pharmacodynamic effects of cannabis. Another emphasis of his work is on characterizing cognitive, psychomotor, and driving impairment associated with cannabis, when used alone and in combination with alcohol. Overall, Dr. Spindle's work is intended to inform policy decisions involving cannabis such as product standards and accessibility, dosing guidelines, and procedures for detecting cannabis impairment. Because the overarching goal of his research is to inform policies and regulatory actions for cannabis, his work can be best described as "cannabis regulatory science."

CHRONIC VOLUNTARY CONSUMPTION OF CANNABINOIDS IN C57BL/6 MICE

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Introduction: The majority of pre-clinical cannabinoid work to date has focused on injection or oral gavage of cannabinoids. However, in humans, cannabis is primarily consumed by inhalation or oral ingestion. Recently, English et al. (2024) developed a voluntary oral consumption model that used Δ9-tetrahydrocannabinol (THC) dissolved in Ensure chocolate gelatin to monitor the acute effects of a cannabinoid in mice. The long-term, or chronic, pharmacodynamic and pharmacokinetic effects of cannabinoids in pre-clinical models remain understudied. Therefore, advancement of the model proposed by English et al. into a chronic treatment is needed to assess behavioural and physiological effects, drug tolerance, and bioaccumulation. The purpose of these experiments is to assess the chronic effects of voluntary oral cannabis use. **Methods**: Mice were individually housed and given Ensure chocolate pudding that contained 10 mg/15 mL of cannabinoids [THC or cannabidiol (CBD)] daily for 14 days. There were 6 groups of 12 mice, split between sex and drug that received [control (olive oil), THC or CBD]. Each Ensure cup was weighed before and after placement in the cage to measure voluntary consumption. Mice underwent behavioural and physiological testing and blood collection on alternating days (every 48 hours). Behavioural and physiological tests included the bar holding test for catalepsy; body temperature by rectal thermometer; warm water tail-flick test for nociception; and open field test for locomotion (in that order). Mice also underwent saphenous vein blood collection every 48 hours and cardiac puncture (at the end of the experiment) for final blood and tissue (liver and brain) collection. Data were analyzed using repeated measures 2-way ANOVA (treatment x sex), followed by Tukey's post-hoc for multiple comparisons. **Results**: Male and female mice voluntarily consuming CBD or THC gained significantly less weight than their control counterparts ($F_{treatment}(5,65)=4.465$, $p=0.0015$). However, mice in the THC group consumed significantly less Ensure than either control or CBD groups ($F_{treatment}(5,64)=161.6$, $p<0.0001$). Among the physiological measurements made, reduced body temperature ($F_{treatment}(5,65)=33.67$, $p<0.0001$) and nociception ($F_{treatment}(5,65)=9.017$, $p<0.0001$) were observed in THC-consuming males and females compared to control. Analyses of blood CBD and THC levels from these mice are ongoing. **Conclusions**: Establishing this animal model will allow our group to explore the long-term harms and benefits of cannabis using an approach that more closely resembles real-world cannabis use. Planned future work will utilize this model to assess changes in metabolism and sleep.

Reference: English A, Uittenbogaard F, Torrens A, Sarroza D, Slaven AVE, Piomelli D, Bruchas MR, Stella N, Land BB. A preclinical model of THC edibles that produces high-dose cannabimimetic responses. eLife 12: RP89867, 2024. doi: 10.7554/eLife.89867.

Funding: This research was supported by a GlaxoSmithKline Saskatchewan Endowed Research Chair to RBL.

DELTA-9-TETRAHYDROCANNABINOL-SPARING EFFECTS OF CANNABIGEROL: A PLACEBO-CONTROLLED HUMAN LABORATORY STUDY

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Introduction: Delta-9-tetrahydrocannabinol (THC) demonstrates therapeutic potential for pain relief and appetite stimulation, but its intoxicating and abuse-related effects limit clinical utility. Cannabigerol (CBG), a non-intoxicating cannabinoid, exhibits orexigenic and analgesic effects in preclinical models. This was the first human study to directly compare the effects of vaporized THC, CBG, and their combinations across varying ratios on appetite, pain, and subjective experience in healthy human volunteers.
Methods: Healthy adults (N=19; aged 21-55 years) who reported recent non-medical cannabis use completed nine experimental sessions following 12-hour abstinence. Participants inhaled vaporized THC (0, 5, 15 mg) and CBG (0, 5, 15 mg) alone and co-administered in various dose combinations. An intravenous catheter was placed to measure blood levels of THC, CBG, and their respective metabolites. Subjective and appetite-stimulating effects were assessed using visual analog scales, and analgesic effects were measured via Cold Pressor Test.
Results: THC (5 and 15 mg) significantly increased subjective ratings of a good drug effect, hunger, anxiety, perceived heart rate, and actual heart rate compared to placebo. CBG (15 mg) reduced anxiety and perceived heart rate without affecting appetite or producing abuse-related effects. Critically, CBG co-administration attenuated THC-induced increases in anxiety, perceived heart rate, and actual heart rate. Neither cannabinoid produced acute analgesic effects. Blood sample assays are ongoing.
Conclusion: CBG demonstrated an anxiolytic profile and mitigated several adverse THC effects without producing intoxication or abuse-related subjective effects, supporting its potential as a THC-sparing agent to minimize risks associated with cannabinoid therapeutics. Future work will examine pharmacokinetic interactions.

DETECTION OF PRENATAL CANNABIS EXPOSURE: IS MECONIUM A RELIABLE BIOMARKER?

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Introduction

Understanding the neurodevelopmental consequences of prenatal cannabis exposure requires accurate exposure assessment. Although meconium is widely used as a cumulative biomarker of in utero exposure, its sensitivity relative to detailed maternal biological measures remains poorly characterized. This study evaluates the concordance between maternal urinary phytocannabinoid measurements, monthly self-reported cannabis use, and phytocannabinoid detection in meconium within the Cannabis and Neurodevelopment of Babies (CAN-B) birth cohort.

Methods

A subset of 94 mother–infant dyads (61 cannabis-exposed, 33 controls) were included. Eighteen phytocannabinoids were quantified in maternal urine and blood collected during the first, second, and third trimesters, and in meconium. Cannabis use was prospectively assessed monthly using online questionnaires, including frequency of use. Samples were classified as positive when ≥1 phytocannabinoid was detected. Descriptive analyses, Spearman correlations, and non-parametric comparisons were performed.

Results

No meconium-positive sample was observed in the absence of a positive maternal urine/blood. Among participants with positive urine samples in the 3rd trimester, 60% have positive meconium results. Phytocannabinoids in meconium were observed among women reporting regular cannabis use (≥2 times per week to daily) in the second and third trimesters, whereas meconium remained negative in occasional users. Urinary THC-COOH-glucuronide concentrations were significantly higher in meconium-positive participants (Mann–Whitney U test), and urine–meconium correlations present among regular users ($r^2 \approx 0.3–0.9$).

Conclusions

While reliably identifying sustained exposure later in pregnancy, meconium lacks sensitivity for detecting low-level or occasional prenatal cannabis exposure. These findings highlight the need for robust, multimodal exposure characterization before assessing developmental outcomes associated with prenatal cannabis exposure.

ADOLESCENT THC EXPOSURE INDUCES A MONOCYTE PHENOTYPE PRIMED FOR ENHANCED IMMUNE RESPONSE LATER IN LIFE

Rodrigo M. Klein*[1], Saeed Al Masri[1], Heidi C. Avalos[1], Dominick J. D'Agosta[1], Daphne Williamson[1], Kwang-Mook Jung[1], Daniele Piomelli[1]

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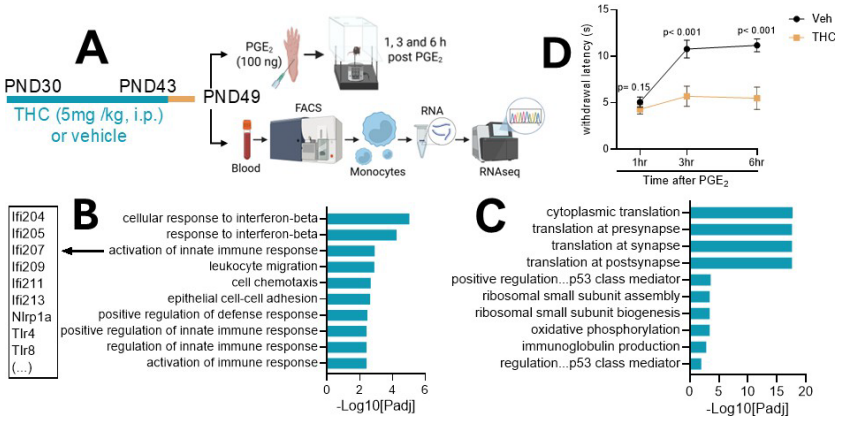
Introduction: Adolescent exposure to cannabis' psychotropic constituent, Δ9-tetrahydrocannabinol (THC), has enduring effects on immune function and may increase vulnerability to inflammatory challenges. In this study we aimed to determine whether adolescent THC exposure alters the phenotype and responsiveness of circulating monocytes, a key cellular component of innate immunity.

Methods: Adolescent C57BL/6 mice received vehicle or THC (5 mg/kg, i.p.) from postnatal day (PND) 30 to 43 and were evaluated at PND49 (Fig. 1A). Pain behavior was assessed by intraplantar prostaglandin E_2 (PGE_2; 100 ng) injection followed by paw withdrawal latency measurement using the Hargreaves test at 1, 3, and 6 h post-injection. Data were analyzed using two-way ANOVA with treatment and time as factors. Sidak's correction was applied and $p < .05$ considered significant. In a separate cohort, blood monocytes were isolated by fluorescence-activated cell sorting (FACS) and analyzed by RNA sequencing (Novogene, USA). Differential gene expression and gene ontology (GO) enrichment were considered significant at Padj < .05.

Results: Transcriptomic analyses of FACS-purified monocytes revealed, in THC-exposed mice, significant upregulation of genes involved in innate immunity (Fig. 1B) and downregulation of genes involved in mitochondrial respiration (Fig. 1C), consistent with a phenotype primed for enhanced responsiveness to innate immune and painful triggers. Supporting this conclusion, THC-exposed mice exhibited prolonged heat hyperalgesia after intraplantar injection of low-dose PGE_2, compared to vehicle-treated animals (Fig. 1D).

Conclusions: Adolescent THC exposure induces a primed monocyte phenotype and a persistent sensitized pain state in late adolescent mice, suggesting increased susceptibility to subsequent inflammatory challenges.



CANNABIS EFFECTS ON OPIOID SELF-ADMINISTRATION: PILOT STUDY IN NON-DEPENDENT, OCCASIONAL OPIOID USERS

Shanna Babalonis*, PhD; Paul Nuzzo, MA; Michelle Lofwall, MD; Laura Fanucchi, MD; Sharon Walsh, PhD

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Introduction: Cannabis and opioids are widely used for both therapeutic and non-medical purposes; however, no studies to date have evaluated the effects of cannabis on opioid self-administration in humans.

Methods: This completed randomized, double-blind, placebo-controlled, inpatient, 5.5-week study enrolled participants (*n*=6) with occasional non-medical use of cannabis and opioids (no current opioid/cannabis use disorder). Participants sampled doses of intranasal oxycodone (0, 15, 45 mg; one dose/session). On the following days, cannabis (0, 30 mg vaporized THC) was administered prior to opioid self-administration sessions. Participants worked for the available opioid dose (1/7th increments, up to the full dose) or a money alternative ($21 total; $3 increments). Primary outcomes were the number of trials completed for the opioid doses, as a function of cannabis pretreatment. Secondary outcomes included abuse potential, physiological, pain and psychomotor outcomes.

Results: Active doses of oxycodone (with placebo cannabis pretreatment) were self-administered (15 mg oxycodone: 4.3 ± 0.7 trials; 45 mg oxycodone: 4.0 ± 0.4 trials). Cannabis pretreatment (30 mg) decreased oxycodone self-administration (15 mg oxycodone: 2.3 ± 0.8 trials; 45 mg: 3.3 ± 0.9 trials). This represents a 47% decrease in opioid-taking at the low dose and a 18% decrease at the higher opioid dose.

Conclusions: This controlled study suggests that acute exposure to cannabis may have the potential to decrease the drive to take opioids in those with infrequent opioid misuse. These effects will be further examined in an upcoming trial with participants with severe opioid use disorder/physical dependence in a model of repeated cannabis exposure.

Funding: NIDA (R01DA045700)

EVIDENCE OF BRAIN INJURY AND DYSREGULATION OF HOMEOSTATIC INFLAMMATORY MEDIATORS IN A PRECLINICAL MODEL OF PRENATAL CANNANOID EXPOSURE

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Introduction. Use of cannabis during pregnancy has escalated, with smoking/vaping being the most common mode of use. We examined the effects of prenatal cannabinoid exposure (PCE) on neurodevelopment, with a focus on structural and functional brain injury and homeostatic inflammatory mediators in brain regions associated with cognitive and motor function.

Methods: To model PCE, pregnant female rats were exposed to vaporized 9-tetrahydrocannabinol (THC) or vehicle during gestation and with offspring through postnatal day (P)10 (human equivalent full term). Separate groups of offspring (n=6-10/group) were assessed for: Brain concentrations of THC and metabolites on P10, DigiGait analysis and open field tests on P30 (adolescent), immunohistochemistry and inflammatory assays (biomarkers, flow cytometry), magnetic resonance imaging (DTI, fMRI) and cerebral immune cell activation on P60 (adult). Data were analyzed with Student's T-tests or Mann-Whitney U-tests; α=0.05.

Results: Fetal brain concentrations of the THC metabolite 11-OH-THC were 46-48% of maternal brain concentrations. Adolescent PCE rats showed abnormal gait and hyperactivity compared to controls ($p< 0.01$). Adult PCE rats had global hyperconnectivity within and between cortical and subcortical networks ($p=0.0001$), reduced fractional anisotropy in the corpus collosum ($p<0.05$), and diminished myelin basic protein expression in the cortex and fimbria ($p< 0.0001$) consistent with impaired myelin development and/or degradation. Systemic TNFα and CXCL1 and CXCR2+ neutrophils were increased, with reduced systemic anti-inflammatory mediators and fewer protective cerebral T cells (all $p< 0.05$).

Conclusions: PCE via vaped THC led to a hyperactive behavioral phenotype in adolescence and immune function dysregulation in adulthood with widespread white matter abnormalities and global alterations in functional connectomes.

OMEGA-3 SUPPLEMENTATION COUNTERACTS PERSISTENT BEHAVIOURAL AND GUT–BRAIN ALTERATIONS PRODUCED BY ADOLESCENT EDIBLE THC EXPOSURE

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Introduction: Adolescent cannabis use remains prevalent despite its association with increased vulnerability to psychiatric disorders later in life. Exposure to Δ9-tetrahydrocannabinol (THC) during this developmental window may produce enduring consequences. Psychiatric and gastrointestinal (GI) disturbances frequently co-occur, and the endocannabinoid (eCB) system plays a role in regulating both brain and gut function, suggesting gut–brain axis dysregulation could be involved in THC's long-term effects. We previously showed that adolescent THC edibles induce sex-dependent behavioural alterations, accompanied by prefrontal cortex (PFC) and ventral tegmental area (VTA) hyperactivity. Given the limited interventions available to mitigate these neurodevelopmental effects, omega-3 polyunsaturated fatty acids represent a promising therapeutic strategy due to their influence on eCB and dopamine signalling, and neuronal membrane integrity. We hypothesize that omega-3 supplementation during adolescence will prevent THC-induced behavioural and gut-brain alterations.
Methods: Adolescent male and female rats received THC edibles (escalating 1–5 mg/kg; mixed in Nutella®) twice daily for 11 days and were maintained on a control or omega-3 enriched diet for 21 days. In adulthood, affective and cognitive behaviours were assessed, and *in vivo* electrophysiology quantified PFC glutamatergic and VTA dopaminergic activity. Intestinal and brain tissues were collected for PCR analysis of eCB, monoamine and inflammation-related gene expression.
Results: Omega-3 supplementation prevented adolescent THC induced long-term cognitive and affective deficits in both sexes and countered PFC glutamatergic and VTA dopaminergic hyperactivity. In the small intestine, adolescent THC reduced DAGLα and PPARγ while increasing COX2 expression, indicating altered eCB and inflammatory signalling, which were normalized by omega-3 treatment.
Conclusions: These findings demonstrate that adolescent THC exposure produces persistent behavioural, neuronal and gut alterations, supporting gut–brain axis involvement in THC's developmental impacts. Omega-3 supplementation represents a promising strategy to protect against the consequences of adolescent cannabis exposure.

CANNABIDIOL EFFECTS UPON EXECUTIVE FUNCTION IN A CLINICAL TRIAL OF PEDIATRIC EPILEPSY AND COMORBID ANXIETY

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Introduction: Anxiety disorders are common among youth with epilepsy. However, many anti-seizure medications are associated with adverse cognitive effects, including sedation and cognitive slowing, which may limit treatment efficacy for neuropsychiatric comorbidities. Medications that can effectively treat both seizures and anxiety without cognitive burden are therefore of clinical interest. An open-label clinical trial targeting both epilepsy and anxiety was conducted with pharmaceutical grade cannabidiol, Epidiolex®, a USFDA-indicated treatment for some complex epilepsy syndromes.

Methods: Participants were 20 children and adolescents aged 6-17 (mean = 12.9 years; 12 female), with active epilepsy and clinically impairing anxiety symptoms. Titration from 5-20 mg/kg/day occurred with a flexible dosing paradigm. Executive function, anxiety and seizure characteristics were measured at baseline, 8 and 16 weeks. Outcome measures included Clinical Global Impression Improvement (CGI-I), and the Behavior Rating Inventory of Executive Function (BRIEF).

Results: Sixteen of the 20 participants completed the 16-week clinical trial. Among completers, mean CGI-I scores at end of study (EOS) were 1.7 (SD = 0.8) for overall improvement and 1.6 (SD = 0.8) for anxiety-specific improvement. The Global Executive Composite score on the BRIEF notably improved from baseline to EOS ($p = 0.03$). Both anxiety and epilepsy measures improved as well, in a near simultaneous time course.

Conclusions: Epidiolex was associated with significant improvement in both seizures and comorbid anxiety in pediatric patients. Executive functioning did not worsen and appeared to improve in most cases. Epidiolex® is a well-tolerated option for pediatric epilepsy and comorbid anxiety, warranting further investigation in controlled trials.

DOES PRENATAL/PERINATAL ACETAMINOPHEN TREATMENT INHIBIT DIACYLGLYCEROL LIPASE ALPHA TO CAUSE ASD-LIKE BEHAVIORS IN MICE?

Michaela Dvorakova[*1,2], Ella Sodunke[1], Taylor Woodward[1,2], Wenwen Du[1,2], Alex Straiker[1,2], Heather Bradshaw[1,2], Ken Mackie[1,2]

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Introduction: Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental condition characterized by impairments in social interaction, sensory processing abnormalities, and repetitive behaviors. Comorbid conditions such as anxiety and memory deficits are also common. ASD etiology reflects complex interactions between genetic susceptibility and environmental factors. Among proposed environmental contributors, prenatal exposure to acetaminophen (APAP) has emerged as a potential risk factor. APAP is used by over 50% of pregnant individuals, and some epidemiological studies associate prenatal APAP exposure with increased ASD incidence. The neurobiological pathways underlying APAP's potential effects on brain development have not yet been determined.

Methods: Based on our identification of diacylglycerol lipase alpha (DAGLa) as a molecular target of APAP, we tested the hypothesis that APAP-induced DAGLa inhibition disrupts developmental endocannabinoid signaling and contributes to ASD-related phenotypes. DAGLa synthesizes 2-arachidonoylglycerol (2-AG), which is essential for axonal growth, guidance, and neural circuit formation. Pregnant and lactating mouse dams were administered APAP, and behaviors were assessed in their offspring. To probe circuit-level mechanisms, we employed a chemogenetic strategy to selectively enhance interneuron activity in the prefrontal cortex.

Results: Developmental APAP exposure produced ASD-relevant behavioral alterations in offspring, including social deficits and increased anxiety-like behaviors. Some behavioral abnormalities were rescued by chemogenetic enhancement of prefrontal cortex interneuron activity.

Conclusions: These results identify DAGLa inhibition as a novel mechanistic link between prenatal APAP exposure and ASD-relevant neurodevelopmental outcomes, highlighting the endocannabinoid system as a potential target for understanding environmentally mediated ASD risk.

Disclosures: The authors have nothing to disclose.
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ICRS Presidential Plenary Lecture 2:
12:00-13:00, Thursday, July 2, 2026

SHINING FLUORESCENT LIGHT ON COLORFUL CANNABINOID SIGNALING

Istvan Katona, PhD

Naus Family Chair of Addiction Sciences, Professor, Psychological & Brain Sciences, Indiana University

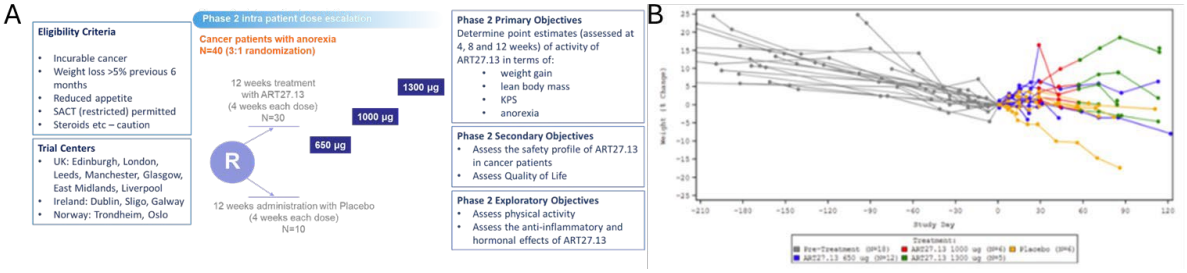
Istvan Katona works as Naus Family Chair in Addiction Sciences at Indiana University Bloomington and as senior consultant at HUN-REN Institute of Experimental Medicine, Budapest. His research team focuses on the adaptive and maladaptive plasticity mechanisms underlying cannabis effects and endocannabinoid signaling in the brain. His main contributions include the molecular architecture for retrograde endocannabinoid signaling in synapses; the synaptic circuit-breaker model for the role of endocannabinoids in epilepsy; and the development of STORM super-resolution imaging methodologies for nanoscale imaging of cannabis effects. He is a lifetime member of the Academia Europea and EMBO. In his talk, he will present new super-resolution microscopy approaches for cell-type-specific cannabinoid nanopharmacology in complex tissues.

INTERIM ANALYSIS OF THE PHASE 2 CANCER APPETITE RECOVERY STUDY (CARES) EVALUATING ART27.13, A PERIPHERALLY SELECTIVE CANNABINOID AGONIST

Andrew Yates, Paula Daunt, Saoirse E O'Sullivan*, Steve Reich, Barry Laird[1]

Artelo Biosciences Limited, Alderley Edge, UK

Introduction: ART27.13 is a peripherally selective CB_1/CB_2 agonist, originally developed by AstraZeneca. Evidence from the Multiple Ascending Dose study suggested a dose-dependent increase in body weight. This is supported by a large body of preclinical data showing peripheral CB_1 activation promotes weight gain. Our hypothesis was that ART27.13 would increase weight in cancer patients who have unintentionally lost >5% body weight in the previous 6 months. **Methods**: Cancer Appetite Recovery Study (CAReS) is a Phase 1/2 dose-ascending, multi-center trial (ISRCTN: 15607817, ART27.13-100, IRAS 278450)(see Figure 1A). **Results:** In Phase 1, ART27.13 was escalated from 150–650 µg and was well-tolerated for 12 weeks, with no serious adverse events (SAEs) and preliminary evidence of a positive impact on weight. Interim analysis of Phase 2 was carried out in 25 patients who received at least one dose of ART27.13 (650–1300 µg). Seven patients (32%) had AEs considered related to study drug, which were mostly mild to moderate with no SAEs. The most common (>2 patient) AEs related to trial drug were vomiting (12%) and dry mouth (12%). Stabilisation or an increase in weight on ART27.13 was observed in patients at all doses explored in the study (Figure 1B) compared to a decline in weight on placebo. After 3 cycles of treatment, patients on the top dose of 1300 µg had a mean increase in weight of 6% compared to a mean loss in weight of 5% for placebo. After 1 cycle of treatment, changes in lean body mass aligned with changes in weight; however, limited data was available at the end of treatment. Activity data captured by MotionWatch showed an increase in total activity for patients on ART27.13 treatment compared to those on placebo. Larger and more frequent increases in activity were observed for patients on active treatment.



Conclusions: ART27.13 is an orally active, once daily, peripherally selective, CB_1/CB_2 agonist being trialed in cancer patients with anorexia and cachexia. Interim analysis of the CAReS Phase 2 indicated strong quantitative signals of ART27.13's efficacy in weight gain, lean body mass, and activity levels. In cancer cachexia patients, ART27.13 was well-tolerated up to 1300 µg/day. Currently, there is no FDA approved treatment for cancer anorexia cachexia syndrome.

CANNABINOID RECEPTOR β-ARRESTIN SIGNALING ALTERS CYTOKINE CONTROL OF METABOLIC AND INFLAMMATORY PATHWAYS

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Introduction: In specific physiological and pathological scenarios, CB_1 and CB_2 receptors engage phosphatase pathways and may therefore limit cytokine receptor signaling. We hypothesized that such a mechanism contributes to the central orexigenic effects of CB_1R by opposing leptin signaling, and to the peripheral anti-inflammatory effects of CB_2R by opposing inflammatory cytokine signaling.

Methods: Leptin signaling and cytokine receptor activity were analyzed by immunohistochemistry and Western blotting in mouse hypothalamus and psoriatic skin samples. For the quantification of *in vitro* cytokine and leptin receptor activity, we developed a semi-automated, confocal microscopy assay measuring the nuclear translocation of multiple STAT isoforms. Protein-protein interactions were assessed by co-immunoprecipitation and Western blotting.

Results: CB_1R agonists reduced leptin-induced STAT3 activation in GT1-7 and N2a immortalized neurons and neuroblasts, and this effect was abolished by inhibition of T cell protein tyrosine phosphatase (TC-PTP) or by deletion of β-arrestin1. Upon CB_1R activation, β-arrestin1 associated with both STAT3 and TC-PTP. When examined in vivo, CB_1R agonism consistently suppressed hypothalamic leptin signaling in wild-type but not in $CB_1R^{-/-}$ or β-arrestin1$^{-/-}$ mice. Similarly, CB_2R agonists attenuated IL-6-induced STAT1 and STAT3 signaling in cultured cells and reduced STAT3 activation in psoriatic skin.

Conclusions: Cannabinoid receptors can limit cytokine/leptin receptor signaling through β-arrestin-dependent inhibition of key STAT isoforms. CB_1R activity reduces hypothalamic leptin signaling, while CB_2R suppresses inflammatory cytokine signaling in psoriatic skin, indicating a shared regulatory mechanism of cannabinoids across central and peripheral tissues.

CB2 RECEPTOR ACTIVATION DRIVES MITOCHONDRIAL METABOLIC STRESS AND LIMITS AGGRESSIVE POTENTIAL IN COLORECTAL CANCER CELLS

Daniela Esposito*[1], Salvatore Sicco Carandente[1], Marco Lambiase[1], Alessandro Nicois[1], Debora Paris[1], Eduardo Maria Sommella[2], Nella Prevete[3], Alessia Ligresti[1]

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Introduction: Mitochondrial function is a key driver of cancer metabolic rewiring, shaping energy production and cellular plasticity linked to invasive traits. In colorectal cancer (CRC), as in other malignancies, disease progression relies on profound alterations in energy metabolism. Emerging evidence suggests that cannabinoid signaling may directly influence cellular bioenergetics, yet the role of the cannabinoid receptor type 2 CB2 in mitochondrial metabolism remains largely unexplored. Here, we investigated whether CB2 activation directly rewires mitochondrial energy metabolism and invasive traits in CRC cells.

Methods: CB2 expression was assessed in a panel of human CRC cell lines (DLD-1, HT-29, HCT-116) using a selective fluorescent ligand. Mitochondrial respiration was analyzed by high-resolution respirometry (O2k, Oroboros), with CB2R specificity evaluated using the antagonist SR144528. Integrated metabolomic, lipidomic, and proteomic analyses were performed in HCT116 cells following non-cytotoxic JWH-133 treatment defined by SRB. Cell migration and invasion were assessed using Transwell assays.

Results: In all CRC cell lines, CB2R expression was confirmed, and its activation with JWH-133 significantly suppressed oxidative phosphorylation and maximal respiratory capacity. In HCT-116 ($p<0.001$), this effect was dose-dependent and fully reversed by SR144528, demonstrating direct CB2-mediated control of mitochondrial respiration. Multiomics profiling revealed a coordinated metabolic stress response characterized by mitochondrial dysfunction, increased oxidative stress, and lipid remodeling, supported by elevated mitochondrial ROS production (MitoSOX, $p<0.001$) and increased neutral lipid storage with lipid droplet accumulation (BODIPY, $p<0.001$). Pathway analysis identified downregulation of thermogenesis, redox regulation, and motility programs. Consistently, CB2 activation significantly reduced cell migration and invasion (Transwell assay, $p<0.01$).

Conclusions: CB2R activation induces a coordinated mitochondrial metabolic stress program that constrains invasive behavior in CRC cells. These findings reveal CB2R-driven bioenergetic rewiring as a previously underappreciated metabolic vulnerability with potential translational relevance.

NEW THERAPEUTIC TARGETS FOR HEPATIC FIBROSIS IN MASLD: THE SGLT2 COTRANSPORTER AND THE CANNABINOID-1 RECEPTOR.

Maéva Loriot[1*], Océane Pointeau[1,2], Romain Barbosa[1], Amélie Lainé[1], Alexia Rouland[1,3], Audrey Geissler[4], Patricia Passilly-Degrace[1], Julia Leemput[1], Laurent Demizieux[1], Malliga Iyer[5], Resat Cinar[6], Pascal Degrace[1], Bruno Vergès[1,3] and Tony Jourdan[1].

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Introduction: Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is the leading cause of chronic liver disease worldwide, affecting ~30% of the population. It is highly prevalent in individuals with obesity or Type 2 Diabetes (T2D) and is driven by hepatic fat accumulation, insulin resistance, and inflammation, progressing from steatosis to fibrosis and cirrhosis. Current therapeutic options remain limited. This study aimed to assess whether a poly-pharmacological approach combining SGLT2 inhibition and CB1 receptor antagonism could represent an effective strategy to limit hepatic fibrosis.

Methods: C57Bl/6J mice were fed a Western Diet (WD) and high fructose corn syrup water to induce obesity, insulin resistance, and MASLD. Mice received daily for 28 days either a vehicle, the CB1 receptor antagonist MRI-1867, the SGLT2 inhibitor empagliflozin, or their combination. Metabolic parameters, liver histology, and gene expression were evaluated.

Results: WD-fed mice developed significant weight gain, insulin resistance, and hepatic steatosis, with elevated ALT and AST levels. Empagliflozin improved glucose tolerance but worsened insulin resistance, while MRI-1867 had minimal metabolic effects. Combination treatment markedly reduced hepatic steatosis and lipid droplet size, accompanied by decreased expression of lipogenic genes (*Acc1, Fasn*). Gene expression of inflammatory markers (*Cd68, Clec4f, Tnf, Il1b*) were reduced, indicating attenuated liver inflammation. Sirius red staining and fibrotic gene expression (*Tgfb1*, *Col1a1*, *Col1a2*, *Col3a1*) were significantly decreased, demonstrating fibrosis regression.

Conclusion: Dual SGLT2 inhibition and CB1 receptor antagonism reduces steatosis, inflammation, and fibrosis in MASLD, supporting the potential of poly-pharmacological strategies for disease management.

ICRS 2026

Dijon

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DATABLITZ & POSTER PRESENTATION ABSTRACTS

CB2 AGONIST HU-910 RESOLVES PAIN EQUALLY TO MORPHINE IN MOUSE MODEL OF CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN

Natalia Zemliana*[1], Natalya M. Kogan[1]

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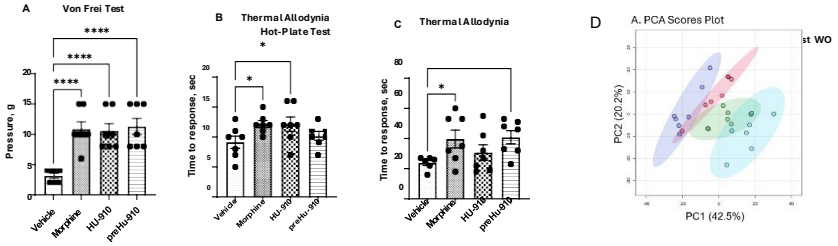
Introduction: Main strategies for pain management rely primarily on activation of opioid receptors. The global public health crisis caused by widespread opioid abuse, addiction, and overdoses highlights the urgent need for alternative pain relief methods. In addition to the opioid system, the endocannabinoid system also contributes to pain sensing, importantly, not only through the psychotropic CB1 receptor, but also through the CB2 receptor. In the present study, we aimed to compare the analgesic efficacy of HU-910, a potent and selective CB2 agonist, with the standard opioid treatment morphine in a mouse model of chemotherapy-induced neuropathic pain. **Methods:** Paclitaxel (PTX) was intraperitoneally administered to the ICR male at days 0, 2, 4, and 6, n=7 per per group, total dose of 8 mg/kg (2 mg/kg/day). The treatment with HU-910 and morphine (10 mg/kg dissolved in a vehicle of 1:1:18 ethanol:tween80:saline) administered at days 11, 13, 15, 17, and pre-treatment group obtained HU-910 at days 0,2,4,6,8 1h before PTX additionally. Pain perception was assessed by Von Frei filaments, Hot Plate and Acetone Drop tests on day 17. Behavioral tests (Elevated Plus Maze, Open Field and Forced Swim Test) were performed on day 18, while mice were treated with vehicle, morphine, or HU-910 i.p. 1h before the tests. Dissections were performed on day 21, while mice were treated with vehicle, morphine, or HU-910 i.p. 1h before the tests. Brain, blood and spinal cord were collected and stored in -80. Hippocampus tissue was extracted for metabolomics analysis. Samples were analyzed by HPLC/MS chromatography, the processing was performed with Progenesis QI software (Nonlinear). MetaboAnalyst software was used to analyze differences in metabolic profiles and distinguish significantly altered metabolites, and further, the key metabolites were identified by comparing the masses and the fragments to databases such as HMDB and LipidBlast. Statistical Analysis was performed using Graphpad Prism 10 (San Diego, CA). Results are represented as means ± SEM. P values of < 0.05 were considered significant by one-way ANOVA. **Results:** 2 weeks after the onset of PTX treatment, the symptoms of nociceptive pain develop: the mice express more sensitivity to mechanical and to thermal stimulation. In Von Frei test the antinociceptive effects of HU-910, the pre-treatment with HU-910 and morphine were equipotent, while all the treatments significantly elevated mechanic pain threshold. In Hot Plate test, the treatment with morphine and HU-910 was equipotent in reducing the thermal pain, while HU-910 treatment was less effective. In Aceton Drop test, the pre-treatment with HU-910 was equipotent to morphine in reducing cold allodynia, while HU-910 treatment was less effective. Metabolomics studies revealed significant differences in lipid metabolism between the groups. In the PCA plot, there is a clear difference on PC1 between the groups, while the control group is the furthest from the HU-910 pre-treated group. Important metabolites of diacylglycerols, ethanolamides, phospholipids, ceramides and fatty acyl esters of fatty acids were identified.



Conclusion: The HU-910 is the CB2R selective synthetic agonist which exerted comparable to the morphine anti-inflammatory and peripheral antinociceptive activities in a paclitaxel (PTX)-induced pain model. As HU-910 was equipotent to morphine, but not psychotropic and not addictive, we suggest the treatment, or even pre-treatment by HU-910 during the chemotherapeutic treatment to reduce, and potentially even prevent neuropathic pain.

PHARMACEUTICAL STABILIZATION OF ACIDIC CANNABINOIDS: ENABLING TRANSLATIONAL RESEARCH AND DEVELOPMENT

Benjamin Cameransi

Mingowood Pharmacal

Background: Acidic cannabinoids (e.g., CBDA, THCA, CBGA) exhibit superior pharmacological potential, including significantly higher potency and bioavailability than their neutral counterparts. However, their clinical utility is critically limited by inherent chemical instability, primarily due to rapid autocatalytic decarboxylation and oxidation.

Methods: Patented LDH™ (Layered Double Hydroxide) technology was utilized to formulate CBDA, THCA and CBGA. Stability was rigorously confirmed under accelerated conditions (40C/75%RH) over 90 days. Purity and degradation kinetics were quantitatively monitored using HPLC and ATR-FTIR.

Results: Both CBDA, THCA and CBGA formulated via LDH™ demonstrated exceptional stability, retaining over 95% purity after 90 days at 40C. Crucially, the stabilization process successfully suppressed decarboxylation rates compared to unformulated raw material controls, meaning that even the new 0.4mg THC limit does not pose any limitations to the use of these materials.

Conclusion: LDH™ offers a reliable and reproducible platform technology for the pharmaceutical stabilization of acidic cannabinoids. This breakthrough enables the rigorous pharmacological evaluation necessary to advance these compounds into translational research across multiple therapeutic areas, capitalizing on potent activity at PPAR receptors, 5HT-1A, COX2, and others, with pain and metabolic disorders representing.

COMBINED AND SEPARATE EFFECTS OF CANNABIS AND TOBACCO: PSYCHOMOTOR, SUBJECTIVE AND PHYSIOLOGICAL OUTCOMES (CASE-CT): A PROTOCOL

Patricia Di Ciano*, Ahmed Hassan, Bernard Le Foll, Sergio Rueda, Michael Chaiton, Wei Wang, Christine Wickens,
Justin Matheson, Pamela Kaduri, Sampson Zhao, Julia Migas

*Presenting Author
Centre for Addiction and Mental Health

Introduction: Co-use of tobacco with cannabis ('spliffs', 'blunts', 'chasing') is increasing in prevalence, but there is little experimental evidence into the effects of co-administration of these two substances.

Methods: This is a crossover, double-blind, placebo-controlled study in 60 participants (anticipated 30 males). Participants are administered: 1) an active cannabis 'joint' (26% in 350-400 mg pre-roll) + an active tobacco cigarette (standard nicotine; NIDA drug supply program)'; 2) an active cannabis 'joint' + low nicotine cigarette; 3) a placebo cannabis 'joint' (NIDA Drug Supply Program) + standard nicotine cigarette; 4) placebo cannabis 'joint' + low nicotine cigarette. Driving, cognition and subjective assessment are conducted 15 minutes and 3 hours after smoking the 'joint'/cigarette. Blood is also collected at the time of the drive to measure THC and its metabolites.

Results: At present, 29 males and 18 females have completed all session procedures. Study completion is anticipated in the summer of 2026. Interactions between the effects of cannabis and tobacco will be analysed, as well as the effects of cannabis or tobacco alone. In addition, any relationship between blood THC, metabolites, nicotine/cotinine and driving/cognitive variables will be assessed.

Conclusions: The present study will provide important information about the effects of tobacco on the health-related effects of cannabis. Evaluation of the relationship of blood THC to driving variables will provide evidence-based information into the utility of current cut-offs of THC in blood while driving.

INVESTIGATION OF THE PSYCHEDELIC EFFECTS OF HIGH-DOSE DELTA-9 THC

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Introduction: High-dose delta-9-tetrahydrocannabinol (THC) exposure has been associated with "psychedelic" effects but has yet to be evaluated in a controlled study using the set and setting typical of psychedelic clinical trials.

Methods: Healthy adults completed four randomly ordered drug administration sessions, receiving oral doses of either placebo, 25 mg THC, 50 mg THC, or 25 mg psilocybin. THC was either derived from a cannabis distillate extract or synthesized (dronabinol) and randomly assigned for each participant. After drug administration, participants lay on a couch and wore eyeshades while listening to a program of music through headphones under the supervision of two session monitors. 8 hours post-dosing, participants completed questionnaires related to the subjective drug experience, including the Mystical Effects Questionnaire (MEQ-30). Both participants and session monitors guessed what they received from a list of drugs known to produce psychedelic experiences.

Results: 4 participants have completed the study—two randomized to dronabinol and two to cannabis distillate. 25 mg dronabinol yielded scores on the MEQ30 akin to psilocybin in both participants who received it and was mistaken for a classic psychedelic by both the participants and session monitors. THC was associated with a longer time course of effects compared with psilocybin.

Conclusions: THC may evoke psychedelic-like experiences in the context of psychedelic assisted therapy. Additional data will determine whether THC can produce mystical effects associated with positive psychedelic therapy outcomes and whether it would be a suitable positive control for psychedelic clinical trial

CONTROL OF CLINICAL SIGNS OF EARLY INFLAMMATORY BOWEL DISEASE IN NON-HUMAN PRIMATES USING CANNABIDIOL AND PREDNISOLONE

Tim Lefever[1*], Diana Scorpio[2], Daniela Schwotzer[2], Judith Meriwether[2], Jacob McDonald[2], Trina Hazzah[1], Hunter Land[1]

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Introduction: Captive Non-human primates (NHPs) suffer from pervasive gastrointestinal issues (e.g., diarrhea) that cause frequent hospitalizations and can lead to long-lasting and treatment resistant inflammatory bowel disease (IBD) or related enteropathies. With no currently approved treatment for NHPs and limited long-term success of traditional approaches, a combination of cannabidiol and low dose prednisolone is being investigated in a phased approach as a potential early intervention. This initial exploratory study is ongoing with NHPs presenting with acute diarrhea.

Methods: Cynomolgus macaques (n=20, ~3kg) identified with diarrhea are enrolled in the study. Subjects are randomly assigned to 1 of 4 blinded treatment groups, n=5/group: placebo, prednisolone (0.28mg total daily dose (TDD)), cannabidiol (6.9mg TDD), prednisolone (0.28mg TDD) + cannabidiol (6.9mg TDD). Subjects are given marshmallow flavored treats containing the drug(s) (BioServ, NJ USA), two times per day for 7 days, followed by a 5-day post-treatment observation period. Animals are monitored twice daily, and detailed observations and body weight collections are performed once weekly. Fecal samples and blood for clinical chemistry analysis are collected prior to treatment start and at the end of the treatment period. Fecal scoring is performed daily.

Results: Thus far, the treatment has been well tolerated by all groups to date, and no drug-related adverse events were reported or expected in subsequent efforts.

Conclusions: This initial exploratory study shows some promise in the treatment of early IBD clinical signs in NHPs. Future research will focus on treating NHPs that exhibit chronic/severe IBD related clinical signs or clinical presentations.

CB_1 ANTAGONIST AM251 INHIBITS LOCOMOTION AND AFFECTS STEP KINEMATICS DURING CLIMBING IN MARKER-BASED 3D MOTION CAPTURE OF MALE MICE

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Introduction: In our previous studies, we demonstrated that moderate doses of potent CB_1 and CB_2 agonist CP55,940 affect step kinematics during open field exploration, vertical climbing, and treadmill running. The most prominent effects were decreased swing heights across all tasks, with the most pronounced effects observed during climbing, despite general locomotion being unaffected in that task. In the present study, we used the same method to evaluate the effects of CB_1 antagonist AM251 in the open field and climbing tasks.
Methods: We used adult male C57BL/6J mice in a within-subject, randomized design (n=12). We recorded voluntary locomotor behavior during open field exploration and climbing behavior on a spoked mesh wheel, in mice treated with vehicle (1:1:18 EtOH, Kolliphor, saline) or a moderate dose of AM251 (3 mg/kg), which fully blocked the effects of CP55,940 at high dose (1 mg/kg) but doesn't produce significant effects in standard open field test. Mice were implanted with permanent markers located on the hips, shoulder blades, hindlimb knees, and ankles. A high-speed, high-resolution 3D motion capture system (Qualisys, Sweden) was used to track 3D trajectories and velocity of markers in behaving animals.
Results: AM251 significantly reduced general locomotion in the open field and vertical climbing tasks but had no effect on step kinematics in the open field. However, significant effects on step kinematics were found during climbing. Both in the open field and during climbing, AM251 significantly reduced distance traveled, time spent locomoting, and the motion index (average velocity of all markers) compared to vehicle treatment. AM251 decreased climbing speed but had no effect on the speed of locomotion during exploration of the open field. Surprisingly, only during climbing, ankle swing parameters were affected, including: mean and maximum swing speed, swing height, and direct distance, which were all decreased by AM251, while duration was unaffected. No significant effects on swing kinematics were observed during open field exploration despite inhibited locomotion. The total number of steps was reduced during open field exploration but not during climbing.
Conclusions: The results suggest that general locomotion parameters, such as distance and time spent locomoting, do not align with step kinematics control mechanisms. This is consistent with our previous observations of other pharmacological perturbations. The results indicate that cannabinoid ligands exert complex effects on general locomotion and step kinematics that are task dependent.

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

INTEGRATIVE TRANSFORMER BASED MULTI-OMICS MODELING OF CANNABINOID AND ENDOCANNABINOID PATHWAYS FOR PRECISION PREVENTIVE HEALTH

Umesh Gangadhar, J Singh and P Kumar

Centre for Biomedical Research and Multi-omics Foundation

Background and Aims: The endocannabinoid system (ECS) is a primary regulator of physiological homeostasis, influencing systemic inflammation, metabolic flux, and neuro-immune signaling. Despite its critical role, the molecular heterogeneity and non-linear interactions between ECS genetic variants and environmental factors remain inadequately defined. This study implements an AI-driven bioinformatics framework to decode these complex regulatory networks and identify high-fidelity biomarkers for disease prevention.

Methods: We engineered a transformer-based multi-task learning architecture to synthesize high-dimensional multi-omics datasets. The analytical pipeline integrated: Genomic & Epigenomic Discovery: Automated extraction of salient ECS-associated mutations and chromatin modifiers using scRNA-seq and ATAC-seq data. Predictive Systems Modeling: Application of Graph Attention Networks (GAT) to map temporal homeostatic stability and characterize dynamic cellular states within the fibroimmune microenvironment. Causal Inference & Simulation: Computational modeling of early-stage cannabinoid modulation to predict individualized responses and metabolic reprogramming.

Results: The Machine Learning (ML) framework demonstrated superior predictive performance, achieving an AUC-ROC of 0.82–0.94 and 94.1%–95.1% sensitivity in stratifying individuals by disease susceptibility. The model identified distinct cellular subpopulations ($P < 0.003$) acting as biological triggers for intervention. Multi-omics integration enhanced risk assessment accuracy by 23.2% over traditional metabolic biomarkers. Simulation-based analyses isolated key targets capable of mitigating subclinical declines and addressing "Cognitive Knowledge Deficits" in preventive adherence.

Conclusion: This research represents a paradigm shift in precision health by merging functional genomics with real-time predictive analytics. By leveraging "humanized" digital surveillance, the system effectively bridges the gap between static metabolic snapshots and the dynamic reality of ECS-driven disease progression. These AI-enabled strategies provide a scalable platform for personalized risk assessment and the rational design of cannabinoid-based preventive interventions.

THE SUBJECTIVE EFFECTS OF CANNABIS: A DOSE-RESPONSE META-REGRESSION

Isabella Goodwin[1],Dominic Oliver[2], Edward Chesney[3], Alexandra Gaillard[4], Simiao Wang[5], Andrea Wong Koo[6], Kat Petrilli*[3], Martine Skumlien[3], Amir Hossein Dakhili[1], Stina Wigroth[3], Stiliyana Obreshkova[3], Suhal Yusufzai[3], Ryan Vandrey[7], Michael Krausz[6], Tom P Freeman[8], Philip McGuire[2], John Strang[5], Valentina Lorenzetti[1,9], Amir Englund[3]

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Introduction: Global shifts towards the liberalisation of cannabis policies have increased the accessibility, diversity, and potency of cannabis products worldwide. Despite an increase of people who use cannabis for the first-time and an increase in the diversity of cannabis products in legal markets, there is a lack of evidence-based communication on the subjective effects of delta-9-tetrahydrocannabinol (THC) consumption. We therefore aimed to systematically review and meta-analyse the evidence from randomised, double-blind and placebo-controlled experimental studies on the subjective acute effects of cannabis according to THC dose in healthy individuals who use cannabis infrequently. **Methods:** We included 46 studies (total n=1,075). Pairwise and variability ratio meta-analyses estimated the mean difference of peak subjective effects between THC and placebo. Meta-regressions of dose were stratified by route of administration. **Results:** Inhaled dose of THC showed a significant positive association with feeling high (b=1.06, p=0.0024) and contentedness (b=1.06, p=0.025), and a negative association with calmness (b=-0.96, p=0.048). There were no associations with anxiety (b=-0.03, p=0.845), tiredness (b=0.39, p=0.129), or alertness (b=0.11, p=0.837). As inhaled dose increased, there was a significant decrease in the individual variability of feeling high (b=-0.05, p=0.021) and contentedness (b=-0.04, p<0.0001), meaning that these effects became more consistent between subjects as the THC dose increased.
Conclusions: After 5mg inhaled THC, there were percentage point increases in peak feelings of: high, anxiety, tiredness, and calmness by metrics 34.2, 14.6, 26.2, 4.5, respectively; and decreases in alertness and contentedness by 40.2 and 71.0 respectively. Overall, greater inhaled doses of THC produced both stronger and more consistent experiences of intoxication. This evidence can inform public health messaging, experimental study design, and guidance on recreational cannabis use.

THE ROLE OF SEX AND THE PERIPHERAL CANNABINOID RECEPTOR (CB2R) ON NEUTROPHIL EGRESSION AND MATURATION IN MICE WITH SYSTEMIC *C. ALBICANS* INFECTION

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Introduction: *Candida albicans (C. albicans),* a yeast commonly found in normal flora, is an opportunistic pathogen that can lead to mucocutaneous and systemic infections. Neutrophils are the main defense against C. *albicans* infections. Neutrophils, produced in the bone marrow (BM), transition from immature neutrophils to mature neutrophils and egress into the blood. Mature neutrophils are most effective against C. *albicans*. The peripheral cannabinoid receptor (CB2R), mainly expressed in immune cells, regulates neutrophil functions such as migration and inflammatory responses. Sex hormones affect neutrophil maturation and activation. We investigated the role CB2R and sex have on neutrophil maturation in mice with systemic *C. albicans* infection.

Methods: CB2R+/+ and CB2R-/- male and female mice were either uninfected or infected intravenously with *C. albicans* ($7.5x10^6$yeast cells/mL). BM and blood were collected 3 days post infection. Neutrophils ($CD62L^{hi}$ and $CD16^{hi}$) were identified as immature ($CD62L^{hi}$ and $CD16^{low}$), mature ($CD62L^{hi}$ and $CD16^{hi}$) and aged ($CD62L^{low}$ and $CD16^{hi}$) using flow cytometry.

Results: In uninfected mice, sex and CB2R did not seem to affect neutrophil maturation levels in the BM or blood. In infected mice, there seem to be more immature neutrophils and less aged neutrophils in BM than in blood. The levels of mature neutrophils seem to be the same in BM and blood. Sex and CB2R may also play a role in neutrophil maturation levels.

Conclusions: Systemic *C. albicans* infection in mice seems to increase production and alter maturation state of neutrophils compared to uninfected mice. CB2R and sex may also play a role in neutrophil maturation.

OPTIMIZATION OF PERMEATION AGENTS FOR TRANSDERMAL DELIVERY OF THERAPEUTIC CANNABINOIDS

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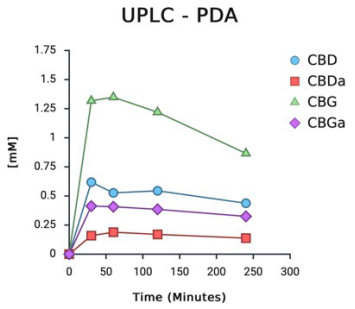
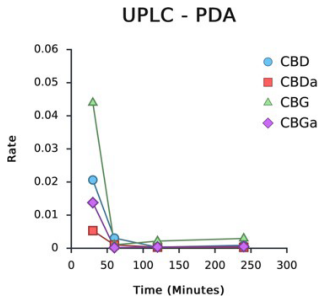
Introduction: Transdermal delivery of cannabinoids is a critical field of study for improving therapeutic outcomes. Amphiphilic formulation with novel deep eutectic solvents (DESs) is a potent subject of study for cannabinoid delivery agents due to their increased efficacy in navigating complex absorption barriers. Of these compounds, choline geranate (CAGE), is known to be an effective transdermal carrier, thus serving as a viable means of overcoming historical limitations of administration for therapeutic cannabinoids with the aim of improved systemic delivery, bioavailability, and administration accessibility.

Methods: Novel materials were synthesized with individual cannabinoids CBG, CBGa, CBD, and CBDa in CAGE eutectic materials of various ratios. Evaluation of transport efficacy was determined through diffusion modeling with synthetic membranes in a Franz Diffusion Apparatus. Diffusion and rate of delivery for each cannabinoid was analyzed via UPLC analysis of time interval sampling.

Results: Concentrations of cannabinoids were determined in the acceptor solutions at several time intervals during replicated diffusion trials and documented as an average represented in total concentration and rate of diffusion (Figures 1 and 2 respectively).





Conclusions: UPLC data shows findings consistent with delivery kinetics and confirmation of membrane permeation of CAGE-Cannabinoid compounds. Developments in this work aim to guide future optimization of transdermal permeation agents targeting novel cannabinoid delivery focused on increasing efficacy and clinical accessibility of cannabinoids as therapeutics.

PHYTOCANNABINOID BIOMARKER DIFFERENCES BY PSYCHEDELIC USE STATUS AMONG DAILY CANNABIS CONSUMERS IN THE HERBAL HEART STUDY

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INTRODUCTION: Whether cannabinoid biomarker concentrations differ by psychedelic use status among cannabis consumers remains unclear despite increasing reports of co-use. Clarification may improve the interpretation of biological exposure measures in studies. We examined whether phytocannabinoid/metabolite concentrations differ by psychedelic use status among daily cannabis consumers.

METHODS: Data are from daily cannabis consumers in the Herbal Heart Study (N=159; M_{age}: 26.1 years (SD=4.9), 51.1% female, 55.6% Hispanic/Latino, 31.1% Non-Hispanic Black. Whole blood and urine Δ9-THC, 11-OH-Δ9-THC, THC-COOH, and CBD were quantified using GC-MS/MS. Lifetime and past-year psychedelic use were self-reported. Cannabinoid concentrations were log-transformed [log(1+concentration)] to address skewness and zeros. Differences were evaluated using Welch's two-sample t-tests.

RESULTS: Half (56.5%, n=87) the sample reported lifetime psychedelic use; of these, 54.0% reported past-year use. Lifetime psychedelic consumers had higher log-transformed cannabinoid concentrations than non-consumers across most THC-related analytes. In blood, lifetime psychedelic use was associated with significantly higher log(1+Δ9-THC) (p = 0.003), log(1+11-OH-Δ9-THC) (p = 0.006), and log(1+carboxy-Δ9-THC) (p = 0.019), while blood log(1+CBD) did not differ (p = 0.331). In urine, lifetime psychedelic users had higher log(1+Δ9-THC) (p = 0.018) and log(1+11-OH-Δ9-THC) (p = 0.028); differences in log(1+THC-COOH) (p = 0.059) and log(1+CBD) (p = 0.062) did not meet statistical significance. Biomarker concentrations did not differ by past-year psychedelic use status.

CONCLUSIONS: Lifetime, not past-year, psychedelic use was associated with higher THC-related biomarkers among daily cannabis consumers. Future studies should examine larger samples of past-year psychedelic consumers. Accounting for lifetime and current use may improve the interpretation of cannabinoid biomarker variability.

SUPPORT: R01HL153467 (Vidot); T37MD008647 (Diggs); T32 DA007292 (Baral).

ASSESSMENT OF IN.VITRO GENOTOXICITY AND HEPATOTOXICITY AND IN.VIVO SUBACUTE ORAL TOXICITY OF SYNTHETIC AND PLANT-DERIVED CANNABIDIOL

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Introduction: Although cannabidiol (CBD) has recognized therapeutic potential, comparative toxicology between synthetic and plant-derived sources remains underexplored. Most existing studies examine only one CBD type, leaving uncertainty about whether origin, purity, or extraction method influence safety. We assessed the *in vitro* genotoxicity and hepatotoxicity, and *in vivo* subacute oral toxicity of CBD from different sources.

Methods: Two synthetic and two plant-derived CBD preparations (one high-purity isolate, one lower-purity distillate) were tested. *In vitro* genotoxicity was evaluated using Ames and micronucleus assays, with and without S9 mix. Hepatocytotoxicity was assessed in HepaRG™ cells through cell viability, albumin, alanine aminotransferase, and Cytochrome P450 (CYP) activities. Subacute toxicity was determined in Sprague Dawley rats, dosed orally with low (15 mg/kg), mid (50 mg/kg), or high (150 mg/kg) CBD from each source, or vehicle, for 7 days.

Results: All four CBD samples (purity 84.3–99.5%) demonstrated similar safety profiles, with no genotoxicity or mutagenicity *in vitro*. EC_{50} values for cytotoxicity ranged from 27 to 30 µM. At higher concentrations, all CBDs induced comparable acute cell damage and similarly inhibited CYP activities after 14 days. In vivo, repeated 150 mg/kg dosing increased liver and adrenal weights, with associated microscopic changes (hepatocyte hypertrophy, adrenal vacuolation) across all CBDs, although the number of incidences varied among APIs. These histopathological findings were not linked to degeneration or inflammation and considered non-adverse.

Conclusions: The data indicated that synthetic and plant-derived CBDs display comparable safety profiles in the battery of tests used in this study.

DEVELOPMENT OF SOLID CBD-LOADED PRO-NANO LIPOSPHERES WITH PRESERVED RELEASE AND BIOAVAILABILITY

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Introduction: Enhancing the oral bioavailability of poorly water-soluble drugs such as cannabidiol (CBD) has driven the use of lipid-based delivery systems; however, their solidification is often limited by low drug loading. This study aimed to develop pro-nano liposphere (PNL) systems that enable high CBD loading while retaining nano-dispersion performance in solid oral dosage forms.

Methods: Three liquid CBD-PNL formulations containing 25% (w/w) CBD were prepared to form nano-dispersions upon aqueous dilution. Solid PNLs were produced by adsorption onto Fujicalin® and compressed into lozenges containing 75 mg CBD. Liquid and solid formulations were characterized with respect to particle size, polydispersity, stability, CBD content, and emulsification behavior. *In vivo* bioavailability was assessed in fasted Sprague Dawley rats, and lozenges were evaluated for hardness, disintegration, and *in vitro* CBD release.

Results: All CBD-PNL formulations formed stable nano-dispersions and maintained CBD solubility at 25% (w/w) loading. Liquid PNLs remained physically stable for at least three months at room temperature and exhibited enhanced thermal stability following solidification. The solid PNLs enabled robust lozenge manufacture, showed rapid dispersion, and achieved >90% CBD release under simulated gastric conditions. Pharmacokinetic evaluation demonstrated rapid systemic absorption and high exposure, with PNL-1 providing the highest dose-normalized exposure (AUC_{inf}_D = 1948.2 min·kg·ng/mL/mg). Solidification preserved the exposure profile and resulted in the earliest time to maximum plasma concentration (T_{max} = 38.9 min).

Conclusions: PNL formulations enabled high-loading CBD nano-dispersions that were successfully solidified without loss of stability, release performance, or systemic exposure, supporting their utility as a solid oral delivery platform for poorly water-soluble drugs.

AFFECTIVE RESPONSES TO EXERCISE WHILE USING CANNABIS TRANSLATE TO SOCIAL COGNITIVE DETERMINANTS OF EXERCISE

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Introduction: Use of cannabis concurrent with exercise is very common, but the reasons for and implications of this practice are not well understood. The Theory of Planned Behavior (TPB) argues that people's behaviors are driven by their intentions, which are shaped by their attitudes, norms, and perceived behavioral control. Thus, the TPB can be used to understand the association of cannabis use with exercise engagement and maintenance. Specifically, cannabis may improve affective responses during exercise (e.g., runner's high) which may, in turn, change attitudes towards, perceived behavioral control (PBC) over, and intentions for exercise.

Methods: The present data came from a parent study investigating the acute effects of cannabinoid content (THC vs CBD) on responses to exercise. Participants (n = 40) engaged in two treadmill runs: one after acute use of legal-market cannabis flower (THC- or CBD-dominant), and one without cannabis.

Results: Separate sets of linear regressions indicated that across both the THC and CBD conditions, affective valence (β =.19, p=.03) and tranquility (β = .28, p=.02) during exercise were positively associated with attitudes toward exercise. Affective valence (β = .17, p=.02), tranquility (β = .28, p=.01), and runner's high symptoms (β = .33, p=.01) during exercise were positively associated with PBC over exercise.

Conclusions: Results suggest that affective responses during a cannabis exercise session are associated with subsequent attitudes toward and PBC over exercise, helping to explain the positive relationship between cannabis use and exercise behavior.

AN EXPLORATORY SENSORY EVALUATION OF CANNABINOID PRODUCTS WITH DIFFERENT FLAVOR VARIANTS

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Introduction: Despite its wide application in the food industry, the use of Sensory and Consumer Science in the development of cannabinoid (CB) products remains limited. To explore the application of sensory evaluation on CB products, this study compared sensory profiles across Oral Dissolvable Cannabinoid containing products with different flavor variants and additionally examined each product relative to its placebo counterpart. Results were expected to be used to support further product screening ahead of the end-user study.

Methods: Generic descriptive analysis was conducted following standard procedures, including panel training, the development of a sensory vocabulary, and final quantitative intensity ratings for predefined attributes. In this study, a total of thirteen predefined sensory attributes were assessed by seven trained panelists in two sensory evaluation sessions. At the end of evaluation, they were asked to give comments for each product.

Results: Although the flavor variants of CB containing products differed in their sensory flavor profiles, they showed comparable intensities of bitterness, throat irritation, and off-notes, indicating that the CB taste and sensations were similarly masked across products. Additionally, when compared with their placebo counterparts, CB products had lower flavor intensity and sweetness, and relatively higher bitterness, off-notes, and throat irritation, although these differences were not always statistically significant.

Conclusion: Overall, the results indicate that effective masking of the CB was achieved and confirm that generic sensory analysis can be successfully applied to guide further product development.

COMPREHENSIVE CHARACTERIZATION OF THE AORMA LANDSCAPE: INTEGRATING SENSORY ANALYSIS AND BIOSYNTHETIC PRECURSORS TO MAP THE VOLATILE PROFILEDS OF HEMP AND HIGH THC CANNABIS

Thi Khanh Linh Tran, Amandine André, Elodie Gillich, Dániel Árpád Carrera, Irene Chetschik and Leron Katsir

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Cannabis sativa L. produces a complex matrix of secondary metabolites, yet the pharmacological focus has historically remained centered on cannabinoids and major monoterpenes. While the aroma profile is a defining characteristic of different cultivars, the molecular drivers of these sensory experiences and their contributions to the plant's broader chemical identity remain under-characterized.

We expand upon our recent molecular characterization of CBD-rich hemp (Type 3) by performing a comparative analysis of high-THC (Type 1) cultivars using gas chromatography–olfactometry (GC-O) and aroma extract dilution analysis (AEDA). Our findings in hemp identified 52 odor-active compounds, including 38 reported for the first time in dried cannabis flowers and six constituents previously undocumented in any *C. sativa* matrix. While terpenes such as linalool, pinene and myrcene with high flavor dilution (FD) factors (256–1024) are sensory relevant, our data reveals a significant contribution from trace, highly potent non-terpenoid classes. Specifically, sulfur-containing odorants including 3-methylbut-2-ene-1-thiol, 4-methyl-4-sulfanylpentan-2-one, 3-sulfanylhexan-1-ol, and 3-sulfanylhexyl acetate, were identified as dominant sensory contributors in Type 3 as well as Type 1 cultivars despite their low abundance. Subsequent quantitation studies using isotopically labeled standards and the calculation of the dose over threshold values confirmed the importance of the odor active compounds as key contributors to the aroma of hemp.

Crucially, we have extended this work by identifying novel biosynthetic precursors for some of these sulfur-containing odorants, providing a roadmap explore the biology and volatilization route of these aroma compounds. These findings underscore the necessity of sensory-guided analysis, demonstrating that quantitative abundance does not inherently equate to sensory impact. By identifying the specific molecules and their precursors that drive the "aroma experience," this research provides a framework to explore how biology and post-harvest handling can define the chemical profile of *C. sativa*. Additionally, this provides a necessary foundation for investigating the potential contribution of these minor, high-potency odorants to the plant's broader therapeutic landscape.

INVESTIGATION OF CBD-ACCUMULATING CANNABIS SATIVA CULTIVARS AND SAFETY CONSIDERATIONS ON THE EFFECTS OF MINOR CANNABINOIDS

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Introduction: Cannabinoids are the primary components of *Cannabis sativa*. Beside the major cannabinoids (THC, CBD) found in the plant, the effects of minor cannabinoids are less understood. However, the study of cannabinoid composition and minor compounds is of increasing importance with the growing use of hemp and hemp-extract products, including foods. In this study, we investigated the cannabinoid composition of CBD-accumulating hemp cultivars and the antiproliferative effects of CBD and a minor cannabinoid (CBG).
Methods: A reversed-phase UPLC-PDA method was developed based on the ICH Q2(R2) guideline for the analysis of 14 cannabinoids. Six hemp cultivars were examined, and changes in cannabinoid content were monitored from July to October. The antiproliferative effects of CBD and CBG, as well as their interactions, were tested on tumour cell lines (Colo205, Colo320, MCF-7, PC-3, and A549).
Results: The cannabinoid profiles of the hemp varieties showed significant variation depending on cultivation conditions. CBD content typically peaked between the end of August and mid-September, while THC content reached its maximum by mid-October. In varieties selected for high CBD content, a significant CBD level (2.56%) was achievable alongside a low THC level (0.11%). Among the minor cannabinoids, CBN, CBNA, CBGA, and CBG were detectable in the samples. Interaction studies between CBD and CBG revealed synergistic effects at a 2:1 ratio in the Colo320 cell line and a 1:1 ratio in the A549 line. An additive effect was observed at an 8:1 ratio in the MCF-7 line, while an antagonistic effect was noted at a 1:1 ratio in the PC-3 line.
Conclusions: The method developed in this study is suitable for monitoring the cannabinoid content of hemp varieties. Based on the interaction studies, it can be hypothesized that minor cannabinoids influence the effects of CBD, which raises important safety considerations.

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UHPLC-PDA BASED SURVEY OF COMMERCIAL CBD PRODUCTS: METHODOLOGICAL VALIDATION AND LABEL ACCURACY ASSESSMENT

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Introduction: Cannabidiol (CBD) is a non-psychotropic phytocannabinoid used in medicines and increasingly found in commercial food supplements. Due to the lack of harmonized daily intake safety guidelines and potential adverse effects, there is a significant need for market surveillance. This study aimed to develop a reliable analytical method to verify the accuracy of CBD labelling and the safety of products available on the market.
Methods: A reversed-phase ultra-high performance liquid chromatography coupled with a photodiode array detector (UHPLC-PDA) method was developed and validated for the identification of cannabigerol (CBG), CBD, and Δ^9-THC. The method was validated for CBD quantification according to ICH Q2(R2) guideline. A variety of commercial products, including nine CBD-enriched oils, one capsule, and five hemp seed oils, were analysed.
Results: The analytical method demonstrated high accuracy (recovery: 95.2% to 100.42%) and precision (3.89%). Analysis of the products revealed that all samples were free of CBG and THC. CBD concentrations in the supplements ranged from 12.87 to 51.57 mg/mL. Out of the food supplements tested, only two were accurately labelled; two were under-labelled, and eight were over-labelled.
Conclusions: While the majority of tested supplements contained less CBD than indicated on their labels (over-labelled), in case of five products the detected levels exceed 2 mg/day CBD provisional safe daily intake set up by EFSA. Furthermore, the high frequency of incorrect labelling and poorly described components highlights a need for stricter regulatory oversight and standardized quality control to protect consumers.

Acknowledgement: This research was funded by Hungarian National Research, Development and Innovation Office (2021-1.2.6-TÉT-IPARI-MA-2022-00021) and supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00102/25).

ADDRESSING THE CANNABIS EDUCATION GAP IN HEALTHCARE TRAINING PROGRAMS

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Introduction: Despite the expanding legalization of medical cannabis across the United States, major Pharmacy accrediting bodies do not require formal education about cannabis and/or the endocannabinoid system within their curricula. Thus, pharmacy students must independently seek knowledge or face entering practice without adequate education. The goal of this study was to investigate the efficacy of a structured medical cannabis course in our School of Pharmacy program.

Methods: Pharmacy students enrolled in our Medical Cannabis Elective course completed pre and post-tests, which included seven categories: Neurotransmitter Overview, Cannabis Pharmacokinetics/Pharmacodynamics (PK/PD), Medical Uses of Cannabis, Cannabis Adverse Effects (AEs), Clinical Counseling, Dosage Forms, and Regulations. Change in students' scores were analyzed to measure knowledge improvement.

Results: Twenty-eight students were enrolled in the course; the average pre-test score increased from 33% at the start of the course to 95% at the conclusion. Gains were observed in all categories, and in particular in the areas of "Regulations" and "Medical Uses of Cannabis," which are critical to clinical practice.

Conclusions: Structured medicinal cannabis training is effective and important to improve learner competency. Without formal instruction, many students may graduate without foundational knowledge in an area that is increasingly relevant to patients. As legalization expands and public interest in alternative medicine grows, healthcare professionals must be prepared to provide accurate, evidence-based counseling. Integrating at least one structured module on medicinal cannabis into healthcare curricula nationwide could meaningfully improve baseline competency, reduce misinformation, and enhance patient safety.

WITHDRAWN

HEALTHCARE PROVIDER COMMUNICATION AND CANNABIS USE DURING PREGNANCY BY TRIMESTER: RESULTS FROM THE COVID-19 CANNABIS MOM STUDY

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INTRODUCTION: Cannabis use during pregnancy remains prevalent, yet evidence on how healthcare provider communication (HPC) influences use is limited. We examined the relationship between HPC and cannabis use during pregnancy among women who report cannabis use for medical reasons.

METHODS: Data are from a cross-sectional study of women in the United States who reported cannabis use prior to or during pregnancy. Participants self-reported discussion of cannabis use with a healthcare professional and whether the provider was supportive of use during pregnancy. Cannabis use by trimester was self-reported. Chi-square tests examined differences in use by HPC status.

RESULTS: In the sample (N=254), 5.1% discussed cannabis use with their provider before pregnancy, 25.6% discussed it during pregnancy; 46.7% of providers were perceived as supportive of use during pregnancy. Cannabis use frequency varied by trimester: 42.9% consumed daily in the first trimester, 35.0% in the second, 24.4% in the third. HPC was associated with daily cannabis use in the first (72.6% vs 42.1% among no-HPC p=0.02) and second trimesters (60.2% vs 36.8%, p=0.04), but not the third (42.5% vs 25.4%, p=0.28). There were no differences in cannabis use frequency before pregnancy by HPC-status (p=0.72); nor use for medical reasons (83.6% HPC vs 79.8% no-HPC, p=0.23).

CONCLUSIONS: Provider communication represents a modifiable clinical touchpoint to promote harm-reduction strategies in perinatal care.

SUPPORT: R01HL153467 (Vidot); T37MD008647 (Diggs); American Cancer Society (Brown)

IMPACT OF CANNABIS USE ON THE VIRAL RESERVOIR AND IMMUNE CELL GENE EXPRESSION IN PEOPLE WITH HIV ON ANTIRETROVIRAL THERAPY

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Introduction: Cannabis (CB) use is frequent amongst people with HIV (PWH), but the impact of CB on infection, antiviral immunity and viral persistence during therapy is unknown. PWH on antiretroviral therapy (ART) maintain a viral reservoir that is resistant to ART as well as elevated levels of chronic inflammation and comorbidity.

Methods: We performed detailed HIV reservoir analysis and immunophenotyping on two cohorts of people with HIV on antiretroviral therapy (ART), including cannabis users and non-users. 25 color flow cytometry indicated that cannabis using PWH exhibit elevated levels of naïve T cells and reduced levels of activated and exhausted T cells compared to non-using PWH. Bulk RNA sequencing (RNA-seq) on peripheral CD4+ T cells, as well as quantified plasma immune marker levels identified three distinct participant transcriptomic clusters, defined by differential expression of genes regulated by the inflammatory transcription factor NF-κκB.

Results: Moderate cannabis use was associated with a lower inflammation profile, while heavy use was associated with a proinflammatory profile. Strikingly, cannabis use was associated with a significantly smaller HIV reservoir and with reduced levels of viral reservoir expression during ART. In vitro stimulation of peripheral blood mononuclear cells (PBMCs) from people with HIV indicated that THC exposure rapidly downregulates a module of inflammasome-regulated gene expression in monocytes, including IL-1 beta.

Conclusions: These findings reveal that cannabis use is associated with distinct alterations to the viral reservoir and with the transcriptomic phenotype of immune cells in people with HIV, and that these changes may be regulated by inhibition of inflammasome activity in monocytes.

TEMPORAL AND CELL-SPECIFIC FUNCTIONS OF CANNABINOID 1 RECE¨TOR (CB1R) DRIVE THE TRANSITION FROM ACUTE KIDNEY INJURY TO FIBROSIS

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Introduction: Chronic kidney disease (CKD) affects millions of individuals worldwide and is mainly driven by diabetes and hypertension. Current therapies remain non-curative, underscoring the need to identify emerging therapeutic targets. **Methods:** Using murine renal ischemia–reperfusion (I/R), we dissected the role of the cannabinoid receptor type 1 (CB1R, encoded by *Cnr1*) through global genetic deletion (Cnr1-/-), cell type-specific targeting in tubular epithelial cells (CnroPax8LC1), myofibroblasts (Cnro.P0-Cre), and pharmacological inhibition. *Cnr1* expression was assessed by RT-qPCR and RNAscope. Renal function and histopathological injury were quantified. Transcriptomic analyses (nCounter) were performed in vitro on primary murine tubular cells and myofibroblasts. **Results:** *Cnr1* expression increased significantly 72 h after I/R (n=6, $p<0.05$). Global deletion did not impact the acute phase (n=6) but significantly reduced fibrosis and tubular atrophy at day 17 (n=8, $p<0.01$). Pharmacological inhibition provided complete protection, lowering serum creatinine (n=10, $p<0.001$) and histological damage (n=7, $p<0.05$). In contrast, tubular-specific deletion worsened CKD, increased serum creatinine (n=6, $p<0.05$), renal fibrosis (n=8, $p<0.01$), macrophage infiltration (n=7, $p<0.05$), and tubular injury (n=7, $p<0.01$). Myofibroblast-specific deletion attenuated fibrosis (n=7, $p<0.05$) without improving renal function or inflammation (n=7). In vitro, CB1R deletion reprogrammed pro-fibrotic signaling pathways in both tubular cells and myofibroblasts. Importantly, in human renal biopsies, *CNR1* expression correlated with CKD stages according to KDIGO 2012 classification ($p < 0.01$) supporting its clinical relevance. **Conclusions:** Collectively, CB1R exerts cell type–specific effects in CKD, orchestrating pathogenic crosstalk between tubular cells and myofibroblasts. Peripheral CB1 inhibition emerges as a promising therapeutic strategy in CKD.

A PILOT RANDOMISED, CONTROLLED, SINGLE-CENTRE, OPEN-LABEL STUDY TO ASSESS THE PHARMACOKINETICS OF A NANO-EMULSIFIED CANNABIDIOL EDIBLE FORMULATION IN HEALTHY ADULTS

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Introduction: Traditional edible cannabis formulations suffer from poor intestinal absorption due to high cannabinoid lipophilicity. There is an emerging use of emulsion technologies (e.g. nanoemulsion) in the market that aims to augment solubility and absorption of orally ingested cannabinoids. However, the literature to support the performance of such technologies are limited. Therefore, we sought to characterize the pharmacokinetic profile of cannabidiol (CBD) in a nanoemulsion formulation.

Methods: A randomized, controlled, single-centre, open-label crossover study in healthy adults (n = 36) was utilized to compare the pharmacokinetics (PK) of 60 mg CBD in a nanoemulsion gummy formulation to traditional oil-formulated gummies. Following oral ingestion, blood was sampled at specified time points (0 min, 15 min, 30 min, 1 h, 1.5 h, 2 h, 2.5 h, 3.5 h, 4 h, 4.5 h, 6 h, 12 h, 24 h). Plasma samples were analyzed for CBD, 7-OH-CBD, and 7-COOH-CBD using LC-MS.

Results: Acute ingestion of nanoemulsion gummies resulted in a significantly (adjusted $p<0.05$) higher $AUC_{0\text{-}4hr}$ (~1.5-fold) and C_{max} (~1.7-fold) relative to traditional oil-formulated gummies. Interestingly, the median T_{max} in the traditional oil-formulated gummy was ~50% slower compared to the nanoemulsion gummy ($p<0.05$). Likewise, the nanoemulsion gummy had a significantly (adjusted $p<0.05$) higher 7-OH-CBD $AUC_{0\text{-}4hr}$ and C_{max} as well as an earlier T_{max} ($p<0.05$), relative to the comparator.

Conclusions: This data demonstrates that nanoemulsions may provide an effective formulation strategy for modifying the pharmacokinetics of orally ingested cannabinoids such as CBD in a healthy adult population. Future PK studies are warranted to examine different cannabinoids.

DELTA-TETRAHYDROCANNABINOL ALTERS CELL TYPE-SPECIFIC TRANSCRIPTOME AND CHROMATIN ACCESSIBILITY IN HUMAN IMMUNE CELLS

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*Presenter

Medical and recreational cannabis use are increasing worldwide. Evidence suggest that cannabis modulates immune function, which medical treatment. However, the underlying genomic mechanisms of cannabis's immunomodulation effects remain unclear. Previous pre-clinical studies have largely been conducted *in vitro* or in bulk cells, resulting in inconsistent findings.

We conducted an *ex vivo* study to identify genes affected by Tetrahydrocannabinol (THC) by profiling single nucleus (sn) RNA-seq and chromatin accessibility in peripheral blood mononuclear cells (PBMCs) from nine healthy participants following THC, a principal psychoactive component of cannabis. Blood was collected before dosing and at 70 and 300 minutes following intravenous THC administration. After quality control, a total of 93,355 nuclei, 30,284 genes, and 229,907 chromatin peaks were analyzed.

We identified 604 differentially expressed genes (DEGs) that changed over time following THC administration, of which 85.9% were unique to a single cell type. The significant genes included transcription regulation and transcription factor (e.g. *CREB1*), immune function (e.g. *ITGA4*), and cell cycle and proliferation (e.g. *CDK6*). We identified 2,389 differential chromatin accessibility regions (DARs), including 2,107 were cell type specific. A proportion of DEGs and DARs overlapped, suggesting that THC alters gene expression regulated by chromatin accessibility. Together, our findings demonstrate that THC has profound cell-type-specific effects on transcriptomic alteration that partially regulated by chromatin accessibility, providing new insights on dynamic cannabis-immune interactions in humans.

PERCEPTIONS REGARDING THE USE OF CANNABIS, ALCOHOL, AND TOBACCO DURING PREGNANCY AMONG WOMEN OF CHILD-BEARING AGE

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Introduction: Prenatal cannabis use is increasing, yet limited research has systematically characterized patterns of use during pregnancy, including product types, motivations, and perceived safety relative to established teratogens such as alcohol and tobacco. Improved understanding of pregnant individuals' attitudes and behaviors regarding cannabis use is critical for informing evidence-based clinical guidance and public health interventions.

Methods: A national U.S. sample of women of childbearing age (N = 622) completed an anonymous online survey assessing prenatal use of cannabis, alcohol, and tobacco. Validated measures assessed perceived safety and risk to fetal development, birth outcomes, and infant health. Analyses included descriptive statistics, McNemar's tests, and repeated-measures ANOVA with post hoc comparisons.

Results: Among participants with a history of pregnancy (n = 352), 25.9% (n = 91) reported cannabis use during pregnancy, 39.3% (n = 83) reported tobacco use, and 9.5% (n = 29) reported alcohol use. Approximately half of prenatal cannabis users reported use at least twice weekly, with joints and blunts the most common routes of administration. The most frequently reported reasons for cannabis use were relief of nausea, anxiety, sleep disturbances, and pain. Across the full sample, cannabis was perceived as significantly safer ($M = 3.85$, $SD = 1.46$) than either alcohol ($M = 4.86$, $SD = 0.54$; $p < .001$) or tobacco ($M = 4.79$, $SD = 0.63$; $p < .001$).

Conclusions: Women of childbearing age in this sample commonly viewed cannabis use during pregnancy as low risk, particularly when used to manage pregnancy-related symptoms. These perceptions underscore the need for targeted educational efforts to equip clinicians with clear, evidence-based guidance that supports informed decision-making and reduces substance use during pregnancy.

NAPE-PLD ACTIVATION BY MUSCARINIC RECEPTORS

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Introduction: The endogenous cannabinoid signaling system consists of G protein-coupled receptors, messengers – 2-arachidonoylglycerol (2-AG) and anandamide – and the enzymatic machinery to synthesize and metabolize these messengers. Anandamide is physiologically important, but its synthesis is incompletely understood. N-arachidonoyl-phosphatidylethanolamide phospholipase D (NAPE-PLD) synthesizes acylethanolamines including anandamide, but how is NAPE-PLD activated?

Methods: We used genetically encoded GRAB sensors for endocannabinoids (eCBs) and immunohistochemistry to investigate.

Results: HEK293 cells natively express G_q-coupled muscarinic M_3 receptors. The muscarinic agonist oxotremorine-M stimulated the $GRAB_{eCB}$ sensor only when HEK293 cells were cotransfected with NAPE-PLD. This signal was reduced by the NAPE-PLD inhibitor LEI401 and required both phospholipase-C and internal calcium stores. G_q-coupled mGluR5 glutamate receptors effectively substitute for M_3 receptors. Thus, M_3 receptors stimulate NAPE-PLD synthesis of acylethanolamines such as anandamide via G_q signaling pathways resembling those for 2-AG. Parasympathetic activation stimulates tearing and salivation via M_3 receptors on myoepithelial cells. A CB1 receptor-based signaling system may act as a feedback inhibitor to inhibit acetylcholine release, but the identity and source of the endogenous cannabinoid messenger are uncertain. NAPE-PLD and M_3 proteins colocalize in myoepithelial cells while FAAH resides in glandular acinar cells. Oxotremorine-M stimulation of lacrimal or submandibular salivary gland explants co-cultured with HEK293-$GRAB_{eCB}$ cells stimulated a $GRAB_{eCB}$ response. This response was diminished by LEI401 and was also present in cells expressing $GRAB_{AEA}$, an anandamide-specific sensor, but not those expressing $GRAB_{2AG}$.

Conclusions: We conclude that muscarinic M_3 receptors stimulate NAPE-PLD to produce anandamide in cell lines and exocrine glands. G_q-coupled GPCR activation of NAPE-PLD may be a general mechanism to induce the synthesis of anandamide.

WITHDRAWN

EVALUATING THE TUMOR ALTERING EFFECTS OF CANNABINOID AGONISTS (ACEA, JWH-133, CP55,940 and CANNABIDIOL) IN OUR BREAST (AT-3 CANCER CELL LINES) CANCER WILD-TYPE (C57BL/6J) MICE MODEL

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Introduction: There are approximately 2 million new cancer diagnoses and 600,000 cancer related deaths in the United States each year. Breast cancer is the leading cancer diagnosis, representing approximately 16 of all new cancer diagnoses, and is a leading cause of cancer death in women worldwide. Cannabis is frequently used as an anti-emetic in cancer patients and has recently been explored for its antinociceptive potential in patients with cancer- or chemotherapy-related pain. Previous studies have demonstrated that cannabinoid compounds produce an anti-tumor effect *in vitro* while *in vivo* studies show conflicting results. In this project, we evaluated the tumor altering effects of the phytocannabinoid cannabidiol (CBD) along with selective CB_1 (ACEA), selective CB_2 (JWH-133), and mixed CB_1/CB_2 (CP-55,940) agonists in our AT-3 murine model of breast cancer.

Methods: This project was performed using adult C57BL/6J female mice. Mice received subcutaneous injections of AT-3 breast cancer cells or HBSS into the 4th mammary fat pad. Tumor growth was assessed daily via daily measurement using a digital caliper (formula estimation of tumor volume, Dubey et al., 2015). After 20 days of unaltered tumor growth, mice received daily injections of CBD, ACEA, JWH-133, CP-55,940, or vehicle for 14 days.

Results: Following fourteen days of chronic administration, the different treatment groups involving CB_2 activation (whether selective JWH-133 or mixed CP55,940) resulted in a significant increase in tumor growth, while CB_1 selective (ACEA) agonism did not. Furthermore, chronic administration of two different doses of CBD didn't significantly alter tumor growth.

Conclusions: These results demonstrate the need for robust preclinical studies investigating the potential pro-tumor effects of cannabinoids in murine cancer models that closely mimic human cancer progression.

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A PHASE 1 STUDY TO ASSESS THE SAFETY AND PHARMACOKINETICS OF ART26.12; AN ORALLY DOSED FATTY ACID-BINDING PROTEIN 5 (FABP5) INHIBITOR

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Introduction: Fatty acid-binding protein 5 (FABP) 5 is a cytosolic chaperone that mediates endocannabinoid and lipid 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 inflammatory (including osteoarthritis) and neuropathic pain models via activation of cannabinoid molecular targets (Warren et al., 2024; 2025). In 2024, the FDA cleared Artelo Biosciences' Investigational New Drug application for ART26.12 for the treatment of chemotherapy-induced peripheral neuropathy.

Methods: The Phase 1 Single Ascending Dose (SAD) study was designed to assess the safety, tolerability, and pharmacokinetics of ART26.12 in healthy volunteers. The SAD study enrolled 49 subjects. Wide dose-exposures of 50–1050 mg were successfully explored. A separate food effect cohort assessed 600 mg of ART26.12 in healthy volunteers under high and low-fat fed and fasted conditions. The selected dose was based on previously established safety and pharmacokinetic data from the SAD study. Participants received three single doses of ART26.12 separated by 7-day intervals.

Results: Plasma analysis confirmed dose-dependent, linear absorption of ART26.12 across the 50–900 mg dose range. All adverse events (AEs) were mild, transient, and self-resolving. No drug-related AEs were observed in the blinded dataset, and no tolerability issues or safety signals were detected across multiple assessments (vital signs, ECGs, clinical laboratory tests, physical examinations, and visual analogue mood scales). Non targeted plasma proteomic analysis identified multiple proteins upregulated in response to ART26.12 (300 mg) which may be biomarkers of target engagement. In the food effect cohort, no serious AEs, safety concerns, or tolerability issues were identified. ART26.12 T_{max} was delayed, and C_{max} and $AUC_{0\text{-}24hr}$ were decreased, with increasing fat/calorie content in the meal.

Conclusions: The first-in-class FABP5 inhibitor ART26.12 was very well tolerated in its primary clinical study. A wide safety margin was observed between estimated therapeutic plasma analgesic concentrations and the highest exposure levels achieved, supporting potential titration for maximum efficacy in future studies. ART26.12 represents a new therapeutic class with a non-opioid, non-steroidal analgesic approach designed to target a novel mechanism in pain modulation.

MACHINE LEARNING METHODS TO INTEGRATE MULTI-OMIC DATA AND IDENTIFY TREATMENT MOLECULAR SIGNATURES IN A PSORIASIS MODEL

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Introduction: We and others have validated fatty acid-binding protein (FABP) 5 as an interesting target in psoriasis, with pharmacological inhibition or genetic knockout resulting in disease amelioration. Here, we present the efficacy of a second FABP5 inhibitor, SBFI-103, in the imiquimod (IMQ) model of psoriasis. To shed light on the role of FABP5 in psoriasis, we used two complementary multidimensional approaches (MOFA and DIABLO) on proteomic and lipidomic datasets, evaluating the disease-modifying effects of two structurally-related FABP5 inhibitors (ART26.12 and SBFI-103) and a tyrosine kinase (TYK) 2 inhibitor (BMS-986165; standard of care treatment).
Methods: Male Balb/C mice were given either vehicle or SBFI-103 (25 or 100 mg/kg PO BID) for two days prior to application of IMQ (62.5 mg of a 5% cream for 7 days) on their backs, and throughout IMQ treatment. This was done concurrently with the previously published work on ART26.12 (25 or 100 mg/kg PO BID) and BMS-986165 (10mg/kg QD). Skin samples were taken 2h after last dosing and flash frozen at −80 °C. Identification of proteins and lipids was done by Creative Proteomics using tandem mass tag-based quantitative proteomics and UHPLC MS/MS, respectively. Bioinformatics analyses were carried out using agentic AI platform ScienceMachine. Differential analysis, pathway analysis, MOFA, and DIABLO were run using the Limma, enrichR, MOFA, and mixOmics packages in R.
Results: Chronic dosing of SBFI-103 significantly attenuated total PASI scores on Day 7 of IMQ administration when compared with IMQ-Vehicle animals (25 mg/kg = $P \leq 0.01$; 100 mg/kg = $P \leq 0.05$). In skin samples, 50 differentially expressed proteins were common to all treatment groups, including epidermal alarmins, neutrophil effector pathways and keratinocyte–immune interface components. Integration of proteomic and lipidomic data using MOFA identified a latent 'Factor' associated with PASI slope score (disease severity). This was dominated by proteomic features associated with metabolic enzymes, innate immune effectors, as well as barrier and stress proteins. Both MOFA and DIABLO were able to robustly separate and predict treatment group, indicating clear molecular differences. Features that predicted FABP5 inhibition by ART26.12 converged upon metabolic restoration, anti-inflammatory effects, and barrier repair. SBFI-103 was well separated from the other treatments by a Factor dominated by proteins involved in mitochondrial oxidative phosphorylation and lipid biosynthesis, indicating divergent metabolic effects. Compared with TYK2 inhibition, ART26.12 modulated a diverse range of lipids supporting restoration of epidermal lipid architecture and metabolic reprogramming of keratinocytes and immune cells, while BMS-98616 modulated a narrower subset focussed on reprogramming immune cell fate. Similarly, only ART26.12 seemed to have a direct effect on metabolic rewiring and mitochondrial stress, whereas BMS-986165 had a strong effect on ECM remodelling. **Conclusions:** The efficacy of an alternate FABP5 inhibitor SBFI-103 adds to the growing body of evidence supporting a pathological role of FABP5 in psoriasis. Unsupervised and supervised AI analysis of multi-omic datasets identified key similarities and differences in FABP5 inhibition as compared to standard-of-care TYK2 inhibition. This analysis supports development of FABP5 inhibitors for psoriasis and further work exploring combination therapy approaches with TYK2 inhibitors.

UNLOCKING THE ENDOCANNABINOID SYSTEM (ECS) WITH CHEMICAL PROBES

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Introduction: Fluorescent probes play a crucial role in exploring G-protein-coupled receptor (GPCR) pharmacology, including processes such as kinetic ligand binding, internalization and interaction. Cannabinoid receptors 1 and 2 (CB_1R and CB_2R) are key proteins of the endocannabinoid (eCB) system, an essential signaling network present in all vertebrates.[1] Dysfunction in the eCB system has been linked to various disorders, including those affecting the CNS and immune system. Despite its critical role in the (patho)physiology, the downstream signaling mechanisms of the eCB system are still not well characterized.[2] Furthermore, tissue and cell-type-specific expression profiles remain largely unexplored due to the lack of high-quality, specific biological and chemical tools.[2] This knowledge deficiency has hindered drug development programs. Therefore, high-quality fluorescent probes are urgently needed to bridge these gaps. **Methods:** Utilizing a reverse-design approach[3], combined with computational modeling and extensive SAR and linkerology studies, we designed and synthesized a set of novel fluoroprobes. The resulting compounds were extensively validated and evaluated by radioligand displacement, FACS, live cell imaging, FRET, fluorescence polarization and other assays. **Results:** We obtained a series of highly potent and selective fluoroprobes with varying dyes (e.g. cell-permeable and cell-impermeable) and varying recognition elements (agonists and inverse agonists matched pairs) and modes of action (classical and traceless) suitable for diverse biological settings. These probes are used to localize proteins of interest in different systems and measure ligand-binding kinetics and the potency of drug candidates. **Summary:** We have developed a modular platform of tool probes that highly specifically and selectively target either CB_1R or CB_2R[4-6] and are suitable for time-lapse confocal and super-resolution imaging. We believe that these findings will help characterize the eCB system and validate the roles of its main components under inflammatory conditions.

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CHEMICAL BIOLOGY TOOLS TO STUDY GLYCEROPHOSPHODIESTERASE GDE4 AS A THERAPEUTIC TARGET IN PEDIATRIC NEURAL CANCERS

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Introduction:

The glycerophosphodiesterase GDE4 generates N-acylethanolamine signaling lipids from lyso-NAPE and glycerophospho-NAE precursors in the brain. Our recent work shows that inactivity of GDE4 causes accumulation of its GP-NAE substrates, which function as a novel class of PPAR agonists and promote differentiation of oligodendrocyte progenitor cells. Consequently, GDE4 inhibition may hold promise as differentiation therapy in pediatric neural cancers, in particular subtypes that transcriptionally resemble OPCs.

Methods:

For hit-to-lead optimization of the first-in-class GDE4 inhibitor, structure-guided design was performed using an AlphaFold model. In parallel, model predictions led to the design of covalent probes with a N-acyl-N-alkyl sulfonamide (NASA) warhead to irreversibly modify reactive surface lysines on GDE4. Depending on warhead orientation, these probes either inhibit GDE4 through an irreversible, covalent mechanism, or act as fluorescent tracers after dissociation of the reversible recognition element. In addition, we engineered GDE4-NanoLuc constructs and synthesized reversible fluorescent probes to establish a live cell NanoBRET target engagement assay.

Results:

Structure-guided design led to a >10-fold increase in inhibitor potency, resulting in low nanomolar K_i cell-active inhibitors. The modification site and specific enrichment of GDE4 by the NASA probes were confirmed by proteomic analysis. In the NanoBRET assay, competitive reversible inhibitors dose-dependently displaced the probe in situ. Moreover, preliminary patient-derived organoid testing showed subtype specific efficacy in several pediatric neural cancers.

Conclusions:

Together, this chemical biology toolbox, comprising inhibitors, and both reversible and irreversible probes contributes to the further characterization of GDE4 as an understudied member of the endocannabinoid system.

CANNABINOID SUBTYPE-2 (CB2R) RECEPTOR AGONISTS AS A STRATEGY TO FACE INFLAMMATION-BASED DISEASES: PROMISING IN VIVO EVIDENCE IN ALZHEIMER'S DISEASE AND IN GASTRIC CANCER

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The cannabinoid subtype 2 receptor (CB2R) belongs to the endocannabinoidome, together with the other canonical receptor CB1R, the non-canonical receptors (GPRs, TRPVs, and PPARs), the endogenous agonists N-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG), their lipid precursors, and the enzymes responsible for their biosynthesis and degradation. Unlike CB1R, which is predominantly expressed in the central nervous system (CNS) and associated with psychotropic effects, CB2R exhibits limited physiological expression in the brain but is strongly upregulated under pathological conditions, particularly in inflammatory states such as neuroinflammation and cancer. CB2R Activation has been consistently associated with anti-inflammatory and immunomodulatory effects, making it an attractive target for drug discovery.

To develop novel anticancer and neuroprotective agents, we have designed and synthesized libraries of ligands targeting CB2R, including both single-target and multi-target molecules. Among these, dual-acting compounds that combine CB2R agonism with inhibition of fatty acid amide hydrolase (FAAH) have attracted great interest, as simultaneously able to enhance endocannabinoid tone and directly and indirectly CB2R activity. From these efforts, three compounds—**ASF151**, **Fi8**, and **CC48**—have emerged as particularly promising candidates.

ASF151, **Fi8**, and **CC48** exert neuroprotective effects in an in vivo model of Alzheimer's disease, supporting their potential as therapeutic agents in neurodegenerative disorders. Importantly, **CC48** displayed potent anticancer activity in vivo by blocking gastric cancer progression and overcoming paclitaxel resistance, one of the major challenges in current chemotherapeutic regimens.

These results suggest that CB2R agonists, particularly dual-acting agents, may represent innovative pharmacological strategy to counteract inflammation-driven diseases, as cancer and neurodegeneration.

BOTANICALS: A KNOWLEDGE GRAPH-DRIVEN AI PLATFORM FOR NATURAL PRODUCT DRUG DISCOVERY

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Introduction: Natural compounds possess medicinal and nutritional properties, and understanding their intricate associations with human biological systems can reveal meaningful correlations with healthcare outcomes. Here, we present BOTANICALS, an integrative, AI-enabled platform designed to elucidate the multiscale relationships between experimentally validated bioactive plant compounds and human health.

Methods: We focus exclusively on compounds identified through experimentally validated studies and further analyze them using DRIFT. This neural network-based algorithm predicts and scores each compound's most relevant molecular targets. DRIFT has been extensively validated using benchmark datasets from previously characterized compounds. This framework has been applied to develop CANDI. This publicly available web server identifies synergistic combinations of molecular targets and their associated pathways, revealing patterns that were previously considered too complex to detect through conventional experimental studies. We further employ knowledge graphs as a central analytical framework, enabling the integration and discovery of previously unrecognized connections between compounds, their targets, pathways, and other relevant biomedical entities.

Results: By mapping compound–target interactions alongside these diverse health data, our platform captures complex cascade patterns that are not readily apparent through standard analyses. This well-curated dataset and the constructed knowledge graphs form the backbone of a system in which the trained LLM leverages the graph structure to refine query outputs, ensuring specificity and accuracy based on verified information.

Conclusions: This integrative approach enables a more comprehensive understanding of the molecular and systemic effects of natural compounds, potentially transforming the discovery, validation, and repurposing of plant-derived therapeutics in precision medicine.

SIMULTANEOUS QUANTIFICATION OF FOUR ENDOCANNABINOIDS AND ENDOCANNABINOID CONGENERS TOGETHER WITH DELTA-9 TETRAHYDROCANNABINOL AND RELATED METABOLITES IN DRIED BLOOD SPOTS (DBS)

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Introduction: In recent years, dried blood spots (DBS) have been increasingly used in analytical methods in the fields of biochemistry, pharmacology and toxicology. In addition to being minimally invasive, this blood sampling method improves the stability of certain compounds. Our objective is to present the development and application of an analytical method enabling the simultaneous quantification of endocannabinoids and phytocannabinoids in DBS samples.

Methods: Based on the use of volumetric absorption micro-sampling (VAMS) systems, an LC-MS/MS method for the simultaneous quantification of four endocannabinoids and their congeners (AEA, 2AG, OEA and PEA) as well as THC and its main metabolites (THC-COOH and 11-OH THC) was developed and validated. This analytical method has been used in particular to determine endocannabinoids and cannabinoids in the blood of healthy volunteers and in a *post-mortem* study.

Results: This validated method reported LLOQs of 0.1µg/L for AEA, 0.5µg/L for THC and 11-OH THC, and 1µg/L for OEA, PEA, 2AG and THC-COOH. Intra- and inter-assay accuracy and precision are <15% and <20% at the LLOQ. In particular, the use of DBS improves the acceptable *in vitro* stability of endocannabinoids in whole blood. Endocannabinoid concentrations in DBS are generally higher than those observed in plasma and serum. The results of endocannabinoid testing in *post-mortem* blood show higher concentrations of OEA, 2AG and PEA compared to living subjects.

Conclusion: The proposed method for quantitative analysis of endogenous ligands of the endocannabinoid system and THC (and associated metabolites) in DBS can be used in future clinical studies investigating the effects of cannabis use.

METABOLIC SIGNATURE OF PHYTOCANNABINOIDS IN KERATINOCYTES STUDIED BY ^{1}H-NMR

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Introduction:
Cannabis has been used since antiquity for its different properties due to molecules called phytocannabinoids, the best known of which are tetrahydrocannabinol (THC) and cannabidiol (CBD). More than 200 phytocannabinoids have been identified in *Cannabis sativa* plant, but their roles in the human skin remain poorly understood. In this project, we are investigating the impact of four different phytocannabinoids — cannabidiol (CBD), cannabidivarin (CBDV), cannabigerol (CBG), and cannabichromene (CBC) — on keratinocyte metabolism.

Methods:
Immortalized keratinocytes cells (HaCaT) were treated during 24h with 1 µM (non-cytotoxic) of each cited phytocannabinoids. Metabolites of treated or non-treated cells were extracted to performed metabolomic analyses using proton nuclear magnetic resonance (^{1}H-NMR). Statistical analyses were performed to discriminate variables between treated cells and non-treated cells. Metabolites associated with these variables have been identified.

Results:
Treatments with phytocannabinoids impact several metabolic pathways such as antioxidant pathway that are currently being studied. Most of the discriminant metabolites identified are common to CBD, CBDV, CBG and CBC treatments: these are mainly amino acids and antioxidants (glutathione, taurine). However, other discriminant metabolites are specifically linked to CBD or CBG alone treatments which would indicate a specific effect of each phytocannabinoid.

Conclusions:
Our studies provide a better understanding of the effects of phytocannabinoids on skin cells and highlight their benefits for potential applications in the dermocosmetic field.

A CANNABIDIOL COCRYSTAL (ART12.11) TABLET HAS COMPARABLE PHARMACOKINETICS TO EPIDIOLEX

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Introduction: Development of solid cannabidiol (CBD) formulations has been limited by CBD's physical properties. Cocrystallisation is a strategy to improve physicochemical properties of difficult pharmaceutical ingredients. Artelo Biosciences have developed a patented cocrystal of CBD with the co-former tetramethylpyrazine (TMP; also called ligustrazine), designated ART12.11. Artelo previously reported that an unoptimised oral solution of ART12.11 has improved pharmacokinetic (PK) and pharmacodynamic properties compared to CBD in multiple species. The aim of the present work was to develop an optimised solid tablet of ART12.11.

Methods: Prototype formulations were manufactured using common pharmaceutical techniques for compression tableting. The excipients evaluated are common and accepted by regulators, with the majority having GRAS status. Differences in formulations were related to drug loading (up to 30%), precipitation inhibitor, disintegration agent, glidant, and filler. Following initial screening, three lead prototypes (A, B, and C) were taken forward for dissolution studies (comparing against an Epidiolex-like formulation) and *in vivo* PK studies, where male Beagle dogs (n=3, ~10 kg) were administered a single tablet of ART12.11 (100 mg CBD PO, equivalent to 10 mg/kg) or Epidiolex® (10 mg/kg PO) in the fed state. Plasma samples were analysed for CBD and TMP by Liquid chromatography–mass spectrometry (LC-MS/MS).

Results: Prototype ART12.11 tablets contained 100 mg CBD per tablet. In FaSSIF, CBD release from prototype tablets was ~20%, reflecting CBD's poor solubility in the absence of surfactants. In FeSSIF, CBD dissolution was enhanced, and greater in all ART12.11 tablet prototypes compared to an Epidiolex-like solution. *In vivo* dog PK studies showed that Tablets B and C led to similar overall plasma CBD exposure (AUC) to Epidiolex®. Tablet C had a similar PK profile to Epidiolex®, while Tablet B showed a later T_{max} and lower $C_{max,}$ with longer lasting CBD levels. Tablets B and C led to higher levels of two CBD metabolites (7-OH and 7-COOH CBD) compared to Epidiolex®. Optimisation of the ART12.11 tablets is ongoing in preparation for human studies.

Conclusions: ART12.11 tablets represent a revolutionary formulation approach using a patented form of CBD. ART12.11 shows similar CBD, and enhanced CBD metabolite exposure, compared to the oral solution Epidiolex®. For pharmaceutical companies, this represents a scalable, protected, low-cost approach for CBD in conditions previously seen as "out-of-scope". For patients, ART12.11 offers a simple, conventionally sized tablet that is familiar and easy to take.

INTEGRATIVE MULTI-OMICS ACROSS DIVERSE INDICATIONS IDENTIFIES LINOLEIC ACID AS CENTRAL LINK IN FATTY ACID-BINDING PROTEIN 5 INHIBITION

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Introduction: Fatty acid-binding protein (FABP) 5 inhibition is beneficial in multiple preclinical models, with disease-modifying effects in cancers, dermatological conditions and metabolic disorders, as well as analgesic effects in osteoarthritis and neuropathies. To understand how FABP5 inhibition operates in diverse indications, we undertook comparative multi-omics analysis to uncover consensus mechanisms using non-targeted data. These data were derived from efficacious studies with the FABP5 inhibitor ART26.12, which included the imiquimod (IMQ) model of psoriasis in mice, the destabilization of the medial meniscus model (DMM) of osteoarthritis in rats, and oxaliplatin-induced peripheral neuropathy (OIPN) in rats. **Methods:** In the OIPN model, untargeted proteomic analysis of plasma samples was undertaken by a commercial partner, Somalogic. Targeted lipidomics were undertaken on dorsal root ganglion, spinal cord, periaqueductal gray, and prefrontal cortex by HPLC MS/MS. On skin tissue from the IMQ model and knee synovium from the DMM model, untargeted proteomic and lipidomic analyses were undertaken by commercial partner, Creative Proteomics, using tandem mass tag-based quantitative proteomics and UPLC MS/MS, respectively. **Results:** Within OIPN, endogenous lipids were modulated within the spinal cord. This included some known and many undescribed FABP5 substrates, with molecular modelling of the latter class supporting accommodation into the FABP5 binding pocket. Among free fatty acids in the spinal cord, only linoleic acid (18:2) was upregulated ($p=0.024$). In the DMM and IMQ models, linoleic acid and derivatives were a statistically enriched metabolite set ($p<0.0018$ and $p<0.014$, respectively). Across the DMM and IMQ models, ceramides were consistently upregulated, consistent with CB_1 activation and/or modulation of fatty acid trafficking to biosynthetic enzymes. Within the proteome, diverse outcomes were observed, with generally specific differentially expressed proteins identified in each tissue/indication. Nevertheless, these pathways have previously been linked with upstream effects on lipid metabolism and signalling. These included the PI3K/AKT/MTOR pathway in DMM; and PKC and PPARβ/δ in IMQ. This suggests modulation of similar classes of lipids by FABP5 inhibition can lead to diverse effects on the proteome, giving rationale for efficacy across multiple indications. **Conclusions:** FABP5 inhibition with ART26.12 consistently modulates the pro-inflammatory ω-6 lipid linoleic acid (18:2), and its derivatives. This is consistent with other literature identifying the linoleic acid – FABP5 axis as a pathologically relevant signalling pathway. The directionality and identity are disease and/or tissue specific. Downstream changes in the proteome were unique in each indication. FABP5 remains an interesting, novel target across a range of indications, and its interaction with linoleic acid may be of interest.

WITHDRAWN

PRENATAL CANNABIS USE IS ASSOCIATED WITH INCREASED CIRCULATING ENDOCANNABINOIDS IN LATE PREGNANCY: FINDINGS FROM THE PROSPECTIVE CAN-B COHORT

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Introduction: The endocannabinoid system (ECS) plays a critical role in pregnancy, including embryo implantation, placentation, neurodevelopment, and parturition. Phytocannabinoids contained in cannabis interact with the ECS and may induce adaptive changes. While chronic cannabis use has been shown to alter circulating endocannabinoid levels in non-pregnant populations, no study has investigated these alterations during pregnancy. This study aimed to compare circulating endocannabinoid-related lipid concentrations in pregnant women according to cannabis exposure during late gestation.

Methods: This cross-sectional analysis included 45 pregnant women from the prospective CAN-B cohort who provided plasma samples during the third trimester. Participants were classified as cannabis users (n = 23) or controls (n = 22) based on self-report and the detection of phytocannabinoid biomarkers in plasma or urine. Plasma concentrations of endocannabinoids and related N-acylethanolamines were quantified using liquid chromatography–tandem mass spectrometry. Group comparisons were performed using Mann–Whitney U tests, and linear regression models were used to adjust for nicotine exposure using plasma cotinine levels.

Results: Pregnant cannabis users exhibited higher circulating concentrations of 2-arachidonoylglycerol (2-AG), 2-linoleoylglycerol (2-LG), linoleoylethanolamide (LEA), palmitoylethanolamide (PEA), stearoylethanolamide (SEA) compared with controls. In contrast, arachidonic acid (AA), a degradation product of endocannabinoids, showed lower concentrations in cannabis users. These findings are partly consistent with observations in non-pregnant chronic cannabis users and may reflect adaptive modulation of the endocannabinoid system, cannabinoid receptor downregulation and/or reduced endocannabinoid degradation, as suggested by lower arachidonic acid concentrations.

Conclusions: Prenatal cannabis use is associated with increased circulating endocannabinoid-related lipids during late pregnancy. These alterations may reflect adaptive ECS responses to phytocannabinoid exposure and could have implications for maternal and fetal physiology. Ongoing analyses aim to further characterize relationships with maternal inflammation and pregnancy outcomes.

ADDRESSING BOTANICAL COMPLEXITY IN PURIFIED PHYTOCANNABINOID EXTRACTS THROUGH ADVANCED ANALYTICAL CHARACTERISATION

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Introduction: As enthusiasm grows for the therapeutic potential of phytocannabinoids, sourcing materials, particularly botanical extracts, presents unique challenges. Understanding potential variability is essential to ensure final product consistency, quality, and reproducibility of intended effects. Accurate mass balance in these extracts requires characterization of both cannabinoid and non-cannabinoid fractions; however, most available analytical methods are application-specific, limiting comprehensive profiling and direct comparison across materials.

Methods: An efficient, multi-technique analytical framework was developed and validated, supporting broad and consistent applications across materials manufactured from different source biomass and processing conditions. The framework included UPLC-UV (cannabinoids), GC-FID-MS (terpenes and sterols), HS-GC-FID (residual solvents), ICP-OES (elemental impurities), and KF titration (water content). The methods demonstrated strong performance, meeting validation requirements and complying with ICH guidelines. This framework was subsequently applied to assess variability in purified cannabinoid extracts sourced from commercial medical suppliers.

Results: Mass balance greater than 95% was achieved in the majority of samples. Minimal variability was observed in the predominant cannabinoid, consistent with suppliers' certificates of analysis (relative standard deviation [RSD] = 2.5%). Notable variability was observed in the presence and abundance of minor cannabinoids (identified 4.6–14.9% w/w and unidentified 0.5%–1.9% w/w), terpenes (0.0–0.3% w/w), residual solvents, and water content. Elemental impurities were below internationally accepted safety limits; however, levels varied between suppliers.

Conclusion: Material variability was consistent with differences in starting biomass and supplier-specific processing conditions. Accordingly, the validated multi-technique analytical framework provides a robust and comprehensive approach for characterizing cannabinoid materials, supporting reliable product development, quality control, and regulatory compliance.

BRAIN SELECTIVE SELENIUM-BASED MGL INHIBITION VIA AN ALLOSTERIC MECHANISM

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Introduction: Allosteric modulation of monoglyceride lipase (MGL) represents an effective approach to regulate MGL function and enhance 2-arachidonoylglycerol (2-AG)-mediated signaling at CB1 receptors, offering a way to bypass the limitations of active site-directed inhibitors. Benzothiazolinone molecules, able to sulfenylate redox-sensitive cysteines Cys201 and Cys208, have been identified as potent allosteric MGL inhibitors. Based on this previous knowledge, we hypothesized that ebselen, a clinically safe antioxidant, could act as an allosteric MGL inhibitor through bioisosterism, with the potential for therapeutic application in diseases that may benefit from a controlled increase in 2-AG signaling.

Methods: MGL activity in the presence of ebselen was assessed in HeLa cell lysates expressing human MGL (*h*MGL). The mechanism of action of ebselen was investigated using site-directed mutants and LC/MS-MS to identify cysteine adducts. Rapid dilution assays were performed to assess the reversibility of ebselen binding. C57BL/6J mice were treated once with ebselen (10, 30, or 100 mg/kg, i.p.) and tissues were collected to evaluate pharmacokinetics, metabolism, and MGL inhibition *in vivo*.

Results: Ebselen is a potent, reversible allosteric MGL inhibitor acting on regulatory cysteines C201 and C208. *In vivo* treatment with ebselen at 30 mg/kg increased 2-AG levels in the brain, but not in peripheral tissues (white adipose and heart). Inactive Se-methylated metabolites of ebselen were detected in both tissues and circulation.

Conclusions: We identified ebselen as a novel allosteric MGL inhibitor, targeting the regulatory cysteines C201 and C208. Our results demonstrate potent, reversible inhibition with a specific increase in 2-AG levels in the brain. The mechanism underlying this selectivity is currently under investigation.

DEVELOPMENT OF MONOACYLGLICEROL LIPASE DEGRADING PROTEOLYSIS TARGETING CHIMERAS

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Introduction: Monoacylglycerol lipase (MAGL) is a serine hydrolase that degrades 2-arachidonoylglycerol (2-AG), an endogenous agonist of the CB_1 and CB_2 receptors. Covalent and reversible MAGL inhibitors elevate 2-AG levels and have demonstrated analgesic effects in preclinical models of neuropathic pain. Here, we present a class of compounds with a novel mode of action that induce degradation of the MAGL protein, termed Proteolysis Targeting Chimeras (MAGL-PROTACs).

Methods: 8 MAGL-PROTACs were designed based on a versatile probe platform and previously developed CRBN-based MAGL PROTACs.[1,2] The reporter tag was replaced with various linkers and E3 ligase ligands. These E3 ligase ligands included VH032 (VHL), idasanutlin (MDM2), and bestatin (IAP)-derived ligands. The PROTACs were synthesized, and their biochemical activity was determined using a natural substrate assay. Cellular activity was assessed by activity-based protein profiling (ABPP) and MAGL protein levels were evaluated by Western blot analysis.

Results: All compounds were active in the biochemical and cellular assays with high potency. All PROTACs were highly potent in a natural substrate assay (pIC50; 9.4-10.1). *In situ* ABPP with LEI-463-Cy5 showed that all PROTACs are cell permeable and engaged with MAGL (pIC50 > 9). The IAP and MDM2-based PROTACs showed minimal MAGL degradation (D_{max}; 2-41%). In contrast, the VHL-based PROTACs degraded MAGL to a maximum of 61%.

Conclusion: The synthesized MAGL-PROTACs are cell permeable, highly potent MAGL inhibitors and the VHL-based PROTACs degrade MAGL with a D_{max} of 61%.

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RATIONAL OPTIMIZATION OF THE N-ADAMANTYL-ANTHRANIL AMIDE STRUCTURAL CORE FOR THE DEVELOPMENT OF NEW SELECTIVE LIGANDS FOR THE CANNABINOID SUBTYPE 2 RECEPTOR (CB2R)

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Introduction: CB2R is a Gi-protein-coupled receptor (GPCR) forming part of the endocannabinoid system (ECS), which includes CB1R, endocannabinoids, and related enzymes. CB2R is expressed in immune tissues and upregulated in microglia cells during pathological states, suggesting its involvement in inflammatory and neurodegenerative diseases. Recent research has focused on developing selective CB2R ligands due to their therapeutic potential. **Methods:** We synthesized a series of *N*-adamantyl-anthranil amide derivatives and evaluated their binding affinity and selectivity for CB2R over CB1R. The design was based on the "three-arm pose" binding mode of the CB2R antagonist AM10257. We employed molecular docking simulations to rationalize the observed pharmacodynamic profiles and conducted functional studies to assess the biological activity of the most promising compounds. **Results**: Compounds with a phenyl ring or hydrogen on arm 1 and a five-carbon alkyl chain on arm 2 demonstrated the highest affinity. The carboxy-adamantyl amide group on arm 3 was critical for interaction with CB2R. Selected compounds exhibited significant selectivity for CB2R over CB1R, with promising pharmacodynamic profiles. Functional assays confirmed their potential as CB2R modulators, and molecular docking provided insights into their binding mechanisms (1). Interestingly in vivo neuroprotection studies have been performed in the pharmacological model of Alzheimer Disease induced in mice after injection of oligomerized Aβ25-35 peptide, obtaining really interesting results. In order to improve the pharmacokinetic properties of our published derivatives that were not ideal because of their high lipophilicity, we started a systematic lead optimization by introducing polar substituents around the anthranyil amide scaffold. **Conclusion**: We were able to identify new selective CB2R ligands endowed with better pharmacokinetic properties. The evaluation of their functional activity, of their anti-inflammatory properties and of their effects on selected cellular lines let us to be confident for their possible therapeutic applications.

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RTICBM-303, A CB_1 RECEPTOR ALLOSTERIC MODULATOR, REDUCES CUE- AND DRUG-PRIMED REINSTATEMENT OF METHAMPHETAMINE SEEKING IN RATS

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Introduction: The cannabinoid type 1 receptor (CB_1R) plays a critical role in several physiological processes, including regulation of reward pathways within the central nervous system. As an alternative strategy to modulate CB_1R while potentially minimizing the psychiatric adverse effects associated with orthosteric antagonists/inverse agonists, our group has been developing negative allosteric modulators (NAMs). In this study, we explored modifications on the left 4-chlorophenyl group of di-aryl urea analogues bearing a biphenyl moiety and evaluated the effects of one such analogue, RTICBM-303, on attenuating the reinstatement of methamphetamine-seeking behavior in rats. **Methods**: A series of novel urea-based CB_1R allosteric modulators were designed, synthesized, and characterized by ^{1}H and ^{13}C NMR, LCMS, and HPLC. All compounds were evaluated for their CB_1R modulating activity in calcium mobilization assays using CHO-RD-HGA16 overexpressing human CB_1R or CB_2R and [^{35}S]GTPγS binding assays using human CB_1-expressing HEK293 cell membranes or mouse cerebellar membrane. Compounds demonstrating potent activities were assessed for ADME properties, including metabolic stability against rat liver microsomes, kinetic solubility, and CNS permeability using the MDCK-MDR1 assay. RTICBM-303 was further evaluated for in vivo pharmacokinetic properties and efficacy in attenuating cue-induced and drug-primed reinstatement of methamphetamine seeking behavior in male Sprague-Dawley rats.**Results**: Multiple compounds demonstrated nanomolar IC_{50} values at CB_1R with minimal activity at CB_2R. These compounds also showed efficacy in [^{35}S]GTPγS binding assays at both human and mouse CB_1R. RTICBM-303 exhibited excellent metabolic stability in rat liver microsomes and demonstrated favorable central nervous system penetration in pharmacokinetic studies. Notably, RTICBM-303 significantly reduced cue- and drug-primed reinstatement of methamphetamine-seeking behavior at 10 mg/kg (i.p.) without affecting food-maintained responding. **Conclusions**: We have identified CB_1R allosteric modulators with good in vivo efficacy against methamphetamine reinstatement in rats. This work supports that CB1R NAMs may be further developed as potential medications for the treatment of drug addiction and other CB1R-mediated conditions.

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WITHDRAWN

CHEMICAL PROBES TO VISUALIZE DIACYLGLYCEROL LIPASE

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Introduction: Psychiatric disorders are increasingly prevalent, yet their underlying molecular mechanisms remain incompletely understood. The endocannabinoid system is a key regulator of synaptic signalling and neuropsychiatric function. One of its main endocannabinoids, 2-arachidonoylglycerol (2-AG), is produced by diacylglycerol lipase-a (DAGLA). Dysregulation of DAGLA activity has been implicated in multiple psychiatric disease states. Spatially and temporally resolution of DAGLA activity in human brain and brain organoid models is therefore of high importance. Here, we report the design and synthesis of novel DAGL-targeting chemical probes to map DAGL activity in psychiatric disease-relevant organoids and postmortem human brain tissue.

Methods: Based on previously reported DAGL inhibitors, including DO34, DH376, and LEI-105, we designed hybrid small molecules to obtain both reversible and irreversible, selective DAGL probes. Structural guidance was obtained from AlphaFold 3-derived DAGL models combined with molecular dynamics simulations. Following synthesis, probe potency was evaluated using a DAGL natural substrate assay, while selectivity and target engagement were assessed by gel-based activity-based protein profiling (ABPP) in mouse brain proteome.

Results: We present an optimized DAGL structural model from which we designed and synthesized a library of 8 reversible inhibitors and 2 probes, of which compound 1 had a pIC_{50} of 6.1 ± 0.2 (n=2) and showed selectivity over FAAH, MAGL, and ABHD6. Additionally, we report 3 irreversible inhibitors with $pIC_{50} > 7.5$ which showed selectivity over MAGL, FAAH.

Conclusions: This work demonstrates a set of optimized DAGL chemical probes enabled by activity-based protein profiling, functional assays, and AlphaFold 3-guided modelling, providing new tools to study endocannabinoid signalling in psychiatric disease models.

PROBING THE RAT BRAIN SERINE HYDROLASE REPERTOIRE FOR ENDOCANNABINOID HYDROLASES

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Introduction: Many enzymes associated with endocannabinoid turnover are serine hydrolases, which can be labelled with the fluorescent probe TAMRA-FP. This binds irreversibly to allow simultaneous quantification of multiple activities in small quantities of complex proteomes (Activity-Based Protein Profiling). As much of the analysis of brain serine hydrolase activities has focussed on mouse tissues or recombinant enzymes, we set out to assess the repertoire of serine hydrolases in the rat brain.

Methods: Rat brain regions were homogenised and centrifuged to provide particulate and soluble fractions and were probed with TAMRA-FP prior to SDS-PAGE protein separation and fluorescence scanning.

Results: 27 bands were identified in the soluble and particulate fraction of rat cerebral cortex and the other brain regions examined. None of the bands in the soluble or particulate fractions were significantly altered in the presence of 2OG, PEA, AEA, or ODA. However, 2AG inhibited bands consistent with a MAGL doublet in the soluble fraction, while inhibiting only two bands of ~50 and~55 kDa from the particulate fraction. JZL184 and JJKK048 failed to alter enzyme activities in the soluble phase, but JZL184 enhanced a band at ~75 kDa, while JJKK048 inhibited bands consistent with a MAGL doublet in the particulate fraction. KT203 also failed to alter enzyme activities in the soluble fraction, but inhibited a particulate band of ~34 kDa.

Conclusions: These data provide pharmacological evidence for MAGL and ABHD6 activities in the rat brain (quelle surprise!), as well as potentially novel 2AG-sensitive serine hydrolases in rat brain particulate fractions.

IN VIVO PROFILING OF PHYTOCANNABINOIDS IN MEDICINAL CANNABIS PLANTS USING SOLID-PHASE MICROEXTRACTION APPROACH

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Introduction: The profile of phytocannabinoids (PCs) in a given *Cannabis spp*. variety depends on the cultivation conditions and should be tightly monitored throughout the plant's growth to obtain a final medicinal cannabis plant with known composition of major and minor PCs. In the analysis of complex biological matrices, miniaturized extraction techniques like solid-phase microextraction (SPME) are well established as they require minimal sample handling and facilitate monitoring low molecular weight compounds at trace levels directly in the living systems (*in vivo*).

Methods: SPME was optimized for direct isolation of PCs from growing *Cannabis spp*. plants under *in vivo* conditions. Biocompatible C18 probes were inserted into the inflorescences of CBD- and THC-dominant varieties. Analysis was performed using liquid chromatography-coupled with mass spectrometry (LC-MS). For comparison, extracts were also analyzed via HPLC-UV according to US Pharmacopeia recommendations.

Results: The developed SPME-based method was comprehensively optimized with respect to parameters related to *in vivo* SPME and instrumental analysis, which was critical in evaluating its validity for targeted and untargeted analyses of PCs in growing plants. The developed method proved to be a precise and efficient for rapid isolation and analysis of acidic and neutral forms of PCs without plant material collection. The results facilitated monitoring PC content and composition at different stages of plant growth.

Conclusions: The proposed SPME-based methodologies serve as a valuable quality tool for real-time monitoring of PCs in medicinal cannabis cultivation facilities. This approach offers a concise and non-destructive alternative to traditional sampling methods.

DESIGN AND SYNTHESIS OF NATURE-INSPIRED ALKYLAMIDES FROM ZANTHOXYLUM PIPERITUM AND ACMELLA OLERACEA AS DUAL CB1/TRPV1 MODULATORS WITH ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY

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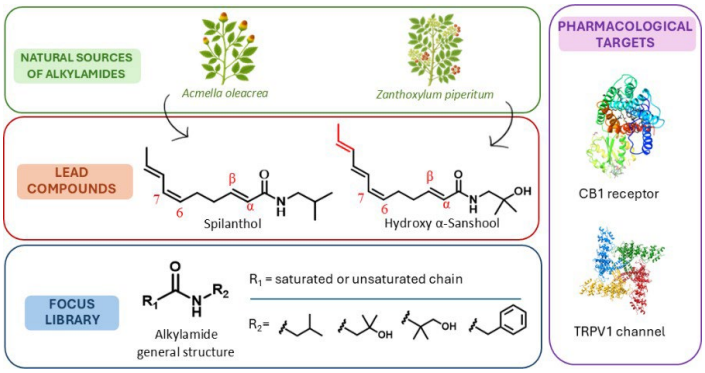
Natural alkylamides from *Zanthoxylum* species and *Acmella oleracea* have long been associated with analgesic and anti-inflammatory effects in traditional medicine.[1,2] Key representatives, such as hydroxy-α-sanshool and spilanthol, exert their activity through modulation of molecular targets involved in nociception, including the transient receptor potential vanilloid 1 (TRPV1) channel and the cannabinoid type 1 (CB1) receptor.[3,4]

Here, we report the rational design and synthesis of a novel series of alkylamide derivatives inspired by these natural scaffolds, aiming to achieve dual modulation of CB1 and TRPV1. A systematic structure–activity relationship (SAR) investigation enabled the identification of key structural determinants governing target interaction and functional activity. Complementary receptor interaction analyses and molecular modelling studies will be presented to provide further insight into ligand–target recognition and binding modes. Overall, this study reinforces the relevance of natural product-inspired approaches in drug discovery and highlights alkylamides as a promising chemotype for the development of innovative cannabinoid- and vanilloid-targeting therapeutics.



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HEALTH IMPACT OF NEWLY STARTING MEDICINAL CANNABIS USE AMONG ADULTS WITH ANXIETY AND/OR PTSD – A LONGITUDINAL OBSERVATIONAL STUDY

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Introduction: First-line treatments for anxiety or posttraumatic stress disorder (PTSD) are often ineffective or cause adverse effects that are unacceptable to patients. Cannabis is an exploratory therapeutic of interest, but requires more data related to safety and efficacy.

Methods: A longitudinal observational research study is ongoing in which adults newly initiating cannabis use to treat anxiety and/or PTSD are followed prospectively. Surveys of general health, anxiety (GAD-7), and PTSD symptom severity (PCL-5) are collected at baseline and for 1 year after cannabis initiation.

Results: Participants (N=62) were 42 years of age on average, 65% female, and 80% White/Caucasian. Cannabis product use was diverse with respect to route of administration and chemical composition, 39% used more than one product, and 9% stopped using cannabis by 3-months. Initiation of cannabis was associated with clinically significant mean reductions in anxiety, PTSD severity, and insomnia as well as improvements in quality of life and functioning. 55% of those with PTSD showed a PCL-5 score reduction of at least 10 points from baseline, but symptom management regressed for some patients while others showed continued improvement over time. 10% were significantly worse at the 3-month follow-up compared with baseline. Few major changes in concomitant medications were noted.

Conclusions: Initiation of cannabis use was associated with aggregate improvements in health among adults with anxiety and/or PTSD, but considerable diversity in the trajectory of health effects was observed. Differential outcomes were not clearly related to cannabis product type. Additional research is needed to identify predictors of treatment response.

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FIRST REPORT OF OFF-LABEL CBD-RICH BROAD-SPECTRUM CANNABIS THERAPY IN RUBINSTEIN–TAYBI SYNDROME: CLINICAL OUTCOMES FROM A RARE GENETIC DISORDER

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Introduction: Rubinstein-Taybi syndrome (RTS) is a rare genetic disorder caused by mutations in the CREBBP and EP300 genes, primarily characterised by intellectual disability, distinct facial features, limb anomalies, and symptoms overlapping with autism spectrum disorder (ASD). Evidence suggests that cannabidiol (CBD)-rich cannabis products may benefit behavioural and social aspects in ASD. However, individuals with THC sensitivity may benefit from the entourage effect of the crude extract, which contains phytocannabinoids, terpenes, and flavonoids.

Methodology: This study evaluated the impact of a broad-spectrum (BS), THC-free, CBD-rich cannabis extract in an 11-year-old Brazilian girl with RTS confirmed by genetic testing. The treatment involved a gradual increase in CBD dosage, starting at 5 mg/day and reaching 110 mg/day over 14 months, administered twice daily after meals. The patient was assessed using the Vineland Adaptive Behaviour Scale-3 at baseline and after 14 months, along with the DEMC® Protocol and semi-structured interviews.

Results: Genetic testing showed an alteration on chromosome 16p13.3 (CREBBPx2), without microdeletions. Before treatment, the patient showed speech and linguistic delays, cognitive impairment, and behavioural symptoms such as aggressiveness, anxiety, and motor coordination difficulties. After 14 months, significant improvements were observed in communication (from 44.8% to 57.9%), daily living activities (18.5% to 22.4%),
socialisation (40.2% to 41.1%), and motor skills (51.9% to 59.7%), with overall adaptive functioning improving from 53.3% to 62.5%. Attention and school behaviour improved within 2 months of therapy, and no adverse effects were reported.

Conclusion: CBD-rich cannabis extract may improve behavioural symptoms and quality of life in RTS without significant side effects.

Financial support: The product used in this project was donated by REVIVID Brazil to UNILA.

Ethics Committee: CAAE No. 81351624.6.0000.0107.

EVALUATING THE INDEPENDENT AND COMBINED EFFECTS OF THC AND CBD ON CHRONIC PAIN: PRELIMINARY EVIDENCE FROM A PROSPECTIVE COHORT OF OLDER ADULTS

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Background: Whether consuming THC-CBD combined medical cannabis (MC) are more beneficial than THC alone remain unclear. This study examined the associations between plasma THC/CBD and pain intensity/interference in a prospective cohort of older adults with chronic pain.

Method: We analyzed baseline, 3- and 6-month data from 147 participants (age=65□9, 62% female) from an ongoing cohort following older adults as some initiate MC and others do not. Blood tests were conducted at each timepoint for THC, CBD and their metabolites, along with self-reported pain severity assessed by the Brief Pain Inventory (BPI) and pain interference measured by the 4-item PROMIS Pain Interference short form. MC consumption was categorized into four groups based on blood test: 1)no THC/CBD, 2)THC only, 3)CBD only, and 4)THC+CBD. Linear mixed effects model (LMM) was used to examine how MC consumption was associated with chronic pain severity/interference at the follow-ups while controlling for baseline demographics and pain severity/interference.

Results: At 3 months, 96 had no THC/CBD, 24 had THC only, 1 had CBD only, and 26 had THC+CBD in their blood. At 6 months, the breakdown was 81 none, 22 THC only, 1 CBD only, and 21 THC+CBD based on results received so far. LMM indicated that THC only was associated with lower pain intensity (□=-2.99, p<.01), while THC+CBD was associated with lower pain interference (□=-.93, p<.05).

Conclusions: Preliminary findings suggest consuming THC may be more effective in reducing pain severity, but consuming THC with CBD may be more helpful in improving functioning therefore reducing pain interference.

Disclosure/Funding
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TOPICAL CANNABIDIOL ENHANCES AFFECTIVE TOUCH AND AUTONOMIC MEASURES WITHOUT CLEAR EVIDENCE OF PERIPHERAL AFFERENT MODULATION: A PLACEBO-CONTROLLED INVESTIGATION

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Introduction: While the effects of oral or systemic Cannabidiol (CBD), have been extensively studied, the impact of topical administration remains less well understood, particularly concerning its influence on peripheral somatosensory pathways. Therefore, this study aims to evaluate the effects of topical CBD on tactile pleasantness and heart rate variability (HRV) and to ascertain whether these effects are mediated by changes in peripheral afferent units.

Methods: Employing a randomized, double-blind, placebo-controlled design, participants received either topical CBD or a placebo cream applied to the forearm. A total of 53 healthy subjects (30 women) participated in the study, although not all completed every component. Tactile pleasantness was assessed using the Robotic Tactile Stimulation (RTS) test. HRV was measured through electrocardiographic recordings. Single nerve electrophysiological recordings using the microneurography technique were conducted to assess the activity of peripheral afferent units.

Results: Administration of CBD significantly increased ratings of tactile pleasantness ($p < 0.05$) and HRV indices (SDNN) indicating improved parasympathetic tone ($p < 0.01$). Notably, no significant changes were observed in the firing rates or response profiles of peripheral afferent units, except for the field units (with a small sample size of recordings), suggesting that the effects were not mediated by direct modulation of primary somatosensory input.

Conclusion: CBD enhances affective touch perception and autonomic regulation without modulating peripheral afferent neural activity. These findings suggest that CBD's effects are likely mediated by central mechanisms rather than peripheral sensory pathways, although the study is limited by the small number of successful afferent recordings. Further research is warranted to elucidate the specific central processes involved and to determine the clinical relevance of these findings for conditions characterized by altered sensory or autonomic function.

Conflict of Interest: This study is sponsored by ThisWorks LTD

BASELINE STATES AS MODERATORS OF THE ACUTE EFFECTS OF CANNABIS ON ANXIETY AND MOOD IN THE LAB AND FIELD

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Introduction: Medical cannabis patients commonly use cannabis to reduce anxiety and improve mood, yet evidence on the acute effects of cannabis on these outcomes remains mixed. This study examined whether baseline anxiety and mood moderate the acute effects of cannabis on changes in anxiety and mood.

Method: Data from two studies were analyzed. The first was a field study with 80 participants randomly assigned to remain sober or to use one of three different cannabis products (THC flower, THC+CBD flower, THC+CBD concentrate). The second was a randomized, double-blind, placebo-controlled lab investigation with 120 participants randomly assigned to vape placebo, 20mg THC, or 40mg THC. In both studies, mood and anxiety were rated at baseline (T0), as well as immediately (T1), 25 minutes (T2), and 50 minutes (T3) after use.

Results: In the field study, baseline mood significantly moderated changes in mood in both flower conditions, with lower baseline mood predicting greater mood enhancement relative to the sober condition at T1 and T3. Higher baseline anxiety predicted greater anxiety reductions in the THC+CBD flower condition at T1 and T2. At T3, higher baseline anxiety predicted greater anxiety reductions across all cannabis conditions relative to placebo. In the laboratory, results indicated that lower baseline mood predicted greater mood enhancement in the 40mg THC condition relative to the placebo condition at T2. Baseline anxiety didn't moderate anxiety outcomes.

Conclusion: Baseline state, product, and environment appear to interact to influence acute effects of cannabis. These findings will inform therapeutic applications for depression and anxiety.

D-LIMONENE ATTENUATION OF THE ACUTE ADVERSE EFFECTS OF HIGH-DOSE ORAL Δ9- TETRAHYDROCANNABINOL (Δ9-THC)

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Introduction: Prior research showed that inhaled d-limonene selectively mitigated anxiety induced by high dose inhaled delta-9-tetrahydrocannabinol (Δ9-THC). The present study aims to evaluate whether this effect is also observed with oral administration.

Methods: Healthy adults completed 6 double-blind, outpatient drug administration test sessions during which each participant received a single acute oral dose of Placebo, 30mg Δ9-THC, 30mg Δ9-THC + 25mg d-limonene, 30mg Δ9-THC + 50mg d-limonene, 30mg Δ9-THC + 100mg d-limonene, or 30mg Δ9-THC + 200mg d-limonene in a random order across test sessions. Outcomes assessed before and for 8 hours after drug administration included subjective drug effects, vital signs, and cognitive performance assessments.

Results: Four participants have completed the study and an additional 5 participants are currently enrolled (target n=20). Acute administration of 30mg Δ9-THC produced strong interoceptive drug effects, increased heart rate, and impaired cognitive performance. The co-administration of d-limonene was associated with an attenuation of self-reported ratings of overall unpleasant drug effects, feeling anxious, paranoid, sad, and sick as well as reduced ratings of having trouble with memory, dry mouth, and red/irritated eyes compared with Δ9-THC alone. D-limonene had no effect on overall drug effect severity, cognitive performance, vital signs, or other subjective drug effects.

Conclusion: Preliminary data suggest that co-administration of oral d-limonene with high-dose oral Δ9-THC is associated with reduced adverse effects, especially those related to anxiety, compared with Δ9-THC alone. This is consistent with prior research using inhaled dosing and suggests that adding d-limonene to pharmaceutical Δ9-THC products should be further explored.

CANNABIS EFFECTS ON CANCER BURDEN: RANDOMIZED CONTROLLED TRIAL PROTOCOL

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Introduction: Cancer-related pain and symptom burden are incompletely addressed by current pharmacologic strategies. Cannabinoids (THC, CBD) engage signaling cascades that may modulate cancer-related symptoms and treatment-related toxicities; however, controlled trials defining therapeutic dose ranges and real-world safety in oncology populations remain limited.

Methods: This ongoing randomized, double-blind, placebo-controlled, Phase I trial evaluates the safety and tolerability of daily oral cannabis administration (gummy formulation). Patients are randomized 1:1:1:1 to one of four arms: (1) THC 5 mg, (2) THC 15 mg, (3) THC 15 mg + CBD 15 mg, or (4) placebo. Eighty adults (n=20/arm) with locally advanced/metastatic solid tumors, receiving active anticancer therapy (excluding immune checkpoint inhibitors) are enrolled. Participants complete remote, observed daily dosing for 16 weeks; if escalation to the target dose is not tolerated, participants remain on their highest tolerated dose. Safety data (HR, O2) are monitored for 4 hrs post-dose. Daily phone app questionnaires assess cancer/treatment related symptoms including pain, sleep, appetite, mood, physical activity and hot flashes/night sweats. Primary outcomes include the proportion of participants discontinued due to adverse events and the highest tolerated dose within each arm. Cancer burden outcomes, adverse event frequency and severity and post-dose safety violations are also assessed.

Results: This ongoing trial (NCT06601218) began enrollment in June 2025 (n=2 completed).

Conclusions: This trial was designed to meet all federal regulatory requirements (DEA Schedule I, FDA IND, safety monitoring) and can serve to help inform others how to conduct rigorous outpatient Schedule I dosing trials.

Funding: University of Kentucky Cannabis Center

CANNABIS NURSE-LED AI HARM REDUCTION INTERVENTION DEVELOPMENT: MULTIDIMENSIONAL PERSPECTIVES OF ACCEPTANCE AND BARRIERS

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Introduction: Technology-supported education, including artificial intelligence (AI), may provide scalable solutions; however, acceptability and trust in AI-enabled cannabis education remain insufficiently characterized across age groups and professional roles.

Methods: A national cross-sectional electronic survey was administered in June-July 2025 among healthcare providers caring for patients who consume cannabis and medical cannabis patients. Participants were recruited via listservs, targeted email outreach, and social media. Measures assessed patient/provider attitudes, preferences, and openness toward technology-based and AI-assisted cannabis educational interventions.

Results: The analytic sample (46.9% female; 48.3% Non-Hispanic Black, 38.3% Non-Hispanic White) had a median age of 30 years (IQR: 28–35) and included nurses (39.5%), patients (39.5%), and health professionals (HP; 21.0%). Among patients, cannabis use was most frequently reported for anxiety/depression (78.1%), pain (34.4%), and sleep (31.3%). Nearly half of participants reported strong comfort collaborating with an AI cannabis nurse (49.9%); however, skepticism remained regarding AI's ability to provide personalized advice (60.5%) and emotional support (50.6%). Preferences favored expert-led (27.2%) and technology-based education (34.6%) over clinician-only delivery. Acceptability varied significantly by age: participants aged 30–40 reported the highest comfort (70%) and likelihood of collaboration (73.3%), whereas 50% of those >40 reported strong discomfort ($p=0.0002$) and very low collaboration likelihood ($p<0.0001$). Hesitancy toward AI emotional-support functions was highest among health professionals (70.6%; $p=0.20$).

Discussion: AI-supported cannabis education shows promise as a scalable adjunct, particularly among younger adults, but hesitancy remains regarding personalization and emotional support. Age and professional role shape acceptability. Hybrid, human-guided AI models may preserve clinical credibility and trust.

PROBING THE IMMUNE, ANALGESIC, ABUSE-RELATED, AND DELTA-9-THC-SPARING EFFECTS OF TWO TERPENES, BETA-CARYOPHYLLENE AND MYRCENE, IN A PLACEBO-CONTROLLED HUMAN LABORATORY STUDY

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Introduction: Cannabis is frequently used for pain, but adverse effects limit its clinical potential. Preclinical studies show that beta-caryophyllene (BCP) and myrcene (MYR), two terpenes in cannabis, have analgesic and anti-inflammatory effects without abuse-liability. This study assesses the immune, analgesic, and abuse-related effects of these terpenes (alone and with Δ9-tetrahydrocannabinol [THC]) in healthy volunteers.

Methods: A subset of participants (N = 12, 33 ± 9 yrs) from a placebo-controlled, within-subject study inhaled vaporized BCP (7.5 mg), MYR (12.0 mg), THC (15.0 mg), alone and in combination, or placebo (PBO), across 6 sessions. Pain response was measured using Cold Pressor Test (CPT), subjective pain ratings and subjective drug effects with visual analog scales. Seven plasma cytokines (MesoScale V-Plex Viral Panel 2) and plasma cortisol (R&D systems ELISA) were measured.

Results: THC increased ratings of 'High' ($p<0.001$), 'Liking' ($p<0.05$), and 'Good Effect' ($p<0.01$) (alone and with either terpene) compared to PBO. Neither terpene alone elicited these subjective effects. THC alone elicited analgesic effects in the CPT (increased pain threshold at 15-min, $p<0.05$) and reduced ratings of 'Bothersomeness' ($p<0.01$) compared to PBO. Neither terpene alone nor with THC elicited these analgesic effects. Peak concentrations of IL-1β and IL-8 were lower with THC+MYR compared to PBO ($p<0.05$).

Conclusions: THC produces subjective drug and analgesic effects, whereas terpenes alone do not produce these effects. Two pro-inflammatory cytokines were reduced after THC+MYR administration. The effects of cortisol and correlations between immune, stress, and analgesic outcomes are ongoing and may clarify these results.

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LEVERAGING LARGE LANGUAGE MODELS TO CAPTURE CBD USE IN ELECTRONIC MEDICAL RECORDS

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Introduction: Millions of patients use CBD, yet its effects on individual health and healthcare system burden remain poorly understood. Electronic medical records (EMRs) offer valuable clinical data, but medicinal cannabis use is inconsistently coded or absent from structured fields. This study applies LLM-based NLP methods to identify CBD use from free-text provider notes across the Johns Hopkins Health System (JHHS).

Methods: Using OpenAI's large language models (LLMs) GPT-5 and GPT-5-mini within a secure, HIPAA-compliant Azure environment, we developed preprocessing routines and classification schemas through iterative analysis of ~2.1 million clinical notes from over 800,000 patients to identify current CBD users.

Results: Preliminary validation between two independent reviewers indicated high accuracy of the model in distinguishing CBD use from other cannabis products. The NLP tool identified 11,282 current CBD users with a mean age of 53 years, 67% female and 74% White. Approximately one-third of current CBD users identified were ≥ 65 years old. Overall, 51% had diagnosed pain syndromes (neuropathic, inflammatory/degenerative, fibromyalgia, migraine) and 46% had a sleep disorder diagnosis. Our analysis on 600 examples by four expert annotators yielded F1 scores exceeding 95% for both affirmative and negative classifications.

Conclusions: The LLM-based NLP tool applied to routine clinical documentation was able to reliably identify current CBD use at scale within a large, diverse health system. Pending analyses will compare healthcare utilization and clinical characteristics between CBD users and matched non-users. This pipeline is scalable to existing global EMR infrastructure for large-scale implementation.

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SYNERGISTIC EFFECTS OF THC AND CBD ARE ASSOCIATED WITH REDUCED CYTOKINES IN A CLINICAL SAMPLE WITH CHRONIC PAIN

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Introduction: Despite pre-clinical evidence of the immunomodulating effects of delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), clinical evidence remains elusive. Dysregulated inflammation plays a key role in the pathology of many chronic diseases, including chronic pain. This study aimed to assess if concentrations of cytokines interleukin(IL)-1ß, IL-6, and IL-10 changed after 14 days of naturalistic cannabis use in participants with chronic low back pain.

Methods: Participants (N=207) self-selected a legal-market, edible cannabis product to use ad.libitum for 14 days to self-manage symptoms of chronic low back pain. Plasma cytokine concentrations for IL-1ß, IL-6, and IL-10 were assessed at baseline and again after 14 days of naturalistic use. Self-administered total dose (average milligrams: THC 52±203; CBD 130±227) over the 14 days was assessed via timeline follow-back. Generalized estimating equations assessed whether dose of THC and CBD was associated with longitudinal changes in cytokines. Models controlled for participant age, gender, body mass index, typical dose, and cannabis health expectancies.

Results: There was a significant THC×CBD dose interaction for IL-1ß ($p=0.008$), with lower IL-1ß concentrations associated with increasing THC doses at average ($b=-0.004$, 95%CI[-0.007,-0.001]) and high ($b=-0.006$, 95%CI[-0.010,-0.002) CBD doses. IL-6 significantly decreased over time ($p<0.001$), and lower concentrations of IL-10 were associated with higher doses of THC ($p=0.04$).

Conclusions: Decreases in IL-1ß over 14 days of edible cannabis use were dependent on THC and CBD dose. The potential THC and CBD synergy to affect inflammation may inform future research on the health effects of cannabis.

LONG-TERM PATTERNS AND COGNITIVE EFFECTS OF MEDICAL CANNABIS IN CHRONIC MUSCULOSKELETAL PAIN: A PROSPECTIVE COHORT STUDY

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Introduction: Medical cannabis (MC) is increasingly utilized for chronic pain management, yet questions remain regarding long-term adherence patterns, optimal formulations, and cognitive safety profiles. While its efficacy in pain relief is promising, concerns about patient retention and long-term compliance warrant further investigation. This study aimed to evaluate long-term MC use patterns, perceived efficacy, and its impact on cognition among patients with chronic musculoskeletal noncancer pain.

Methods: This prospective study included patients who were certified for MC between October 2022 and December 2024. A total of 78 patients certified for MC under Pennsylvania Medical Marijuana Program guidelines were followed, tracking their usage for at least one year utilizing the Inventory of Medical Cannabis Use (IMCU) questionnaire assessing usage patterns, dosage knowledge, efficacy, cognitive effects, mood changes, and tolerance changes.

Results: At one year, only 41% of patients (32/78) maintained consistent MC use. Among long-term MC users, the majority (77.5%) reported using MC daily or near-daily. Topical formulations were most frequently used (63.6%), followed by oral products (27.1%), while more potent concentrates were less common (9.3%). Approximately half of the respondents were uncertain of their exact oral THC/CBD dosage. High levels of perceived efficacy were reported, with over 93% of respondents agreeing that MC improved their primary symptoms. Cognitive and motor effects were minimal for most users (72.1% reported no impact; Figure 1), although a minority experienced some impairment. Product-specific effects varied significantly. Mood changes were reported, predominantly positive (42.6% improved mood). Furthermore, 79.8% of respondents indicated stable usage patterns over the prior three months.

Conclusions: These findings highlight the heterogeneous nature of MC use among chronic musculoskeletal pain patients. While a large proportion (59%) of patients discontinued MC use, a notable subset maintained long-term use. Among the long-term users, substantial symptom relief was reported with minimal cognitive compromise. The variability in dosing awareness underscores a need for improved patient education and standardized labeling to optimize therapeutic outcomes. Future research should focus on identifying predictors of sustained benefit and refining individualized MC treatment strategies.

CANNABINOID THERAPY IN BRODY'S DISEASE: A TRANSLATIONAL CASE REPORT OF A RARE GENETIC MYOPATHY

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Introduction: Brody's disease (BD; ICD: G71.8) has a prevalence of <1/1,000,000 and is considered an ultra-rare disease. It's a genetic condition characterised by a biallelic mutation in the ATP2A1 gene on chromosome 16p11, which encodes the SERCA1 protein responsible for calcium transport in skeletal muscle. This manifests as difficulty in muscle relaxation after contraction, due to dysregulation of calcium in the muscle, leading to stiffness, intense myalgia, and myofibrillar damage. Cannabis has demonstrated potential in relieving muscle spasms, a common symptom in BD. The goal is to evaluate, off-label, the therapeutic efficacy of a high-THC cannabis product for chronic pain, vomiting, and weight loss in BD.

Methods: A balanced full-spectrum cannabis product was used, 30 mg/ml (1:1), rich in cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC), with an initial dose of 0.25 ml (0.75 mg) per day, increasing by 0.25 ml every 7 days for 3 months. Vaporised inflorescences with high THC from the following strains were also used: Monkey fever, Winter bike and Gorilla zkittlez, with doses of 0.3 g in case of crises.

Results: Patient, a 30-year-old man, presented with initial symptoms in adolescence, including muscle stiffness, severe chronic pain, and recurrent episodes of rhabdomyolysis. The crises were associated with elevated CPK levels. Episodes of hospitalisation: In 2017, rhabdomyolysis with CPK of 204,000 U/L; in 2024, CPK > 23,000 U/L; in January 2025, with CPK > 15,000 U/L. Pharmacokinetic profile includes relevant polymorphisms: CYP2D6 (ultra-rapid metaboliser), SLCO1B1, and CYP2C9 (toxicity or therapeutic failure). Refractory to multiple drugs (opioids, muscle relaxants, and antidepressants). Symptoms: weight loss, nausea, lack of appetite, fatigue, insomnia, and chronic pain. The investigation included a muscle biopsy and genetic and molecular analysis, confirming the diagnosis of BD.

Conclusions: The use of full-spectrum 1:1 cannabis oil and vegetal vapourised inflorescences was able to reduce the clinical symptoms presented by the patient with Brody's myopathy. This report is the first case of BD in South America and highlights the use of cannabinoids as therapy.

Approval by the Research Ethics Committee: CAAE - 81351624.6.0000.0107.

PRELIMINARY EVIDENCE FOR THE SAFETY AND TOLERABILITY OF A TOPICAL CANNABIGEROL FORMULATION IN MUSCULOSKELETAL PAIN MANAGEMENT

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Introduction: Chronic musculoskeletal pain is a contributor to global disability and associated with physical, emotional, and functional burdens. Conventional pharmacological treatments have variable efficacy and adverse effects. Cannabigerol (CBG) has demonstrated preclinical analgesic potential. This study evaluated the safety, tolerability, and preliminary efficacy of a topical CBG-containing transdermal gel (CBG-TDG) in adults with musculoskeletal pain.

Methods: An open-label, prospective observational study was conducted at a medical cannabis clinic in Montreal, Canada. Adults with localized musculoskeletal pain were enrolled. Phase 1 (n=114) assessed CBG-TDG as adjunct to oral cannabinoid therapy (1-month follow-up). Phase 2 (n=19) evaluated CBG-TDG as standalone therapy (1-week follow-up). Primary outcomes were patient-reported musculoskeletal health (MSK-HQ) and symptom burden (ESAS-r). Secondary outcomes included adverse drug reactions (ADRs).

Results: In Phase 1, participants completing follow-up (n=67 MSK-HQ; n=66 ESAS-r) showed significant improvements in MSK-HQ scores (mean change +9.22, 95% CI [7.20, 11.24], d=1.11) and multiple ESAS-r domains, including pain (-1.41, 95% CI [-0.87, -0.34], d=-0.6), anxiety (Z=-5.65, $p<0.001$), sleep (-1.32, 95% CI [-0.65, -0.14], d=-0.39), and well-being (-1.47, 95% CI [-0.73, -0.21], d=-0.47). Mixed-effects models confirmed these changes. ADRs occurred in 13.0% of participants, with one event attributed to CBG-TDG. In Phase 2, participants completing follow-up (n=17) showed significant improvements in MSK-HQ scores (+6.12, 95% CI [1.78, 10.46], d=0.72) and day- and night-time pain/stiffness ($p<0.01$), with minimal ADRs.

Conclusions: CBG-TDG was well-tolerated and associated with improvements in musculoskeletal health and symptom burden. These findings support further controlled studies to confirm efficacy, explore mechanisms, and identify predictors of response.

CANNABIS USE AND OBJECTIVELY MEASURED SLEEP AND CIRCADIAN ACTIVITY AMONG NON-HISPANIC BLACK WOMEN: RESULTS FROM CANNA-ESSENTIAL STUDY

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Introduction: Cannabis use for sleep difficulties is increasing. There is a gap in cannabis studies with objectively measured sleep and circadian activity, particularly among women who are disproportionately affected by sleep difficulties.

Methods: Data are from the ESSENTIAL Sleep Study ancillary (Canna-ESSENTIAL), a cross-sectional study on the profiles of Non-Hispanic Black (NHB) adults at risk of insufficient sleep (≤6 hours) through home-based and clinical visits. Participants self-reported cannabis use. Total Sleep Time (TST; minutes), Sleep Efficiency (SE; %), and Wake after Sleep Onset (WASO; minutes) were collected through a 7-day actigraph (WGT3X-BT). Descriptive statistics, Wilcoxon tests, and Cosinor analysis were applied using RStudio with an alpha of 0.05.

Results: The analytic sample consisted of 66 NHB women (mean age 41.3 years, SD=11); 42.4% reported cannabis consumption in the past year, of which 19.7% were daily consumers and 34.8% consumed to manage sleep quality and/or duration. Overall, median TST was 349.8min, median sleep efficiency was 90%, and median WASO was 23.2min. Compared to non-consumers, past-year cannabis consumers had lower TST (345.46min vs. 354.59min), lower SE (93.0% vs. 94.0%), and higher WASO (25.48min vs. 19.94min). In cosinor analyses of 7-day actigraphy data, past-year cannabis consumption was found to have significantly lower Mesor (1.53 vs $1.70x10^6$; p=0.05), higher amplitude (415,978.2 vs. 323,214.2), and lower acrophase (0.01 vs. 0.59).

Discussion: Differences in objectively measured sleep and circadian activity were observed by past-year cannabis use among NHB women. Longitudinal studies are needed to examine the causal relationships between these factors and better inform future interventions.

INNOVATIVE OROMUCOSAL NANOEMULSION FOR THE DELIVERY OF CANNABIS OILY EXTRACTS: FROM FORMULATION AND CHARACTERIZATION TO LONG-TERM CLINICAL ADHERENCE

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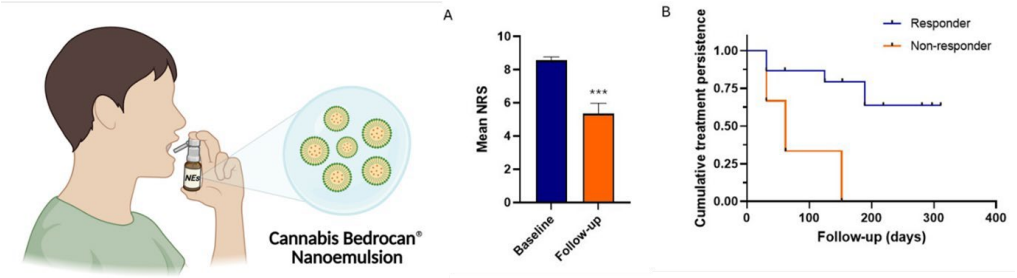
Introduction: Cannabis has established therapeutic potential; however, conventional oral oil formulations are limited by chemical instability, erratic gastrointestinal absorption, and marked interindividual pharmacokinetic variability due to hepatic first-pass metabolism. Oromucosal nano-delivery represents a rational strategy to bypass first-pass metabolism and improve absorption kinetics while reducing exposure variability.
Methods: A buccal oil-in-water nanoemulsion (THC-NE) was developed using Bedrocan® extract within a medium-chain triglyceride (MCT) lipid core stabilized by Tween 80/Labrasol ALF. Physicochemical characterization was performed using Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM). Drug loading and in.vitro release kinetics were quantified by HPLC (PBS pH 6.8:isopropanol 1:3 v/v). Stability was evaluated at 4, 25, and 37°C, after dilution in simulated buccal fluids, and following spray nebulization. Clinical translation was explored in an observational cohort of 18 treatment-resistant chronic pain patients.
Results: THC-NE exhibited a mean hydrodynamic diameter of 72.8 nm (PDI 0.214) and remained stable for 60 days without phase separation. In. vitro assays demonstrated accelerated yet controlled THC release. Sustained clinical benefit was observed over a median follow-up of 189 days. A clinically meaningful response (≥ 20% NRS reduction) was achieved by 83% of patients, with responders showing a mean 45% pain reduction. Treatment persistence was 64% at 6 months and no serious adverse events were reported (Fig.1).



Conclusions: Buccal nanoemulsion-based THC delivery mitigates the pharmacokinetic variability of conventional formulations, supporting precision-oriented cannabinoid therapy for refractory chronic pain. These findings support further controlled translational studies aimed at defining the role of nano-enabled cannabinoid delivery in precision pain management.

EFFECTS OF CANNABIDIOL ON THREAT ANTICIPATION IN ALCOHOL USE DISORDER: A FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY

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Introduction: Cannabidiol (CBD) has previously been suggested to be an emerging pharmacotherapy for Alcohol Use Disorder (AUD), however, results from clinical studies remain mixed and potential mechanisms remain elusive. The aim of the present study is to investigate the potential for CBD to modulate neurobiological, cognitive and psychological processes during anticipation of threatening stimuli.

Methods: In a cross-over, double-blind, randomized trial, 22 non-treatment seeking individuals diagnosed with AUD (DSM-V) were recruited to first receive 3 doses of 800mg of CBD or matched placebo, completing two respective fMRI sessions. Region of interest (ROI) and whole-brain analysis were utilized to examine treatment effects on neural activity in response to high vs low threat anticipation stimuli during a fear anticipation continuous performance task (FCPT).

Results: No main treatment effects were observed across cognition, regional or whole-brain activation. Recency of drinking was associated with longer latencies in responses on the continuous performance task.

Conclusion: The present findings indicate that CBD did not produce measurable effects on brain activity or cognitive performance during threat anticipation in individuals with AUD suggesting that previously reported neural effects may be more closely related to direct responses to salient stimuli rather than anticipatory processes.

Trial Registration: This trial was registered at ClinicalTrials.gov (NCT05387148)

EFFECTS OF A CANNABIDIOL/TERPENE FORMULATION ON SLEEP IN AN ADOLESCENT WITH AUTISM SPECTRUM DISORDER

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Introduction: Studies have shown that cannabidiol (CBD) may improve some features of autism spectrum disorder (ASD), though no studies have examined its effects on sleep. The primary objective of this case study was to evaluate the effects of an oral CBD-terpene formulation on sleep physiology in an adolescent with ASD.

Methods: A wrist-worn sleep-tracking device was used to measure baseline total sleep time (TST) and architecture in a 16-year-old male with ASD (73 days), followed by oral dosing with highly purified CBD (150 mg/day; 18 days); then a formulation of CBD and terpenes (300 and 8 mg, respectively; 59 days). The study medication was devoid of Δ9-Tetrahydrocannabinol (Δ9-THC).

Results: The study participant tolerated all treatments without any adverse events. CBD alone did not have any significant effects on sleep. The CBD and terpene formulation did not influence TST, but decreased light sleep [mean (SEM), -0.30 (0.11) h), 95% C.I. -0.04 to -0.58 h, $P = 0.0185$]; increased SWS sleep [mean (SEM), 0.47 (0.12) h, C.I. 0.78 to 0.18 h, $P = 0.0006$]; and increased REM sleep [mean (SEM), 0.28 (0.08) h, C.I. 0.48 to 0.08 h, $P = 0.0029$]. These benefits persisted with daily dosing for >18 months.

Conclusions: A select CBD-terpene ratio was well tolerated and increased SWS and REM (restorative) sleep in an adolescent with ASD. Future studies will be required to determine if CBD-terpene mediated increases in SWS and REM sleep influence problematic behavioral features of ASD.

CARDIAC SURGERY-INDUCED ACUTE KIDNEY INJURY IN PEDIATRIC PATIENTS DISPLAYS EARLY SYSTEMIC ENDOCANNABINOID SYSTEM ELEVATION

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Introduction: Acute kidney injury (AKI) is a frequent and serious complication of pediatric cardiac surgery. Because serum creatinine rises only after significant renal damage, early predictive biomarkers are needed. The endocannabinoid system (ECS) is increasingly recognized as a regulator of renal pathophysiology, yet its role in pediatric AKI remains undefined.

Methods: Sixty children (0–18 years) undergoing cardiac surgery with cardiopulmonary bypass were enrolled. Serum metabolites were analyzed before and 6, 12, 24, and 48 hours after surgery. AKI severity was defined and graded by KDIGO criteria (serum creatinine elevation and urine output). Time-course profiles of endocannabinoids and related lipid mediators were compared between AKI and non-AKI groups.

Results: Thirty-three patients (55%) developed AKI, 13 (22%) with severe AKI (stage 2–3). Although pre-surgery 2-arachidonoylglycerol (2-AG) levels were similar, they rose markedly within 6 hours post-surgery in severe AKI, and remained elevated throughout. Severe AKI patients displayed significantly higher levels in total 2-AG levels post-surgery. Baseline *N*-acylethanolamines were significantly higher in patients who developed AKI, and stayed elevated early postoperatively, along with other endocannabinoid-like lipids sharing related metabolic routes.

Conclusions: Cardiac surgery-induced AKI in children is accompanied by early systemic ECS activation. These findings identify the ECS as a potential early biomarker and mechanistic contributor to pediatric AKI.

MONLUNABANT : A BETTER STRATEGY TO TACKLE TYPE 1 DIABETES?

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Introduction: Cannabinoid type 1 receptor (CB1R) blockade in islets prevents immune cell infiltration (insulitis) in type 1 diabetes (T1D), an autoimmune disease characterized by beta (β)-cell loss leading to insulinodeficiency and hyperglycemia. Mechanistically, CB1R blockade prevents islet nitric oxide (NO) production and lowers chemokine expressionsecretion. While it improve β-cell function, it also drives glucose-stimulated insulin secretion (GSIS) beyond normal levels, possibly due to excessive Gαi–adenylyl cyclase–cAMP signaling, which could be detrimental over time. We hypothesize that the β-arrestin–biased CB1R-inverse agonist monlunabant may prevent insulitis without causing insulin oversecretion.

Methods: Human islets ± same organs donor's immune cells (*ex vivo* models of insulitis), were treated with 1-100nM monlunabant (*S*-MRI-1891) or vehicle in the presence of proinflammatory cytokines (IL1β, TNFα, and IFNγ). B-cell function and insultis were assessed by GSIS, Seahorse Analyzer, continuous cell imaging, qPCR, and ELISA.

Results: Monlunabant at 1 and 10nM preserved human β-cell function in proinflammatory conditions, without enhancing GSIS beyond control levels. Monlunabant protected mitochondrial function, preserving ATP production and further enhancing it 2-fold over control conditions. Monlunabant (10nM) also ameliorated insulitis : 1) prevented cytokine-induced NO production, 2) reduced 50±4% *IL1B* and *CXCL10* expression/secretion, and 3) prevented immune cell infiltration into islets.

Conclusion: These findings demonstrate that β-arrestin–CB1R signaling modulates islets inflammation, mitochondrial metabolism and GSIS. Its blockade succesfully preserves functional β-cell responses without leading to insulin oversecretion and potential β -cell exhaustion. Monlunabant therefore represents a promising therapeutic candidate to preserve functional β-cell mass longterm in T1D.

ULTRASTRUCTURAL AND FUNCTIONAL EVIDENCE FOR MITOCHONDRIAL CANNABINOID-1 RECEPTORS MODULATING RENAL PROXIMAL TUBULE CELL METABOLIC HOMEOSTASIS

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Introduction: Mitochondrial dysfunction within renal proximal tubule cells (RPTCs) is a major driver of chronic kidney disease (CKD) progression. Although the endocannabinoid system (ECS) influences mitochondrial bioenergetics in several tissues, the presence and role of mitochondrial cannabinoid-1 receptors (mtCB1R) in kidney cells remain uncharacterized. Given the high energetic demands of RPTCs, we investigated whether mtCB1R contributes to metabolic regulation.

Methods: Kidneys from wild-type (WT), total CB1R knockout (CB1R-KO), and mitochondria-specific CB1R knockout (mtCB1R-KO) mice were processed using pre-embedding and post-embedding immunogold transmission electron microscopy (TEM) to localize CB1R at the ultrastructural level. Mitochondrial function in isolated primary RPTCs was analyzed using Seahorse XF mitochondrial stress tests, applying oligomycin, FCCP, and antimycin A/rotenone to assess oxygen consumption rate (OCR) parameters.

Results: Immunogold TEM revealed distinct CB1R labeling on RPTC mitochondria and apical membranes in WT kidneys, absent on mitochondria from mtCB1R-KO mice, confirming antibody specificity and mitochondrial localization of CB1R. Quantitative analysis showed an enrichment of gold particles along mitochondrial membranes compared to cytoplasmic regions. Functionally, WT RPTCs exposed to CB1R agonists exhibited changes in basal and maximal OCR, indicating modulation of mitochondrial respiration and ATP coupling efficiency, whereas CB1R-KO and mtCB1R-KO RPTCs were resistant to these effects. Conversely, CB1R antagonism affected respiratory capacity in WT cells, suggesting that mtCB1R exerts an influence on oxidative metabolism.

Conclusions: These findings provide direct ultrastructural and functional evidence for mtCB1R in RPTCs, identifying a previously unrecognized regulator of renal bioenergetics with potential implications for acute and chronic kidney disease pathogenesis.

TESTING TOPICAL OR SYSTEMIC ADMINISTRATION OF ART26.12 – A FATTY ACID-BINDING PROTEIN 5 INHIBITOR – IN A MURINE MODEL OF ATOPIC DERMATITIS

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Introduction: Atopic dermatitis (AD) is a chronic inflammatory skin condition characterised by dry, itchy skin, and redness. Fatty acid-binding protein (FABP) 5 is upregulated in AD skin samples and is correlated with disease severity. However, Skadow et al. (2025) showed that an FABP5 global knock-out exacerbated symptoms in the MC903 AD mouse model. ART26.12 is a novel, selective FABP5 inhibitor that has shown efficacy in a preclinical model of psoriasis, where FABP5 is also upregulated. This study developed a lead topical formulation for ART26.12 and assessed whether topical or oral administration of ART26.12 was efficacious in a murine model of AD.

Methods: A pilot pharmacokinetics study assessed three topical formulations in Balb/c mice. Test formulations (0.018 mg/ml in 30 µl; ~1 µM tissue concentration) were applied to back skin BID for six days, with QD dosing on the final day before sacrifice. Blood sampling was conducted 4, 8, 12, and 24h post-last dose, with skin samples collected 24h post-last dose. The MC903 model of AD was conducted in Balb/c mice. MC903 was administered at 1 nM in 25 µL/ear QD for 15 days. Treatments were dosed concurrently for 15 days and included clobetasol (standard-of-care; 0.05%, 15 µL/ear, QD), topical ART26.12 (0.012, 0.036, 0.108 mg/mL, 15 µL/ear, BID), and oral ART26.12 (10, 25, 50 mg/kg, BID). Endpoints included ear thickness, redness, and scaling, as well as scratching. Terminal plasma and ear tissue samples were collected 2h post-last dose.

Results: Formulation 1 (0.02 mg/mL) led to pharmacologically relevant levels of ART26.12 in the back skin 24h after dosing, without skin irritation. None of the topical formulations caused detectable plasma levels of ART26.12. Formulation 1 was taken forward to the AD preclinical study. In this experiment, neither topical nor oral ART26.12 influenced skin erythema, scaling, thickness, or scratching. Clobetasol showed a significant reduction in all AD-related symptoms. Tissue and plasma analysis showed that ART26.12 was present in the ear (~2–70 µM) and plasma (~0.2–1.2 µM) following topical or oral administration, respectively.

Conclusion: This study shows that ART26.12 is suitable for topical formulation, with detectable drug in the skin up to 24h after application. ART26.12 did not show efficacy in the MC903 model, suggesting FABP5 has alternate roles in dermatological conditions with different underlying immune and barrier dysregulation. However, ART26.12 did not exacerbate AD symptoms, as seen with FABP5 deficiency in the MC903 model (Skadow et al., 2025), showing that pharmacological inhibition of FABP5 does not have deleterious effects in AD.

ENDOCANNABINOID RESPONSES TO RELIEF OF UPPER URINARY TRACT OBSTRUCTION (KIDNEY STONES)

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Introduction: The endocannabinoid system (ECS) plays a key role in regulating renal hemodynamics, and its dysregulation has been implicated in chronic kidney disease (CKD). However, the involvement of the ECS in acute kidney injury (AKI) remains poorly understood. This study aims to characterize changes in circulating endocannabinoid (eCB) levels before and after relief of upper urinary tract obstruction (UUTO) due to urolithiasis (kidney stones), in order to elucidate ECS dynamics during AKI.

Methods: Patients presenting with acute renal colic (pain) secondary to obstructive urolithiasis who underwent kidney decompression within 24 hours of admission were prospectively enrolled. Clinical data was collected, along with blood samples obtained before and after drainage for measurement of circulating eCBs. Patients were divided into two subgroups based on the presence of AKI at presentation. Changes in eCB levels were analyzed and compared between groups.

Results: Twenty-two patients enrolled (10 AKI and 12 non-AKI controls). Serum N-acylethanolamines (NAEs) exhibited distinct post-drainage patterns between groups. In AKI, N-arachidonoylethanolamine (AEA), N-palmitoylethanolamine (PEA), and N-oleoylethanolamine (OEA) increased following obstruction relief ($p = 0.06$, 0.008, and 0.08, respectively). Conversely, patients without AKI demonstrated a reduction in NAE levels, with a significant AEA decrease ($p = 0.03$). The fold change in NAE levels post-drainage was significantly higher in patients with AKI compared to non-AKI.

Conclusions: Relief of UUTO is associated with increased circulating NAEs in patients with AKI, suggesting ECS activation may contribute to the pathophysiology of obstruction-related kidney injury. These findings identify the ECS as a potential target for future mechanistic and therapeutic studies in AKI.

MÜLLER GLIAL CELLS ARE INVOLVED IN THE RETINAL ENDOCANNABINOID SYSTEM, POTENTIALLY THROUGH PLASMALOGEN-DEPENDENT MECHANISMS

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Introduction: The retina is a lipid-rich tissue containing high levels of polyunsaturated fatty acid (PUFA). PUFA-derivative endocannabinoids (ECs) are present in the retina, yet their physiological role and cellular origins remain elusive. Müller glial cells, the main retinal glia, are highly enriched in plasmalogens, considered as PUFA reservoirs. We therefore investigated whether Müller cells produce ECs and if plasmalogens modulate this process.

Methods: Expression of genes and proteins involved in EC synthesis and degradation was analyzed by transcriptomics and proteomics in human iPSC-derived Müller cells (hiMGC). Primary mouse Müller cells from wild-type and plasmalogen-deficient mice ($DAPAT^{-/-}$) as well as hiMGC were cultured *in vitro* and treated with JZL195, an inhibitor of EC-degrading enzymes. ECs levels in cell lysates and supernatants were determined by LC-MS.

Results: Both transcriptomic and proteomic analyses showed that hiMGC express the enzymatic machinery for EC metabolism. Mouse Müller cells and hiMGC produced major ECs, including 2-arachidonoylglycerol (2-AG), anandamide (AEA), oleoylethanolamide (OEA), palmitoylethanolamide (PEA), and docosahexaenoylethanolamide (DHEA), which accumulated over time following JZL195 treatment. Interestingly, plasmalogen deficiency reduced the synthesis of 2-AG, OEA, PEA, and DHEA.

Conclusions: Müller cells are an active source of retinal ECs in both humans and mice, supporting the relevance of mouse models for studying retinal EC biology. Our data further identify plasmalogens as potential regulators of EC production, opening new avenues for understanding lipid–glia interactions in the retinal endocannabinoid system.

MATERNAL OBESITY DECREASES CIRCULATING ENDOCANNABINOIDS IN PREGNANT RATS AND DIFFERENTIALLY REGULATES PLACENTAL CANNABINOID RECEPTOR EXPRESSION

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Introduction: Maternal obesity (MO) during gestation and lactation is related with the developmental origins of metabolic and behavioral dysfunctions in the offspring. We have demonstrated that MO induces overactivation of the endocannabinoid system (ECS) in rat offspring associated with metabolic syndrome and anxiety. In the present study, we investigated the effect of an obesogenic diet on maternal circulating endocannabinoids and placental cannabinoid receptors (CB1 and CB2).

Methods: Female rats were fed a control diet (C; 10% lipids) or an obesogenic diet (OD; 37% lipids, 11% sucrose) for 9 weeks before and during pregnancy (Ethics protocol n° 147/21). On the 20th gestational day, cesarean section was performed to collect placentas and fetuses. Maternal, placental and fetal anthropometric and metabolic parameters were recorded. Endocannabinoids were evaluated by UPLC-MS, and placental CB1 and CB2 by immunohistochemistry. Data were analyzed by Student's t-test or Two-way ANOVA (p<0.05).

Results: OD increased body weight, glucose intolerance and visceral adiposity, while decreased serum levels of the endocannabinoid 2-arachidonoylglycerol (2-AG) p<0.05) in pregnant rats. OD increased placental triglycerides and the fetal/placental weight ratio (p<0.05). OD decreased CB1 in the junctional zone (Jz) (p<0.05) and increased in the labyrinth zone (Lz) (p<0.05) of male placentas, with no changes in females. OD decreased CB2 in the Jz regardless of fetal sex (p<0.05).

Conclusions: Diet-induced obesity decreased maternal 2-AG levels, which may have a potential deleterious impact on fetal neurodevelopment. OD increased placental triglycerides, and lipotoxicity has been associated with placental dysfunction and negative metabolic outcomes in offspring. An increased fetal/placental weight ratio suggests an adaptive response aimed at preserving fetal growth. Increased Lz CB1 expression may be part of this adaptation, as it stimulates the expression of placental growth factors. However, CB1 induces lipogenesis and oxidative stress. Additionally, decreased Jz CB2 may be associated with a proinflammatory environment, potentially affecting on placental hormone production.

CB2 RECEPTOR OF THE YOUNG PROMOTES INFLAMMATION IN PERIPHERAL NERVES DURING OBESITY

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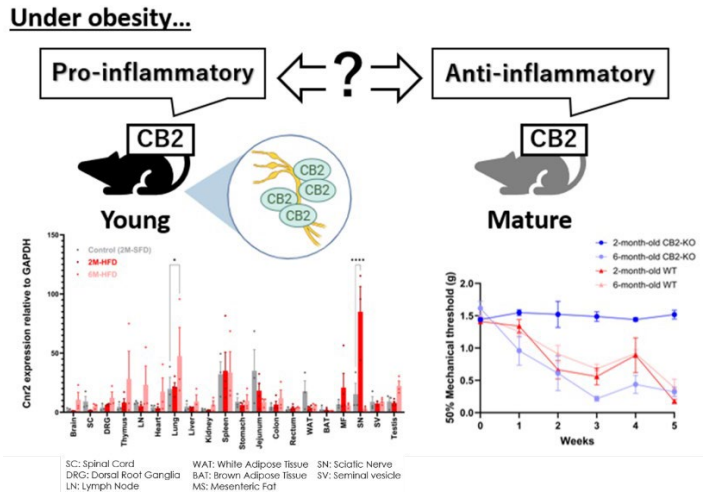
Introduction: Childhood obesity is closely linked to later-life inflammatory disease, yet the mechanisms connecting age, obesity, and inflammation remain unclear. Although the type 2 cannabinoid receptor (CB2) is generally anti-inflammatory, we have found that it can exert pro-inflammatory effects in obese mice, possibly in an age-dependent manner. This study aims to identify the factors that determine the inflammatory role of CB2 under obesity. **Methods:** We compared young (2-month-old) and mature (6-month-old) mice, with or without CB2 receptors. Obesity-associated inflammation was induced by feeding them a high-fat diet (HFD) for 5 weeks. Obesity-associated inflammation was evaluated using behavioral tests, flow cytometry, and gene expression measurements. **Results:** CB2 receptor deficiency suppressed hyperalgesia under HFD-induced obesity-associated inflammation only in young mice. While differences between genotypes were observed, no significant differences were found in the immune cell populations across ages. CB2 receptor gene expression levels were further measured across various organs, resulting in a marked and typical increase in the sciatic nerve of young obese mice. **Conclusions:** This study demonstrates that the CB2 receptor may act as an age-dependent regulator of obesity-associated inflammation, and, furthermore, that the mechanism of CB2 receptor accumulation in the peripheral nerve is present.



FOOD, SEX AND STRESS - SEASONAL DIFFERENCES OF BLOOD ENDOCANNABINOIDS AND ENDOCANNABINOID SYSTEM-ASSOCIATED LIPIDS IN VERVET MONKEYS

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Introduction: Vervet monkeys (*Chlorocebus pygerythrus*) display pronounced seasonal changes in reproductive activity, and stress physiology related to food availability. We investigated whether these seasonal differences are reflected in blood endocannabinoids and endocannabinoid system–associated lipids.

Methods: Whole blood samples were collected from wild vervet monkeys at the INKAWU Vervet Project, in Mawana Game Reserve, South Africa, during the dry (mating) season in June–July 2022 (13 females, 25 males) and the wet (birth) season in October 2023 (9 females, 25 males). A subset of individuals (3 females and 11 males) was sampled in both seasons. Blood was collected into EDTA tubes and endocannabinoids, endocannabinoid system–associated lipids, prostaglandins, polyunsaturated fatty acids (PUFAs), glucocorticoids, and testosterone were quantified by liquid chromatography–electrospray ionization–tandem mass spectrometry.

Results: During the dry season, monkeys exhibited elevated testosterone and cortisol concentrations. This was accompanied by higher levels of the endocannabinoid 2-arachidonoylglycerol and its precursor 1-stearoyl-2-arachidonoyl-sn-glycerol. The endocannabinoid Anandamide and the related lipids *N*-linoleoylethanolamine and *N*-palmitoylethanolamine were increased exclusively in males, while *N*-oleoylethanolamine and *N*-stearoylethanolamine were elevated in both sexes. Several PUFAs showed seasonal differences, partly in a sex-dependent manner. Additionally, males exhibited lower prostaglandin D2 levels during the wet season.

Discussion: These findings demonstrate pronounced seasonal modulation of blood endocannabinoids and lipid mediators in wild vervet monkeys. The observed patterns parallel seasonal changes in food availability, reproductive behavior, and stress hormones, suggesting a potential integrative role of the endocannabinoid system in coordinating metabolic, reproductive, and stress-related processes in natural ecological settings.

LOW-DOSE THCA PRESERVES PANCREATIC ISLET FUNCTION IN PROINFLAMMATORY CONDITIONS

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Introduction: Tetrahydrocannabinolic acid (THCA), the non-psychotropic acidic precursor of tetrahydrocannabinol, exhibits anti-inflammatory properties. Although THCA has been shown to reduce inflammation and immune cell infiltration in metabolic disorders, its therapeutic potential has not yet been evaluated in human pancreatic islets. Given the central role of inflammation in islet dysfunction, we investigated the effects of THCA *in vitro* in human islets exposed to proinflammatory cytokines.

Methods: Human islets isolated from n=3 brain-dead organ donors were cultured for 24 hours in the presence (or absence) of a proinflammatory cytokine cocktail (IL-1β, TNFα, and IFNγ), with increasing concentrations of THCA (10^{-9} – 10^{-6} M) or vehicle (DMSO). Insulin-producing beta cell function was assessed by dynamic glucose-stimulated insulin secretion (GSIS) using perifusion system and insulin ELISA.

Results: THCA alone had no effect on insulin secretion. Islet stress and dysfunction, as it happens with proinflammatory cytokines, led to a 4.5-fold increase in basal insulin secretion (at 3 mM glucose) and reduced GSIS (at 15 mM glucose). Low-dose THCA (10^{-9} M) reduced basal insulin secretion by 1.6-fold in islets treated with cytokines, and preserved glucose responsiveness. THCA presented a U-shaped dose-response; at 10^{-6} M, it worsened the proinflammatory cytokine effect leading to islet dysfunction.

Conclusion: Low concentrations of THCA preserved GSIS under inflammatory *in vitro* conditions, whereas higher doses induced beta cell dysfunction. These findings highlight low-dose THCA as a promising candidate to protect functional islet mass in diabetes. Further studies are warranted to evaluate its immunomodulatory potential in islets.

THE INHIBITORY EFFECTS OF A PERIPHERALLY RESTRICTED CB1 RECEPTOR ANTAGONIST ON MYOFIBROBLAST TRANSDIFFERENTIATION OF HUMAN RETINAL PIGMENT EPITHELIAL CELLS

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Introduction: Myofibroblasts derived from retinal pigment epithelial (RPE) cells play a key role in the pathogenesis of retinal fibrotic conditions such as proliferative vitreoretinopathy (PVR). Upon exposure to growth factors and cytokines such as TNFa and TGFb (TNT), RPE cells undergo epithelial-mesenchymal transition and subsequent transdifferentiation to contractile myofibroblasts.

Methods: In this study, the effects of JD5037, a peripherally restricted CB1 antagonist, on myofibroblast transdifferentiation of primary cultures of human RPE cells was assessed. The collagen matrix contraction assay was used to assess myofibroblast function. Western blot analysis was used to measure the myofibroblast markers.

Results: JD5037 significantly reduced TNT-induced, RPE cell-mediated collagen gel contraction, an indicator myofibroblast function, in a concentration-dependent manner. Western blot analysis showed that JD5037 attenuated TNT-induced expression of α-SMA and fibronectin, two molecular markers of myofibroblasts. Furthermore, siRNA knockdown of CB1 cannabinoid receptor partially inhibited TNT-induced myofibroblast transdifferentation of human RPE cells and eliminated the inhibitory effects of JD5037 on myofibroblast transdifferentiation.

Conclusions: These data demonstrate, for the first time, that peripherally restricted antagonists such as JD5037 targeting CB1 cannabinoid receptor has therapeutic potential for PVR and other retinal fibrotic conditions.

CB1 RECEPTORS ON ADVILLIN- AND GPR65-POSITIVE SENSORY AFFERENTS OF THE GASTROINTESTINAL TRACT CONTROL VOLUNTARY ALCOHOL DRINKING IN MICE

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INTRDUCTION: The vagus nerve transmits caloric and rewarding values of food from the gut to the brain and represents a potential therapeutic target for alcohol use disorder (AUD). Our previous work indicated that cannabinoid-1 receptor (CB1R) on ghrelin-producing cells of the gastrointestinal (GI) tract rather than central CB1Rs contribute to the control of voluntary ethanol intake (VEI) in mice via a gut-brain axis (Cell Metab 2019;29:1320-33).

METHODS: VEI was tested in C57Bl6J mice using 2 paradigms: two-bottle/free choice and drinking in the dark. VEI and its inhibition by the peripheral CB1R antagonist JD5037 were tested in mice with selective deletion of markers of subsets of sensory afferent neurons and their wild-type littermates.

RESULTS: Here, we show that selective deletion of CB1R from ghrelin-producing cells only partially occluded the inhibition VEI by the peripheral CB1R antagonist JD5037 in $Ghrl^{Cre/wt}$;$Cnr1^{lox/lox}$ mice, indicating that CB1R at additional sites along the gut-brain axis may control VEI. Therefore, we generated transgenic lines deleting CB1R from dorsal root ganglia (DRG) ($Wnt^{Cre/wt}$;$Cnr^{lox/lox}$) or nodose ganglia (NGA) ($Phox2b^{Cre/wt}$;$Cnr^{lox/lox}$), finding that JD5037's effect was occluded only in the latter. The requirement for CB1R on vagal sensory neurons was further substantiated by: (i) chemical ablation of CB1R-expressing NGA neurons with CB1R-targeted saporin immunotoxin, which markedly attenuated JD5037's ability to reduce VEI; and (ii) conditional deletion of CB1R from advillin$^+$ NGA neurons ($Avil^{Cre/ERT2/wt}$;$Cnr1^{lox/lox}$), which completely abolished JD5037's modulatory effect. Next, we explored which of two functionally distinct NGA afferents control VEI. We selectively deleted CB1R from either Glp1r$^+$ neurons ($Glp1^{riresCre/wt}$;$Cnr1^{lox/lox}$) that innervate the muscular layer of the gut, or Gpr65$^+$ neurons ($Gpr65^{riresCre/wt}$;$Cnr1^{lox/lox}$), which innervate the mucosa. Deletion from Glp1r$^+$ afferents had no impact on CB1R antagonist efficacy. In stark contrast, deletion from Gpr65$^+$ afferents completely abolished JD5037's ability to reduce VEI. JD5037 inhibited VEI in both male and female mice, demonstrating that peripheral CB1R control of VEI is conserved across sexes.

CONCLUSIONS: Our findings establish CB1Rs on Avil$^+$/Gpr65$^+$ mucosal sensory afferent neurons of the vagus nerve as key regulators of alcohol-seeking behavior. Selective targeting of these peripheral receptors represents a promising strategy for treating AUD without side effects mediated by central CB1R. Studies are ongoing to determine if the same pathway is involved in the control of palatable feeding.

CHRONIC CBD MITIGATES AGE-ASSOCIATED DYSREGULATION OF NEUROINFLAMMATION IN A MOUSE MODEL OF GULF WAR ILLNESS

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Introduction: Gulf War Illness (GWI) is a chronic disorder characterized by debilitating physical and cognitive symptoms associated with chronic inflammation. Research suggests the etiology involves veteran's exposure to toxic chemicals during the Gulf War, leading to immune pro-inflammatory priming. Our previous study found that GW chemical exposure disrupts the brain endocannabinoid (ECB) system, which serves critical neuroprotective functions. Since many veterans are now >50 years old, focus should be placed on increased inflammation associated with aging.

Methods: In the current study, we profiled the abnormal brain transcriptome of aged GWI model mice using bulk- and cell-type-specific RNA sequencing (RNAseq) of microglia. We also tested the effectiveness of chronic treatment with cannabidiol (CBD) on the brain transcriptome. Additionally, molecular and cellular landscapes of brain cells from the hippocampus of aged GWI model mice were assessed using single-nucleus RNA sequencing (snRNAseq) under basal and immune-stimulated conditions.

Results: GWI is associated with heightened age-associated increases in the transcription of pro-inflammatory cytokines but decreases in anti-inflammatory cytokines. At 17 months, GWI brains displayed increased transcription of select cytokine-related genes in both brain-wide and microglial transcriptomes, implying immune sensitization. Consistently, select subtypes of hippocampal immune cells displayed heightened pro-inflammation-related transcription under basal conditions and abnormal responses to systemic lipopolysaccharide (LPS). Chronic administration of CBD (30 mg/kg, i.p. daily for 28 days) reverted these abnormalities in select immune cell types including microglia.

Conclusions: Our results characterize the abnormal molecular and cellular landscape of aged GWI brains, which could be potentially normalized using CBD.

CHRONIC FAAH INHIBITION MODULATES NEUROINFLAMMATION IN AD MICE

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Introduction: The endocannabinoid system (ECS), a central regulator of neuroimmune balance, has emerged as a potential modulator of glial-driven pathology. In particular, fatty acid amide hydrolase (FAAH), the primary enzyme responsible for anandamide degradation, influences microglial and astrocytic activity, positioning it as a candidate target for intervention in AD.

Methods: Here, we investigated whether sustained pharmacological blockade of FAAH modifies neuroinflammatory dynamics in a sex-dependent manner using the 5xFAD mouse model. Six-month-old male and female transgenic mice were chronically treated with the irreversible FAAH inhibitor PF-04457845 (10 mg/kg, oral administration) for three months. Behavioral performance was assessed to evaluate cognitive and affective domains. Comprehensive molecular and cellular profiling—including flow cytometry, qPCR, Western blotting, and immunohistochemistry—was conducted to characterize glial reactivity, inflammasome-related pathways, and synaptic integrity.

Results: Female 5xFAD mice exhibited a more pronounced pro-inflammatory phenotype and heightened microglial activation compared to males. FAAH inhibition markedly attenuated these alterations in females, dampening inflammatory signaling, reducing glial reactivity, and preserving synaptic markers. In contrast, males showed comparatively limited responsiveness to treatment, underscoring differential ECS engagement across sexes.

Conclusions: Collectively, our findings reveal a robust sexual dimorphism in ECS-mediated regulation of neuroinflammation and provide experimental support for incorporating sex as a key biological variable in the development of FAAH-targeted strategies for AD.

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EFFECTS OF CANNABINOIDS ON BIOMARKERS ASSOCIATED WITH COGNITION AND INFLAMMAGING IN OLDER ADULTS

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Introduction: Research suggests the endocannabinoid system is highly involved in processes of cognitive decline, inflammation, and aging, but the field is still working to understand these mechanisms and pathways. Meanwhile, highly multiplexed quantification of both high- and low-abundance molecules in plasma is making it increasingly possible to study numerous biomarkers of aging and inflammation in the blood proteome, that in turn may help elucidate how the endocannabinoid system impacts these important processes. **Methods:** The data for these analyses were drawn from a patient-centered, observational study examining effects of edible cannabis of varying compositions (THC-only vs. CBD-only vs. THC+CBD) compared to a control group among older adults (NCT05188404). Participants provided blood samples at baseline and again after using their assigned product *ad libitum* for 4 weeks. Plasma was separated from whole blood and underwent analysis with a nucleic acid linked immune-sandwich assay (NULISA) panel for 120 central nervous system-associated markers of cognitive decline, inflammation, and aging processes (Alamar Biosciences, Fremont, CA). Intra- (5.23%) and inter-plate (6.68%) median CV was good and detectability was very high (94.7%). Linear mixed effect models that were false discovery rate (FDR) adjusted and likelihood ratio non-parametric tests were used to examine interaction effects of time and cannabinoid formulation on biomarker plasma concentrations. **Results:** 304 participants completed the study and had blood samples available for analysis (M_{age}=69.7, *SD*=6; 52.3% female, 84% bachelors degree or higher). Results revealed a significant timeXgroup interaction for brain derived neurotropic factor (BDNF; χ^2(3, *N*=604)=15.6, *p*=0.042), chemokine ligand 26/eotaxin-3 (CCL26; χ^2(3, *N*=604)=22.6, *p*=0.003), interleukin-6R (IL-6R; χ^2(3, *N*=604)=20.1, *p*=0.007), and nerve growth factor (NGF; χ^2(3, *N*=604)=24.4, *p*=0.003) but for no other biomarkers in the assay. These differences appear to be driven by use of products containing THC; Specifically, while concentrations of biomarkers did not significantly change over time compared to the control group for CBD only, BDNF, CCL26, and IL6R concentrations significantly increased and NGF concentrations decreased for the group using THC compared to the control group (*p*s<0.23), and concentrations of CCL26 significantly increased and NGF decreased compared to the control group for the group using THC+CBD (*p*s<0.001). **Conclusions:** Among older adults using varying ratios of THC and CBD compared to those who did not use cannabis, changes in both protective and risk-associated biomarkers were associated with use of products containing THC but not necessarily CBD. These associations were varied in their impact. THC use was positively associated with concentrations of neuroinflammatory biomarkers IL-6R and CCL26 (both known to accelerate synaptic dysfunction and associated neurodegeneration and cognitive decline) and negatively associated with the protective neurotropic factor NGF. The positive relationship between THC and BDNF concentrations is suggestive of contributing to synaptic resilience processes. As it is increasingly believed that the tone of endocannabinoid system shifts with aging processes, future work is needed to better understand how exogenous cannabinoid use is related to inflammaging processes among older adults.

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MICROGLIAL CANNABINOID RECEPTOR 2 INFLUENCES ALPHA-SYNUCLEIN CLEARANCE

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Introduction: There is widespread evidence that cannabinoid type 2 receptor (CB2) is a driver of anti-inflammatory responses. Brain expression of CB2 is increased in Parkinson's disease (PD) patients and in animal models of proteinopathies, suggesting it's functional relevance under disease conditions. We've previously shown that pharmacological targeting of CB2 can reduce alpha-synuclein (α-syn) aggregation in a rat model of synucleinopathy. However, it remains unclear whether this effect was mediated specifically by microglia.

Methods: Therefore, mice with CB2 conditionally deleted from CX3CR1+ cells (CB2 cKO) and WT mice (n=4/group) were stereotaxically injected with AAV-human-WT-α-syn into the substantia nigra. The brains were collected after 2 months and immunostained for phosphorylated α-syn (pSer129) and proteinase-k resistant α-syn to evaluate α-syn aggregation, tyrosine hydroxylase to evaluate dopamine nigral neurons, and microglial markers (TMEM119, IBA1, MHCII, CD68) to identify microglia phenotypes.

Results: Preliminary findings reveal a trend toward reduced pSer129 pathology ($p = 0.089$) and proteinase-k resistant α-syn ($p=0.053$) in CB2 cKO mice, suggesting that CB2 in CX3CR1+ immune cells may influence early α-syn aggregation. Furthermore, nigral TH+ cell counts reveal an average of 24.7% loss in WT mice and 8.7% loss in CB2 cKO when compared to the uninjected nigra. CD68-immunoreactivity was significantly reduced in CB2 cKO mice compared to WT ($p<0.05$). Further analyses are underway to complete microglia phenotypic evaluation.

Conclusions: Altogether, these data suggest that removal of CB2 from microglia and other CX3CR1+ cells alters immune phenotypes which may in turn increase α-syn clearance resulting in the protection of nigral TH+ neurons.

CHRONIC FAAH INHIBITION REDUCES HIV-1 TAT-INDUCED NEUROINFLAMMATION AND MEMORY IMPAIRMENTS IN FEMALE MICE

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Introduction: HIV-1 invades the central nervous system shortly after infection, releasing viral proteins like the transactivator of transcription (Tat). Prolonged viral exposure drives neuroinflammation and HIV-associated neurocognitive disorders, characterized by memory impairments in the prefrontal cortex (PFC) and hippocampus (HPC). Drugs targeting endocannabinoids (eCBs) exert neuroprotective and anti-inflammatory effects, making them promising therapies for neurodegenerative disorders. One such target is fatty acid amide hydrolase (FAAH), the primary catabolic enzyme of anandamide/AEA.

Methods: We evaluated whether the selective FAAH inhibitor, PF3845, ameliorates neuroinflammation in HIV-1 Tat transgenic mice. Mixed-sex mice received chronic PF3845 or vehicle (i.p., 10mg/kg, 12d) before sacrifice 2hr post-final injection. Cytokines and chemokines were quantified in the PFC and HPC via Bio-Plex immunoassay. A separate female-only cohort underwent behavioral testing to assess spatial working memory (T-maze) and recognition memory (novel object recognition; NOR) following chronic PF3845 treatment.

Results: Vehicle-treated Tat(+) females showed elevated proinflammatory cytokines, including IL-1β, in the PFC but not the HPC, and these increases were significantly reduced by chronic PF3845 treatment. Behaviorally, Tat(+) females displayed impaired working memory, reduced locomotion, and increased freezing, however, PF-3845 increased novelty preference in NOR. To further examine the cellular basis of cytokine changes, ongoing IHC studies examine IL-1β colocalization with astrocytes in the PFC and HPC to assess region-and cell-type-specific alternations.

Conclusions: Chronic FAAH inhibition provides sex-dependent protection against Tat-induced neuroinflammation and may improve memory-related cognitive outcomes. These findings highlight FAAH inhibition as a promising therapeutic strategy for mitigating HIV-driven neuroinflammation and cognitive dysfunction.

CANNABIDIOL (CBD) PROMOTES ASTROCYTE VIABILITY AND SUPPRESSES INJURYINDUCED ASTROCYTE STRESS RESPONSES IN ZEBRA FINCH SONG CONTROL NUCLEI

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Background: Cannabidiol (CBD), a non-euphorigenic phytocannabinoid, is FDA-approved for childhood epilepsies often linked to developmental delays in vocal/sensorimotor skills. In the zebra finch songbird model, CBD promotes recovery of learned vocalizations after focal injury to HVC, a pre-motor cortical-like nucleus (Tripson et al., 2023). Prior work showed CBD reduces neuroinflammation, oxidative stress, microglial activation (TMEM119), and synaptic loss in the song circuit. The present study investigated astrocytic contributions to these protective effects.

Methods: Adult male zebra finches received daily CBD (10 mg/kg, i.p.) or vehicle treatments followed by a unilateral electrolytic microlesion of HVC, (~10% volume). Astrocyte viability and apoptosis were assessed via immunofluorescence using co-localization of cleaved caspase-3 with glutamine synthetase (GS; astrocyte marker) and NeuN (neuronal marker). Stress responses were evaluated by lysosomal burden (LAMP1/LC3 expression), reactivity markers (C3, S100a10, aromatase), and neuroprotective markers (GS, GCLM) using immunohistochemistry and/or qRT-PCR in HVC, RA, and Area X.

Results: HVC microlesions induced significant cell loss in HVC and projection targets (RA, Area X), with a substantial proportion of apoptotic cells (cleaved caspase-3+) identified as astrocytes. CBD treatment significantly reduced lesion-induced apoptosis and preserved astrocyte populations across regions. Lesions elevated astrocyte stress, including increased lysosomal burden (LAMP1/LC3) and reactivity marker induction (C3, S100a10, aromatase). CBD attenuated these stress signatures while enhancing expression of metabolic/antioxidant markers (glutamine synthetase, GCLM), consistent with improved resilience to excitotoxicity and oxidative challenge.

Conclusions: Astrocyte homeostasis is a key mechanism underlying CBD's preservation of song circuitry and recovery of a complex learned sensorimotor behavior. Given that sensorimotor skills (vocal, manual, or otherwise) depend on learning-dependent establishment and ongoing maintenance of specialized neural circuits vulnerable to TBI disruption, the zebra finch model provides a valuable preclinical platform for investigating glial-targeted interventions to promote circuit resilience and functional recovery after focal CNS trauma.

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ENDOCANNABINOIDS, MILD COGNITIVE IMPAIRMENT, AND DEMENTIA: A SPRINT COHORT STUDY

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Introduction: Cardiovascular disease significantly increases the risk of developing Mild Cognitive Impairment (MCI) and its progression to dementia. Endocannabinoid system (ECS) is emerging as crucial regulator of cerebral blood flow and neuroinflammation. We hypothesized that alterations of ECS will associate with occurrence of MCI and dementia in patients with hypertension.

Methods: The Systolic Blood Pressure Intervention Trial (SPRINT) enrolled participants >50 years with hypertension and increased cardiovascular risk. An extended battery of cognitive function tests was performed during the trial. Protocol-defined MCI required two consecutive, adjudicated classifications of MCI to ensure the condition was persistent rather than transient. Targeted liquid chromatography-mass spectrometry was used to quantitate endocannabinoids in 550 patients' plasma samples collected at baseline.

Results: In the chosen cohort of 550 patients, 58 were diagnosed with MCI during the main trial. Lower baseline plasma anandamide (AEA) levels significantly associated with occurrence of MCI and diagnosis of probable dementia (adjusted for kidney function, sex, age and race). Kidney function itself (expressed as estimated glomerular filtration rate (eGFR)) followed the same association pattern. Logistic regression with the composite of MCI or probable dementia as outcome, revealed additional positive association with baseline plasma 2-arachidonoylglycerol (2-AG) levels.

Conclusions: In adults with hypertension and increased cardiovascular risk, lower AEA and higher 2-AG associated with cognitive impairment, defined as MCI or probable dementia. Early cognitive impairment is currently difficult to diagnose, and our results suggest that peripheral levels of endocannabinoids may be indicative of early stages of MCI.

CANNABIS USE AND SUBJECTIVE COGNITIVE DECLINE AMONG MIDLIFE AND OLDER ADULT CANCER SURVIVORS

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Introduction: Research shows that cannabis use may lead to cognitive decline among midlife and older adults, but evidence is limited among cancer survivors, despite widespread use of cannabis by survivors for symptom management. Moreover, cancer and its treatment may affect the cognitive health of cancer survivors. Given this group's vulnerability, our study investigated the association between cannabis use and subjective cognitive decline (SCD) among cancer survivors aged ≥45 years living in the USA.

Methods: Our sample included 5,889 cancer survivors from the 2024 Behavioral Risk Factor Surveillance System survey. The outcome was self-reported SCD and related functional limitations in the past 12 months, and the predictor was self-reported cannabis use in the past 30 days. We conducted weighted descriptive and multivariable logistic regression analyses.

Results: Among participants, 63.0% were aged ≥65 years, 55.3% were female, 36.0% had a high school education or lower, and 87.0% lived in urban areas. About 11.4% used cannabis in the past 30 days. Nearly 22.0% self-reported experiencing SCD, and 10% reported SCD-related functional limitations during the past 12 months. Those who used cannabis in the past 30 days had 78% higher odds of reporting SCD than those who did not use cannabis (OR 1.78, 95% CI 1.30-2.44).

Conclusions: Our findings indicate that many cancer survivors use cannabis, further increasing their risk of SCD. Hence, early identification of SCD, the formulation of personalized support strategies, and careful monitoring of cannabis use are necessary to balance the risks and benefits of cannabis use among cancer survivors.

THE EFFECTS OF ENDOCANNABINOID AND PHYTOCANNABINOID MODULATION IN AN ACUTE MOUSE MODEL OF PARKINSON'S DISEASE

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Introduction: Parkinson's disease (PD) involves nigrostriatal dopamine loss and motor dysfunction. Although L-DOPA remains the gold standard therapy, chronic treatment produces Levodopa Induced Dyskinesia (LID). The endocannabinoid system, particularly 2-arachidonoylglycerol (2-AG) signaling at CB1 receptors, modulates striatal neurotransmission and may influence L-DOPA responses. We examined how bidirectional manipulation of 2-AG alters L-DOPA induced motor activity in dopamine deficient mice.

Methods: Dopamine transporter knockout mice rendered dopamine deficient using alpha methyl p tyrosine received acute or chronic L-DOPA. Acute studies assessed locomotor activity following L-DOPA with MAGL inhibitors (MJN110 or ABX-1431), the DAGL inhibitor DO34, or a CB1 antagonist. Chronic experiments used daily L-DOPA for 14 days, measuring horizontal distance and vertical activity, a proxy of dyskinesia. Prevention studies paired ABX-1431 with L-DOPA. Intervention studies were conducted after vertical activity increased and horizontal activity decreased, testing amantadine, DO34, and ABX-1431 with standard or reduced L-DOPA doses. Delta-9-THC combined with CBD is being evaluated acutely and in the LID paradigm. Data were analyzed using repeated measures ANOVA.

Results: Acutely, MAGL inhibition potentiated L-DOPA induced locomotion, whereas DO34 reduced output. Chronically, L-DOPA increased vertical activity and decreased horizontal activity. ABX-1431 with reduced L-DOPA decreased vertical activity and increased horizontal distance. In intervention studies, ABX-1431 reduced vertical activity only with lower L-DOPA but worsened it at higher doses. DO34 reduced vertical activity and increased horizontal distance in the chronic paradigm despite suppressing locomotion acutely.

Conclusion: These findings demonstrate dose and stage dependent effects of 2-AG modulation on L-DOPA responses in parkinsonian mice.

DIFFERENTIAL REGULATION OF ERK1/2 AND MKP-3 BY JWH-133 AND 2-AG REVEALS LIGAND-BIASED CANNABINOID TYPE 2 RECEPTOR SIGNALING IN MICROGLIA

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Introduction: The cannabinoid type 2 receptor (CB2R) is a G protein coupled receptor that influences neuroinflammation via effects on microglia. However, the temporal and ligand-specific regulation of microglial signaling pathways remain poorly understood. Here, we investigated how the synthetic CB2R agonist JWH-133 and the endocannabinoid 2-arachidonoylglycerol (2-AG) differentially regulate activation of pathways previously implicated in the anti-inflammatory effects of CB2R in human microglia.

Methods: Human microglia were treated with vehicle, JWH-133, or 2-AG and harvested at timepoints less than 60 minutes. Western blotting was used to quantify ERK1/2, MKP-3, Akt, and p38. Densitometry was used to calculate phospho/total ratios and protein expression relative to total protein.

Results: JWH-133 induced an acute transient increase in MKP-3 at early timepoints, with MKP-3 significantly elevated compared to that of vehicle. ERK1 phosphorylation also rose transiently in both vehicle and JWH-133 conditions, consistent with brief ERK activation driving MKP-3-dependent negative feedback. In contrast, 2-AG reduced ERK2 phosphorylation during early timepoints, and combined ERK1/2 analysis confirmed an overall decrease in ERK signaling, but MKP-3 was not increased under 2-AG treatment. Neither AKT nor P38 were affected across conditions.

Conclusions: These data potentially support a model in which JWH-133 elicits an ERK/MKP-3 negative feedback loop, whereas 2-AG preferentially dampens ERK1/2 activity through MKP-3-independent mechanisms. This data may reveal ligand-biased CB2R signaling in human microglia and could indicate that ERK-centric microglial responses can be selectively modulated in neuroinflammatory contexts.

PHARMACOLOGICAL STUDY OF CANNABICHROMENE AND CANNABIGEROL AT THE SEROTONIN 1A (5-HT1A) AND ADENOSINE 2A (A2A) RECEPTORS

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Introduction: Minor cannabinoids cannabichromene (CBC) and cannabigerol (CBG) exhibit antinociceptive and anxiolytic effects not fully explained by cannabinoid receptor signaling. Prior research suggests other G protein-coupled receptors (GPCRs) such as 5-hydroxytryptamine 1A (5-HT1A) and adenosine A2A receptors are responsible. This study characterizes CBC and CBG binding and functional activity at these non-cannabinoid GPCRs.

Methods: Radioligand competition binding was conducted at human 5-HT1A and A2A receptors. Functional signaling at 5-HT1A was evaluated in Chinese hamster ovary (CHO) cells using a luminescence-based calcium assay and A2A signaling in human embryonic kidney (HEK) cells using a fluorescence-based cAMP assay. In vivo behavioral assays will be completed in wild-type and knockout C57Bl/6 mice.

Results: CBC and CBG displaced radioligand at 5-HT1A receptors (Ki = 13.9 nM, 95% CI: 1.86–163 nM and 7.39 nM, 95% CI: 1.50–58.5 nM, respectively). CBC displayed measurable binding at A2A (Ki = 26.0nM), with CBG displaying no activity. At 5-HT1A, CBC was a partial agonist in the calcium assay (EC50 = 1.98 μM; Emax = 80.8% vs 8-OH-DPAT), while CBG elicited robust activation (EC50 = 73.3 nM; Emax = 91.1%) and attenuated 8-OH-DPAT responses, consistent with a 5-HT1A agonist. A2A functional signaling and in vivo studies are ongoing.

Conclusions: These data support direct modulation of 5-HT1A by CBC and CBG with distinct efficacy profiles. Ongoing work aims to extend profiling to A2A receptor and assess pharmacology in receptor knockout mice. Determination of CBC and CBG's non-cannabinoid pharmacology may lead to novel anxiolytic and pain-relieving compounds that circumvent the intoxicating effects of cannabis.

NEURONAL *VERSUS* GLIAL CB2 RECEPTOR: INSIGHTS FROM A NEW STRAIN OF CB2-KO-EGFP REPORTER MICE

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Introduction: The cannabinoid CB2 receptor (CB2R) has emerged as a promising therapeutic target for pain and central nervous system disorders, yet its brain expression has remained controversial due to low basal levels and the lack of reliable antibodies. Previous green fluorescent protein (GFP) reporter mouse lines have produced conflicting findings, possibly because GFP was either randomly inserted into the genome or placed in the 3'-untranslated region of the CB2R gene (*Cnr2*), complicating interpretation.

Methods: We generated a new strain of CB2-KO-eGFP mice, in which the endogenous CB2-coding sequence was replaced by eGFP while preserving the remaining CB2R gene structure. To examine GFP expression in the brain, we performed fluorescence-based flow cytometry and immunohistochemistry (IHC) using a GFP antibody. We then used RT-PCR and RNAscope in situ hybridization (ISH) to confirm CB2R deletion at the mRNA level. Lastly, we compared behavioral phenotypes between wild-type (WT) and CB2-KO-eGFP mice.

Results: Loss of CB2R expression was confirmed by qRT-PCR, RNAscope in situ hybridization, and cannabinoid pharmacological assays. GFP-immunostaining was detected across multiple brain regions, including cingulate cortex, hippocampus, red nucleus, and cerebellum, and in several cell types such as microglia, astrocytes, and neurons. Flow cytometry revealed strong GFP signals in spleen and blood cells and quantifiable GFP expression in brain tissue. Notably, ~70% of microglia and ~4% of neurons in cortex and hippocampus expressed GFP under normal physiological conditions. Functionally, CB2R deletion reduced cannabinoid-induced analgesia, hypothermia, and catalepsy, confirming the receptor's physiological relevance.

Conclusion: These findings demonstrate that CB2R is indeed expressed in healthy brain tissue and across multiple neural and glial cell types, resolving long-standing uncertainty regarding CB2R localization. This new mouse line provides a reliable and highly informative tool for studying CB2R expression and function in both the brain and peripheral immune system.

PHARMACOLOGICAL CHARACTERIZATION OF Δ⁹-THC, Δ⁸-THC, AND HHC SIDE-CHAIN HOMOLOGS AT CB1R AND CB2R CANNABINOID RECEPTORS

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Introduction: Structural modifications of phytocannabinoids have been shown to alter their pharmacological properties at cannabinoid receptors. However, systematic comparisons of side-chain length, double-bond isomerism, and HHC stereochemistry across multiple signaling pathways remain limited. We examined how these features influence CB1R and CB2R activation and pathway preference.

Methods: A panel of Δ⁹-THC, Δ⁸-THC, (9*R*)-HHC, and (9*S*)-HHC analogs bearing C3-C8 alkyl side-chains was evaluated in HEK293 cells expressing CB1R or CB2R. Bioluminescence resonance energy transfer assays quantified activation of individual $G_{i/o}/G_z$ protein isoforms and recruitment of β-arrestin1 and β-arrestin2. Concentration-response curves were analyzed to determine potency, efficacy, receptor selectivity, and signaling bias.

Results: Extension of the alkyl side chain increased agonist potency in a stepwise manner, with maximal effects observed for C7-C8 derivatives. Longer homologs displayed strong efficacy in $G_{i/o}$ signaling at both receptors, particularly at CB1R, while producing comparatively weaker β-arrestin recruitment, consistent with partial agonism in arrestin pathways. In contrast, relocation of the double bond (Δ⁹ vs Δ⁸) or hydrogenation of the cyclohexene ring produced variable, pathway-dependent changes. Epimeric configuration at atom 9 in HHC created a clear pharmacological separation, with 9*R* analogs consistently showing higher potency and efficacy than the corresponding 9*S* analogs.

Conclusions: Small structural changes in THC- and HHC-based scaffolds translate into marked differences in receptor coupling and pathway output. Alkyl chain elongation primarily governs potency and G protein efficacy, while HHC stereochemistry modulates intrinsic activity.

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EFFECTS OF β-ARRESTIN AND GRK OVEREXPRESSION ON CANNABINOID RECEPTOR TYPE-1 (CB_1) $G_{\alpha i3}$ ACTIVATION AND DESENSITIZATION KINETICS

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Introduction: CB_1 signaling is regulated through phosphorylation by G protein receptor kinases, followed by recruitment of β-arrestins. G protein activation and regulation is inherently kinetic, which is why we sought to investigate how overexpression of β-arrestin1,2 and GRK2,3,5,6 influence the temporal dynamics of CB_1 mediated G protein signaling.

Methods: HEK293 cells were transfected with pcDNA3.1(+) plasmids that were empty (control) or encoded human CB_1, TRUPATH $G_{\alpha i3}$ $BRET^2$ biosensors, and either β-arrestin1, β-arrestin2, GRK2, GRK3, GRK5, or GRK6. Real-time BRET responses to the endocannabinoid 2-arachidonylglycerol or synthetic cannabinoid 5-fluoro MDMB-PICA were recorded. Data were fit to published kinetic models.

Results: Both β-arrestins increased the rate of desensitization compared to pcDNA3.1(+) for both ligands with β-arrestin2 showing a greater effect. β-Arrestin2 overexpression reduced the steady state of activated G protein and lowered the peak activation for both ligands. All GRKs decreased the peak compared pcDNA3.1(+) for both ligands with GRK2/3 showing a greater effect than GRK5/6. The baseline activation was increased by all GRKs compared to pcDNA3.1(+) and steady state was decreased by GRK2/3 for 2-AG.

Conclusions: Kinetic parameters were sensitive to β-arrestin and GRK overexpression, suggesting these models provide a useful platform for investigating GPCR regulatory mechanisms. Additionally, data showed a dominant role for β-arrestin2 and a greater desensitization by the cytosolic GRK2/3 family. Although the initial transduction rate was not immune to regulation, as previously suggested, it will be important to confirm in systems with natural stoichiometry. Ongoing studies are evaluating the effects of β-arrestin and/or GRK knockdown.

DISTINCT FUNCTIONAL PROFILES OF EMERGING SYNTHETIC CANNABINOIDS AND THEIR METABOLITES AT THE CANNABINOID RECEPTOR 1 (CB1R)

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Introduction: Synthetic cannabinoid receptor agonists (SCRAs), commonly marketed as ”Spice”, represent a rapidly expanding class of psychoactive substances associated with severe intoxications and toxic effects. According to the EU Early Warning System, the emergence of structurally diverse synthetic cannabinoids poses a public health risk. Unpredictable pharmacology complicates clinical and forensic identification. This study aimed to synthesize and characterize synthetic cannabinoids and rationally designed analogues and examine their pharmacological properties.

Methods: The most prevalent designer cannabinoids, indazole and indole carboxamides, were selected for structural modification. Analogues were synthesized by transesterification of the terminal methyl ester to introduce alkyl side chains, and carboxylic acid metabolites formed by ester hydrolysis. Compounds were characterized *in vitro* using CB1R binding assays with a [^{3}H]JWH-018 radioligand and functional [^{35}S]GTPγS assays. Off-target profiling was performed at selected non-cannabinoid receptors.

Results: The synthesized compounds showed very high affinity for the CB1R (K_i = 0.01–40 nM). FUBINACA derivatives exhibited 5–5000-fold, while PICA analogues showed 1.3–128-fold higher affinity than Δ^{9}-THC. Most ligands acted as potent full agonists in [^{35}S]GTPγS assays (E_{max} 151–173%; EC_{50} 0.6–21 nM), whereas analogues with longer alkyl side chains showed reduced affinity and efficacy. Carboxylic acid metabolites showed lower affinity and antagonist or inverse agonist profiles. Off-target screening showed no activation at opioid, 5-HT_2A, D_1/D_2 dopamine, NMDA or sigma-1 receptors, indicating high selectivity for CB1R.

Conclusions: These findings improve the pharmacological understanding of emerging synthetic cannabinoids and their metabolites, supporting the identification, risk assessment and forensic monitoring of emerging psychoactive substances.

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TUBEROUS SCLEROSIS COMPLEX–ASSOCIATED NEUROPSYCHIATRIC DISORDERS (TAND) OUTCOMES FOLLOWING ADJUNCTIVE CANNABIDIOL TREATMENT: 6-MONTH INTERMEDIATE ANALYSIS OF THE EPICOM TRIAL

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Introduction: EpiCom (NCT05864846) is a phase 3b/4 study evaluating behavioural and co-occurring outcomes following adjunctive cannabidiol (CBD) treatment in participants with tuberous sclerosis complex (TSC)-associated seizures.

Methods: Participants with TSC (aged 1–65 years) received open-label CBD (Epidyolex® [EU]/Epidiolex® [US], 100 mg/mL oral solution; ≤25 mg/kg/day) plus standard of care (SOC) for 26 weeks, followed by CBD with SOC or SOC alone for ≤26 additional weeks. Most problematic behaviour (MPB), TAND Self-Report Quantified Checklist (TAND-SQ), Aberrant Behaviour Checklist (ABC), and Caregiver/Clinician Global Impression of Severity scales were assessed at baseline, Weeks 13 and 26.

Results: Participants with ≥1 postbaseline assessment (n=62/79; median age: 16.0 years) were analysed. Baseline median MPB numerical rating scale (NRS) was 9.0/10, suggesting severe TAND problems. At Week 26 (n=23), median (95% CI) MPB NRS change from baseline was −2.0 (−3.0, −1.0). Greatest changes in TAND-SQ cluster severity scores were in eat/sleep (−2.0 [−3.5, −0.0]) and overactive/impulsive scores (−1.3 [−3.3, −0.0]). Greatest changes in ABC subscales were in irritability (−8.0 [−10.0,−2.0]) and hyperactive noncompliance (−7.0 [−13.0, −2.0]). At Weeks 13 and 26, fewer caregivers and clinicians reported severe/very severe behavioural problems compared with baseline. Treatment-emergent adverse events occurred in 62% participants, most commonly diarrhoea, infections, and psychiatric disorders.

Conclusions: Reductions were observed in TAND-SQ and ABC subscale scores and in severity of caregiver and clinician-reported behavioural problems 26 weeks after CBD initiation. The safety profile remains consistent with previous studies.
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ENDOCANNABINOID MODULATION OF CUE-EVOKED FEAR IN A MOUSE MODEL OF ELEVATED DOPAMINE TONE

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Introduction: Endocannabinoid (eCB) signaling regulates threat responding and interacts with dopamine (DA) systems implicated in stress related psychopathology. Dopamine transporter heterozygous (DAT-HET) mice exhibit two-fold elevated extracellular DA and altered eCB sensitivity. We tested whether monoacylglycerol lipase (MAGL) inhibition modulates cued fear expression in this moderate hyperdopaminergic model.

Methods: Adult wild type (DAT-WT) and DAT-HET mice underwent auditory cued fear conditioning. Freezing was quantified during tone presentation 24-hours after conditioning and across a 0–90s post-tone interval. Baseline cued fear was analyzed in a fully powered cohort using two-way ANOVA. In a pilot study, mice received vehicle or a selective MAGL inhibitor (MJN110;5mg/kg) to elevate 2-arachidonoylglycerol prior to testing. Group sizes ranged from n=3–4 per condition.

Results: In the baseline cohort, cued fear recall showed no genotype×sex interaction, $F(1,50)=0.0206, p=0.8864$, and no main effects of genotype, $F(1,50)=1.114, p=0.2964$, or sex, $F(1,50)=1.215, p=0.2757$, indicating intact cue driven learning in DAT-HET mice. In the pilot study, peak tone freezing was comparable across genotype and drug conditions. During the post-tone period, DAT-HET mice exhibited sustained freezing under MAGL inhibition relative to DAT-WT controls. As a proportion of tone freezing, DAT-HET mice maintained approximately 78% of tone responding under MAGL inhibition, compared to approximately 62% in DAT-HET vehicle controls and 53–68% in DAT-WT groups.

Conclusions: Elevation of 2-AG via MAGL inhibition may enhance sustained cue evoked freezing selectively in DAT-HET mice without altering peak tone responses. These findings support interaction between eCB signaling and elevated DA tone in regulating threat disengagement.

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CHRONIC SOCIAL STRESS ON FEAR MEMORY GENERALIZATION

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Introduction: Fear memories promote survival by guiding avoidance of threat, yet their utility depends on precision. Overgeneralization leads organisms to avoid harmless contexts, which is maladaptive. In mice, a fear memory is normally stored in a *sparse* ensemble of neurons ("engrams") in the lateral amygdala (LA). We previously showed acute stress (restraint stress or administration of corticosterone) induces fear memory generalization by increasing size of the fear memory trace (number of neurons in engram). This work indicated acute stress elevates endocannabinoid levels in the LA, suppressing GABA release from CB1R-expressing parvalbumin interneurons. Although these findings support a mechanistic link between acute stress and engram size, they do not necessarily predict what occurs under chronic stress, where endocannabinoid tone and CB1R signaling decline. The present study tests whether chronic stress promotes generalization through these adaptations.

Methods: Adult male/female mice undergo *chronic social defeat stress* (CSDS), involving repeated aggressive bouts with a CD1 male (10 min/d for 10 consecutive d). Mice will then be trained in auditory fear conditioning (CS^+ tone paired with mild shock; CS^- unpaired). Increased CS^- freezing 24h later will indicate fear memory generalization. Using activity-dependent labeling (TRAP2), we will quantify the size of the LA engram. Interneuron subclasses will be manipulated using cell-type specific Cre lines and adeno-associated-virus-based chemogenetics. CB1R contributions will be probed using CRISPR-based knockdown and pharmacological modulation.

Results: Preliminary data indicate enlarged engrams and generalized fear following CSDS.

Conclusions: This work aims to delineate how chronic stress reshapes inhibitory control to
degrade fear memory specificity.

POLYGENIC RISK PREDICTORS OF INDIVIDUAL DIFFERENCES IN EFFECTS OF Δ-9-THC IN CONTROLLED LABORATORY STUDIES

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Introduction: Cannabis is one of the most widely used drugs globally. Δ-9-tetrahydrocannabinol (THC), the principal psychoactive constituent of cannabis, acutely induces euphoria, anxiety, and psychotomimetic effects, and is metabolized by several hepatic enzymes, including CYP3A4. There are interindividual differences in the acute effects of cannabis/THC, which may have substantial genetic contributors.

Methods: We examined the influence of polygenic risk score (PRS) for cannabis lifetime use (CanLU), cannabis use disorder (CanUD), and CYP3A4 expression on THC-induced effects in human laboratory studies.

Results: CYP3A4 expression PRS was significantly associated with THC-induced psychotomimetic effects, explaining 5.0-7.7% of the variance in THC-induced effects (r^2=0.05-0.77; significant after FDR correction), suggesting that higher genetic liability to produce CYP3A4 may be associated with faster THC degradation and therefore, blunting of its effects. CanLU PRS nominally predicted enhanced THC-induced "high" (r^2=0.048, $p<0.05$), while CanUD PRS predicted lower "high" (r^2=0.062, $p<0.05$), suggesting that CanUD subjects need higher THC doses to experience euphoria ("high").

Conclusions: Genetic liability to cannabis use and misuse is potentially associated with lower THC-induced psychotomimetic symptoms which, being aversive, might reduce cannabis use and therefore use disorder liability. These findings warrant further study of the genetic factors affecting THC metabolism that may in turn influence the interindividual variability in THC effects.

ESTABLISHING SELF-ADMINISTRATION OF CANNABIS VAPOR IN MALE AND FEMALE ADOLESCENT AND ADULT RATS

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Introduction: With the recent rise of cannabis use, investigating cannabis-seeking behaviors becomes increasingly urgent, with approximately one-fifth of users developing a cannabis use disorder. In both humans and animals, sex-dependent effects of cannabis use have been observed, with females self-administering more rapidly. Despite this, females are underrepresented in animal research, resulting in lack of understanding in cannabis use within females.

Methods: Animals will be evaluated for drug-seeking behavior using a vapor self-administration paradigm, trained to nose-poke for either cannabis or vehicle under a fixed ratio (FR)-1 reinforcement schedule in daily 1-hour sessions for 11-consecutive days. They progressed to an FR-2 schedule (days 12-18) then to an FR-4 schedule (days 19-25). For each valid nose-poke, animals received a 3-second puff following a cue-light and 60-second timeout period. A control tablet records the number of valid pokes, total pokes, and total vapes per animal.

Results: In a small cohort of adult animals, females self-administered cannabis significantly more compared to their male counterparts. Within an adolescent cohort early into the paradigm, self-administration rates are lower than what was seen in adults; however, females continue to self-administer at faster rates. In adolescence, females receiving cannabis self-administer more compared to females receiving vehicle.

Conclusion: With this project in its infancy, these findings infer females self-administer vapor (regardless of cannabis or vehicle) at a faster rate compared to males in both adulthood and adolescence, with adolescent females self-administering cannabis at greater rates. Continuation of this project will deepen our understanding of cannabis-seeking behaviors in both sexes.

DEPRESSIVE SIDE EFFECTS OF RIMONABANT EXPLAINED, AND REVERTED BY MDMA MICRODOSES

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Introduction: Depression associated with anti-obesity drugs remain a major concern despite their effectiveness. The most striking case is Rimonabant, a CB1 antagonist that was highly effective for weight loss but withdrawn due to severe psychiatric side effects, which preclinical testing failed to predict. In this research we seek to understand whether individual traits, such as stress-coping abilities, may shape vulnerability or resilience to depressive side effects of anti-obesity drugs, and whether the use of psychedelics microdoses can be a rescue in this case, as at microdoses, MDMA may exert mood-stabilizing effects without inducing psychoactivity. **Methods:** Dominant (Dom, stress-resilient) and Submissive (Sub, stress-vulnerable) mice were used. Drugs: Mice were treated with Rimonabant (3 mg/kg. i.p.), MDMA (0.01, 0.03 or 0.1 mg/kg), co-treatment with Rimonabant and MDMA, or vehicle. Behavioral tests: Dominant-Submissive relationship (DSR), Three-Chamber Test (TChT), Open Field (OF) and Elevated Plus Maze (EPM). mRNA levels of CB1R, CB2R, FAAH, D1R, D2R, and D5R were assessed in the hippocampus (HIP) and prefrontal cortex (PFC). **Results:** DSR test reveals differential responses rimonabant based on personality types and on gender of mice. Female (F) mice exhibit more pronounced depressive-like behaviors following Rimonabant administration than male (M), and Dom animals are more sensitive to Rimonabant administration than Sub animals. In DSR, TChT and FST, clear depressive-like effect of Rimonabant is observed, while no differences in EPM and OF suggest no anxiogenic-like effect. In the experiments with MDMA, Dom mice were more sensitive to the antidepressant effect of MDMA and males (M) were more sensitive than females (F), DomM responding to lowest doses, and SubF to the highest, highlighting a critical role of sex in modulating drug response. Interestingly, the antidepressant effect of MDMA is best assessed by the DSR test, and not significant in the TChT and FST tests; MDMA also possesses the anxiolytic effect by the EPM test. When co-administered in Dom females, MDMA helps to rescue the Rimonabant-treated mice by TchT and FST. Although the antidepressant effect of MDMA alone is not significant in these tests, when co-administered with Rimonabant, it ablates its significant depressing effect. **Conclusions:** Dom and Sub mice differentially react on the treatment with both Rimonabant and MDMA, while Dom being much more susceptible to both Rimonabant's depressive effects, and MDMA antidepressant effects. As for the gender differences, females are more susceptible for Rimonabant depressive effects, but react less to MDMA rescue. The results can explain why in the preclinical assays on Rimonabant, which used standard wild-type mice, heterogeneity diluted the effects and led to mixed or negative results. In future, careful patient stratification based on vulnerability traitsshould be used. In practical terms, this line of work could help revive the therapeutic potential of CB1-targeting anti-obesity agents by reducing the psychiatric risk that previously limited their use. MDMA microdoses co-treatment can further help to reduce the risk of depression, and may be suggested in the especially vulnerable patients.

CANNABIGEROL (CBG) ENHANCES DIVIDED ATTENTION IN RATS

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Introduction: Cannabigerol (CBG) is a non-intoxicating phytocannabinoid; its pharmacological profile includes alpha-2 adrenoceptor agonist activity. This suggests untapped potential in treating symptoms of various psychiatric conditions. Clonidine and guanfacine are also alpha-2 agonists and are nonstimulant medications for attention deficit-hyperactivity disorder (ADHD). We sought to compare CBG to these more established compounds in a preclinical animal model of sustained and divided attention.

Methods: Female and male adult Long-Evans rats were trained on an operant response task. The sustained attention version reinforced accurate reporting of the presence or absence of an unpredictable light cue. In the divided attention task, subjects completed this component, as well as a secondary auditory distractor task simultaneously. CBG, clonidine, and guanfacine were administered systemically (i.p.) once or twice per week, prior to testing.

Results: CBG enhanced divided attention with a moderate effect size (η_G^2 = .061), but had no effect on the sustained attention task. Improvements in accuracy were seen at doses as low as 0.3 mg/kg. There were no signs of sedation, assessed via a rating scale. In contrast, clonidine impaired performance on both tasks at sedative doses. Guanfacine impaired divided attention, but was not sedating.

Conclusions: CBG significantly enhanced divided attention. Surprisingly, its effects were distinct from other alpha-2 agonists; therefore, the mechanism behind this beneficial effect is unclear. Nevertheless, the ability of CBG to enhance divided attention in wild type animals (i.e., not a model of ADHD or other impairment) indicates a pro-cognitive effect worthy of further exploration.

β-CARYOPHYLLENE ATTENUATES MK-801–INDUCED BEHAVIORAL DEFICITS IN A RAT MODEL OF SCHOZOPHRENIA

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Introduction Schizophrenia is a chronic psychiatric disorder affecting about 240 million people worldwide, characterized by positive symptoms (e.g., hallucinations), negative symptoms (e.g., social withdrawal), and cognitive deficits (e.g., memory impairment). Although current antipsychotic medications effectively reduce positive symptoms, their impact on negative and cognitive domains remains limited. Moreover, nearly 30% of patients are treatment-resistant, underscoring the need for novel therapeutic approaches. Growing evidence indicates that modulation of the endocannabinoid system (ECS) may offer a promising therapeutic approach for neuropsychiatric disorders, including schizophrenia, due to its involvement in neuroinflammation, synaptic plasticity, and emotional regulation. β-Caryophyllene (BCP), a natural terpene found, among others, in Cannabis sativa L., is a selective cannabinoid receptor type 2 (CB2R) agonist with anti-inflammatory, antioxidant, and anxiolytic properties. Preclinical and clinical studies indicating ECS dysfunction further support its potential role in the pathophysiology of schizophrenia.

Methods Male Wistar rats received intraperitoneal injections of BCP (25 mg/kg) and MK-801 (0.1 mg/kg), a well-established pharmacological model that reproduces behavioral alterations associated with schizophrenia. BCP was administered 60 minutes prior to behavioral testing, whereas MK-801 was injected 30 minutes before the assessments. Behavioral evaluation included the open field test (locomotor activity and anxiety-related behavior), the elevated plus maze (anxiety-like behavior), the social interaction test (social behavior), and the novel object recognition test (recognition memory).

Results BCP attenuated MK-801–induced hyperlocomotion. In the elevated plus maze, BCP increased the percentage of time spent in the open arms, indicating an anxiolytic-like effect. In the novel object recognition test, MK-801 induced marked memory deficits, which were significantly reversed by BCP, restoring performance to control levels. However, BCP did not counteract the MK-801–induced reduction in the number or duration of social interactions.

Conclusions BCP exhibits procognitive and anxiolytic-like effects and attenuates hyperactivity in an MK-801–based rat model of schizophrenia. These findings suggest that modulation of the endocannabinoid system may represent a promising therapeutic approach for selected symptom domains of schizophrenia.

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COMPARATIVE ANALYSIS OF WITHDRAWAL PROFILES IN NATURAL AND SYNTHETIC CANNABINOID USERS: INSIGHTS FROM A PROSPECTIVE CLINICAL STUDY AND SYSTEMATIC REVIEW

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Introduction: Cannabinoid withdrawal is an increasingly recognized clinical syndrome, yet comparative characterization of withdrawal from natural cannabis versus synthetic cannabinoids (SCs) remains limited. SCs act as full agonists at CB1 receptors with 2–100-fold greater potency than Δ^9-THC, suggesting more severe withdrawal. This study provides an integrated analysis combining prospective clinical assessment of time-dependent natural cannabis withdrawal with a systematic review of SC withdrawal case reports.

Methods: For natural cannabis, 45 male ICD-10-diagnosed cannabis-dependent participants underwent 28-day inpatient detoxification with prospective symptom monitoring using the Marijuana Withdrawal Checklist (MWC). For SCs, a PRISMA-compliant systematic review of PubMed, Scopus, EMBASE, and PsycINFO databases (inception–March 2025) identified eligible case reports, assessed using CARE guidelines.

Results: Natural cannabis withdrawal (n=45; 100% male, mean age 29.68±7.74) showed a biphasic trajectory: irritability, nausea, restlessness, and depressed mood peaked at Day 6, then declined, while cravings, appetite loss, sleeplessness, and abnormal dreams peaked at Day 14 before resolving. SC withdrawal (n=11 case reports; 82% male, mean age 28.08±7.78 years) presented with psychosis (82%), agitation (73%), seizures (45%), and tachycardia (36%), with onset within 24–48 hours in 62% of cases. SC withdrawal additionally featured delirium, rhabdomyolysis, and hallucinations not typically observed with natural cannabis.

Conclusions: Natural cannabis withdrawal follows a predictable, self-limiting course amenable to outpatient management, whereas SC withdrawal manifests with severe neuropsychiatric complications, including psychosis and seizures, often requiring acute inpatient intervention. These findings underscore the need for distinct clinical protocols and further research into the neurobiological mechanisms underlying differential CB1 agonist withdrawal severity.

SYT-510, A CLINICAL CANDIDATE FOR A FIRST-IN-CLASS SELECTIVE ENDOCANNABINOID REUPTAKE INHIBITOR, DEMONSTRATE EFFICACY IN MULTIPLE PRECLINICAL CNS DISEASE MODELS AND A FAVORABLE PHASE 1 SAFETY PROFILE

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SYT-510 is the first clinical candidate of a new class of ECS modulators, called Selective Endocannabinoid Reuptake Inhibitors (SERIs). Preclinically, SYT-510 binds to the newly identified endocannabinoid (eCB) membrane transporter ACSL4 isoform 2 and reversibly inhibits the cellular uptake of both major eCBs. In brain slices, SYT-510 effectively modulates CB1 receptor-mediated short-term synaptic plasticity in regions relevant to movement, learning and memory, anxiety. SYT-510 is effective in animal models of anxiety, compulsive behavior, drug-induced muscle spasticity and acute pain without other negative cannabimimetic effects. The pharmacological effect is evident after single administration as well as upon chronic treatment without any signs of tolerance. It correlates with moderate increases in eCB levels, which are sufficient to fully activate the ECS without eliciting sedation. Clinically in Phase 1 single and multiple ascending dose studies, SYT-510 was safe and well tolerated across all doses tested. SYT-510 showed a linear and dose-proportional PK, reaching and exceeding estimated pharmacologically active concentrations. Furthermore, SYT-510 CSF measurements demonstrated dose-proportional central exposure. SYT-510 is in clinical development for neuropsychiatric disorders and currently in Phase 2a for Generalized Anxiety Disorder.

AGE MEDIATES EFFECT OF CB1 RECEPTOR DELETION ON FINE MOTOR LEARNING, SPINE DYNAMICS, AND NEURON–MICROGLIA PROXIMITY

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Introduction: Age-related alterations in neuronal plasticity and microglial function are key contributors to cognitive decline and neurodegeneration. In mice, deletion of cannabinoid receptor 1 (CB1) accelerates aging-associated phenotype, including neuroinflammation and premature learning deficits, with early impairments in gross motor learning. However, the impact of CB1 deletion on fine motor skill learning and on neuronal and microglial plasticity within the primary motor cortex remains unclear.

Methods: We performed longitudinal *in vivo* two-photon imaging of dendritic spine dynamics in the primary motor cortex of *Cnr1*$^{-/-}$; Thy1-GFP-M and littermate wild-type male mice at 3, 6, and 12 months of age. In the same mice, we conducted histological analyses to evaluate microglial morphology, microglia–dendrite colocalization, and CD68 levels. Fine motor skill learning was assessed in separate cohorts using a skilled reaching task.

Results: *Cnr1*$^{-/-}$ mice exhibited age-dependent alterations in learning and neuronal and microglial parameters. At 3 months of age, they exhibited increased stability of newly formed dendritic spines, followed by elevated spine density at 6 months. Microglia displayed progressive changes: enlarged territory and increased ramification at 3 months; elevated microglia–dendrite colocalization at 6 months; and increased CD68 levels at 12 months. Behaviorally, *Cnr1*$^{-/-}$ mice demonstrated impaired fine motor learning, with fewer animals qualifying for training and a significantly reduced proportion of learners at 6 months.

Conclusions: CB1 deletion leads to age-dependent disruptions in neuronal and microglial function in the primary motor cortex, suggesting a critical role of CB1 in maintaining neuron–microglia interactions and supporting fine motor learning across the lifespan.

DISTINCT IN VIVO SHORT-TERM PLASTICITY OF HIPPOCAMPAL CCK- AND PV-EXPRESSING BASKET CELL SYNAPSES

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Introduction: There are two major basket cell (BC) types: cannabinoid-sensitive, cholecystokinin (CCK) and CB1 receptor-expressing; as well as cannabinoid-insensitive, parvalbumin (PV)-expressing cells. While previous in vitro work revealed several differences in their synaptic properties, how their inhibitory functions differ in vivo remains unknown.

Methods: We investigated inhibitory synaptic transmission in the hippocampus of awake, behaving mice using an all-optical method based on 2-photon voltage imaging in postsynaptic pyramidal neurons (PNs) combined with optogenetic stimulation of presynaptic CCK/CB1- or PV BCs to record cell-type-specific optogenetically evoked inhibitory postsynaptic potentials (oeIPSPs). Mice were treated with intraperitoneal injections of drug or vehicle solution before head-fixed recording on a treadmill.

Results: Both exogenous and endogenous CB1 agonists modulated CCK/CB1- (but not PV-) IN synapses onto PCs. Surprisingly, unlike in vitro, a CB1 inverse agonist did not unmask tonic endocannabinoid signaling at CCK/CB1 BC synapses in vivo. Quantifying single-cell, single-trial oeIPSP amplitudes suggested that at low stimulation frequency (<1 Hz), both BC types hyperpolarize the PN regardless of the PN membrane potential, but CCK/CB1 oeIPSPs are more variable compared to PV oeIPSPs. However, at increased stimulus frequencies, PV BC oeIPSPs were rapidly suppressed, while CCK/CB1 BC oeIPSPs were stable.

Conclusions: Altogether, these data suggest that PV BC synapses can generate large initial amplitudes at response onset; however, despite their high presynaptic firing rates, their synaptic output cannot be sustained. In contrast, CCK/CB1 BC synapses are relatively weaker and more variable, yet they can sustain long-lasting inhibition in vivo.

ENDOCANNABINOID AND FATTY ACIDE METABOLISM AS THERAPEUTIC TARGETS AFTER INTRAVENTRICULAR HAEMORRHAGE IN NEWBORNS

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Background: Intraventricular Haemorrhage (IVH) is a frequent complication in extremely low gestational age newborns, increasing the risk of Cerebral Palsy due to White Matter injury after enhanced neuroinflammation. The endocannabinoid system (ECS), in particular CB_2 receptor (CB2R), ligands as 2-AG, 2-DHG and DHEA; and its metabolic machinery (DAGLα, DAGLβ, MAGL, and COX-2), play an important role modulating neuroinflammation in rat adult brain. **Aim:** As there is no information regarding lipid signalling and ECS responses to acquired brain damage in very immature rat brain, therefore our aim is to assess ECS responses to IVH-induced brain damage in immature newborn rats. **Methods:** IVH was induced in newborn Wistar rats (P1) by injecting Chlostridium collagenase into the left Germinal Matrix by stereotactic surgery (GMH). Non-injected animals served as controls (SHAM). Western Blot was performed at 5 time points (3hours post-injury (3HPI), 12HPI, PND2, PND5 and PND7) on samples from ipsilateral striatum to determine CB2R, DAGLα, DAGLβ, MAGL, and COX-2 expression. Brain inflammation was assessed by TLR-4 expression. Brain concentration of endocannabinoids (eCBs) was quantified using liquid chromatography coupled to mass spectrometry. MAGL enzymatic activity was assessed by a MAGL activity assay kit. Arachidonic acid concentration was determined by an ELISA assay. **Results:** IVH led to a dynamic and interdependent endocannabinoid and inflammatory response in striatum, 2-AG concentration increased at 3HPI then decrease to levels lower than SHAM by PND2. In parallel, there was an increase of the protein expression and activity of MAGL and the protein expression of DAGLα and DAGLβ, resulting in a faster metabolism of this lipid. From 12HPI onwards, we found a maintained augmentation of arachidonic acid concentration increased progressively, which, together with the increase in COX-2 protein expression from PND2 n, suggest a shift in lipid metabolism towards a pro-inflammatory profile. There was an increase in DHEA concentration at 12HPI and PND2, together with an increase in f 2-DHG concentration at PND2, suggesting a compensatory activation of lipid pathways related with neuroprotection and anti-inflammatory activities. Interestingly, 2-DGH concentration decreased by PND5, at the same time when increased concentration of COX-2, AA and TLR-4 was found, pointing to enhanced inflammatory activity. Finally, at PND5 and PND7, CB2 receptor protein expression was increased, likely as a part of a process aimed to modulate inflammation. **Conclusions:** IVH-induced immature brain injury induced an early but transitory activation of the ECS, followed by a progressive dysregulation characterized by the high degradation rate of 2-AG, accumulation of AA and sustained activation of inflammatory pathways, with compensatory anti-inflammatory mechanisms mediated by 2-DHG and DHEA, which appear to be insufficient. Thus, lipid transmitter metabolism emerged as an interesting target for IVH-induced brain damage treatment.
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INTEGRATED BEHAVIORAL AND MOLECULAR ANALYSIS OF PSILOCYBIN EFFECTS IN C57BL/6 WT MICE

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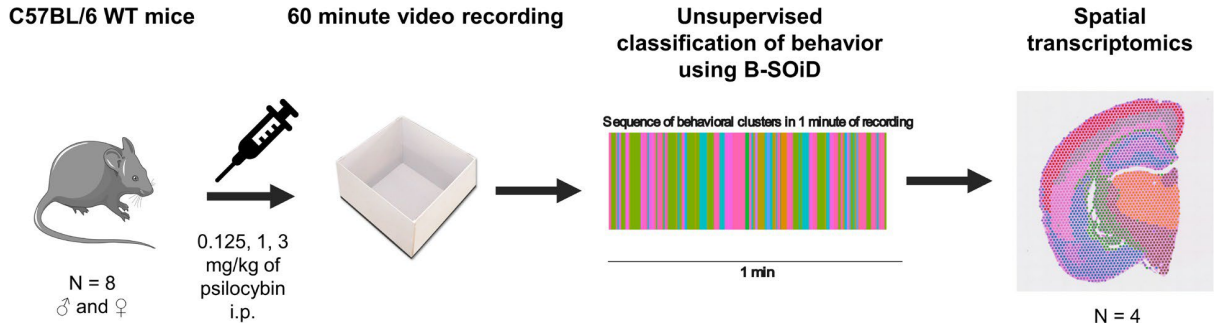
Introduction: Psilocybin, a serotonergic psychedelic, induces marked behavioral and molecular changes, yet how altered behavior relates to the underlying transcriptome remains poorly understood. Here, we integrate high-resolution behavioral phenotyping with spatial transcriptomics in the same animals to map psilocybin-induced behavioral states onto their molecular signatures across key brain regions.
Methods: C57BL/6 mice received 0.125, 1, or 3 mg/kg psilocybin (i.p.) and underwent 60-minute Open Field Tests (OFT), followed by brain collection for 10x Genomics Visium spatial transcriptomics. Behavioral videos were processed using DeepLabCut. Classical OFT metrics were quantified with DLCanalyzer, while distinct behavioral motifs were identified using the B-SOiD. Spatial transcriptomic data were analyzed in Seurat, and Canonical Correlation Analysis was used to link gene expression with behavior. **Results:** OFT revealed dose- and time-dependent effects: 1 mg/kg psilocybin induced early hyperlocomotion, whereas 3 mg/kg produced delayed hypolocomotion with increased peripheral occupancy, consistent with anxiogenic-like behavior. B-SOiD identified over 10 behavioral motifs, with sniffing and rearing selectively modulated in a dose-dependent manner. Spatial transcriptomics resolved molecular clusters corresponding to different neuroanatomical structures. We identified 1,397 cluster-specific psilocybin-induced differentially expressed genes (DEGs), with DEGs abundance positively correlated with regional expression of *Htr2a*. Canonical correlation analysis revealed transcriptomic signatures associated with behavioral patterns, including downregulation of the endocannabinoid transporter *Fabp7* in the medial habenula. **Conclusions:** These findings implicate medial habenula endocannabinoid signaling in psychedelic-induced anxiogenic effects and provide a framework for linking behavior with transcriptional changes induced by psychoactive compounds. **Acknowledgments:** Supported by SciLifeLab, the Wenner-Gren Foundation, the Swedish Research Council (I.P.D.), and the SciLifeLab RED Postdoctoral Fellowship (J.M.).



INTEGRATING GRAB SENSORS FOR THE STUDY OF ENDOCANNABINOID AND PURINERGIC SYSTEMS IN ASTROCYTES OF FAAH-DEFICIENT-ALZHEIMER'S DISEASE MICE

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Introduction: The endocannabinoid system (ECS) modulates neuroprotection and glial inflammatory responses. In Alzheimer's disease (AD) models, inhibiting fatty acid amide hydrolase (FAAH) to elevate anandamide (AEA) levels reduces beta-amyloid deposition and enhances synaptic plasticity. However, the specific roles of astrocytes and microglia in these beneficial effects require further characterization.

Methods: G protein-coupled receptor activation-based (GRAB) sensors were used to track AEA, 2-AG, and ATP dynamics in real-time. Following AAV-mediated, cell-specific expression, the sensors were validated in GL261 cells and primary astrocytes from WT and FAAH-/- mice. Fluorescence recordings captured ligand fluctuations in response to pharmacological stimuli.

Results: The results confirm the efficient expression and robust performance of GRAB sensors in GL261-based glial models. In primary astrocytes, the absence of FAAH resulted in distinct response profiles in the presence of pharmacological stimuli. These findings validate the experimental platform for discerning genotypic differences in molecular signaling and provide a foundational step for future in vivo experiments. Such studies will involve viral injections into live mice to track the real-time molecular dynamics of the purinergic system and endocannabinoids (ECBs) involving glial cells in the context of AD.

Conclusions: GRAB sensors enable the first visualization of glial molecular orchestration under FAAH deficiency. This in vitro validation establishes a foundation for future two-photon in vivo studies to explore how ECB modulation redirects neuroinflammation toward neuroprotective phenotypes in AD.

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ENDOCANNABINOID SIGNALING DYNAMICS IN MOTOR CORTEX DURING DEXTEROUS BEHAVIOR

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Introduction: The primary motor cortex (MoP) is crucial for commanding dexterous movements via flexible neural dynamics. MoP dynamics are dictated by ongoing brain states (both internal and external) that are maintained by neuromodulators. While global neuromodulation is well-studied, the role of locally produced endocannabinoids (eCBs) in shaping MoP dynamics remains unknown. Here, we investigated how local eCB signaling influences cortical processing during dexterous tasks.

Methods: We expressed GRABeCB sensors in MoP and recorded eCB dynamics in vivo using fiber photometry while mice performed head-fixed reaches to grab food pellet rewards. To dissect eCB ligand contributions, we pharmacologically manipulated 2-AG and anandamide signaling via i.p. injections, recording dynamics and behavior pre- and post-injections.

Results: We found that MoP eCBs exhibited heterogeneous, preparatory dynamics beginning before the reach initiation, with the peak amplitudes of the average response independent of behavioral outcome. JZL184, a MAGL inhibitor, enhanced, and DO34, a DAGL inhibitor, attenuated eCB dynamics, while anandamide agents had no effect, identifying 2-AG as the primary ligand in MoP.

Conclusions: 2-AG is dynamically modulated in MoP on a fast timescale during reaching, establishing locally produced neuromodulators as active participants in cortical motor processing. This signaling appears to broadly regulate preparatory network states rather than encoding outcome-specific information. Further analysis of kinematics is being explored to elucidate how 2-AG shapes MoP dynamics during dexterous behavior.

SEX-DEPENDENT EFFECTS OF CB_1 SIGNALING ON HIPPOCAMPAL NEUROGENESIS AND STRESS-RELATED BEHAVIOUR

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Introduction: Cannabinoid Receptor $_1$ (CB_1) is implicated in the regulation of multiple neuronal functions, including adult hippocampal neurogenesis and stress resilience. Here, we investigated sex-related differences in the role of CB_1 signaling in modulating both these processes.

Methods: Three-month-old male and female CB_1 knock-out$_1$($CB^{-/-}$) mice were used in the study and compared with wild-type (WT) non-carrier littermates. Open Field (OF), Novel Object Recognition Test (NORT), and Forced Swim Test (FST) were employed as *in vivo* measures of basic cognitive function and stress- or depression-related behaviour. Brain cryosections were analyzed to assess neurogenesis markers in the dentate gyrus of the dorsal hippocampus using anti-Ki67 and anti-DCX immunohistochemistry.

Results: Both male and female $CB^{-/-}$ mice showed unchanged OF and NORT, suggesting preservation of basic cognitive functions. FST revealed increased immobility time in WT female mice compared to WT males, indicating sex-related differences in stress-related or depressive-like behaviour. Moreover, immobility time was further increased in $CB_1^{-/-}$ mice of both sexes compared to their respective WT counterparts. Histological analysis revealed a significant reduction of Ki67-positive cells in $CB^{-/-}$ female mice compared to WT females, whereas DCX-positive cells were decreased in $CB^{-/-}$ male mice compared to WT littermates.

Conclusions: CB_1 modulates stress-related behaviour with distinct roles in the regulation of hippocampal neurogenesis in females and males, thereby highlighting sex-dependent mechanisms in CB_1-mediated neuroplasticity and stress resilience.

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THE SELECTIVE FATTY ACID BINDING PROTEIN 5 INHIBITOR ART26.12 RELIEVES 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 non-steroidal anti-inflammatory drugs (NSAIDs) that exhibit poor long-term efficacy and increase the incidence of gastrointestinal ulceration. Fatty acid binding protein 5 (FABP5) is a cytosolic chaperone that regulates endocannabinoid and fatty acid transport and metabolism. FABP5 additionally regulates pronociceptive cytokine and chemokine expression and the output of inflammatory pathways. ART26.12 is a novel, selective orally active FABP5 inhibitor. The goal of this study was to evaluate the antinociceptive efficacy of ART26.12 in a rat model of OA and profile its downstream targets via lipidomic and proteomic analyses. **Methods:** The experiments were approved by the Stony Brook University Institutional Animal Care and Use Committee (#277150). Female Sprague Dawley rats underwent destabilization of the medial meniscus surgery. Following eight weeks of OA development, the rats received vehicle, naproxen (8 mg/kg), or ART26.12 (10, 25, or 50 mg/kg) via the oral route acutely and BID for four weeks. Static weight bearing/incapacitance was evaluated at defined intervals. At the end of the treatment window, plasma and synovial membranes were collected for lipidomic analyses, synovial membranes for proteomics, and stomachs for evaluation of gastrointestinal pathology. **Results:** ART26.12 dose-dependently increased weight bearing on the ipsilateral (arthritic) hind limb following acute dosing and chronic dosing, which was comparable in magnitude to naproxen. Elevations in plasma 2-arachidonoylglycerol and reductions in anandamide and epoxyeicosatrienoic acid levels were observed in ART26.12-treated rats. Synovial membrane lipidomics identified broad reductions in fatty acids and elevations in ceramides along with bidirectional alterations in phosphoglycerides and sphingolipids after ART26.12 dosing. Proteomic analysis of synovial membranes revealed differential expression of numerous proteins and pathways following the induction of OA, a subset of which were normalized by ART26.12. Naproxen increased the incidence of non-glandular hyperkeratosis in the stomachs of treated rats, which was not observed in the ART26.12 groups. **Conclusions:** Acute and chronic oral dosing with ART26.12 reduces OA pain, which is accompanied by alterations in endocannabinoid levels and broad categories of lipids and proteins. ART26.12 represents a promising non-opioid analgesic for the management of OA pain with a potentially superior safety profile compared to NSAIDs. **Acknowledgements**: This work was funded by Artelo Biosciences

DUALSTERIC CB1 POSITIVE ALLOSTERIC MODULATOR/CB2 AGONIST GAT1102 DISPLAYS BROAD SPECTRUM ANALGESIC EFFICACY WITHOUT PRODUCING CANNABIMIMETIC EFFECTS

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Introduction: CB1 positive allosteric modulators (PAMs) bind to the allosteric site of the CB1 and enhance affinity or efficacy of orthosteric CB1 activation. CB1 PAMS do not produce unwanted on target effects (psychoactive high, tolerance, physical dependence) associated with the CB1 receptor and display analgesic efficacy. CB2 agonists also display analgesic efficacy preclinically with minimal side effects. Here, we assess GAT1102, a novel dualsteric CB1 PAM/CB2 agonist across multiple preclinical pain models and assess potential for producing reward or aversion.

Methods: Anti-allodynic efficacy of GAT1102 was evaluated in models of CFA-induced inflammatory pain, chemotherapy-induced peripheral neuropathy (CIPN), spared nerve injury (SNIt), and hind paw incisional injury. Analgesic efficacy and pharmacological specificity of GAT1102 using AM251 or AM630 in each pain model were evaluated. Conditioned place preference (CPP) was used to compare effects of chamber pairings with GAT1102, morphine or vehicle. Cannabinoid triad was used to assess hypothermia, tail-flick antinocieption and catalepsy.

Results: GAT1102 suppressed mechanical hypersensitivity via a CB1-mediated mechanism in the CFA Model. In incisional injury, CIPN, and SNIt models, both CB1 and CB2 antagonists attenuated efficacy of GAT1102. GAT1102 did not produce tolerance in SNIt or CIPN following chronic dosing. GAT1102 did not produce cannabimimetic effects in the triad test. GAT1102 enhanced the cannabimimetic profile of JZL284, an inhibitor of monoacylglycerol lipase. Lastly, unlike morphine, GAT1102 did not produce reward in CPP.

Conclusions: GAT1102 is a broad-spectrum analgesic with minimal side effects that exhibits dualsteric pharmacological specificity in a pain model dependent manner.

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IMPACT OF CANNABIS USE FREQUENCY ON THE ANALGESIC EFFECTS OF SMOKED CANNABIS

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Introduction: Pain is one of the primary reasons for medical cannabis use. Tolerance to delta-9-tetrahydrocannabinol (THC)-related effects is observed under a range of outcomes, but tolerance to the analgesic effects of THC has yet to be studied in an experimental setting. This study examined differences in the analgesic effects of smoked cannabis in people that use cannabis frequently (≥5 times/week) and occasionally (<5 times/week).
Methods: Healthy people who used cannabis were recruited for this study. Participants smoked 560 mg of 0% (0 mg THC), 4%, (~20 mg THC) and 10% (~60 mg THC) THC cannabis across three outpatient sessions. Objective pain (pain threshold, pain tolerance) and subjective pain (pain intensity and bothersomeness) were measured using Cold Pressor Test, an experimental test with predictive validity for chronic pain therapeutics.
Results: Participants (N=103) were grouped accordingly: "frequent" (N=55, 6.4 ±1.2 days/wk cannabis use) or "occasional" use (N=48, 2.6 ±1.4 days/wk cannabis use). Active cannabis increased pain threshold and reduced subjective pain ratings compared to placebo in the occasional use group only (*p*s<.005). Group differences were observed in active conditions, with greater pain threshold and lower subjective pain ratings observed in the occasional use compared to the frequent use group (*p*s<.05).
Conclusions: Following smoked cannabis, the analgesic effects of THC were observed in the occasional use group only. Group differences suggest that frequent cannabis use may lead to tolerance to the analgesic effects of THC. These findings point to tolerance as an important factor that may influence the analgesic efficacy of cannabis/THC.

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EFFECTS OF ART27.13 IN A PRECLINICAL MODEL OF PACLITAXEL-INDUCED PERIPHERAL NEUROPATHY

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Introduction: Paclitaxel and other chemotherapeutic agents often cause chemotherapy-induced peripheral neuropathy (CIPN), a debilitating and treatment-limiting condition. ART27.13 is a highly potent, peripherally selective CB_1 and CB_2 agonist that was primarily developed as an analgesic. However, development in this indication was terminated and ART27.13 was never evaluated in models of CIPN. ART27.13 is currently in Phase 2 clinical development in cancer patients suffering from anorexia and cachexia (Cancer Appetite Recovery Study [CAReS; ISRCTN: 15607817, ART27.13-100, IRAS 278450]). This study assessed whether ART27.13 has an analgesic effect in a preclinical model of paclitaxel-induced peripheral neuropathy (PIPN). **Methods:** This study used 31 male and 35 female Sprague Dawley rats. Healthy baseline measurements for von Frey paw withdrawal threshold (PWT; mechanical allodynia) and latency to withdraw from a cold plate (thermal hyperalgesia) were measured on Days -5, -4, and -3. On Day -2, prophylactic administration of ART27.13 (3 µmol/kg PO QD) began and continued daily until Day 20. On Days 0, 2, 4, and 6, animals were given paclitaxel (4 x 2 mg/kg IP). Daily dosing of duloxetine (positive control; 30mg/kg PO QD) began on Day 0, ending on Day 20. Active control animals received paclitaxel and vehicle injections QD. On Days 11, 15, 16, 19, and 20, PWT thresholds and withdrawal latencies were reassessed 2h post-dose. **Results:** In both mechanical and thermal assays, vehicle-treated animals showed significant mechanical allodynia and thermal hyperalgesia on all neuropathic test days (i.e., significant differences between neuropathic tests and their healthy baseline on Days 11–20). In the mechanical allodynia assay, males treated with ART27.13 showed no significant difference between neuropathic and healthy baseline von Frey measurements on Days 11, 15, and 16. This indicates that animals treated with ART27.13 did not show mechanical symptoms of neuropathy on these days. Similarly, females treated with ART27.13 showed no significant mechanical allodynia on Days 11, 16, 19, and 20 when compared to their own healthy baseline. Duloxetine prevented the development of mechanical allodynia in both sexes on all test days. In the cold plate test, males treated with ART27.13 showed no significant difference between neuropathic and healthy baseline on Days 11, 15, and 16. This indicates that animals treated with ART27.13 did not show thermal symptoms of neuropathy on these days. Females treated with ART27.13 – as well as all animals treated with duloxetine – did not develop thermal hyperalgesia throughout the trial. **Conclusion:** ART27.13 shows efficacy in a preclinical model of PIPN by attenuating neuropathic symptoms in males and females. Whilst ART27.13 is currently being trialled in cancer anorexia and cachexia syndrome, these results suggest it may have broader utility in cancer supportive care. The CAReS study is evaluating pain, quality of life and opioid usage as secondary and exploratory endpoints.

REPEATED MONOACYLGLYCEROL LIPASE INHIBITION REDUCES ALLODYNIA IN A MOUSE MODEL OF OPIOID-INDUCED HYPERALGESIA

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Introduction:
Opioid-induced hyperalgesia (OIH) is results when repeated opioid use increases pain sensitivity, often manifesting as allodynia. OIH treatments are limited, hindered by perceived patient drug-seeking, and promote continued opioid use. Inhibiting monoacylglycerol lipase (MAGL), the primary catabolic enzyme degrading 2-arachidonoylglycerol, decreases inflammation and central sensitization. Similarly, CB_1 positive allosteric modulation reduce allodynia in multiple models. We hypothesized that MAGL inhibition or CB_1 positive allosteric modulation reduces OIH in mice.

Methods:
Male and female C57BL/6J mice (n = 8–10 per group) were, aseptically implanted with osmotic minipumps delivering morphine (64 mg/mL; 1 µL/hour) and mechanical and acetone-induced cold allodynia were assessed. Once OIH was established, the acute effects of the MAGL inhibitor JZL184 (40 mg/kg) or the CB_1 PAM ZCZ011 (40 mg/kg) were tested. Next, the effects of repeated JZL184 (0.6, 2.5, 10, 40 mg/kg) were assessed. Finally, the potential cannabinoid receptor mechanism was assessed using the selective CB_2 receptor antagonist SR144528 (3 mg/kg) co-administered daily with JZL184 (40 mg/kg).

Results:
Allodynia presented consistently by day 5 of morphine administration. Morphine treatment also induced splenomegaly ($p=0.012$). Acute ZCZ011 reduced mechanical ($p=0.047$) and cold allodynia ($p<0.001$). Repeated, but not acute, JZL184 dose-dependently reduced both mechanical allodynia ($F(12, 123) = 7$, $p<0.001$) and cold allodynia ($F(12,126) = 3$, $p<0.001$) as compared to controls. SR144528 did not block the antiallodynic effect of JZL184 ($p=0.619$), suggesting that JZL184 anti-allodynia occurs via a CB_2-independent mechanism.

Conclusions:
MAGL inhibition has therapeutic potential to attenuate OIH, most likely through a non-cannabinoid receptor mechanism.

MONOACYLGYCEROL LIPASE AS A POTENTIAL BIOMARKER AND THERAPEUTIC TARGET FOR SICKLE CELL DISEASE PAIN

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Introduction: Chronic pain is a major comorbidity of sickle cell disease (SCD), with inflammation serving as a defining component. The standard of care therapy for chronic SCD pain is opioids, which elicit severe side effects and misuse liability. Here, we explore inhibition of monoacylglycerol lipase (MAGL), the primary degradative enzyme of 2-arachidonoylglycerol (2-AG), as a novel therapeutic strategy in SCD that targets both pain and inflammation.

Methods: HbSS (SCD) and HbAA (control) humanized Berkeley mice were administered the selective MAGL inhibitor MJN110 (1.25-10 mg/kg) and tested in von Frey and grip strength assays. To evaluate MAGL activity, spleen (an immune organ particularly vulnerable in SCD) and cerebellum (for comparison) proteomes were taken from HbSS and HbAA mice, incubated with serine hydrolase probe FP-TAMRA, and band intensity was quantified after SDS-PAGE.

Results: MJN110 dose-dependently reduced mechanical hypersensitivity and restored motor function in the HbSS mice, while not affecting either measure in HbAA mice. Grossly enlarged and sclerotic spleens derived from HbSS mice showed twice the MAGL activity as control mice. No genotype differences were found in MAGL from cerebellum. Experiments are underway to determine whether repeated (7 day) MJN110 administrations undergoes antinociceptive tolerance.

Conclusions: Collectively, these data suggest that MAGL represents a potential biomarker of SCD in disease relevant tissues as well as a plausible therapeutic target for the treatment of SCD pain with superior behavioral outcomes compared to opioid monotherapy in HbSS mice[1].

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ASSESSING THE ROLE OF NATURAL AND NATURE-INSPIRED COMPOUNDS FROM *CANNABIS SATIVA* L. AGAINST NEUROPATHIC PAIN

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Introduction: Neuropathic pain, deriving from nerve damage or injury, affects 7-10% of the global population and significantly affects quality of life. Cannabis-based therapies have emerged as promising treatment options, even if the psychotropic effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) limit their long-term clinical usage.
Methods: This study was focused on the investigation of the anti-hyperalgesic potential of non-psychotropic *Cannabis sativa* L. oil extracts in animal models of peripheral and central neuropathic pain. A comprehensive chemical characterization of the extracts was performed using targeted metabolomics, based on UHPLC-HRMS and GC-MS, with key constituents quantified by HPLC-UV and GC-FID. *In silico* modelling was performed on the main compounds and new dualsteric ligands were designed and synthesized.
Results: Oral administration of the extract K2 (25 mg kg^{-1}) significantly attenuated mechanical and thermal allodynia in spared nerve injury (SNI) model, having rapid and sustained effects without impairing locomotor activity. Intranasal administration of hemp essential oil (EO), rich in cannabidiol (CBD) and β-caryophyllene (CAR) and devoid of Δ^9-THC, elicited comparable anti-hyperalgesic effects, which were reversed by a selective CB_2 receptor antagonist. The EO demonstrated also efficacy in the experimental autoimmune encephalomyelitis (EAE) model, reducing hyperalgesia and eliciting anxiolytic- and antidepressant-like behaviours. Complementary *in vitro* studies showed that CBD and CAR protected microglial cells from LPS-induced neuroinflammation. Molecular docking indicated complementary binding of CBD and CAR to CB_2 receptors. Novel hybrid molecules, combining CBD and simplified analogues with a selective CB_2 agonist (COR167), were obtained and their pharmacological activity in neuropathic pain models is under evaluation.
Conclusions: These results highlight the therapeutic potential of non-psychotropic cannabis-derived compounds as new promising agents for neuropathic pain.

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SEX-SPECIFIC REGULATION OF OPIOIDINDUCED ADAPTATION BY MICROGLIAL CB2

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Introduction: Microglia are the primary immune cells of the central nervous system and play a pivotal role in modulating neuroinflammation and synaptic plasticity. CB_2 is highly expressed on these cells and serves as a key regulator of microglial activity; however, its specific role in opioid-induced neuroadaptations remains poorly defined. Because microglia exhibit significant sexual dimorphism in their density and inflammatory responses, we investigate the potential role of microglial CB_2 signaling in regulating opioid responses in a sex-dependent manner.

Methods: Male and female CX3CR1$^{Cre/+}$ CB_2 $^{fl/fl}$ mice (microglial CB_2 deficient) and their CX3CR1$^{Cre/-}$ CB_2 $^{fl/fl}$ control littermates, aged 16-18 weeks old, were used to ensure microglial-specific CB_2 receptor depletion. Antinociceptive tolerance was assessed using a 55ºC thermal tail-flick assay following a 4-day opioid dependence protocol involving repeated-administration of morphine (0–100 mg/kg) or oxycodone (0–30mg/kg). Physical dependence was quantified as naloxone-precipitated (10 mg/kg) somatic withdrawal jumps. Finally, voluntary oxycodone reward and consumption were measured using a two-bottle choice paradigm following a forced-access escalation phase. Data were analyzed via two-way and repeated-measures ANOVA and Spearman's rank correlation to assess estrous cycle influence

Results: Morphine potency was identical across genotypes on Day 1. However, by Day 4 of repeated administration, microglial CB_2 deletion exacerbated the magnitude of morphine tolerance and increased naloxone-precipitated withdrawal jumps in males. Females showed no genotype-dependent differences in morphine tolerance or withdrawal. While oxycodone tolerance developed independently of microglial CB_2 in both sexes, the receptor was found to bidirectionally regulate drug-seeking behavior. Specifically, CB_2-deficient females exhibited increased oxycodone consumption and a higher preference for the drug over water compared to controls, indicating that microglial CB_2 may constrain oxycodone reward in females. Estrous cycle stages did not correlate with oxycodone consumption levels. Conversely, CB_2-deficient males showed reduced oxycodone intake.
Conclusions: These findings identify microglial CB_2 as a critical, sex-specific regulator of opioid adaptations. The loss of this signaling pathway selectively promotes morphine tolerance and physical dependence and lessens oxycodone intake in males while exacerbating oxycodone-seeking and reward in females. These observations highlight the necessity of considering both biological sex and the specific opioid compound when developing endocannabinoid-targeted therapies for pain management and opioid use disorder.

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CANNABIS EXPOSURE AND POSTSURGICAL PAIN: IMPLICATIONS FOR OPIOID USE AND ANALGESIC DEMAND

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Introduction: Cannabis exposure is increasingly prevalent among surgical patients, yet its relationship with postsurgical pain and opioid requirements remains incompletely understood. Cannabinoid modulation of nociceptive, inflammatory, and neuroimmune pathways may influence perioperative analgesic demand. However, clinical findings remain inconsistent across surgical populations.

Methods: A narrative review was conducted using structured searches of PubMed, CINAHL, and Embase to identify observational human studies evaluating associations between preoperative cannabis exposure and postoperative pain or opioid consumption. Studies published within the past decade were included. Due to heterogeneity in exposure definitions, surgical context, and outcome measures, findings were synthesized using a structured narrative approach rather than meta-analysis.

Results: Forty-two observational studies were included across orthopedic, spine, general surgical, and mixed populations. Fourteen studies (33%) reported higher postoperative pain scores among cannabis-exposed patients, while 10 (24%) reported no difference and 2 (5%) reported lower pain; pain outcomes were not reported in 16 studies. Increased postoperative opioid requirements were reported in 18 studies (43%), most consistently in spine and mixed surgical cohorts, whereas arthroplasty populations more frequently demonstrated no difference. Variability reflected nonstandardized exposure ascertainment, differences in surgical invasiveness, and baseline pain burden. Most studies used validated pain scales and standardized opioid metrics; observational designs limited causal inference.

Conclusions: Cannabis exposure is variably associated with postsurgical pain and opioid demand, with stronger signals observed in spine and mixed surgical populations. These findings highlight the need for individualized perioperative analgesic planning and standardized exposure definitions. Prospective, ECS-informed studies are needed to clarify mechanisms and optimize pain management strategies.

CHANGES IN MEDICATION USE IN OLDER ADULTS USING LEGAL MARKET CANNABIS FOR SLEEP, PAIN, AND MOOD

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Introduction: The current study examined changes in self-reported medication counts for older adults (60+) who used or did not use legal market cannabis for sleep, pain, and mood problems.

Methods: Participants completed a baseline assessment, 4-week assessment, and 3 monthly follow-ups. From baseline to the 4-week assessment, participants actively used their cannabis product. During the monthly follow-ups, participants could stop or change their product use.

Results: Generalized estimating equations using Poisson distributions examined whether use versus non-use, time, or their interaction was associated with changes in medication count from baseline to 4-weeks. Results indicated a timeXuse status interaction (β =-0.03, p=0.06). For non-users, medication count increased from baseline to 4-weeks (β=0.03; p=0.039) while for users it did not change (β=0.01; p=0.632). When expanding timepoints to the full study period, there was also a main effect of time wherein medication count increased from baseline to 4 months (β=0.02; p=0.07) with no main effect of cannabis use nor a timeXcannabis use interaction (ps>0.14).

Conclusions: During active cannabis use, marginal differences between cannabis users and non-users were observed, suggesting edible cannabis might help mitigate increases in polypharmacy in older adults. However, during long-term follow-up, where participants potentially discontinued use, there were no longer differences between users and non-users.

PILOT SAFETY OF INTRA-ARTICULAR CANNABIDIOL ALONE AND WITH TRIAMCINOLONE IN RODENTS FOR OSTEOARTHRITIS FEASIBILITY

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Introduction: This pilot study with few animals was performed with the intent of determining the acute safety of direct intra-articular injection of CBD in animals prior to future studies examining the potential of CBD as a possible local treatment for osteoarthritis.

Methods: Adult, female Long-Evans rats, n= 2-3 per group, received a single 25ul injection in the right femorotibial joint with one of: low dose CBD (12.5mg/ml), high dose CBD (25 mg/ml), CBD (12.5mg/mL) + triamcinolone (1.87 mg/ml) or vehicle alone with the left limb serving as an internal control. Rats were monitored for 14 days for changes in ambulation, joint width, ability to vertically extend, and other routine clinical observations. Animals were sacrificed on day 15 and both femorotibial joints examined for cartilage degeneration and synovial reaction.

Results: No animals demonstrated signs of pain, joint swelling, weight loss or clinical signs that could be associated with the injections. As a percentage of the entire articular cartilage, in each animal, there was less than a 5% difference in significant cartilage degeneration between the injected and contralateral joint in animals receiving CBD only injections. Synovial reaction scores were considered mild or absent in all treatment groups.

Conclusions: This small pilot study demonstrated that the CBD products (either alone or in combination with TA) do not elicit a significant amount of acute inflammation of injected joints and did not negatively affect rats clinically post-injection. The demonstration of low risk to animals should allow for expansion of in-vivo discovery studies with these products.

EFFECTS OF CANNABINOIDS ON THE ANTINOCICEPTIVE AND ABUSE-RELATED BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF FENTANYL IN MICE

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Introduction: Prescription opioids remain the most effective treatment for pain management despite their adverse effects. To overcome this problem, studies have suggested that non-opioids like cannabinoid receptor 1 (CB1) agonists could be utilized to enhance opioid analgesia without augmenting their unwanted effects. Here, we appraised the utility of a novel partial CB1 agonist AM11101 to enhance fentanyl's antinociceptive but not its abuse-related behavioural and neurochemical effects.

Methods: Using thermal (warm water tail-withdrawal) and chemical (formalin) antinociception assays, first we documented the ability of CB1 partial (Δ9-THC, AM11101) and full (AM8936) agonists to enhance fentanyl's antinociceptive effects in male mice. Next, conditioned place preference (CPP) and in vivo microdialysis was used to, respectively, determine how AM11101 alters fentanyl-induced increases in rewarding effects and dopamine (DA) efflux in the nucleus accumbens (nAcc) shell of male mice.

Results: Results show that fentanyl and CB1 agonists produced dose-dependent increases in antinociception and, when combined, all CBs produced dose-related leftward shifts in fentanyl's antinociceptive effects. This CB-mediated enhancement of fentanyl antinociception is selective, as doses of AM11101 that have limited reward-related effects did not alter or attenuated fentanyl-induced increases in CPP and DA in the nAcc shell.

Conclusion: The present results are consistent with prior work showing that CBs can enhance the antinociceptive effects of opioids without exacerbating their abuse-related properties. Moreover, we provide further evidence that AM11101 has a unique pharmacological profile with partial CB1 agonist-like actions that ought to be explored when developing opioid-CB-based treatments for pain management.

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EFFECTS OF ESTROUS CYCLE AND CHRONIC THC ON PAIN IN HIV-1 TG26 MICE

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Introduction: Chronic pain in HIV-1 is increasingly linked to neuroendocrine disruption; however, the interaction between biological sex, endocrine status, and cannabis use remains poorly understood. We i) investigated whether estrous phase differentially modulates pain based on HIV-1 genotype, and ii) incorporated an independent chronic THC study to explore its effects on endocannabinoid receptors and enzymes.

Methods: Baseline hot plate and Hargreaves data were collected from HIV-1 Tg26 mice [Tg26+/−,*n*=16(8f)] and controls [Tg26−/−,*n*=20(9f)]. Estrous phase was determined via daily vaginal cytology for 30 days. A separate cohort (Tg26+/−,*n*=24(12f); Tg26−/−,*n*=24(12f)) received vehicle or THC (3mg/kg) subcutaneously for 90 days. After a 7-day drug-free period, rotarod and hot plate data were collected, followed by CB_1R, CB_2R, MAGL, FAAH, and endocannabinoid lipid quantification.

Results: At baseline, Tg26(+/−) mice showed increased hot plate antinociception, and females showed higher thermal pain sensitivity in Hargreaves; a significant genotype by estrous-phase interaction suggested that estrous-dependent modulation of thermal pain sensitivity differs with genotype. Separately, Tg26(+/−) mice, especially females, showed slower cerebellum-dependent motor learning, and chronic THC attenuated their elevated hot plate antinociception and slowed rotarod motor decline. Plasma and cortical THC metabolite data suggest genotype-dependent differences in THC metabolism. Genotype-and drug-dependent variation in 2-AG, MAGL, and CB_1R expression in motor learning and nociception-related CNS regions indicate potential alteration of endocannabinoid tone.

Conclusions: Estrous phase may modulate pain behaviors in Tg26 mice. Separately, chronic THC may attenuate genotype-dependent behavioral differences due to altered endocannabinoid signaling. Future work will examine whether genotype-specific changes in endocannabinoid tone contribute to estrous-dependent changes in pain behaviors.

CHRONIC CANNABIDIOL TREATMENT RESTORES HIPPOCAMPAL NEUROTRANSMITTER BALANCE AND IMPROVES GAIT DYNAMICS IN A MOUSE MODEL OF NEUROPATHIC PAIN

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Introduction: As demonstrated in our previous study (ICRS 2025, poster 45), chronic neuropathic pain is associated with impaired hippocampus-dependent cognition and disrupted synaptic plasticity. Using the spared nerve injury (SNI) model, we previously showed that chronic cannabidiol (CBD) administration alleviated mechanical and cold allodynia, restored long-term potentiation in the entorhinal–dentate gyrus (DG) pathway, and improved memory performance. In vitro experiments further revealed that CBD enhanced neuronal differentiation of hippocampal neural stem cells, supporting its neurogenic potential. In the present study, we extended this line of research by examining hippocampal neurotransmitter dynamics and gait analysis as an objective measure of functional impairment in chronic pain using *in vivo* microdialysis and artificial intelligence–based motion analysis. **Methods:** Neuropathic pain was induced in male C57BL/6J mice using SNI model. 14 days after surgery, CBD (5 mg/kg) was administered orally for 14 consecutive days. 24 hours after the last gavage, microdialysis was performed in the DG, and glutamate (Glu), glycine (Gly), and gamma-aminobutyric acid (GABA) levels were quantified using high-performance liquid chromatography (HPLC). Gait analysis was conducted before and after 14 days of CBD administration. Locomotor patterns were recorded and analyzed using DeepLabCut (DLC) to assess changes in joint flexion dynamics. **Results:** CBD treatment increased Glu levels in the DG in both sham and SNI mice. Gly concentrations were significantly elevated in SNI animals, whereas CBD administration reduced Gly levels to control level. GABA concentration was markedly decreased following SNI, and CBD treatment reversed this deficit. Kinematic analysis showed that SNI animals exhibited impaired gait dynamics, whereas SNI mice treated with CBD displayed a trend toward a locomotor profile resembling that of sham-operated animals. **Conclusions:** Chronic CBD administration modulated hippocampal excitatory and inhibitory neurotransmitter balance while simultaneously improving locomotor deficits associated with neuropathic pain. These findings suggest that CBD exerts multimodal effects on both central neurochemical homeostasis and motor function. The engagement of glutamatergic, GABAergic, and glycinergic signaling may represent a shared mechanistic link between pain processing and cognitive function, highlighting CBD's potential as a therapeutic agent targeting both sensory and affective-cognitive symptoms of neuropathy.

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PHARMACOLOGICAL STUDY OF ALLOSTERIC MECHANISMS AT THE TYPE 1 CANNABINOID RECEPTOR AND THEIR APPLICATION IN A MOUSE MODEL OF PAIN

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Introduction: Positive allosteric modulators (PAM) of the type 1 cannabinoid receptor (CB1R) can reduce pain without the psychoactivity effects of phytocannabinoids such as Δ9-tetrahydrocannabinol. GAT211 has shown dual agonist-PAM properties *in vitro*, alongside antinociceptive and anti-epileptic effects *in vivo*. While GAT211 showed promise, it suffers poor pharmacokinetics (PK). This project investigates the GAT2500 series of compounds, structural derivatives of GAT211 designed to resolve and optimize CB1R PAM pharmacodynamics (PD) and PK. The goal of the present research was to determine the PD of these novel CB1R ago-PAMs to modulate cAMP inhibition and their physiological effects in a mouse model of pain.

Methods: Chinese hamster ovary (CHO-K1) cells expressing CB1R were treated with increasing concentrations of CP55,940 (positive control), GAT2500 compounds (agonism), or combinations therein (allosterism). C57BL/6 mice were treated with a subset of compounds ± CP55,940 then assessed for nociception, as well as movement and body temperature.

Results: Two compounds - GAT2513 and GAT2515 - were efficacious ago-PAMs that shifted the potency of concentration-response curves to sub-nanomolar levels when tested *in vitro*. When tested in mice, these compounds displayed anti-nociceptive properties relative to the vehicle control.

Conclusions: The GAT2500 series of compounds show strong preclinical potential based on their high potency and efficacy as CB1R AMs with minimal psychoactivity. Planned future experiments will assess the PK of these ligands in both acute and chronic treatment models.

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CANNABINOID CB_1 RECEPTOR INVOLVEMENT IN THE BENEFICIAL EFFECTS OF ENRICHED ENVIRONMENT IN A RAT MODEL OF NEUROPATHIC PAIN

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Introduction: Preclinical studies suggest that enriched environments (EE) alleviate chronic pain-related behaviour and associated affective disturbances, although mechanisms remain unclear. The endocannabinoid system regulates nociception and is engaged by exercise. We evaluated the involvement of CB_1 receptors in the beneficial effects of EE on nociceptive and anxiodepressive behaviour in a rat model of neuropathic pain, and examined EE-induced alterations in endocannabinoid levels. **Methods:** Adult male Wistar Han rats were housed in either EE or standard housing. Animals received daily intraperitoneal injections of AM251 (CB_1 receptor antagonist/inverse agonist) or vehicle from start of EE. After one week of EE housing, rats underwent spared nerve injury (SNI) or sham surgery, and mechanical hypersensitivity was assessed (von Frey) on days 3, 7, 10, 14 and 16 post-SNI. Two weeks post-SNI, anxiety- and depression-like behaviours were evaluated. CNS endocannabinoid levels on day 19 post-SNI were quantified using liquid chromatography-mass spectrometry. **Results:** EE increased nociceptive thresholds and reduced anxiety- and depression-like behaviours in elevated plus maze (EPM), sucrose splash (SST) and forced swim tests. AM251 attenuated these effects, increasing mechanical hypersensitivity and anxiety-like behaviour in EPM of EE animals compared to vehicle-treated counterparts. AM251 also decreased preference for sweet pellets and grooming time in SNI EE animals in sweet drive test and SST, respectively. EE increased 2-AG levels in vehicle-treated (but not AM251-treated) SNI rats in prefrontal cortex, amygdala and lumbar spinal cord. **Conclusions:** These findings suggest that CB_1 receptors may contribute to the beneficial effects of EE on pain and related affective comorbidities.

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2-AG AND 2-LG BASELINE AND POST-SHOCK COMPARISON BETWEEN CHRONIC PAIN AND HEALTHY CONTROL SAMPLES

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Introduction: This study examined plasma levels of 2-arachidonoylglycerol (2-AG) and 2-linoleoylglycerol (2-LG), endocannabinoids implicated in nociceptive processing, at baseline and following a controlled acute 4mA (subjectively rated painful) shock.

Methods: Twenty-two HC and twenty-three CP participants underwent blood draws at baseline and post-4mA electrical shock, approximately 10-15 minutes apart. Plasma was analyzed for 2-AG and 2-LG concentrations via mass spectrometric analysis. Signed fold-changes at baseline and post-shock were calculated for each participant to indicate direction and magnitude of response. Group means and standard errors were computed per timepoint and analyte. Two-sample t-tests compared means between groups, and an approximate group-by-time interaction was tested via t-statistic with conservative degrees of freedom. Analyses included and excluded outliers.

Results: No significant group*time interaction emerged for 2-AG or 2-LG (p=0.99), indicating comparable stress responsivity across groups. Including outliers, the CP group exhibited lower 2-AG concentrations compared to HC at baseline (p=0.158) and post-shock (p=0.08). The pattern held at baseline when outliers were removed (p=0.150) but a smaller difference was observed at post-shock (p=0.17). In the case of 2-LG, differences in baseline values were largely similar to differences post-shock values (p=0.12 and p=0.10 respectively), but this pattern changed when excluding outliers, where baseline difference was larger (p=0.05) and post-shock difference smaller (p=0.19).

Conclusions: These findings suggest persistent differences in endocannabinoid tone in chronic pain. Future work will examine additional endocannabinoids and moderators including cannabis use, psychological measures, and pain ratings to clarify these patterns.

LIPIDOMIC PROFILING REVEALS DISTINCT ENDOCANNABINOID AND OXYLIPIN SIGNATURES IN HIP AND RIB FRACTURE TRAUMA

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Introduction
Traumatic injury triggers local and systemic inflammatory and metabolic responses, yet the lipid mediator pathways underlying these changes remain incompletely defined. Bioactive lipid mediators, including oxylipins and endocannabinoids (ECs), regulate inflammation, pain, and resolution. We performed lipidomic profiling to characterise systemic alterations following hip and rib fractures and to determine whether frail and non-frail trauma exhibit distinct endocannabinoid signatures.

Methods
Plasma samples were collected within 48 hours of admission (or pre-operatively at anaesthesia) from hip fracture patients (n=17, 38% female; mean age 59.6), rib fracture patients (n=32, 80% female; mean age 88.7), and healthy controls (n=78, 100% female; mean age 67.6). Thirty-six bioactive lipids were quantified using LC–MS/MS. Associations between lipid concentrations and trauma status (trauma vs control) were assessed using Spearman's correlation to account for non-normal distributions.

Results
Trauma cases showed significantly lower levels of several hydroxyeicosatetraenoic acids (HETEs), common to both fracture types. Soluble epoxide hydrolase (sEH) pathway precursors, including 11,12-EET and 14,15-EET, were also significantly reduced in trauma versus controls ($p<0.001$). In contrast, ECs were elevated. In rib fractures, 2-arachidonoylglycerol (2-AG) was the only EC significantly increased compared with controls. In hip fractures, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) were abundant, and anandamide (AEA) was significantly higher than in rib fractures ($p<0.001$).

Conclusions
Trauma is characterised by suppression of HETE and sEH-related oxylipins alongside activation of the endocannabinoid system. Distinct EC patterns between hip and rib fractures suggest differential engagement of cannabinoid and TRPV1 pathways, highlighting trauma-specific lipid signatures with potential prognostic and therapeutic relevance.

5-HT_{1A} ACTIVITY IS INVOLVED IN ANTINOCICEPTION DUE TO CB_2, BUT NOT CB_1, ACTIVATION IN THE FORMALIN MURINE MODEL OF INFLAMMATORY PAIN

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Introduction: Inflammatory pain is a key component of rheumatic disease, which significantly reduces patient quality of life. Recent research has revealed a potential functional link between the CB_2 receptor and the 5-HT_{1A} receptor, which has been shown to have anti-nociceptive potential. In this project, we evaluated the involvement of 5-HT_{1A} receptor in ACEA (CB_1-selective) and AM1241 (CB_2-selective) antinociception in the formalin inflammatory pain model.

Methods: This project was performed using adult C57BL/6J female and male mice. In the formalin model, mice received intraperitoneal injections with either vehicle, ACEA, ACEA preceded by WAY-100635 (5-HT_{1A} selective antagonist), AM1241, or AM1241 preceded by WAY-100635. Following this injection, mice received a subcutaneous injection with formalin (10 µL) into their left hind paw and their behavior was scored over the following hour. A separate cohort of mice received similar intraperitoneal injections before completion of tetrad behavioral testing (rotarod, open field, rectal temperature).

Results: Both ACEA and AM1241 provided significant antinociceptive benefit, relative to vehicle. In both male and female mice, pretreatment with WAY-100635 did not significantly alter ACEA antinociception, but did significantly decrease antinociception due to AM1241. No significant sex differences were noted nor were significant differences found in rotarod, open field, or rectal temperature assessments.

Conclusions: This study demonstrates the antinociceptive benefit of CB_1 and CB_2 agonism in a murine model of inflammatory pain. Further, it demonstrates that 5-HT_{1A} receptor activity is involved in CB_2-dependent antinociception. Subsequent studies investigating the mechanism of this finding and to further investigate its potential clinical importance are needed.

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EFFECTS OF CHRONIC CANNABIS USE ON STRESS-INDUCED NEURAL AND IMMUNE RESPONSES IN RATS

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Introduction: Stress, a modulator of the immune response is a major reason for chronic cannabis use. Studies have shown that chronic cannabis use dampens stress-induced neuroendocrine response in humans and rodents. However, the central mechanisms subserving chronic cannabis-induced alterations in the stress response, and its downstream impacts on stress-induced immune responses are unknown. Thus, we investigated the effects of chronic cannabis vapor self-administration on 1) stress-induced activation of neurons expressing corticotropin-releasing factor type-1 receptors (CRFR1), and 2) recruitment of pro- and anti-inflammatory cytokines in male and female CRFR1-Cre:tdTom rats that express Cre-recombinase and a fluorescent reporter (tdTomato) in neurons that express CRFR1.

Methods: Rats were trained to self-administer vaporized cannabis extract (150 mg/ml cannabis in PEG-400) or vehicle (PEG-400 only) in daily one-hour sessions for 30 days. On day 31, all rats were acutely restrained for 30 minutes. Ninety minutes after stress termination, rats were euthanized for the collection of brain and cardiac blood. Brains were cryo-sectioned and stained for c-fos immunoreactivity to assess activation of CRFR1 neurons in the mPFC, BNST, PVN, CeA, BLA, MeA, PVT, and CPu. Neurons expressing c-Fos, and CRFR1-expressing neurons colocalized with c-Fos were counted and analyzed using 2-way ANOVA. Plasma levels of the cytokines IL-1β, IL-6, IL-10, and TNF-$\alpha\alpha$ were measured using ELISA and analyzed using 2-way ANOVA.

Results: Chronic cannabis vapor self-administration significantly decreased c-Fos reactivity in CRFR1-expressing neurons in the mPFC of acutely stressed rats compared to the vehicle group ($p < 0.05$). However, no significant effects were observed in other brain regions. Plasma cytokine analysis revealed a significant reduction in IL-10 levels in acutely stressed male (but not female) rats exposed to cannabis vapor compared to vehicle ($p < 0.05$), which suggests attenuated stress-induced recruitment of anti-inflammatory pathways in male rats. IL-1β, IL-6, and TNF-$\alpha\alpha$ levels remained unchanged across all groups.

Conclusion: These findings suggest that chronic cannabis vapor self-administration selectively dampens stress-induced activation of CRFR1-expressing neurons in the mPFC and impairs anti-inflammatory immune responses in males, highlighting a potential mechanism by which chronic cannabis use may dysregulate neuroimmune interactions under stress and raising important considerations for sex-specific health outcomes associated with long-term cannabis use.

CHRONIC DELTA-9-TETRAHYDROCANNABINOL EXPOSURE DURING ADOLESCENCE PRODUCES SELECTIVE BEHAVIORAL ABNORMALITIES IN ADULT NONHUMAN PRIMATES

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Introduction: Chronic cannabis use during adolescence can impair complex behavioral processes. However, the extent to which such deficits persist into adulthood is not well understood. To address this knowledge gap, we have examined the impact of chronic exposure to delta-9-tetrahydrocannabinol (THC) during adolescence on cognitive function during adulthood via a battery of touchscreen-based tasks.

Methods: Female and male squirrel monkeys (n=23) were treated daily for 6 months during adolescence with either vehicle, a low dose (0.32 mg/kg), or a high dose (3.2 mg/kg) of THC. Approximately 6 months after THC administration was discontinued, touchscreen-based tasks examining learning (repeated acquisition), cognitive flexibility (discrimination reversal), and short-term spatial memory (delayed matching-to-position) were used to determine whether these subjects, now adult, would exhibit behavioral deficits and, if so, whether they would be selective among cognitive domains.

Results: All subjects learned to engage in all touchscreen tasks; however, both acquisition and steady-state performance varied among treatment groups. In drug-free control subjects, performance across all three tasks was comparable to that in previous drug-naïve cohorts. Dosage-related but transient deficits in learning were observed in the repeated acquisition task in both THC-exposed groups. However, larger and persistent deficits in cognitive flexibility were observed in the discrimination reversal task. Surprisingly, short-term spatial memory was not impacted by THC treatment during adolescence; performance in the delayed matching-to-position task closely approximated that of drug-free control subjects.

Conclusions: Selective behavioral deficits among cognitive domains that were produced by chronic THC treatment during adolescence endured into adulthood, as assayed by touchscreen-based tasks. These data are of particular concern considering the ubiquitous role that these basic behavioral processes play in everyday life.

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OVARIAN STEROID MECHANISMS OF CANNABIS USE IN PSYCHIATRIC OUTPATIENTS

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Introduction: As cannabis use evolves with changing laws and societal norms, identifying risk factors for harmful use is critical. Female (vs. male) sex is associated with stronger withdrawal and faster progression to use disorder, suggesting sex-specific vulnerability. Cyclical changes in progesterone and estradiol can influence neurobiological systems regulating affect, motivation, and substance use. Although limited evidence suggests cannabis use rises premenstrually, findings are inconsistent, other phases are poorly measured or underexamined, and high-risk samples are understudied. We address these gaps by characterizing menstrual cycle–related cannabis use trajectories in a population at risk for problem use.

Methods: Using data from 220 naturally-cycling psychiatric outpatients during baseline periods of three randomized trials, we applied a novel phase-aligned cycle time scaling approach to standardize hormonal trajectories within and between participants, enabling precise multilevel nonlinear modeling. Daily cannabis use probability was predicted from cycle time via logistic generalized additive mixed models (GAMMs).

Results: The menstrual cycle significantly predicted cannabis use probability at the group level, with a peak at menses, another elevation before ovulation, and a midluteal nadir (edf=4.43, $p<0.001$). No significant individual differences in cyclical change were observed (edf=5.13, $p=0.30$). Effects remained significant after controlling for alcohol use, and physical pain, and were not moderated by weekend status.

Conclusions: Among female psychiatric outpatients, daily probability of cannabis use varies alongside ovarian hormone fluctuations, identifying windows of heightened vulnerability to problem use. These findings inform temporally specific, personalized interventions and provide framework for future work to probe precise neurohormonal mechanisms underlying substance use.

SEX AND GENDER ANALYSIS OF COGNITIVE PERFORMANCE AND CANNABINOID CONCENTRATIONS THE MORNING AFTER SMOKING CANNABIS

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Introduction: Cannabis use and related harms differ by sex/gender, which is understood to be the result of both biological and sociocultural factors. We examined cannabis effects in the human laboratory through an integrated sex/gender lens.

Methods: Adults who used cannabis 4+ times/week came to the laboratory 12-15 hours after smoking cannabis at home the evening prior; their data was compared to a matched control group (both groups: n=65; 32 female at birth, 33 male at birth). Cognitive performance was measured using a verbal free recall (VFR) task and the Trail Making Test (TMT). THC and its 11-OH and COOH metabolites were quantified in blood; THC was also quantified in oral fluid. Sex assigned at birth was self-reported by participants, while gender was measured using the Traditional Masculinities-Femininities (TMF) scale.

Results: There was a significant group X sex X TMF interaction for VFR immediate recall (p=0.014); the association between TMF and VFR differed between males and females in the cannabis user group (male=1.026, female=-0.481, Δ =-1.507 [95% CI 0.570, 2.443], p=0.002), but not in healthy controls. Results were similar for other VFR variables, but there was less evidence of an interaction for TMT performance. There were significant sex X TMF interactions for blood THC (p=0.014) and THC-COOH (p=0.034), as well as oral fluid THC (p=0.036).

Conclusions: We provide the first empirical support that measurement of both sex and gender in the human laboratory can yield essential insights into cannabis effects that are missed when taking a binary sex differences approach.

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THE CANNABIS BUNGEE JUMP STUDY: DOES BUNGEE JUMP STRESS RELEASE FAT-STORED Δ^9-TETRAHYDROCANNABINOL

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Introduction: Δ^9 tetrahydrocannabinol (THC) accumulates in adipose tissue with repeated cannabis use. Pre-clinical studies suggest that acute physiological or psychological stress may stimulate lipolysis and release fat stored THC into circulation, potentially increasing blood THC concentrations in the absence of recent cannabis use. This is of forensic relevance to drug driving legislation, as different countries have implemented various legal limits for THC, while others have zero tolerance. The present study investigated whether acute stress— a bungee jump — produces measurable increases in circulating THC or its metabolites in regular cannabis users. **Methods:** This observational clinical study recruited regular cannabis users (≥ weekly use for ≥3 months). Following screening, 16 participants attended a study session and abstained from cannabis for ≥12 hours beforehand. After informed consent and baseline measures, participants completed a 49-meter indoor bungee jump as an acute physical and psychological stressor. An indwelling venous cannula enabled serial blood sampling at: baseline, immediate post-jump, 15, 30, 60, 90, and 120 min. Plasma concentrations of THC, 11-OH-THC, THC-COOH, and cortisol were analysed using validated GC-MS methods. Subjective 'high' was assessed at each time point. The primary outcome was change in THC concentration relative to baseline. **Results:** Sixteen participants were enrolled (63% male; mean age 22 ± 2.2 years); 15 completed the bungee jump. THC concentrations decreased rather than increased following the jump, with normalised average values of 94% (immediate), 89% (15 min), 83% (30 min), 88% (60 min), 83% (90 min), and 86% (120 min) of baseline. THC-COOH concentrations showed modest increases post-jump, consistent with ongoing metabolism rather than stress-induced mobilisation. No subgroup—sex, cannabis frequency, or engagement in high-intensity sports—displayed a rise in THC post-stress. Notably, the participant who declined the jump displayed a marked increase from baseline. Five participants exceeded the UK legal limit (2ng/ml) for THC at baseline despite abstaining ≥12 hours. Subjective 'high' increased after the jump, although this was consistent with the experience of the jump rather than THC-like effects. **Discussion:** Acute stress induced by a bungee jump did not increase circulating THC concentrations in regular cannabis users, providing no evidence for stress-driven release of fat-stored THC under these conditions. These findings suggest that acute stress alone is unlikely to generate false-positive results in forensic drug-driving assessments. The small sample size and predominance of young adults limit generalisability. Further research should compare different stress modalities and include formal cortisol analyses to better characterise stress–THC interactions.

TRAJECTORIES OF CANNABIS USE DURING PREGNANCY HIGHLIGHT DYNAMIC CHANGES IN BOTH MOTHERS AND PARTNERS: A WINDOW FOR INTERVENTION

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Introduction: Although cannabis use during pregnancy in Canada has increased since legalization in 2018, most studies rely on static exposure categories and fail to capture dynamic changes. Prospective data describing cannabis use trajectories during this period remain scarce. This study characterizes longitudinal patterns of cannabis use during pregnancy and early postpartum within the Cannabis and Neurodevelopment of Babies (CAN-B) cohort.

Methods: We conducted a prospective longitudinal study of 232 pregnant individuals recruited between 2022-2025 in Quebec, Canada, including 153 who reported cannabis use since conception. Monthly electronic questionnaires assessed cannabis use frequency, number of products used, and mode of consumption from preconception through pregnancy and up to 6 months postpartum. Latent class growth models identified use. Multinomial logistic regression examined associations between preconception preference for illicit cannabis and trajectory membership.

Results: Three pregnancy trajectories were identified: abstinent /occasional use (65%), reduced use (23%), and continued daily use (12%). Following pregnancy recognition, cannabis use declined, with most participants reducing both frequency and number of products combined; but continued to primarily smoke high-THC products. In parallel, 76% of partners reduced cannabis use and 18% stopped entirely. Preconception preference for illicit cannabis was associated with higher odds of continued daily use (OR 3.98, 95% CI 1.29–12.30). At 6 months postpartum, a subset of participants showed re-escalation toward daily use.

Conclusions: Cannabis use during pregnancy follows distinct, dynamic trajectories. While pregnancy offers a key opportunity for change, persistence of high-THC smoking and postpartum re-escalation highlight the need for sustained, partner-inclusive interventions beyond pregnancy.

CANNABINOID COMPOSITION DETERMINES THE ANTI-INFLAMMATORY EFFICACY OF HEMP EXTRACTS IN EXPERIMENTAL COLITIS

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Introduction: Patients with inflammatory bowel disease (IBD) frequently report using Cannabis sativa products for symptom management. Despite widespread use, the efficacy of cannabis in IBD remains unclear, and the relative contributions of discrete phytocannabinoids vers whole-plant preparations are poorly defined. Furthermore, the biological activity of minor cannabinoids such as cannabigerol (CBG) and cannabichromene (CBC), alone or in combination with cannabidiol (CBD), remains largely unexplored.

Methods: Using the dextran sodium sulfate (DSS) model of colitis, we previously demonstrated that a hemp extract equal amounts of CBD and CBG attenuates colitis severity in mice following both intraperitoneal and oral administration. Disease severity was assessed by body weight loss, disease activity index, and histopathology. Ongoing studies examine the mechanisms underlying extract efficacy, including effects on immune response, cannabinoid receptor involvement, and comparisons between extracts and purified cannabinoids.

Results: A CBD/CBG hemp extract significantly reduced inflammatory cytokine production both in. vitro and in. vivo and decreased epithelial infiltration by neutrophils and macrophages. In contrast, a CBD/CBC extract failed to ameliorate colitis. When administered as purified compounds, both CBC and CBG independently reduced disease severity, whereas CBD alone exacerbated colitis. Pharmacokinetic analysis thus far has revealed plasma CBG concentrations are approximately five-fold lower than CBD following extract administration, despite CBG's dominant protective effects.

Conclusions: These findings demonstrate that CBG-rich hemp extracts attenuate experimental colitis, even in the presence of CBD. Collectively, these data underscore the importance of cannabinoid composition of hemp-derived products and support the therapeutic potential of CBG-enriched formulations for intestinal inflammation.

BEST PRACTICES FOR GENERATING AND DELIVERING CANNABINOID AEROSOLS FOR IN VIVO SAFETY AND EFFICACY STUDIES

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Introduction: Inhaled cannabinoid aerosols are increasingly evaluated for preclinical safety and efficacy. Yet such studies often lack standardized methods for aerosol generation, delivery, and monitoring, leading to inconsistent or irreproducible results. Here, we outline best practices for producing and delivering high quality cannabinoid containing aerosols for rodent investigations.

Methods: Aerosol generation and delivery require careful selection of the device, delivery system, and monitoring of key parameters such as analyte concentration and particle size. As a test case, an inhalation pharmacokinetics study in Sprague–Dawley rats was conducted using liquid or powder cannabidiol (CBD) formulations to demonstrate the aerosol handling process for different types of formulation. Aerosols were generated by nebulization (liquid) or with the PreciseInhale® powder generator. Target CBD doses were 1–3 mg/kg (powder) or 1–5 mg/kg (liquid). Aerosol concentrations were quantified by HPLC from collected aerosol samples, and particle size distribution (PSD) was measured using a cascade impactor.

Results: For the pharmacokinetics analysis, both nebulized and powder aerosols were stable and robust. The concentration of CBD in the aerosol was within ±10% of the target. Powder aerosols had acceptable PSD, with Mass Median Aerodynamic diameter (MMAD) values of 1.7–2.1, while nebulized liquid aerosols ranged from 2.0–2.1, while all geometric standard deviation (GSD) values were below 3.0. Pharmacokinetic profiles showed dose-dependent CBD concentrations for both aerosol types.

Conclusions: Standardized approaches for generating, delivering, monitoring, and characterizing aerosols in in vivo systems improve the reliability and reproducibility of cannabinoid aerosol research across laboratories and support OECD-compliant testing.

STUDYING CONSUMMATORY BEHAVIOURS AND NEURONAL ACTIVITY FOLLOWING CANNABIS VAPOUR INHALATION IN MICE

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Introduction. Cannabis promotes food intake ("the munchies") which has therapeutic potential; however, the mechanism/s underlying this remain unknown. We thereby validated a mouse model of "the munchies" to investigate the neuronal mechanisms underlying cannabis-induced consummatory behaviours.

Methods. For model validation, mice were exposed to vaporised vehicle (polyethylene glycol) or cannabis extract (10% THC) for 5-, 10- or 15-minutes (8-second vapour puff every 1-minute 22-seconds), then given chow access or temperature and blood THC measured. Experimental mice were exposed to vapour for 10-minutes then subjected to consummatory behavioural assessment using the Open-Source Head-fixed Rodent Behavioral Experimental Training System (OHRBETS) with free access to 30% sucrose, or the brains removed and processed for c-Fos immunohistochemistry to assess neuronal activity.

Results. *Model validation:* 10-minutes of cannabis vapour exposure increased chow intake ($p<0.0001$), induced hypothermia ($p<0.0001$), and produced blood THC concentrations of
38.6±2.3 ng/ml. The 5- or 15-minute vapour protocols did not enhance feeding ($p>0.05$). *Consummatory behaviour characterisation:* Cannabis vapour increased the number of licks to consume sucrose, specifically in the first 5-minutes of access ($p=0.007$). *Mechanistic investigation:* c-Fos expression in the hypothalamic arcuate nucleus (ARC) was higher with cannabis exposure ($p=0.06$), where 18.4% of c-Fos expression colocalized with appetite-stimulating ARC neuropeptide-Y neurons.

Conclusion. We validate a translational vapour model to study "the munchies" in mice to allow for the utilization of head-fixed behavioural apparatus, transgenic mouse lines and selective genetic circuit manipulation tools to delineate the mechanism/s underlying cannabis-driven appetitive behaviours, with focus on appetite and reward circuits such as ARC neuropeptide-Y neurons.

ACUTE CANNABIS VAPOUR EXPOSURE SELECTIVELY ENHANCES SOCIAL INVESTIGATION TOWARD UNFAMILIAR CONSPECIFICS IN MICE

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Cannabis is one of the most widely used psychoactive substances globally, and inhaled tetrahydrocannabinol (THC) consumption through vaping is increasingly common. This growing trend raises important concerns, as vaping enables rapid systemic absorption, fast brain penetration, and pronounced acute psychoactive effects. Despite widespread use, the acute impact of THC on social behaviour remains poorly defined, particularly across distinct social constructs such as social investigation, social preference, and social recognition. Defining these acute effects is important because social behaviour is a key determinant of mental health and is disrupted in multiple neuropsychiatric conditions, and because cannabinoid-based interventions have been proposed for disorders characterized by altered social approach, anxiety, and affective dysregulation.

Mice were exposed to THC vapour or vehicle and assessed using complementary behavioural paradigms, including free social interaction, cup-based social preference, and social recognition assays. Both familiar and unfamiliar conspecifics were used to distinguish novelty-driven investigation from established affiliative interactions. Acute THC exposure significantly increased anogenital sniffing of unfamiliar conspecifics during free interaction and significantly increased investigation of a novel conspecific in the cup-based social recognition assay. These results indicate that acute inhaled THC selectively enhances investigatory behaviour toward unfamiliar social stimuli, consistent with increased social salience or reduced social avoidance rather than generalized increases in sociability. Together, these findings support further investigation of inhaled THC as a modulator of specific social behavioural domains relevant to stress and anxiety-related disorders.

EXPLORING CANNABIDIOL AS A NOVEL THERAPEUTIC STRATEGY IN PROSTATE CANCER

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Introduction: Prostate cancer (PCa) is the second most common cancer in men worldwide, excluding non-melanoma skin cancers. Although androgen deprivation therapy is initially effective, progression to castration-resistant disease is inevitable, rendering advanced PCa incurable. Consequently, novel therapeutic strategies are urgently needed to improve outcomes for patients with aggressive disease. Our lab has previously demonstrated that cannabidiol (CBD), a major non-psychoactive cannabinoid, reduces PCa cell viability and modulates key cell cycle proteins *in vitro*. This study investigates the mechanism of CBD cellular uptake and determines whether CBD can synergise with anticancer therapies in a physiologically relevant *ex vivo* model. **Methods:** Gene expression was quantified using RT-qPCR and normalised to housekeeping genes. PCa cell lines were transfected with increasing concentrations of siRNA (5, 10, and 20 nM) alongside a non-targeting control (10 nM) for 24 hours, with additional time-course experiments conducted at 24 and 120 hours. Cell viability following drug treatment was assessed using MTT assays. Surgical tumour biopsies from radical prostatectomy patients were precision-cut into 200 µm tissue slice cultures (TSCs), incubated for 24 hours, and treated with CBD for 48 or 72 hours. LNCaP cells were used to inform ex vivo dosing with CBD reducing viability at 72 hours with an IC_{50} of 13.31 µM. **Results:** PPAR$\gamma\gamma$ silencing did not significantly alter DU145 sensitivity to CBD. Olaparib, Docetaxel, Abiraterone, Enzalutamide, and CBD reduced viability to varying degrees. In TSCs, CBD decreased proliferation and increased apoptosis. **Conclusions:** Ongoing combination studies aim to identify synergistic regimens for further validation, supporting translational progression beyond *in vitro* models.

THE ANTI-INFLAMMATORY EFFECTS OF FRENCH *CANNABIS SATIVA* EXTRACTS ON CYTOKINE RELEASE IN THP-1 MACROPHAGES-LIKE AND HUMAN PBMCS

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Introduction: A consortium (Overseed, CBM and CHU Orléans) was established to develop a sovereign French pharmaceutical supply chain. As *Cannabis sativa* phytocannabinoids (pCBs) exert their immunomodulation effects via endocannabinoid and non-canonical systems, we evaluated the anti-inflammatory potential of standardized extracts, to support evidence-based therapeutics.

Methods: Extracts were obtained from various stabilized cannabis chemotypes [CBD-rich, □9-THC-rich, 1:1, and poor CBD/□9-THC] via ethanol maceration followed, or not, by controlled baking treatment. All extracts were characterized with liquid chromatography-mass spectrometry. Extract and purified pCB Cytotoxic Concentration 50 (CC50) and anti-inflammatory potential (IL-6 secretion reduction by ELISA) were assessed on the THP-1 macrophage-like cell line (n=9) and PBMCs from healthy donors (n=9).

Results: Baked extracts were rich in decarboxylated PCBs, whereas non-baked extracts contained their corresponding acidic forms. Decarboxylated extracts exhibited twofold lower CC50 values, indicating higher cytotoxicity than acidic extracts which allowed testing across a higher concentration range for the latter. All extracts containing THC(A) and/or CBD(A) showed a significant dose-dependent reduction of IL-6 induction (75 to 40%) in THP-1 model. Thermal treatment had no impact on this effect, highlighting the pharmacological potency of acid forms. Moreover, plant extracts consistently outperform purified pCB standards, validating a robust entourage effect. PBMCs showed encouraging results, but in order to reduce inter-individual variability, larger cohorts are needed.

Conclusions: Extract potency depends on specific phytochemical profiles and thermal processing, surpassing isolated molecules. This pharmacological foundation paves the way for upcoming preclinical trials, expanding immune profiling to PBMCs from patients with inflammatory disorders in future clinical trials.

HETEROGENEOUS TRAJECTORIES OF ANXIETY AND DEPRESSIVE SYMPTOMS AMONG PREGNANT CANNABIS USERS: LONGITUDINAL FINDINGS FROM THE CAN-B PROSPECTIVE COHORT

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Introduction: Cannabis use during pregnancy has increased in recent years and is frequently reported as a strategy to manage anxiety and depressive symptoms. Yet prospective data describing symptom trajectories across pregnancy remain limited. We examined longitudinal trajectories of depressive and anxiety symptoms among cannabis-using and non-using pregnant women and explored heterogeneity within cannabis users within the Cannabis and Neurodevelopment of Babies (CAN-B) birth cohort.

Methods: Pregnant participants (153 cannabis users vs 79 controls) were assessed monthly using the PHQ-9 and GAD-7 through the entire pregnancy. Mixed-effects longitudinal models compared symptom trajectories between groups. Latent class trajectory analyses were conducted within cannabis users to identify distinct depressive and anxiety profiles. Models were adjusted for maternal age, income, and parity.

Results: Cannabis users had higher baseline depressive and anxiety scores compared with controls. Across gestation, both groups showed significant declines in symptoms ($p<0.001$). Cannabis users demonstrated a steeper decrease in both depressive and anxiety symptoms ($\beta=-0.35$, $p=0.02$), with convergence toward control levels by late pregnancy. Among cannabis users, two distinct trajectories emerged for depression (persistently elevated symptoms (20%) and moderate symptoms with gradual decline (80%)), and three distinct trajectories for anxiety (persistently elevated symptoms (~13%), elevated symptoms with gradual decline (~9%), and low and stable symptoms (~78%)). Covariate adjustment did not alter findings.

Conclusions: Our study suggests that prenatal cannabis use is not associated with a single mental health trajectory but with heterogeneous patterns that may reflect different underlying vulnerabilities and behavioral responses. Ongoing follow-up will examine whether prenatal symptom trajectories predict postpartum depression and explore the factors associated to each trajectory's profiles.

CONTRIBUTORS TO INTER-SUBJECT PHARMACOKINETIC VARIABILITY IN ORAL CANNABIDIOL: EFFECTS OF FEEDING AND VEHICLE VOLUME IN RATS

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Introduction: Oral cannabidiol (CBD) absorption and metabolism exhibits considerable variability, even among subjects administered identical products. However, the contributors to this variability are not fully understood. This study investigated the impact of feeding status, vehicle volume, and habituation on plasma pharmacokinetics (PK) of CBD.

Methods: Male Sprague-Dawley rats (n=15–16 per group) were given CBD (6.643 mg/kg) via gavage, with plasma collected at nine time points over 24 hours. Feeding conditions included strict fasting, food re-introduced for 0.5 hours before dosing (pre-meal), or at 0.5 hours after dosing (post-meal). CBD was administered in sesame oil at 100, 25, or 6.643 mg/mL. An additional group did not undergo prior gavage habituation. CBD and metabolites were quantified using LC-MS/MS. Non-compartmental PK analysis was performed.

Results: Inter-subject AUC variability differed across conditions, with interquartile fold-spread (Q3/Q1) ranging from 1.63-fold (25 mg/mL) to 2.98-fold (pre-meal). C_{max} variability was even more pronounced, spanning 1.73-fold (6.643 mg/mL) to 8.07-fold (pre-meal). Shifts in median AUC and C_{max} were not consistently accompanied by changes in dispersion. Median T_{max} was 2 hours across most conditions, with minimal variability in the 6.643 mg/mL group (IQR width 0 hours) and the greatest dispersion under strict fasting (IQR width 11 hours).

Conclusions: In this exploratory preclinical study, increasing vehicle volume exerted a stronger influence on the consistency of oral CBD absorption than food intake or habituation. These data suggest that formulation optimization may enhance reproducibility of oral cannabinoid absorption, and thereby potentially promote consistency of therapeutic effects.

CANNABIS AND EXPERIMENTAL PAIN: EFFECTS ON THE COLD PRESSOR TEST

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Introduction: The Cold Pressor Test (CPT) is an experimental pain assay used to evaluate the analgesic properties of pharmacological agents in humans. This assay requires a participant to immerse their forearm in frigid water (ranging from 0.6 °C to 5.0 °C), thereby activating peripheral nociceptors and central pain pathways and eliciting an autonomic response. Analgesic effects are measured as pain threshold (time to detect pain), tolerance (time to arm removal), and subjective pain intensity ratings. Although the CPT has been used to assess multiple drug classes, analgesic responses vary substantially. Cannabis/delta-9-tetrahydrocannabinol (THC) has been assessed in this model across a wide range of doses and routes of administration. However, cannabis effects on CPT-induced pain are inconsistent, and it is unclear what drives these mixed findings (e.g., dose selection, methodological differences, participant characteristics).

Method: A systematic search was performed to identify published studies evaluating cannabis/THC effects on the CPT. Data from two controlled inpatient studies from our laboratory assessing the effects of inhaled THC (0, 10, and 30 mg) were also included.

Results: The literature reveals mixed findings, with studies reporting analgesic, no effect, and even hyperalgesic responses to cannabis/THC. The majority of the papers reported little to no analgesic effects of cannabis/THC, consistent with our laboratory data. No systematic effects of dose/route, water temperature, or participant drug use status were detected.

Conclusion: The CPT may lack the necessary sensitivity to detect cannabinoid-induced analgesia in healthy participants, and/or cannabinoids may exert greater effects in clinical pain populations. These findings raise questions about the CPT's suitability as a model for screening cannabinoid-modulated analgesia in the human laboratory.

PHYTOCHEMICAL PROFILE AND ENTOURAGE EFFECT: VARIOUS IMPACT OF FRENCH-APPROVED CBD-BASED DIETARY SUPPLEMENTS ON CHRONIC INFLAMMATION IN LONG-TERM VIRALLY SUPPRESSED PEOPLE LIVING WITH HIV (PLWH)

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Introduction: When not classified as an approved drug, CBD-rich product regulations vary: authorized in Canada, state-regulated in the USA, and classified as "novel foods" in Europe (<0.3% Δ9-THC). We evaluated toxicity and anti-inflammatory potentials of three distinct French 20% phytocannabinoid broad spectrum sublingual oils (BV: 20% CBD; VT: 10% CBD/10% CBN; HM: 10% CBD/5% CBG/5% CBN) in long term efficiently treated PLWH, exhibiting chronic inflammation.

Methods Following Liquid Chromatography-Mass Spectrometry phytochemical characterization, the Cytotoxic Concentration 50 (CC50) and IL-6 reduction (ELISA) of BV, VT, and HM oils were assessed in vitro on the macrophage-like THP-1 cell line (n=9). In vivo, plasma cytokines (IL-1□, TNF-α, IFN-γ, IL-8, IL-6, and IL-10 by ELISA) and their corresponding mRNA expression (RT-droplet digital PCR) were compared, in CD4+ T lymphocyte and monocyte-enriched fractions, between 37 phytocannabinoid-exposed PLWH (oil for at least 4 weeks: BV=10, VT=10, HM=7 and 10 regular cannabis users).

Results: In vitro, the CBD-rich BV oil was significantly less toxic (CC50: 48 µg/ml), than VT (41 µg/ml) and HM (44 µg/ml). All oils significantly decreased IL-6 induction in a dose-dependent manner, though the intensity varies depending on the oil tested. Consistent with an entourage effect, in vivo results showed that the most significant anti-inflammatory effects were observed in monocyte-enriched fraction RNAs of PLWH who consumed complex phytocannabinoid compositions.

Conclusions: Potency depends on specific phytochemical profiles, confirming the superiority of full-spectrum formulations over isolated CBD. These translational results pave the way for clinical trials using multi-cannabinoid medicines to combat residual chronic inflammation in PLWH.

ORAL CANNABIDIOL TREATMENT AMELIORATED ALCOHOL-ASSOCIATED LIVER DISEASE THROUGH INTESTINAL AhR ACTIVATION

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Objectives: Cannabidiol (CBD), a non-psychoactive component of the cannabis plant, is approved for epilepsy treatment and has shown potential benefits in experimental alcohol-associated liver disease (ALD). ALD is often linked to intestinal barrier dysfunction and bacterial translocation. This study aimed to evaluate the efficacy of oral CBD administration in mitigating ALD by restoring gut barrier function.

Methods: Male C57BL/6 mice were subjected to the NIAAA binge-on-chronic alcohol feeding model (10 days of 5% ethanol diet followed by a single binge dose of 5 g/kg body weight). Mice were divided into alcohol-fed (AF) and isocaloric pair-fed (PF) control groups. The treatment group received 2 mg/kg body weight of CBD via oral gavage every other day for 10 days. Group of AF mice received AhR inhibitor (CH-223191) every day.

Results: Oral CBD administration significantly reduced alcohol-induced liver injury, as evidenced by decreased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, lower hepatic TUNEL-positive cell counts, reduced neutrophil infiltration, macrophage activation, and decreased pro-inflammatory factor expression. CBD treatment also significantly reduced serum lipopolysaccharide (LPS) levels, hepatic 16s rRNA, and E. coli protein staining, indicating a reduction in alcohol-associated bacterial translocation. Further analysis revealed that CBD prevented alcohol-induced reactive oxygen species (ROS) accumulation, pro-inflammatory cytokine elevation, and inductions in tight junction proteins (occludin and ZO-1) and IgA in the intestine. Mechanistically, oral CBD administration upregulated mRNA expression of intestinal Cyp1a, Cyp1b1, and ARNT, indicating an activation of the aryl hydrocarbon receptor (AhR) pathway. Reporter assays confirmed that CBD activated AhR. In Caco-2 cells, CBD upregulated AhR target gene expression, which was inhibited by an AhR antagonist. Transwell transepithelial electric resistance (TEER) assays showed that CBD prevented acetaldehyde-induced TEER reduction, supporting its role in maintaining epithelial barrier integrity. Importantly, the protective effects of CBD on ALD were significantly diminished when an AhR inhibitor was used.

Conclusions: This study demonstrates that oral CBD administration reduces alcohol-induced liver and gut injury by mitigating hepatic and intestinal apoptosis, restoring gut barrier function, and reducing inflammation through activation of the intestinal AhR signaling pathway. Further investigation into the role of the gut microbiome and intestinal immunoregulation in CBD's protective effects on ALD is warranted. (Supported by NIH-NIAAA).

PROFILING OF ENDO- AND PHYTOCANNABINOIDS IN BRAIN AND BLOOD SAMPLES VIA SPME-LC-MS/MS METHOD

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Introduction: In recent years, there has been an increasing focus on the endocannabinoid system and the impact of various endogenous and exogenous compounds, such as phytocannabinoids, on its function. However, the analysis of these substances in human and animal tissues remains challenging due to their trace concentrations, necessitating the use of high-sensitivity analytical methods.

Methods: Biocompatible SPME probes along with LC-MS/MS analysis were employed for selective isolation and quantification of CBD, THC, their metabolites (7-OH-CBD, 7-COOH-CBD, 11-COOH-THC), and endocannabinoids (AEA, 2-AG) in blood (in.vivo) and brain (ex.vivo) of rats. This procedure enabled monitoring of concentration changes up to
6 h post-administration of jellies containing CBD/THC, providing critical data on pharmacokinetic profiles and endocannabinoid system dynamics in real biological samples.

Results: Low invasive SPME-based approach was employed in this study to monitor levels of phyto- and endocannabinoids in complex matrices. CBD, THC, and their metabolites were detected in blood and brain tissue, exhibiting characteristic pharmacokinetic profiles, while temporal changes in endocannabinoid levels were observed in both matrices, providing important insights into the dynamics of the endocannabinoid system in relation to exposure to plant cannabinoids.

Conclusions: These findings improve understanding of cannabinoid metabolism and mechanisms of action under in.vivo/ex.vivo conditions, supporting further research into their therapeutic potential.

THE EFFECT OF IN UTERO EXPOSURE TO THC ON THE DEVELOPMENT OF MIDBRAIN DOPAMINERGIC NEURONS

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Cannabis use during pregnancy has increased drastically over the past years, coinciding with increased legalization and a substantial rise in the Δ9-tetrahydrocannabinol (THC). THC readily crosses the placenta into the fetal brain where it can disrupt the finely tuned actions of the endocannabinoid system which plays a crucial role in neurodevelopment. Given that many behavioural outcomes following prenatal cannabis exposure are associated with dopamine dysfunction, this system appears particularly vulnerable. However, the mechanisms through which prenatal THC exposure alters brain development, remains unclear. This study aims to investigate how THC affects the development of midbrain dopamine neurons.

Pregnant dams were exposed to THC or vehicle via intraperitoneal injection during gestation, and embryos were isolated at embryonic day 14.5. RNA sequencing was performed on midbrain dopamine neurons isolated by FACS-sorting using the Pitx3-GFP reporter allele. In addition, the expression profile of cannabinoid receptor 1 (CBR1) was assessed in tyrosine hydroxylase (TH) expressing neurons.

This study provides valuable insight into how prenatal THC exposure affects the development of mdDA neurons. Our transcriptome analysis will aid in identifying affected pathways, laying the groundwork for future studies. This study is the first in a series of studies on the short- and long-term consequences of prenatal THC exposure, aiming to map developmental trajectories and vulnerabilities. Collectively, we strive to clarify the risks associated with cannabis use during pregnancy and further elucidate the role of the endocannabinoid system in neurodevelopment.

PIERCING NUCLEAR HERNIAS IDENTIFY ROLE OF ENDOCANNABINOID SIGNALING IN THE CYTOSKELETON FUNCTIONALITY AND DISORDER OF MIGRATING NEURONS

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Introduction: Migrating metastatic cancer cells show nuclear envelope (NE) rupture and herniation of chromatin in the cytoplasm. We identified powerful streams of chromatin rupturing NE together with the plasma membrane in neurons migrating through mouse embryo cerebrum. Such chromatin streams represent a novel form of cell pathology, which we named 'piercing nuclear hernia' (PNH). Catastrophic rupture of the nuclear and plasma membranes exposes nucleoplasm and cytoplasm to the intercellular space and may result in fast cell death, which, in contrast to the programed cell death mechanisms, are not detectable using biochemical or immunochemical markers.
Methods: Mouse embryo cerebrum was analyzed using electron microscopy with 3D reconstruction from serial sections.
Results: About 40% of migrating cells in $CB_1R^{-/-}$ mouse embryos and wild type embryos exposed to two different CB_1R agonists show NE ruptures or/and PNHs. This indicates that deviations from optimal functioning of the endocannabinoid system in under- or over-activity may trigger the membranes vulnerability and chromatin herniation. Rupture of nuclear and plasma membranes provokes ultrastructural pathology in mitochondria and other organelles. Judging from the large volume and the length of herniated chromatin streams, the NE ruptures and PNHs are consequences of increased intranuclear pressure and suboptimal function of the cytoskeleton.
Conclusions: Optimal endocannabinoid signaling is crucial for correct development of brain cytoarchitecture. Discovered pathology of migrating cells and technique of its upregulation may be applied for inducing breaks of the plasma membrane and death of hazardous cells, for example, metastatic tumor cells.

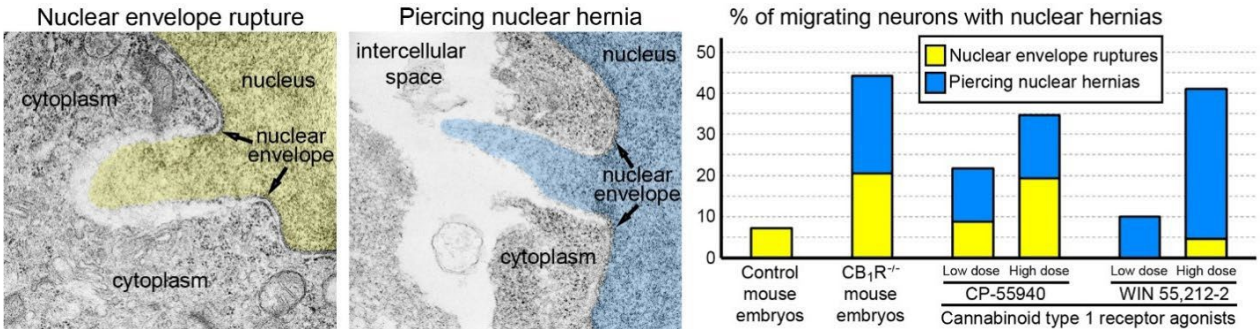

Figure. Electron micrographs and quantifications of cells with nuclear hernias in the embryo cerebrum.

CANNABINOID RECEPTOR TYPE 1 CHARACTERIZATION IN THE EARLY EMBRYO AND INFLUENCE ON CANNABINOID-MEDIATED CRANIOFACIAL DEFECTS

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Introduction: Prenatal cannabinoid exposure (PCE) induces brain and face defects like those observed after prenatal alcohol exposure or inhibition of the sonic hedgehog (SHH) pathway, a key morphogen. Cannabinoids have also been shown to inhibit the Shh pathway by inhibiting a key mediator – smoothened (SMO). It is unclear whether PCE-induced defects are SMO- or CB1-dependent mechanisms. SHH has been widely characterized in embryonic development and is restricted to the midline of the embryo and spans the entire length of the embryo, whereas the endocannabinoid system has not been well characterized. We hypothesize that Cnr7.follows similar restricted expression patterns, and alteration of these patterns underlies cannabinoid-induced defects.

Methods: C57Bl/6J mice were set for timed matings, dams were sacrificed on gestational day 8.25 and embryos were stage-matched and collected. Cnr7.expression was assessed via whole mount in situ hybridization (WISH). Specimens were imaged via confocal microscopy and images analyzed with IMARIS.

Results: Cnr7.expression is concentrated within the hindbrain of the embryo, with minimal expression within the mid and forebrain (n=4 embryos). Cnr7.expression peaks in the midline of the embryo (~200μm), with minimal expression laterally.

Conclusions: These data provide evidence of Cnr7.expression in the embryo and suggest that PCE-induced defects are likely not CB1-mediated. Given the regional expression of Cnr7, we hypothesize that defects from phytocannabinoids are likely due to SMO-dependent mechanisms. We are testing this hypothesis by exposing mice to JWH-018, a cannabinoid that we have shown to be ineffective at inhibiting SMO while maintaining CB1 agonistic effects.

CANNABIDIOL EXERTS A NEUROPROTECTIVE EFFECT ON MICROGLIA VIA REGULATION OF METABOLIC STATE

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Introduction: Cannabinoids exert pleiotropic and context-dependent effects on central nervous system immune signaling. For instance, Δ9-tetrahydrocannabinol (THC), a partial agonist of the cannabinoid receptor type 2 (CB2) expressed on microglia, has been reported to produce divergent outcomes in the literature, ranging from pro-inflammatory and cytotoxic responses to immunosuppressive and neuroprotective effects. These discrepancies likely reflect differences in dose, exposure duration, as well as cell types examined. In contrast, cannabidiol (CBD) has been more consistently characterized as an anti-inflammatory and immunomodulatory compound in the brain, with documented suppression of multiple components of the pro-inflammatory cascade. However, its specific effects on microglial activation states and immunometabolic reprogramming remain incompletely defined. Microglial activation is associated with a metabolic shift from oxidative phosphorylation toward aerobic glycolysis, a hallmark of pro-inflammatory polarization. To interrogate cannabinoid-mediated modulation of this immunometabolic transition, we quantified oxygen consumption rate (OCR) as a proxy for mitochondrial respiratory activity and assessed transcriptional changes in canonical inflammatory markers. These endpoints were evaluated following exposure to the prototypical pro-inflammatory stimulus lipopolysaccharide (LPS) in the presence or absence of CBD co-treatment. These studies begin to address whether CBD can prevent the onset of neuroinflammation, characteristic of neurodevelopmental disorders. **Methods:** To model inflammation associated with neurodevelopmental stages, we established primary mouse microglial cultures from dissociated embryonic day (E)18.5 mouse cortical and hippocampal tissues and exposed them to LPS (10 or 100ng/ml) either alone or in combination with CBD (50-300nM). Using a Resipher system we monitored the oxygen consumption rate of the microglia throughout a 24-hour exposure and examined levels of transcripts encoding key inflammatory markers such as TNFα, IL-1β, Cd86, Cd206 by qRT-PCR after 4 and 24 hours of LPS exposure. **Results:** LPS (100ng/ml) significantly increased OCR at 1 hour (VEH 33% vs LPS 536%, $p = 0.0125$). This was followed by a significant and sustained increase in OCR at 10 hours (VEH -35% vs LPS 150%, $p = 0.0139$) throughout the duration of the 24 hour recording (VEH: -29.97% ± 3.152% vs LPS: 202.8% ± 26.67%, $p < 0.0001$, paired t-test, mean ± SEM), consistent with increased aerobic glycolysis. Notably, 500nM CBD produced no significant effect on OCR at any time point. As expected, LPS also significantly upregulated the expression of Cd68, IL-1β, and TNFα transcripts, while reducing the expression of cell surface markers, TMEM119 and Cd206, consistent with a pro-inflammatory phenotype. When either 50nM or 500nM CBD was given in conjunction with 100ng/mL LPS, the initial OCR was significantly reduced at hour 1 compared to LPS alone (LPS + 50nM CBD 79% vs LPS 536%, $p = 0.0472$ / LPS + 500nM CBD 58.31% vs LPS 536%, $p = 0.0177$). At 11 hours, when the OCR for LPS was significantly increased, the addition of CBD restored OCR to near baseline levels (LPS + 50nM CBD 16% vs LPS 177%, $p = 0.0288$ / LPS + 500nM CBD -34% vs LPS 177%, $p = 0.0051$). This significant stabilization of OCR in the CBD-treated groups persisted for the next 12 hours, illustrating a powerful rescue effect of either 50nM or 500nM CBD on LPS-induced OCR increase. At hour 18 and onward, a small but significant dose response was found between 50nM CBD and 500nM CBD, with 500nM CBD having a slightly more powerful effect on reducing OCR (LPS + 50nM CBD 19% vs LPS + 500nM CBD -36%, $p = 0.0330$). Given the restored metabolic profile, we assessed whether 50nM was sufficient to reduce LPS-induced inflammatory cytokine expression. However, while 50nM CBD significantly reduced pro-inflammatory cytokine expression in response to 10ng/ml LPS at both 4 and 24 hours, it failed to significantly reduce transcript levels of the pro-inflammatory markers at the higher 100ng/ml LPS dose. However higher CBD doses (100nM and 300nM) reduced both 10ng/mL and 100ng/mL LPS-induced proinflammatory cytokine expression. This finding suggests that while low dose (50nM) CBD can prevent disruption to microglia metabolism, higher doses are required to attenuate inflammatory responses.

PLACENTAL ENDOCANNABINOID DYSREGULATION AND MITOCHONDRIAL STRESS IN HUMAN PREGNANCY DISORDERS

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Introduction: The endocannabinoid system (ECS) is expressed in placenta, a temporary organ linking mother and fetus for nutrient/oxygen exchange and hormone production. Gestational diabetes (GD), a diabetes form first-diagnosed during pregnancy, increases the risk of severe complications, including preeclampsia (PE), a disorder with impaired placentation and maternal-fetal risks. The ECS regulates mitochondrial function, and pathological ECS alterations can induce oxidative stress, a hallmark of defective placentation. We theorized that a pathological ECS/oxidative environment exists in placenta during GD and PE.

Methods: Placental villous tree samples, enriched in placental functional cells (trophoblasts), were collected at delivery from women with GD, PE, or healthy controls (n=10-13/group), and total RNA extracted. RT-PCR followed by real-time PCR was performed to assess gene expression.

Results: PE-placentas showed higher upregulation of key antioxidant and redox-regulating genes (i¡e..GPX7?.TXNIP, and COQ8) than GD-placentas compared to control-placentas. Only the expression of NAPE_PLD and ABHD❷was significantly higher (>3.5-fold) in pathological placentas compared to control-placentas. Changes in CNR7 expression were only present in PE-placentas –4-fold higher than control-placentas.

Conclusions: While previous studies reported no changes in placental ECS expression in PE compared to other pregnancy complications, we found that, when compared to healthy placentas, both pregnancy disorders involved a dysregulation of the ECS. Placental oxidative stress was more pronounced in PE, which also exhibited broader dysregulation of the ECS, including altered expression of enzymes and receptor. Our data suggests a stronger pathological phenotype in trophoblasts during PE, potentially impacting placental function, hence pregnancy and fetal development.

CIRCULATING ENDOCANNABINOIDS AND CARDIOVASCULAR DISEASE RISK IN ADULTS WITH OVERWEIGHT OR OBESITY BEFORE AND AFTER A 1-YEAR EXERCISE TRIAL

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Introduction: The CardioRACE trial was a recently completed phase 3 clinical trial that examined the effects of 1 year of supervised exercise (EX) training on cardiovascular disease (CVD) risk factors in 406 middle-aged adults with overweight or obesity. Participants completed 60-minute sessions, three days per week, and were randomized to aerobic EX only (AEX), resistance EX only (REX), combined half aerobic plus half resistance EX (COMBO) or a no-EX control group (CON). Compared to CON, both AEX and COMBO produced comparable improvements in a composite CVD risk score, evident via greater reductions in z-scores derived from four established CVD risk factors (i.e., percent body fat, systolic blood pressure, low-density lipoprotein cholesterol, and fasting glucose). Hyperactivity of the endocannabinoid (eCB) system has been implicated in the development and maintenance of cardiometabolic dysfunction and CVD risk. However, little is known whether different types of EX training differentially influence circulating eCB concentrations or whether changes in eCB tone contribute to improvements in cardiometabolic and cardiovascular health in adults with overweight or obesity. **Methods:** Blood draws were conducted at baseline and 1 year after a ≥ 12-hour overnight fast and ≥ 48 hours after participants' last EX session to minimize acute EX effects. To examine whether EX training (AEX, REX, COMBO) influenced circulating eCBs, linear mixed-effects models (LMEMs) were used to test group × time interactions, adjusted for age and sex, with random intercepts for participants. To determine whether changes in circulating eCBs mediated improvements in composite CVD risk following EX training, LMEMs and bootstrapped mediation analyses were conducted to estimate indirect effects. Additional analyses are ongoing to further characterize the eCB system in relation to participant characteristics (e.g., age, sex, sociocultural factors, physical activity, diet), physiological health (e.g., cardiorespiratory fitness, strength, arterial stiffness), mental health (e.g., sleep, anxiety, stress), and blood biomarkers (e.g., inflammatory markers). **Results:** Preliminary analyses demonstrated a significant reduction in circulating *N*-arachidonoylethanolamide (AEA) from pre-to post-intervention in the AEX group only compared to CON. No significant changes in 2-arachidonoylglycerol (2-AG) were observed in any group. Mediation analyses indicated that reductions in circulating AEA significantly mediated the association between AEX participation and improvements in CVD risk scores. **Conclusions:** Based on the preliminary analyses, circulating AEA decreases following 1 year of AEX training, whereas circulating 2-AG remains unchanged regardless of EX type. Reductions in circulating AEA may partially explain CVD risk improvements among adults with overweight/obesity who engage in regular aerobic EX but not combined half aerobic and half resistance EX. Overall, these findings suggest that basal circulating eCBs are relatively stable and selectively responsive to regular AEX.

DUAL MAGL/HSL INHIBITOR LEI-515 PROTECTS AGAINST ATHEROSCLEROSIS

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Introduction: Atherosclerotic cardiovascular disease (asCVD), mainly caused by combined dyslipidemia and inflammation, is a leading cause of mortality worldwide. The enzymes MAGL and HSL play an important role in lipid metabolism, while MAGL also facilitates the breakdown of anti-inflammatory endocannabinoid 2-AG. Therefore, the aim of this study was to assess the potential anti-atherogenic effect of the potent dual MAGL/HSL inhibitor LEI-515 in a well-established mouse model for human-like atherosclerosis.

Methods: APOE*3-Leiden.CETP mice were fed a Western type diet and treated with LEI-515 (30 mg/kg, i.p., every other day) or vehicle (saline:cremophore:DMSO (18:1:1) for fifteen weeks. Plasma free fatty acid (FFA), total cholesterol (TC) and triglyceride (TG) levels were measured every four weeks. After four weeks, very low-density lipoprotein (VLDL) production and composition were assessed. After fifteen weeks, atherosclerotic lesion size in the aortic root was quantified.

Results: Fifteen-week treatment of LEI-515 did not affect food intake or body weight but reduced plasma FFA (-26%, $p<0.0001$) and TC (-21%, $p<0.05$), mechanistically explained by reduced VLDL-TG production (-34%, $p<0.05$) and the formation of larger VLDL particles (+16%, $p<0.05$) with lower TC/TG ratios (-35%, $p<0.05$). Importantly, LEI-515 treatment largely reduced atherosclerotic lesion size throughout the aortic root (-31%, $p<0.05$). Cholesterol exposure positively correlated with the lesion size ($R^2 = 0.304$, $p<0.0001$).

Conclusions: LEI-515 reduces plasma levels of FFA to attenuate substrate-driven VLDL production, thereby reducing plasma cholesterol levels and consequently atherosclerotic lesion size. Collectively, our findings indicate a protective effect of dual MAGL/HSL inhibition against atherosclerosis development.

USE OF A CANNABIGEROL AND CANNABIDIOL FORMULATION ON MOTOR COORDINATION AND FATIGUE IN THE C56BL/6 MOUSE

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Introduction: Cannabis has perceived medicinal potential, but the pharmacological activity of many components remain poorly characterized compared to Δ^9-tetrahydrocannabinol (THC). Cannabigerol (CBG) and cannabidiol (CBD) are cannabinoids attracting interest for their lack of psychoactivity and purported potential benefits to movement and recovery. The purpose of this study was to assess whether daily oral administration with CBG, CBD, or 1:1 CBG and CBD altered coordination on the rotarod or endurance on a treadmill in mice.

Methods: Phase I assessed 14-day *p.o.* administration of vehicle, CBG (30 mg/kg), CBD (30 mg/kg), or CBG:CBD (30 mg/kg) on latency to fall on the rotarod, anxiety in the elevated plus maze (EPM), and nociception in the tail flick latency test (TFL). Phase II assessed the effects of 14-day *p.o.* administration of vehicle, CBG (30 mg/kg), CBD (30 mg/kg), CBG:CBD (30 mg/kg), THC (3 mg/kg), or bemethyl (50 mg/kg) on rotarod and treadmill scores.

Results: In Phase I, no treatments affected EPM or TFL results, whereas rotarod latency increased over time in mice receiving vehicle, CBG, CBD (males only), and CBG:CBD. Phase II rotarod data showed reduced fall latency in bemethyl (positive control)-treated male mice, but no other groups. Female THC-treated mice spent more time at the front of the treadmill, and male THC-treated mice spent less time at the back of the treadmill compared to vehicle, both indicators of improved performance; whereas neither CBG nor CBD altered performance.

Conclusions: These data present evidence of non-psychoactive cannabinoids displaying no significant impact on motor coordination or fatigue in mice. Overall, CBG and CBD produced little-to-no benefit in rotarod and treadmill tasks, undercutting claims these cannabinoids improve performance.

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EFFECTS OF ACUTE CANNABIS USE ON METABOLISM: A HUMAN METABOLOMICS STUDY

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Introduction: Cannabis influences human metabolism mainly through the endocannabinoid system, which helps regulate energy balance, appetite, and nutrient storage. To investigate the effects of acute cannabinoid consumption on metabolism we performed a metabolomics analysis on a subset of 103 participants from a larger study that investigated the effects of cannabis on metabolism and nutrition.

Methods: Participants purchased and used THC-dominant (23% THC, 0% CBD), THC+CBD (10% THC, 8% CBD), or CBD-dominant (20% CBD, 1% THC) products. Blood samples were taken before, approximately 15 minutes after and 2 hours after cannabis consumption. Blood concentrations of THC and CBD were determined, and a high-performance liquid chromatography – tandem mass spectrometry based targeted metabolomics approach was performed.

Results: The normalized data were fit to a hierarchical model adjusting for age, sex, and BMI. The largest changes in metabolic profiles were observed 2 hours after consumption and included 108, 100 and 130 statistically significantly changed (FDR<0.05) metabolites for the CBD, THC, and THC+CBD study groups, respectively. Eight Acylcarnitines and an additional 16 metabolites belonging to fatty acid β-oxidation and purine metabolite catabolism were changed after CBD-only consumption. In contrast we observed a shift from tryptophan-kynurenine pathway to indole-tryptophan metabolism in THC and THC+CBD groups.

Conclusion: Our targeted metabolomics analysis revealed novel findings regarding the impact of acute cannabinoid consumption on metabolism. This included a possible change in energy production after CBD consumption, as well a shift from organ specific tryptophan-kynurenine metabolism to gut based indole metabolism after THC consumption potentially contributing to its anti-inflammatory effects.

CORRELATION BETWEEN BLOOD AND URINE CANNABINOIDS AND SUB-CLINICAL CARDIOVASCULAR BIOMARKERS IN HEALTHY YOUNG ADULTS IN THE HERBAL HEART STUDY

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Introduction: Reliance on self-report can misclassify cannabis exposure, while blood and urine cannabinoid measurements offer objective confirmation of recent use. Using these biomarkers, we assessed their associations with objective measures of subclinical cardiovascular function in healthy young adult cannabis consumers.

Methods: Data from cannabis consumers enrolled in the Herbal Heart Study [N=159, M_{age}: 26.1 years (SD=4.9), range 18-35y, 57.9% female], were analyzed via Spearman correlations between blood- and urine-quantified Δ9-THC and its metabolites (Δ9-11-OH-Δ9-THC, Δ9-carboxy-Δ9-THC) and cardiovascular biomarkers: central pulse pressure (CPP), systolic/diastolic blood pressure (SBP/DBP), heart rate, flow-mediated dilation (FMD), pulse wave velocity (PWV; femoral and radial) and central augmentation index (cAIx) via applanation tonometry.

Results: Modest but statistically significant positive correlations were observed between concentrations of Δ9-THC and its metabolite and several cardiovascular measures. SBP and DBP were correlated with blood and urine 11-OH-Δ9-THC and Δ9-THC metabolites ($r = 0.165–0.248$, $p \leq 0.046$). Heart rate was correlated with urine 11-OH-Δ9-THC and THC-COOH ($r = 0.207–0.224$, $p \leq 0.019$). Femoral PWV was associated with blood THC-COOH and urine Δ9-THC, 11-OH-Δ9-THC, and THC-COOH ($r = 0.174–0.253$, $p \leq 0.042$), while cAIx was correlated with multiple blood and urine cannabinoid metabolites ($r = 0.199–0.236$, $p \leq 0.016$).

Conclusions: Cannabinoid metabolite concentrations are correlated with some objective measures of central hemodynamics and arterial stiffness. Biomarker-based characterization of cannabinoid exposure may enhance the interpretation of cannabis-related cardiovascular physiology. Future analyses will leverage the study's repeated self-report and biomarker measures to better characterize exposure-physiology dynamics.

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TARGETING BRAIN ENDOCANNABINOIDOME USING UHPLC-ESI-MS/MS

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Introduction: Endocannabinoids (ECBs) are bioactive lipid mediators involved in neuronal communication, and regulation of synaptic transmission in the central nervous system. Acting primarily through CB1/CB2 receptors, they modulate neurotransmitter release and contribute to physiological and pathological processes, such as neuroinflammation, pain and neurodegeneration. Despite the availability of analytical methods for biological fluids, quantitative analysis of ECBs in brain tissue remains a challenging issue, due to the complexity and lipid-rich nature of neural matrices. In the light of this, this study aimed to develop and validate an UHPLC-ESI-MS/MS method for the simultaneous quantification of 11 major ECBs and related lipid mediators in brain tissue, including their assessment in distinct cerebral regions.

Methods: Sample preparation was optimized by evaluating different solvent systems and conditions. Chromatographic separation was achieved on a reversed-phase C_{18} column using a Shimadzu LC-40 X3 UHPLC system coupled to a Sciex 6500+ QTRAP mass spectrometer, operating in the ESI-MS/MS mode. The method was applied to murine specific brain regions to assess the endocannabinoidome.

Results: Ethyl acetate-hexane, tested as a less toxic alternative, showed good performance, supporting its use for sample preparation. The optimized protocol ensured an efficient and reproducible recovery, with calibration linearity and good accuracy and precision. The validated analytical method enabled reliable quantification of multiple eCBs, supporting comprehensive profiling and regional distribution analysis.

Conclusions: The validated UHPLC-ESI-MS/MS method provided an accurate and reproducible quantification of multiple ECBs in brain tissue and specific regions, representing a reliable tool for investigating spatial patterns associated to their signaling.