ICRS2024
SALAMANCA

34TH ANNUAL
SYMPOSIUM
OF THE

INTERNATIONAL CANNABINOID
RESEARCH SOCIETY

SALAMANCA
SPAIN

JUNE 30 - JULY 5, 2024

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JUNE 30 – JULY 5, 2024

Symposium Programming by Cortical Systematics LLC

International Cannabinoid Research Society
Research Triangle Park, NC
USA

ISBN: 978-604-0-00022-4

These abstracts may be cited in the scientific literature as follows:

Author(s), Abstract Title (2024) 34th Annual Symposium on the Cannabinoids,
International Cannabinoid Research Society, Research Triangle Park, NC, USA, Page #.

Funding for this conference was made possible in part by grant 5R13DA016280 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

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ICRS Pre-Conference Activities

Palacio de Congresos de Salamanca, Lower Level
Salamanca, Spain

Sunday, June 30th

9.30 – 16.00	ICRS Pediatric Special Interest Group Meeting **PRECISION CANNABINOIDS:** **A COMPLEX PLANT FOR COMPLEX PEDIATRIC PATIENTS** Hosted by C4T (Canadian Collaborative for Childhood Cannabinoid Therapeutics) Registration desk opens at 9.30
10.00	Welcome and Opening Remarks Lauren Kelly, C4T Director
10.30	History of Medical Cannabis in Children and Future Directions Ethan Russo
11.00	The Complexity of Cannabis Plant; Lessons for Medical Cannabis Patients Dedi Mieri
12.00	Cannabinoid Metabolism and Pharmacogenomic Impacts Rachel Tyndale
12.30	**Lunch and Networking**
13.30	**Workshop:** Development of a Cannabinoid-Related Adverse Event Reporting Tool
16.00	Closing Remarks

Notes:

ICRS Registration: June 30th, 2024 (17.00 – 19.00)

Lobby, Palacio de Congresos de Salamanca

Welcome Reception: 19.00 – 21.00

University de Salamanca, Palacio Fonseca

Day 1
Monday, July 1st

8.30	Welcome and Opening Remarks		
Oral Session 1. Novel Compounds *Chairs:* Mario van der Stelt and Sara Jane Ward			
8.45	Sergiy Tyukhtenko*, Michael Speziale, Huixi Li, Xiaoyu Ma, Olga Tarkhanova, Iryna Kravets, Dmytro Dudenko, Yurii Moroz and Alexandros Makriyannis	DISCOVERY OF ALLOSTERIC MODULATORS FOR MONOACYLGLYCEROL LIPASE VIA THE INTEGRATION OF VIRTUAL SCREENING, LC/MS/MS, KINETICS, AND NMR SPECTROSCOPY	1
9.00	Ka Lai Yip*, Michael Udoh, Thomas S. Harman, Lyndsey L. Anderson, Samuel D. Banister and Jonathon C. Arnold	THE NON-CANNABIS DERIVED PHYTOCANNABINOID-LIKE COMPOUNDS AMORFRUTIN 2 AND MAGNOLOL EXHIBIT ANTI-SEIZURE EFFECTS IN SCN1A +/- MOUSE MODEL OF DRAVET SYNDROME	2
9.15	Michael Ippolito*, William Kinney, Douglas E. Brennemen and Sara Jane Ward	KLS-13019, A NOVEL STRUCTURAL ANALOGUE OF CANNABIDIOL (CBD) AND GPR55 RECEPTOR ANTAGONIST, PREVENTS AND REVERSES CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY (CIPN) IN RATS	3
9.30	Verena Straub, A. Floor Stevens, Tom van der Wel, Na Zhu, Yulong Li, Nephi Stella and Mario van der Stelt*	2-AG IS RELEASED AND TRANSPORTED VIA EXTRACELLULAR VESICLES	4
9.45	**Coffee Break**		

Oral Session 2. Cannabinoids and Cancer

Chairs: Manuel Guzmán and Donald Abrams

10.15	María Rubert*, Marta Seijo-Vila, Isabel Tundidor, Sandra Blasco-Benito, Cecilia J. Hillard, María Teresa Grande, Julián Romero, Cristina Sánchez and Eduardo Pérez-Gómez	ROLE OF CANNABINOID RECEPTOR 2 IN THE BREAST TUMOR MICROENVIRONMENT	5
10.30	William George Warren, Myles Osborn, Andy Yates and Saoirse E O'Sullivan*	ART26.12, A NOVEL FATTY ACID-BINDING PROTEIN 5 INHIBITOR, SHOWS EFFICACY IN BREAST CANCER-INDUCED BONE PAIN	6
10.45	Marisa Weiss, Muath Giaddui, Stephanie Kjelstrom, Joseph Gary, Sara Jane Ward*, Ebuwa Erebor, Sam Meske, Lisa Saeed, Katherine Aliano Ruiz, Arezoo Ghaneie, Juliana Hibbs, John Marks, David Holtz, Paul Gilman, Sharon Larson and Diana Martinez	CANNABIDIOL AND MANAGEMENT OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY: A RANDOMIZED DOUBLE BLIND CONTROLLED TRIAL	7
11.00	Simon Erridge*, Madhur Varadpande, Evonne Clarke, Katy McLachlan, Ross Coomber, James J Rucker, Michael Platt, Shaheen Khan and Mikael H Sodergren	UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS OF MEDICAL CANNABIS FOR CHRONIC CANCER PAIN	8
11.15	**Presidential Plenary Lecture** ANTICANCER ACTIVITY OF CANNABINOIDS: PAST, PRESENT... AND FUTURE? **Manuel Guzmán, Ph.D.** Professor of Biochemistry and Molecular Biology Complutense University of Madrid		

Oral Session 3. Drug Interactions

Chairs: Ryan Vandrey and Linda Klumpers

12.15	Justin C. Strickland*, Hayleigh E. Tilton, Ryan Vandrey, Matthew T. Feldner, Jessica G. Irons and Marcel O. Bonn-Miller	A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED, WITHIN-SUBJECT CROSSOVER STUDY OF THE EFFECTS OF COMBINATIONS OF CANNABINOIDS AND CAFFEINE	9
12.30	Tory Spindle*, C. Austin Zamarripa, Spencer Lin, Madyson Slutzky, Denis Antoine, Thomas Marcotte, Daniel Roche, Elise Weerts and Ryan Vandrey	THE IMPACT OF ORAL CANNABIS ("EDIBLES") INGESTION AND CO-USE OF ALCOHOL ON ACUTE DRIVING AND BEHAVIORAL IMPAIRMENT, SUBJECTIVE DRUG EFFECTS, AND CANNABINOID PHARMACOKINETICS	10
12.45	**Lunch** **NIDA-Supported Trainee Networking Lunch**		
14.00	**2023 ICRS Mid-Career Awardee** TRANSLATIONALLY RELEVANT MODELS OF CANNABIS ADMINISTRATION TO RODENTS **Matt Hill, Ph.D.** Professor, Cumming School of Medicine, Department of Cell Biology and Anatomy University of Calgary, Alberta, Canada		

Oral Session 4. Development

Chairs: Julian Romero and Jonathon Arnold

14.30	Alice Zucchi*, Angelo Onorato, Susanna Dolci and Paola Grimaldi	PRENATAL OVERACTIVATION OF CB2 RECEPTOR AFFECTS OFFSPRING GERM CELLS DEVELOPMENT	11

14.45	Samantha L. Baglot*, Lucia Javorcikova, Savannah H.M. Lightfoot, Andrei. S. Nastase, Cayden Murray, Catherine Hume, Robert J. Aukema, Gavin N. Petrie, Jeffrey F. Dunn, Tamara S. Bodnar and Matthew N. Hill	AN INHALED MODEL OF PRENATAL CANNABIS EXPOSURE IN RODENTS: EFFECTS ON NEURAL-, IMMUNE-, AND ENDOCANNABINOID-SYSTEM DEVELOPMENT, AND LATER-LIFE BEHAVIOUR	12
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Alisha K. Holloway* and Noah Craft	SELF-REPORTED EFFECTS OF TETRAHYDROCANNABIVARIN ON ACTIVITY, ENERGY LEVEL, MOTIVATION, AND APPETITE: A DOUBLE-BLIND, PLACEBO-CONTROLLED, DOUBLE CROSSOVER TRIAL	DB1-3 [P1-8]
Amrit Baral*, Bria-Necole A. Diggs, Yash Agrawal, Sarah E. Messiah, Armando Mendez, Rosa Hernandez, Claudia Martinez and Denise C Vidot	CANNABIS USE AND NON-HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AMONG YOUNG ADULTS WITH A POSITIVE COVID-19 TEST: PRELIMINARY FINDINGS FROM THE HERBAL HEART STUDY COHORT	DB1-4 [P1-11]
Denise C. Vidot*, Bria-Necole Diggs, Amrit Baral, Renessa Williams, Dina Marrakchi El Fellah, Sitara Weerakoon, Ramessu Iyi, Moudou Baqui, Michelle Weiner and Sarah Messiah	PSILOCYBIN AND CANNABIS USE BY MENTAL HEALTH STATUS IN THE UNITED STATES, CANADA, EUROPE, AND NEW ZEALAND DURING THE COVID-19 PANDEMIC: RESULTS FROM THE COVID-19 CANNABIS HEALTH STUDY	DB1-5 [P1-12]

DATABLITZ presenters present *both* a 4-minute presentation *and* a poster at ICRS2024.

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Notes: Presenting Author*

Day 2
Tuesday, July 2nd

8.25	Opening Remarks		
8.30	**2023 ICRS Lifetime Achievement Awardee** CANNABINOID MEDICINES - PAST, PRESENT AND FUTURE **Geoffrey W. Guy** **MB BS, LRCP MRCS, LMSSA, DipPharmMed, BSc, DSc** Founder, GW Pharmaceuticals The Guy Foundation United Kingdom		
Oral Session 5. CBD Mechanism Chairs: Veena Ranganath and Danielle McCartney			
9.30	Gunter van der Walt*, Maria Helena de Donato, Albert Quintana and Emma Puighermanal	DISTINCT GENE REGULATION PATTERNS UNDERLIE CANNABIDIOL BENEFIT IN A MODEL OF MITOCHONDRIAL EPILEPSY	17
9.45	Matthew J. Jones*, Taygun C. Uzuneser, Saoirse E. O'Sullivan, Mohammed H. Sarikahya, Andy Yates, Walter Rushlow and Steven R. Laviolette	A NOVEL CANNABIDIOL: TETRAMETHYLPYRAZINE (ART12.11, CBD:TMP) COCRYSTAL IMPROVES THE EFFICACY AND BIOAVAILABILITY OF CANNABIDIOL TO INDUCE ANXIOLYTIC AND ANTI-DEPRESSANT EFFECTS	18
10.00	Veena K Ranganath*, Holly Wilhalme, Nicolette T Morris, Jenny Brook, Brian Skaggs, David A Elashoff and Ziva D. Cooper	A RANDOMIZED PLACEBO-CONTROLLED TRIAL ASSESSING THE SAFETY AND EFFICACY OF CANNABIDIOL IN RHEUMATOID ARTHRITIS PATIENTS	19

10.15	Danielle McCartney*, Christopher Irwin, Zeeta Bawa, Blake Palmer, Ayshe Sahinovic, Nathan Delang, Gregory R. Cox, Ben Desbrow, Namson S. Lau and Iain S. McGregor	THE EFFECT OF CANNABIDIOL ON SUBJECTIVE RESPONSES TO AEROBIC EXERCISE: A RANDOMISED CONTROLLED TRIAL	20
10.30	**COFFEE BREAK**		
ORAL SESSION 6. ENDOCANNABINOIDS IN DISEASE AND THERAPEUTICS *CHAIRS:* CECILIA HILLARD AND IAIN MCGREGOR			
11.00	Gabriella Smith, Kathleen McCoy, Gonzalo Viana Di Prisco, Brady Atwood, Ken Mackie and Anna Kalinovsky*	ENDOCANNABINOID SIGNALING DEFICITS IN THE CEREBELLUM DISRUPT SOCIAL PREFERENCE	21
11.15	Lola E. Zovko*, Catharine A. Mielnik, Ruth A. Ross and Ali Salahpour	THE EFFECTS OF ENDOCANNABINOID MODULATION ON AN ACUTE MOUSE MODEL OF PARKINSON'S DISEASE	22
11.30	Pavel Powlowski*, Jaehyoung Choi, Lindsay Melhuish Beaupré, Joanna Biernacka, Ana C. Andreazza and Ruth Ross	THE ENDOCANNABINOID SYSTEM IN BIPOLAR DISORDER AND ITS EFFECTS ON MITOCHONDRIAL FUNCTION	23
11.45	A. Matt Reck*, David P. Siderovski and Steven G. Kinsey	THE CB2 RECEPTOR IS AN ANTIPRURITIC TARGET IN MICE	24
12.00	Yumna Abu Ghanem*, Hadas Catane Hovav and Gali Umschweif	REGULATION OF THE CB1 RECEPTOR BY NEURENSIN-2 IN DEPRESSION	25

Oral Session 7A. Research and Development (Preclinical) *Chairs:* Daniele Piomelli and Ruth Ross			
12.15	Marta Gómez- Almería*, Carmen Rodríguez-Cueto, Julián Romero, Benjamin F. Cravatt, Javier Fernández-Ruiz and Eva de Lago	THE EFFECT OF FAAH INACTIVATION IN PRP-TDP43^{A315T} TRANSGENIC MICE: RELEVANCE FOR POSSIBLE NEW ENDOCANNABINOID-BASED THERAPIES IN ALS	26
12.30	Antonella Pacini, Elise Wreven, Alejandro Escamilla Sánchez, François Pattou, Julie Kerr-Conte, Silvina R Villar, Malliga Iyer and Isabel González Mariscal*	UNLOCKING THE THERAPEUTIC POTENTIAL OF THE DUAL INOS AND PERIPHERAL CB1R BLOCKER S-MRI-1867 FOR PRESERVING BETA CELL FUNCTION AND PREVENTING TYPE 1 DIABETES	27
12.45	Yoshihiro Kitaoka, Kyotaro Koshika, Zhiwei Li, Toru Yamamoto, Elisha Haykani, Yatendra Mulpuri, Kyle Whyland, Herbert Seltzman and Igor Spigelman*	PERIPHERALLY-ACTING CANNABINOID PREVENTS MIGRAINE-LIKE PAIN AND SENSITIZATION OF DURAL NOCICEPTORS	28
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14.45	Neta Rimmerman*, Ramit Ravona-Springer, Ziv Sarussi, Noa Bregman, Talya Nathan, Vered Hermush, Nisim Mizrahi, Noa Stern, Talma Gotteiner, Adi Don, Hagay Moshe and Nurit Tweezer Zaks	PART I, OPEN LABEL RESULTS OF THE SAFETY AND EFFICACY OF MEDICANE'S MEDICAL CANNABIS OIL FOR TREATMENT OF AGITATION AND DISRUPTIVE BEHAVIORS IN SUBJECTS WITH DEMENTIA	30

15.00	Karen Chen, Catherine Eliades, Dilara Ertenu and Jay Salpekar*	CANNABIDIOL TREATMENT OF PEDIATRIC EPILEPSY AND COMORBID ANXIETY: INTERIM RESULTS FROM A CLINICAL TRIAL	31
15.15	Audrey Flavin, Paniz Azizi, Natalia Murataeva, Kyle Yust, Ruth Ross, Iain Greig, Yanan Zhang, Ken Mackie and Alex Straiker*	CB1 RECEPTOR NEGATIVE ALLOSTERIC MODULATORS FOR REVERSAL OF CANNABIS TOXICITY	32
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Jenny Chen, Fengyuan Li, Jiyeon Lee, M. Manirujjaman, Lihua Zhang, Zhao-Hui Song, Craig McClain and Wenke Feng*	PERIPHERALLY RESTRICTED CB1 RECEPTOR INVERSE AGONIST JD5037 TREATMENT EXACERBATES LIVER INJURY IN MDR2 DEFICIENT MICE	DB2-1 [P2-8]
Stephanie Patterson,* Robert Laprairie and Stéphane A. Laporte	UNDERSTANDING PHYTOCANNABINOID SIGNALLING PROFILES AT CANNABINOID TYPE I AND TYPE II RECEPTORS	DB2-2 [P2-11]
Natalia Malek*	THE INFLUENCE OF CB2 ACTIVATION IN MICROGLIA ON THE REGULATION OF CALCIUM-DEPENDENT CASPASES ASSOCIATED WITH PYROPTOTIC PATHWAY	DB2-3 [P2-12]
Andrew Yates*, Alison Wilby, William Warren, Myles Osborn and Saoirse E O'Sullivan	ART12.11, A NOVEL CANNABIDIOL:TETRAMETHYLPYRAZINE CO-CRYSTAL, DEMONSTRATES A UNIQUE PHARMACOKINETIC PROFILE	DB2-4 [P2-13]
Beth M. Wiese*, Evgeny Bondarenko and Jack L. Feldman	CANNABIDIOL TO MITIGATE FENTANYL INDUCED PERSISTENT APNEA	DB2-5 [P2-14]

DATABLITZ presenters present *both* a 4-minute presentation *and* a poster at ICRS2024.
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POSTER SESSION 2
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Almudena López-Escobar*, Laura Martín-Pérez, Samuel Ruiz de Martín Esteban, M. Andrea Arnanz, Iván Rodríguez Martín, Ana M. Martínez-Relimpio, Claudia Korn, Catarina Raposo, Roman C. Sarott, Matthias V. Westphal, Erick M. Carreira, Uwe Grether, Cecilia J. Hillard, Julián Romero and M. Teresa Grande	HIGH-SPECIFICITY FLUORESCENT PROBE TO ENABLE CANNABINOID TYPE 2 RECEPTOR STUDIES	P2-2
Nuria G Martínez-Illescas*, Paula Gijón, Marc Pinto, Manuel Luque and María Salazar-Roa	SHORT EXPOSURE TO NANOMOLAR CANNABINOIDS PROMOTES BREAST CANCER CELL DIFFERENTIATION	P2-3
Laura Martín*, M. Andrea Arnanz, M. Teresa Grande, Samuel Ruiz de Martín Esteban, Almudena López, Benjamin F. Cravatt, Ricardo Mostany, Julián Romero and Ana M. Martínez Relimpio	PHARMACOLOGICAL ABLATION OF MICROGLIA REVEALS SPECIFIC MORPHOLOGICAL CHANGES IN PLAQUE-ASSOCIATED MICROGLIA IN 5XFAD/FAAH-/- MICE	P2-4
Valentina Satta*, Álvaro Sierra, José A. Guimaré, Inés Hernández-Fisac, Javier Fernández-Ruiz and Onintza Sagredo	EXPLORING THE IMPACT OF CANNABIDIOL AND β-CARYOPHYLLENE COMBINATION ON MYELINATION STATUS IN A MURINE MODEL OF DRAVET SYNDROME	P2-5
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Notes: Presenting Author*

Day 3
Wednesday, July 3rd

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8.30	Benjamin D. Anderson, Diana E. Sepulveda, Rahul Nachnani, Alonso Cortez, Aviauna Beckett, Jordan Bisanz, Joshua J. Kellogg and Wesley M. Raup-Konsavage*	HIGH CANNABIGEROL HEMP EXTRACT MODERATES COLITIS AND MODULATES THE MICROBIOME IN AN INFLAMMATORY BOWEL DISEASE MODEL	33
8.45	Radka Kočvarová*, Shahar Azar, Ifat Abramovich, Bella Agranovich, Eyal Gottlieb, Liad Hinden and Joseph Tam	TOWARDS A MECHANISTIC UNDERSTANDING OF CBD AND CBG IN THE REVERSAL OF OBESITY-INDUCED FATTY LIVER DISEASE	34
9.00	Myra Rice*, Christine Wu, Akeesha Rodrigues, Ziva D Cooper and Catherine Cahill	CB1 RECEPTORS INVOLVED IN ANTIALLODINIC EFFECTS OF MYRCENE IN RODENT MODELS OF CHRONIC PAIN	35
9.15	Kaylin Ellioff*, Keming Qiu, Anthony English, Sean Piantadosi, Nephi Stella, Michael R. Bruchas and Benjamin B. Land	CHARACTERIZING THE ANALGESIC POTENTIAL OF CANNABIDIOL AND SELECT TERPENES	36

9.30	Isobel Lavender*, Danielle McCartney, Nathaniel Marshall, Ronald Grunstein, Brendon Yee, Iain McGregor and Camilla Hoyos	A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED, SINGLE-DOSE, CROSSOVER, PILOT INVESTIGATION OF CANNABINOL (CBN) 30 MG AND 300 MG EFFECTS ON SLEEP AND NEXT-DAY FUNCTION IN INSOMNIA DISORDER ('CUPID')	37
9.45	**2023 ICRS Mid-Career Awardee** FILLING THE GAPS IN PHARMACOLOGICAL TREATMENT OF CHRONIC PAIN WITH SMART LIPIDS **Katarzyna Starowicz, Ph.D.** Department of Neurochemistry Institute of Pharmacology Polish Academy of Sciences Krakow, Poland		
10.15	**Coffee Break**		
Oral Session 9. CUD and Cannabis Use in Patient Populations *Chairs:* Denise Vidot and Amir Englund			
10.45	Edward Chesney*, Dominic Oliver, Ananya Sarma, Doğa Lamper, Ikram Slimani, Millie Lloyd, Irma Gasparini-Andre, Michael Welds, Natavan Babayeva, Will Lawn, Matilda Kråkström, Alex Dickens, Matej Orešič, Tom P Freeman, Amir Englund, John Strang and Philip McGuire	CBD ALTERS SENSITIVITY TO CANNABIS IN PEOPLE WITH PSYCHOSIS AND COMORBID CANNABIS USE DISORDER:	38
11.00	Erin L. Martin*, Heather B. Bradshaw and Aimee L. McRae-Clark	DIFFERENCES IN STRESS RESPONSE IN INDIVIDUALS WITH CANNABIS USE DISORDER ALONE AND COMORBID CANNABIS USE DISORDER AND MAJOR DEPRESSIVE DISORDER	39

11.15	Conor H. Murray*, Alexa Torrens,Stephanie Lake, Timothy Fong, Elisa Pabon, Daniele Piomelli and Ziva D. Cooper	TOWARD OBJECTIVE BIOMARKERS OF CANNABIS USE DISORDER	40
11.30	Justin Matheson*, Awirut Oon-Arom, Marcos Sanches, Adam Zaweel, Ahmed Hassan, Matthew Sloan, Leslie Buckley, Amy Porath, James MacKillop, Christian Hendershot, Stefan Kloiber and Bernard Le Foll	SEXUAL IDENTITY, SEXUAL MINORITY STRESS, AND CANNABIS USE MOTIVES AND HARMS IN PATIENTS ACCESSING ADDICTION TREATMENT IN ONTARIO, CANADA	41
11.45	David Wolinsky*, Rhiannon E. Mayhugh, Renuka Surujnarain, Johannes Thrul, Marcel O. Bonn-Miller, Ryan Vandrey and Justin C. Strickland	EVALUATION OF ANTIDEPRESSANT AND ANXIOLYTIC EFFECTS OF MEDICINAL CANNABIS USE VIA ECOLOGICAL MOMENTARY ASSESSMENTS	42
12.00	**KANG TSOU MEMORIAL SPEAKER** CANNABINOIDS, STRESS AND PAIN: DOES IT OR DOES IT NOT BLOCK IT? **RAJITA SINHA, PH.D.** Foundations Fund Endowed Professor in Psychiatry and Professor of Neuroscience and of Child Psychiatry Deputy Chair of Psychiatry for Psychology Chief of Psychology Section Director, Yale Interdisciplinary Stress Center Yale University School of Medicine		
13.00	**LUNCH**		
14.00 -	**FREE TIME** **OUTINGS**		

Day 4
Thursday, July 4th

8.25	Opening Remarks		
8.30	**Presidential Plenary Lecture** THE ENDOCANNABINOID SYSTEM IN BRAIN AGING AND NEURODEGENERATION **Javier Fernández-Ruiz, Ph.D.** Department of Biochemistry and Molecular Biology Faculty of Medicine Complutense University of Madrid		
Oral Session 10. Endocannabinoid Mechanism and Signalling *Chairs:* Michelle Glass and Steve Alexander			
9.30	Martin Kaczocha*, Saida Oubraim, Mohammad Fauzan and Samir Haj-Dahmane	ASTROCYTIC FABP5 MEDIATES SYNAPTIC ENDOCANNABINOID TRANSPORT IN THE HIPPOCAMPUS	43
9.45	Eric S. Levine* and Fouad Lemtiri-Chlieh	SYNERGISTIC ROLES OF 2-AG AND ANANDAMIDE IN HIPPOCAMPAL LONG-TERM DEPRESSION	44
10.00	**Coffee Break**		

Oral Session 10. (cont)
Endocannabinoid Mechanism and Signalling

Chairs: Michelle Glass and Steve Alexander

10.30	Robert S. Leddy, Cinthia L. Wilkinson, Carol M. Aherne and Colm B. Collins*	CANNABINOID RECEPTOR 2 SIGNALLING CONSTITUTES A METABOLIC REORGANISATION IN T CELLS, AWAY FROM GLYCOLYSIS TOWARDS THE PENTOSE PHOSPHATE PATHWAY	45
10.45	Alex Kuklish*, Kathleen McCoy, Ken Mackie and Anna Kalinovsky	CANNABINOID SIGNALING MACHINERY REGULATES CEREBELLAR MOSSY FIBER AXON GROWTH	46
11.00	Hannah R. Alton, Emily O. Linz, Guo-Hua Bi, Omar Soler-Cedeño and Zheng-Xiong Xi*	REVISITING THE CANNABINOID-OPIOID INTERACTION HYPOTHESIS USING CONDITIONAL CB1 AND μ OPIOID RECEPTOR KNOCKOUT MICE	47

11.15

2023 ICRS Mechoulam Awardee

CANNABINOID PHARMACOLOGY: THE SHAPE OF THINGS TO COME

Ruth Ross, Ph.D.

Department of Pharmacology and Toxicology
University of Toronto
Toronto, Canada

12.15 — **Lunch**

2023 ICRS William A. Devane Early Career Awardee

14.00

CANNABIS AND THE VULNERABLE BRAIN: IMPACT OF GENETICS, AGE, AND EMERGING ROUTES OF ADMINISTRATION

Jibran Khokhar, Ph.D.

University of Guelph
Ontario, Canada

Oral Session 11. Pain

Chairs: Josee Guindon and Katarzyna Starowicz

14.30	Alex Mabou Tagne*, Yannick Fotio, Kalpna Gupta and Daniele Piomelli	EFFECTS OF THC IN HUMANIZED SICKLE CELL DISEASE MICE	48
14.45	Stephanie Bourke, Laura Boullon, Mary Hopkins, Alba Maria Diego, Maria Redmond, Katie Healy, Ariadni Bella, Chiara Di Marino, Álvaro Llorente-Berzal and David P. Finn*	STRESS-INDUCED ANTINOCICEPTION IN THE RAT SPARED NERVE INJURY MODEL OF NEUROPATHIC PAIN IS MEDIATED BY CANNABINOID CB1 RECEPTORS	49
15.00	Livia Schutz*, Adriana Dibua, Karan Trivedi and Martin Kaczocha	MONOACYLGLYCEROL LIPASE INHIBITION ALLEVIATES POST-INCISIONAL PAIN VIA SUPPRESSION OF DOWNSTREAM EICOSANOID BIOSYNTHESIS	50

Oral Session 12. Metabolism

Chairs: Ilia Karatsoreos and Matt Hill

15.15	Océane Pointeau*, Awa Isma Ba, Audrey Geissler, Abhishek Basu, Arif Muhammad, Marina Nivot, Patricia Passilly-Degrace, Julia Leemput, Sébastien Causse, Laurent Demizieux, Bruno Vergès, Hélène François, Geneviève Gaucher, Michael Harvey, Pascal Degrace, Glenn Crater, Resat Cinar and Tony Jourdan	BLOCKAGE OF CB1 RECEPTORS POTENTIALIZES RENAL ANTI-FIBROTIC EFFECTS INDUCED BY SGLT2 INHIBITION IN DIABETIC MICE	51
15.30	Brennan A. Falcy*, Giancarlo E. Denaroso, Said Akli, Gregory L. Pearson, Jiexin Wang, Catherine Hume, Matthew N. Hill and Ilia N. Karatsoreos	ENDOCANNABINOIDS: A LINK BETWEEN CIRCADIAN DISRUPTION AND WEIGHT GAIN	52

15.45	Asaad Gammal*, Achilleas Fardellas, Noam Freeman, Sharleen Hamad, Yael Soae, Amit Badihi, Taher Nassar, Simon Benita, Niklas K. Björkström and Joseph Tam	MOLECULAR INSIGHTS INTO MITIGATING DIET-INDUCED OBESITY AND METABOLIC DYSREGULATIONS BY A PERIPHERALLY RESTRICTED CB1R ANTAGONIST	53
16.00	Sarah-Catherine Rodan*, Sarah Maguire, Noah Meez and Iain S. McGregor	CANNABIS HAS REMARKABLE SELF-REPORTED EFFICACY IN PEOPLE WITH EATING DISORDERS: FINDINGS FROM THE INTERNATIONAL MED-FED SURVEY	54
16.15	DATABLITZ SESSION 3		DB3
16.45 – 18.45	**Poster Session 3** Reception		P3
20.00	**AWARDS CEREMONY** **AND** **ICRS BANQUET** Casino de Salamanca Ballroom Palacio de Figueroa		

Notes:

Presenting Author*

DATABLITZ SESSION 3

DAY 4, THURSDAY, JULY 4TH: 16:15 - 16:45

Yan Wang*, Kimberly T. Sibille, Zhigang Li, Rene Przkora, Siegfried O. Schmidt, Margaret C. Lo, Ana M. Abrantes and Robert L. Cook	REAL-TIME EFFECTS OF MEDICAL CANNABIS ON OLDER ADULTS WITH CHRONIC PAIN: EARLY RESULTS FROM A PROSPECTIVE COHORT WITH CONTROL	DB3-1 [P3-5]
Harrison J. Elder*, C. Austin Zamarripa, Tory R. Spindle, Ethan Russo, George Bigelow and Ryan Vandrey	INDIVIDUAL AND INTERACTIVE EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL (THC) AND ALPHA-PINENE	DB3-2 [P3-6]
Nephi Stella*, Anthony English, Simar Singh, David Marcus, Fleur Uittenbogaard, Khushi Yadav, Ao Dong, Larry Zweifel, Yulong Li, Benjamin Land and Michael R. Bruchas	STUDYING THC-INDUCED IMPAIRMENT IN MICE: FROM NEW EXPERIMENTAL APPROACHES TO REAL TIME MONITORING OF 2-AG PRODUCTION AND AI ANALYSIS OF BEHAVIORS	DB3-3 [P3-8]
Taygun C. Uzuneser*, Matthew J. Jones, Mohammed H. Sarikahya, Dana Gummerson, Andrew Yates, Saoirse E. O'Sullivan, Daniel B. Hardy, Walter J. Rushlow and Steven R. Laviolette	INHIBITION OF FATTY ACID BINDING PROTEIN 5 PREVENTS STRESS-INDUCED ANXIETY AND DEPRESSIVE-LIKE BEHAVIOURAL SYMPTOMS AND REVERSES STRESS-INDUCED INHIBITION OF HIPPOCAMPAL NEUROGENESIS	DB3-4 [P3-7]

DATABLITZ presenters present *both* a 4-minute presentation *and* a poster at ICRS2024.

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Szabolcs Dvorácskó*, Resat Cinar and Malliga R. Iyer	*IN VIVO* EFFICACY OF NOVEL NEGATIVE BITOPIC/ALLOSTERIC CANNABINOID RECEPTOR 1 MODULATORS IN ALCOHOL DRINKING	P3-2
Kim S. Sugamori*, Claudia Lutelmowski, Catharine A. Mielnik, Ali Salahpour, Iain R. Greig and Ruth A. Ross	CB1 RECEPTOR NEGATIVE ALLOSTERIC MOLECULES: *IN VITRO* PHARMACOLOGY AND *IN VIVO* EFFICACY IN PRECLINICAL MOUSE MODELS OF HYPERDOPAMINERGIA	P3-3
Ashley Brooks-Russell*, Julia Wrobel and Sarah Limbacher	ACUTE USE OF LOW V. HIGH CONCENTRATION CANNABIS ON PSYCHOMOTOR PERFORMANCE AMONG ADULTS WITH DAILY V. MONTHLY USE	P3-4
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Notes:

Presenting Author*

ICRS2024 PRESIDENTIAL PLENARY LECTURE:
11:00 am, ICRS DAY 1, July 1st, 2024

ANTICANCER ACTIVITY OF CANNABINOIDS: PAST, PRESENT... AND FUTURE

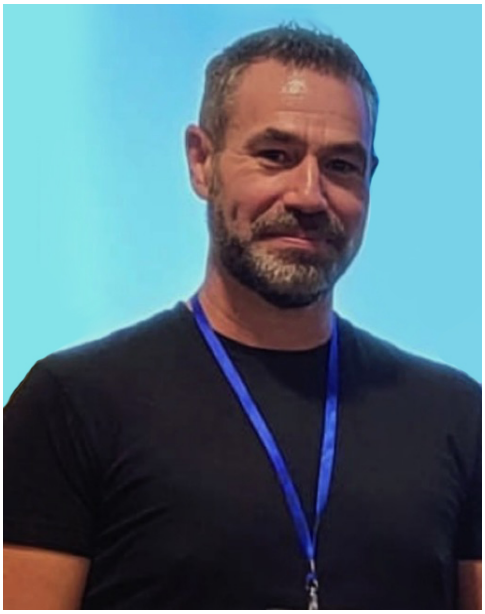
Manuel Guzmán, Ph.D.

Department of Biochemistry and Molecular Biology
Complutense University
Madrid, Spain

In addition to the well-known palliative effects of cannabinoids on cancer-associated symptoms, a large number of studies have shown that these compounds decrease tumor growth in animal models of cancer. They do so by activating cannabinoid receptors located on cancer cells, thereby triggering key signal-transduction pathways that reduce cell survival and proliferation. Cannabinoids can also inhibit angiogenesis, invasion and metastasis in various types of cancer models. In this talk I will make a historical tour of the contributions of our lab to the current understanding of cannabinoids as potential antitumoral agents, focusing on discoveries about their molecular mechanism of action in glioblastoma cells and including future opportunities for their use in combinational therapy. These and other preclinical findings have contributed to the development of clinical studies aimed to evaluate the safety and efficacy of cannabinoids as antitumoral agents in patients with malignant brain tumors.

2023 ICRS MID-CAREER AWARDEE:
2:00 pm, ICRS DAY 1, July 1st, 2024

TRANSLATIONALLY RELEVANT MODELS OF CANNABIS ADMINISTRATION TO RODENTS



Matt Hill, Ph.D.

Professor
Department of Cell Biology and Anatomy and Psychiatry
Hotchkiss Brain Institute
University of Calgary, Alberta, Canada

Understanding the biological impacts of cannabis, its potential therapeutic benefits and harms, has been challenging in humans due to both regulatory and experimental design limitations. Animal models represent an ideal approach to gain further understanding of the impacts of cannabis. Historically, work done using injections of synthetic cannabinoids was the proxy way to study the impacts of cannabis; however, with increased understanding of the pharmacodynamics and differential signaling mechanisms elicited by these drugs, their relevance to representing cannabis has become questionable. Studies utilizing THC, the psychoactive constituent of cannabis, are a better approach to understanding the impacts of cannabis, but recent work has also highlighted that the route of administration of THC can have a pronounced influence on its biological effects. In humans, inhalation and oral consumption represent the two primary modes of cannabis consumption. The overwhelming majority of rodent studies employ injection based approaches of THC, however with the recent introduction of both vapor based and oral consumption based approaches of THC administration to rodents have brought certain issues to light. This talk will cover the development of translationally based approaches to cannabis administration to rodents, how these parallel or differ from traditional injection based approaches, and how the scientific question being tested should determine which route of administration is utilized.

2023 ICRS LIFETIME ACHIEVEMENT AWARDEE:
8:30 am, ICRS DAY 2, July 2nd, 2024

CANNABINOID MEDICINES - PAST, PRESENT AND FUTURE

Geoffrey Guy, MB BS, LRCP MRCS, LMSSA, DipPharmMed, BSc, DSc

Founder and Chairman
The Guy Foundation, United Kingdom

Professor Geoffrey Guy is the founder of GW Pharmaceuticals and served as Chairman from 1998 up to its sale to Jazz Pharmaceuticals in 2021. Prof. Guy is the creator of Sativex and Epidiolex and continues to lead innovation and new product discovery through active involvement in scientific innovation, medical research and global drug development for his entire career. Prior to his role at GW, Prof. Guy served as Chairman and Chief Executive of Ethical Holdings plc, a NASDAQ-quoted drug delivery Company (now Amarin Corporation plc, or Amarin), which he founded in 1985 and led to its NASDAQ listing in 1993. He founded Phytopharm plc in 1989, of which he was Chairman until 1997, and led to its London Stock Exchange listing in 1996. He is now the Founder and Chairman of The Guy Foundation, a U.K. charity which promotes and funds research into Quantum Biology and cellular bioenergetics.

Prof. Guy's talk will cover the earliest references to cannabis in early writings and its subsequent uses as a medicine with special reference to the treatment of epilepsy. The timelines and development of cannabidiol (Epidiolex) for childhood catastrophic epilepsy syndrome will be summarised. Comparisons will be drawn between plant extracted and synthetic cannabidiol.

Geoffrey will the cover new areas of research into cellular bioenergetics and quantum effects in biology that are the focus of the Guy Foundation. These topics are rarely addressed in cannabinoid research but may offer novel avenues of enquiry in the future.

2023 ICRS MID-CAREER AWARDEE: 9:45 am, ICRS DAY 3, July 3rd, 2024

FILLING THE GAPS IN PHARMACOLOGICAL TREATMENT OF CHRONIC PAIN WITH SMART LIPIDS

Katarzyna Starowicz, Ph.D.

Department of Neurochemistry
Maj Institute of Pharmacology Polish Academy of Sciences
Krakow, Poland

Over the past 20 years, our understanding of anandamide (AEA) activation of both CB1 and TRPV1 receptors has significantly evolved. Initially recognized for its role in the endocannabinoid system by activating CB1 receptors, AEA was later found to also interact with TRPV1 receptors. Our studies have highlighted that AEA's dual action is complex; it can activate CB1 receptors to reduce pain, but it also engages TRPV1 [1-4]. Furthermore, the interplay between CB1 and TRPV1 receptors in various physiological processes, including inflammatory responses under chronic pain states, underscores the importance of AEA in modulating these pathways. This dual mechanism is being explored for therapeutic applications, particularly in conditions like arthritis [5-7].

Our research focuses on finding effective pain relief therapies. To achieve this, we are also exploring the endocannabinoid system's (ECS) functional redundancy, meaning multiple receptors and signaling pathways contribute to its regulatory functions. Exploring other receptors, such as CB2, TRPV1, and PPARγ, broadens our understanding of the ECS and enhances the development of targeted treatments for various conditions, including chronic pain and inflammation. This comprehensive approach maximizes therapeutic benefits and minimizes side effects [8-10].

Chronic pain often leads to cognitive impairments, affecting memory and executive function. These cognitive deficits can exacerbate feelings of depression, creating a vicious cycle where pain, cognitive decline, and depressive symptoms reinforce each other [11, 12]. Our group has also been characterizing AEA for its multifaceted role in both pain modulation and cognitive functions [13].

With every advance in our research, we gain a deeper understanding of the complex ECS. We are becoming increasingly aware of how intricate it is and recognize how much more there is yet to explore.

Acknowledgements: This work was supported by the FNP HOMING HOM/ed2007/14b; LIDER/29/60/L-2/10/NCBiR/2011; NCN 2012/07/E/NZ7/01269; NCN 2014/13/B/NZ7/02311, and the Maj Institute of Pharmacology PAS statutory funds.

References: [1-4]: J Neurosci. 2007;27(50):13739-49; Neuropharmacology. 2012;62(4):1746-55; PLoS One. 2013;8(4):e60040; Mol Cell Neurosci. 2015;65:1-10; [5-7] Pain. 2015;156(5):890-903; Pharmacol Res. 2016;111:251-263; Int J Mol Sci. 2018;19(2):342; [8-10] Biomed Pharmacother. 2021;136:111283; Pharmaceuticals 2021;14(10):964; Cannabis Cannabinoid Res. 2023;8(5):779-789; [11,12] Pharmacol Res. 2019;143:40-47.; Pharmacol Rep. 2024;76(1):72-85 ; [13] Neuropharmacology. 2023;222:109304.

2024 KANG TSOU MEMORIAL SPEAKER:
12:00 noon, ICRS DAY 3, July 3rd, 2024

CANNABINOIDS, STRESS AND PAIN: DOES IT OR DOES IT NOT BLOCK IT?

Rajita Sinha, Ph.D.

Foundations Fund Endowed Professor in Psychiatry
Professor of Neuroscience and of Child Psychiatry
Deputy Chair of Psychiatry for Psychology , Chief of Psychology Section
Director, Yale Interdisciplinary Stress Center, Yale University School of Medicine

Endocannabinoids have long been associated with stress coping and with pain reduction. Indeed stress and pain reduction is amongst the most common motivations for cannabis use. This presentation will delve into this link between cannabinoids, stress and pain and addiction with data from clinical and translational research to identify cannabis use/misuse related effects on the multi-level adaptive stress and pain responses peripheral and central stress biology and subjective and behavioral coping measures in controlled experimental studies. It will also identify key stress, trauma and drug related stress pathophysiology markers of risk of cannabis misuse and reduced efficacy of cannabis in reducing stress and pain. The second part of the lecture will present novel data on early phase development and testing of specific combination of cannabinoids with low addictive potential and doses and methods for reducing pain, anxiety and distress in humans. Implications for therapeutic development to target maladaptive stress responses associated with stress, trauma and addiction pathophysiology with cannabis based medicines to address stress and pain will be discussed.

ICRS2024 PRESIDENTIAL PLENARY LECTURE:
8:30 am, ICRS DAY 4, July 4th, 2024

THE ENDOCANNABINOID SYSTEM IN BRAIN AGING AND NEURODEGENERATION

Javier Fernández-Ruiz, Ph.D.

Instituto Universitario de Investigación en Neuroquímica, Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Complutense, Madrid (Spain); Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Madrid (Spain); Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid (Spain)

Several studies carried out in the last 20-30 years have strongly demonstrated that the endocannabinoid system exerts important modulatory functions in the brain that extend along the whole life of animals (including humans), with specific activities during the neurodevelopment (prenatal, postnatal and adolescent periods), the adulthood, and, according to more recent work, also in the senescence. In the aged brain, the activity of this system may be altered (frequently declined although sometimes becomes elevated depending on the age and/or the CNS structure), which contributes to subtle behavioural impairments that typically occur during aging in certain functions, such as learning and memory, motor activity, emotionality, social behaviour and others. Some of these changes in the endocannabinoid activity may represent an attempt to attenuate the age-related impairment in the brain function, which agrees with its proposed role as an endogenous protective system. Such behavioural impairment becomes apparently extreme when normal brain aging acquires pathological characteristics, as happens in chronic progressive neurodegenerative disorders, in which alterations of the endocannabinoid activity (e.g., CB_1 receptor downregulation, deficiencies in its signaling, increase in endocannabinoid-inactivating enzymes) may contribute to the progression of pathogenic events. By contrast, other responses (e.g., upregulation of CB_2 receptors, reduced FAAH and MAGL expression, elevated generation of endocannabinoids) may again serve as endogenous protective responses against brain damage. The present lecture will attempt to collect and update the available information on the status and function of the endocannabinoid system in both the senescent brain in absence of pathology and in the pathological aged brain. This will be followed by the analysis of the experimental evidence supporting the development of neuroprotective and neurorepair therapies based on the pharmacological management of specific endocannabinoid targets in chronic neurodegenerative disorders. The lecture will end with the presentation of some examples concentrated on the amyotrophic lateral sclerosis, in which recent preclinical studies have strongly demonstrated the neuroprotective potential of targeting CB_1 and CB_2 receptors as well as endocannabinoid-inactivating enzymes in experimental models of this disease, and the perspectives and needs for making possible to move these benefits to the clinical scenario.

Supported by grants from CIBERNED (CB06/05/0089) and MICINN (PID2021-128906OB-I00). The author is indebted to all those who participated in the studies carried out by his group that were mentioned in this lecture.

2023 ICRS MECHOULAM AWARDEE:
11:30 am, ICRS DAY 4, July 4th, 2024

CANNABINOID PHARMACOLOGY: THE SHAPE OF THINGS TO COME

Ruth A. Ross, Ph.D.

Department of Pharmacology and Toxicology
University of Toronto
Toronto, Canada

Since the discovery of the CB_1R allosteric site in 2005, and identification of Org275 as the first CB_1 negative allosteric modulator, work has progressed towards the further development of both CB_1 negative and positive allosteric modulators. The positive allosteric modulators have shown efficacy in models of pain and other therapeutic indications. We have recently gathered promising preliminary data in mouse models indicating the potential of CB_1 negative allosteric modulators in models of hyperdopaminergia (hylerDA) (e.g. psychosis, schizophrenia and bipolar disorder). CB_1 negative modulators attenuated the dysregulated behaviours in both a genetic and pharmacological model of hyperDA. This offers the prospect of amelioration of hyperDA states without direct antagonism of D2, which is associated with problematic side-effects. In line with this we find that increasing 2-AG pharmacologically via acute administration of a metabolism inhibitor leads to a pronounced exacerbation of hyperDA behavioural phenotypes and decreasing 2-AG with acute administration of a synthesis inhibitor ameliorates hyperDA behavioural phenotypes. These preliminary findings highlight the importance of CB_1, 2-AG, and its synthesis/degradation enzymes, as a key signalling hub associated with high DA pathologies. It is important to further elucidate the potential clinical implications of CB_1 and 2-AG modulation in hyperDA pathologies.

2023 ICRS WILLIAM A. DEVANE EARLY CAREER AWARDEE:
2:00 pm, ICRS DAY 4, July 4th, 2024

CANNABIS AND THE VULNERABLE BRAIN: IMPACT OF GENETICS, AGE, AND EMERGING ROUTES OF ADMINISTRATION

Jibran Khokhar, Ph.D.

University of Guelph
Ontario, Canada

Cannabis use and its long-term impacts remain critical areas of research amidst increasing legalization and shifting social norms. This talk will delve into three different aspects of cannabis neurobiology. First, we will explore the causal role of the Cadm2 gene in predisposing individuals to cannabis use, shedding light on genetic factors that may influence susceptibility. Next, we will examine the long-term impacts of vaporized cannabis flower exposure during adolescence on behavioural and brain network outcomes, focusing on different chemovars and their unique cannabinoid profiles. Through this, we aim to elucidate how varying chemical compositions can differentially affect neurodevelopment and behavior. Finally, we will present findings from a study characterizing withdrawal symptoms in rats exposed to cannabis flower vapor, providing insights into the physiological and behavioral challenges associated with abstinence. Collectively, these studies will offer a comprehensive understanding of the genetic, developmental, and withdrawal-related facets of cannabis use, with a special focus on routes of administration and pharmacokinetics.

DISCOVERY OF ALLOSTERIC MODULATORS FOR MONOACYLGLYCEROL LIPASE VIA THE INTEGRATION OF VIRTUAL SCREENING, LC/MS/MS, KINETICS, AND NMR SPECTROSCOPY

Sergiy Tyukhtenko*[1], Michael Speziale[1], Huixi Li[1], Xiaoyu Ma[1], Olga Tarkhanova[2], Iryna Kravets[3], Dmytro Dudenko[3], Yurii Moroz[3] and Alexandros Makriyannis[1]

[1]Center for Drug Discovery, Northeastern University, Boston, MA, USA;
[2]Chemspace LLC, NJ, USA; [3]Enamine Ltd, Kyiv, Ukraine

Introduction: The discovery of allosteric modulators offers potential for new therapeutics against enzymes implicated in diseases such as pain, inflammation, and neurodegenerative disorders. However, numerous scientific challenges accompany their discovery and development. Addressing these challenges requires interdisciplinary approaches integrating virtual screening, medicinal chemistry, and biophysical methods. Here, we present the identification of an allosteric binding pocket in the gate region of Monoacylglycerol Lipase (MGL), along with the discovery of lead compounds demonstrating allosteric activity. These findings came as a result of successful integration of existing structural data, Virtual Screening Pipeline (VSP), Mass spectrometry (LC/MS/MS), Michaelis-Menten kinetics, and Nuclear Magnetic Resonance (NMR) Spectroscopy.

Methods: Initial Screening includes virtual screening of 1.3 million molecules from the Enamine in-stock collection, high-throughput docking to identify frequent binding modes and exclusion of compounds not matching binding pocket criteria. At the Docking Model Refinement stage, the top 100,000 molecules were further docked using three models for specific sub-pockets and selection was done based on docking score, hydrogen bond criteria, and key residue interactions. Expansion of Hit Selection was achieved by alignment-free USRCAT method applied to Enamine collection. 17 compounds with shape similarity were selected. A total of 23 compounds, including six hits from docking screens and 17 from shape similarity analysis, underwent validation via LC/MS/MS to assess their affinity and activity against hMGL using enzyme and binding assays. Validation of predicted ligand binding modes, identification of allosteric binding, and gaining insights into the mechanism of action were achieved through Michaelis-Menten kinetics and NMR experiments.

Results: Analysis of hMGL structures from PDB revealed an enlarged primary binding pocket, prompting a virtual screening that identified 23 potential inhibitors for further assessment of their affinity and activity. 3 out of 23 compounds exhibit exceptional activity towards hMGL, with IC_{50} values of 0.4, 5.0 and 200 μM. The effects of varying inhibitor concentrations on V_{max} and K_m provide compelling evidence for the mixed binding mode of identified hits, suggesting an allosteric modulation of hMGL consistent with their calculated binding mode. Target-based and ligand-based NMR binding experiments confirmed the binding of 3 validated hits to the allosteric binding pocket. All acquired data strongly indicate that allosteric modulators bind to the site in the gate region of hMGL lid domain, inducing conformational changes that propagate through this domain, reducing substrate affinity and impacting enzyme activity.

Conclusions: Employing cutting-edge structure-based drug design technologies we have identified promising allosteric modulators targeting hMGL. The remarkable activity exhibited by these modulators suggests their potential as lead candidates for therapeutic development. Our findings not only confirm the presence of an allosteric binding pocket within the gate region of the hMGL lid domain but also provide crucial insights into their mechanism of action. Further optimization and mechanistic studies are warranted for clinical translation.

THE NON-CANNABIS DERIVED PHYTOCANNABINOID-LIKE COMPOUNDS AMORFRUTIN 2 AND MAGNOLOL EXHIBIT ANTI-SEIZURE EFFECTS IN *SCN1A* $^{+/-}$ MOUSE MODEL OF DRAVET SYNDROME

Ka Lai Yip*[1,2], Michael Udoh[1,2], Thomas S. Harman[1,2], Lyndsey L. Anderson[1,2], Samuel D. Banister[1,2] and Jonathon C. Arnold[1,2]

[1]Lambert Initiative for Cannabinoid Therapeutics, The University of Sydney, Australia
[2]Discipline of Pharmacology, School of Pharmacy, Faculty of Medicine and Health, The University of Sydney, Australia

Introduction: Following the clinical approval of cannabidiol (CBD), recent studies have shown that additional phytocannabinoids including cannabigerolic acid (CBGA), a major biosynthetic precursor in cannabis, has anti-seizure effects in preclinical models. Hindering its further drug development, CBGA has suboptimal drug-like properties and is not synthetically tractable. Interestingly, CBGA is structurally analogous to amorfrutin 2, a compound found in the legume *Glycyrrhiza foetida*. Amorfrutin 2 has better synthetic tractability than CBGA, but its effect in seizure models is unknown. Therefore, we aimed to assess its anti-seizure activity in preclinical models. Other non-cannabis cannabinoid-like molecules derived from natural products, such as magnolol and honokiol, are also of interest given their increased synthetic tractability. These molecules resemble biaryl cannabinoids and the synthetic cannabinoid CP 55,940. We then chose to examine the anti-seizure activity of these compounds in preclinical seizure and epilepsy models. Given that phytocannabinoids inhibit T-type calcium channels which may contribute to their anti-seizure effects, we also examined the effects of amorfrutin 2, honokiol and magnolol at T-type Ca_V 3.1, Ca_V 3.2 and Ca_V 3.3 channels.

Methods: The compounds were first tested for activity against hyperthermia-induced generalized tonic-clonic seizure (GTCS) seizures in the *Scn1a*$^{+/-}$ mouse model of Dravet syndrome, an intractable childhood epilepsy. We also examined anti-seizure effects against GTCS in the maximal electroshock seizure (MES) model in mice. Additionally, we investigated the compounds actions on T-type calcium channels using HEK T-rex cells stably expressing Ca_v 3.1, Ca_v 3.2, or Ca_v 3.3 channels via patch clamp electrophysiology.

Results: Consistent with an anti-seizure effect, amorfrutin 2 dose-dependently increased the body temperature threshold against GTCS at a dose of 30 and 100 mg/kg. Magnolol also was effective against hyperthermia-induced seizures but only at 100 mg/kg. Honokiol was ineffective at the doses tested (10-100 mg/kg). In the MES model, 100 mg/kg but not 30 mg/kg of amorfrutin 2 and magnolol protected against hindlimb extension consistent with anti-seizure activity. Both magnolol and amorfrutin 2 inhibited Ca_v3.1, Ca_v3.2 and Ca_v3.3 channels with low micromolar potency.

Conclusions: The study demonstrates for the first-time anti-seizure activity of the phytocannabinoid-like compounds magnolol and amorfrutin 2 in a mouse model of drug-resistant epilepsy. We also provide unprecedented evidence that these compounds inhibit T-type channels, suggesting a new potential mechanism for their anti-seizure effects. These compounds and their synthetic analogues could be further developed in drug discovery campaigns aiming to introduce novel anti-seizure agents for Dravet syndrome.

KLS-13019, A NOVEL STRUCTURAL ANALOGUE OF CANNABIDIOL (CBD) AND GPR55 RECEPTOR ANTAGONIST, PREVENTS AND REVERSES CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY (CIPN) IN RATS.

Michael Ippolito*, William Kinney, Douglas E. Brennemen and Sara Jane Ward

Center for Substance Abuse Research, Temple University, Philadelphia PA, USA

Introduction: Neuropathic pain is a form of chronic pain that develops as a consequence of damage to the nervous system. Treatment of neuropathic pain is often incompletely effective, and most of the available therapeutics have only moderate efficacy and present side effects that limit their use. Opioids are commonly prescribed for the management of neuropathic pain despite equivocal results in clinical studies and significant potential for addiction and abuse. Thus, neuropathic pain represents an area of critical unmet medical need to be addressed by the global medical and pharmaceutical communities. Novel classes of therapeutics with improved efficacy and safety profiles are urgently needed for the treatment of neuropathic pain.

Methods: Novel antagonist of GPR55, KLS-13019, was screened in rat models of neuropathic pain with three dosing strategies: acute reversal, chronic reversal, and prevention. Tactile sensitivity associated with chemotherapy exposure was induced in rats with once daily 1mg/kg paclitaxel injections for 4 days or 5 mg/kg oxaliplatin every third day for one week. Rats were then administered KLS-13019 or comparator drugs on day 7 in an acute dosing paradigm or days 7-10 in a chronic dosing paradigm and mechanical or cold allodynia was assessed. Five classes of comparator drugs were assessed: phytocannabinoids, morphine, clinically used drugs for CIPN, and commercially available GPR55 ligands. In the prevention paradigm, KLS -13019 was co-administered with paclitaxel and mechanical allodynia was assessed for up to 6 weeks.

Results: Allodynia was reversed in a dose dependent manner in the rats treated with KLS-13019, with the highest dose reverting the response to pre-paclitaxel injection baseline levels in both oral and intraperitoneal administration in males and female animals. With chronic dosing, allodynia was reversed with both doses tested for the entire duration of the CIPN phenotype in males and females if administered shortly after chemotherapy treatment. Shifting this window of intervention to a later timepoint resulted in reversal but was not permanent. In the prevention paradigm, allodynia did not develop in the rats who received KLS-13019 during paclitaxel treatment.

Conclusions: Together, these data suggest that KLS-13019 represents a new class of drug that would be potentially useful for the treatment of neuropathic pain, and these animal models in combination with molecular techniques will describe the role of GPR55 in neuropathic pain to provide the proof-of-concept for a novel therapeutic strategy for this affliction. Here we show that KLS-13019 can promote a durable reversal and prevention of neuropathic pain symptoms with better performance than comparator drugs tested here.

2-AG IS RELEASED AND TRANSPORTED VIA EXTRACELLULAR VESICLES

Verena Straub[1], A. Floor Stevens[1], Tom van der Wel[1], Na Zhu[1], Yulong Li[2], Nephi Stella[3] and Mario van der Stelt*[1]

[1]Dept. Molecular Physiology, Leiden University, The Netherlands; [2]Chinese Institute for Brain Research, China; [3]Department of Pharmacology, University of Washington School of Medicine, USA

Introduction: 2-AG acts as a retrograde messenger produced by the postsynaptic neuron upon depolarization or activation of metabotropic glutamate receptors. It traverses the synapse and activates presynaptic cannabinoid CB_1 receptors (CB_1R), thereby modulating neurotransmitter release, synaptic plasticity, and behaviours. Diacylglycerol lipase-α, a biosynthetic enzyme of 2-AG, plays a crucial role in this 'on-demand' signalling**.** While we understand many features of 'on-demand' endocannabinoid signaling model, it falls short in providing a molecular understanding of 2-AG released by neurons and implications in tonic CB_1R signaling. Hence, a deeper comprehension of the molecular processes governing endocannabinoid release and transport is required.

Studying endocannabinoid dynamics has, however, been challenging due to the lack of assays capable of tracking endogenous release and transport in a spatially, quantitatively, and time-resolved manner. Liquid chromatography coupled to mass spectrometry and the use of radiolabeled endocannabinoids have been pivotal techniques for measuring endocannabinoid levels in the context of their transport; however they provide snapshot results and fail to capture the spatiotemporal and cellular specific nature of endocannabinoid signaling.

Recently, a genetically encoded fluorescent sensor, $GRAB_{eCB2.0}$, has been developed for spatiotemporal resolved imaging of endocannabinoid dynamics in cells and behaving animals. It allows to monitor the evoked and spontaneous changes in endocannabinoid levels in the brain of freely moving mice. Inspired by this emerging powerful technique, we describe herein the use of the $GRAB_{eCB2.0}$ to study the molecular processes responsible for the paracrine endocannabinoid release and transport.

Methods: We developed an assay in which neuronal cells are co-cultured with HEK293T cells that stably express $GRAB_{eCB2.0}$. Coupled with detailed biochemical analysis, targeted lipidomics, proteomics, pharmacological interventions, and confocal microscopy monitoring, our approach enabled to unravel the molecular mechanism of real-time endocannabinoid release.

Results: Our results demonstrate that acute stimulation of mouse neuroblastoma Neuro2A cells with ATP and primary mouse hippocampal neurons with an mGluR1/5 agonist triggers the release of 2-AG (but not anandamide) via extracellular vesicles derived from the plasma membrane. The release of these vesicles was inhibited by interfering with ADP-ribosylation factor 6 (ARF6) function and by blocking 2-AG production using DAGL inhibitor DH376. Upon an ATP stimulus, an average of 25 vesicles were released per cell and each vesicle contained approximately 2600 molecules of 2-AG. Inhibitors of endocannabinoid transport and FABP5 reduced sensor activation, whereas Protein Kinase C inhibition enhanced sensor activity.

Conclusion: ATP or glutamate-induced activation of neuronal cells increases the production and release of 2-AG via extracellular vesicles derived from the plasma membrane, a molecular mechanism that involves kinase and ARF6 signaling. Significantly, 2-AG is incorporated into extracellular vesicles via an unknown process, possibly involving the unidentified endocannabinoid transporter and FABP5.

ROLE OF CANNABINOID RECEPTOR 2 IN THE BREAST TUMOR MICROENVIRONMENT

María Rubert*[1], Marta Seijo-Vila[1], Isabel Tundidor[1], Sandra Blasco-Benito[2], Cecilia J. Hillard[3], María Teresa Grande[4], Julián Romero[4], Cristina Sánchez[1] and Eduardo Pérez-Gómez[1]

[1]Department of Biochemistry and Molecular Biology, School of Biology, Complutense University, and Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), Madrid, Spain. [2]Cancer Research Program, Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain. [3]Neuroscience Research Center, Medical College of Wisconsin, 53226, Milwaukee, WI, USA. [4]Faculty of Experimental Sciences, Universidad Francisco de Vitoria, 28223, Pozuelo de Alarcón, Madrid, Spain.

Introduction: Breast cancer (BC) is a heterogeneous disease in which cancer cells establish complex and dynamic interactions with their surrounding stroma (tumor microenvironment, TME), which determines tumor progression and response to therapy. In oncology, cannabis is used with therapeutic purposes due to its palliative properties, and potential anti-tumoral actions have been also described in preclinical models of this disease. Specifically in BC, cannabinoid receptor 2 (CB_2R) is overexpressed by BC cells and associated with poor patient prognosis, and its activation by cannabinoids (CBs) triggers antitumor effects. On the other hand, immune cells, one of the main components of the TME, are known to express CB_2R, whose activation has been linked to immunosuppressive actions. Here, we aimed to analyze the contribution of this receptor in each of these compartments to tumor generation and response to CBs.

Methods: We analyzed the expression of CB_2R in the BTME of tumors generated by orthotopic injection of mouse BC cells into immunocompetent female mice (CB_2R-GFP mouse model), and the consequence of its genetic inactivation on tumor progression (CB_2R-KO mouse model). For a deeper understanding of the specific role of CB_2R expressed in each cell population, we performed *in vitro* experiments with mouse BC cells and CB_2R^+-immune cells, alone or in co-culture, modulating the activity of the receptor in each cell compartment (with different cannabinoid agonists and antagonists) and analyzing the corresponding functional impact in terms of cell viability (determined by crystal violet assay) and immunosuppressive capacity (determined by immunofluorescence characterization of specific markers).

Results: We found that immune cells are the main CB_2R^+-infiltrating population of the BTME and that the lack of the receptor in the stroma tended to reduce tumor growth and alter the TME composition. In cell cultures, all CB_2R ligands tested decreased BC cell viability in a concentration-dependent manner and reduced the killing capacity of immune cells, possibly due to the induction of an immunosuppressive phenotype. However, when both cell types were co-cultured, the impact of the compounds on the viability of BC cells was still observed, suggesting that the contribution of CB_2R in the cancer cell compartment outweighs that of the immune population.

Conclusion: Our preliminary results indicate that CB_2R is expressed in different cell compartments in the TME, having a different function in each of them. This cell-type specific effect should be considered when combining therapies based on cannabinoid ligands with antitumor treatments focused on reactivating the immune system to kill or control tumor cells (i.e. immunotherapy).

ART26.12, A NOVEL FATTY ACID-BINDING PROTEIN 5 INHIBITOR, SHOWS EFFICACY IN BREAST CANCER-INDUCED BONE PAIN

William George Warren, Myles Osborn, Andy Yates and Saoirse E O'Sullivan*
Artelo Biosciences Limited, Mereside, Alderley Park, Alderley Edge, UK

Introduction: Inhibitors of fatty acid binding protein 5 (FABP5) are effective in multiple models of pain, inhibited by antagonists of CB_1, TRPV1 and PPARα. The potent (Ki 0.77 ± 0.08 µM) and selective FABP5 inhibitor ART26.12 is under development at Artelo Biosciences under a licence agreement with Stony Brook University (Warren et al., 2024). The aim of the present study was to establish a potential role for ART26.12 in an as yet untested neuropathy; cancer-induced bone pain.

Methods: On day 0, murine breast cancer cells were injected into the tibial bone cavity of female Sprague Dawley Rats. On day 15, rats were randomly assigned to groups (n=10/group) using a computer-generated randomization procedure based on body weight and baseline Von Frey (VF) measurement (pain behavior assay). Animals were treated orally with ART26.12 (25 and 100 mg/kg BID) for seven days. VF measurements were taken 1 and 4 h post-dosing on days 1, 3, 5 and 7 of drug treatment.

Results: On day 14, all groups animals had reduced VF values, indicating the induction of neuropathy (see Fig 1). On the first day of test drug treatment, pain behaviour was significantly improved by oral treatment with ART26.12 at 100 mg/kg at 1 and 4 h post-dosing (Fig 1B). On day 3, pain behaviour was significantly improved by ART26.12 (25 and 100 mg/kg) at 1 h post-dosing. On days 5 and 7, pain behaviour was significantly improved by 25 mg/kg at 1 and 4 h post-dosing; however, the 100 mg/kg dose was no longer effective. Terminal plasma samples taken 4 h post-dosing (day 21) show a mean ART26.12 plasma level of 13.5 ± 1.5 µM and 46.3 ± 8.1 µM in the 25 and 100 mg/kg groups respectively. Bone mineral density was not affected by drug treatment.

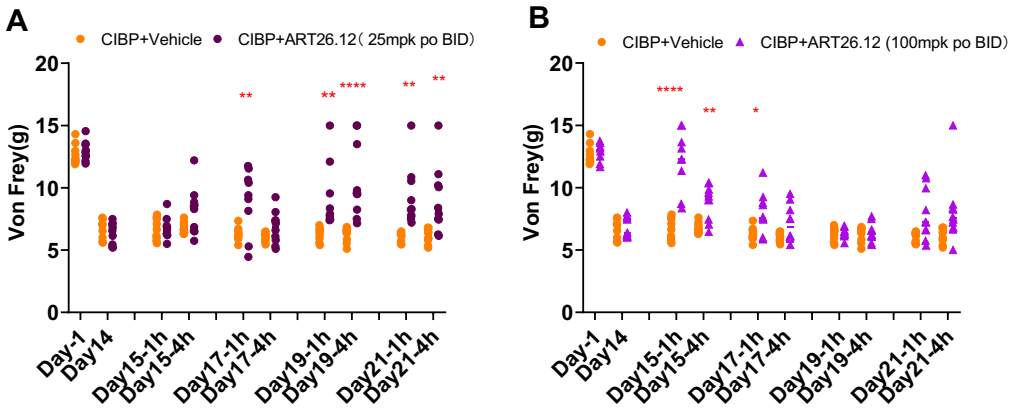


Figure 1. The effects of the FABP5 inhibitor ART26.12 in breast cancer induced bone pain (CIBP). Animals were dosed twice daily at 25 (A) and 100 (B) mg/kg p.o. for seven days with pain behaviour assessed using Von Frey filaments.

Conclusions: In a model of breast cancer induced bone pain, ART26.12 reduces pain behaviours. At the higher dose, the effect is rapid, but reduces over time. At 25 mg/kg, an analgesic effect is apparent on day 3 and persists through the 7-day treatment. This study continues to support the ongoing development of ART26.12 as a novel, non-opioid, non-steroidal analgesic effective in multiple models of pain, now including chemotherapy-induced neuropathy (oxaliplatin or paclitaxel), diabetic neuropathy, and cancer-induced neuropathy, in male and female rodents.

Reference: Warren et al. (2024) Discovery and preclinical evaluation of a novel inhibitor of FABP5, ART26.12, effective in Oxaliplatin-induced Peripheral Neuropathy. Journal of Pain doi: 10.1016/j.jpain.2024.01.335.

CANNABIDIOL AND MANAGEMENT OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY: A RANDOMIZED DOUBLE BLIND CONTROLLED TRIAL

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Background: Chemotherapy-induced peripheral neuropathy (CIPN) is a disabling pain syndrome associated with cytotoxic agents that can impair quality of life and impede patients' ability to complete curative therapy. Most treatment approaches provide insufficient pain relief. Our laboratory has shown that cannabidiol (CBD) can prevent CIPN-like symptoms in rodents treated with taxane- or platin- based chemotherapeutic agents. The goal of the present study was to assess the effect of CBD gel capsules in human participants on symptoms of CIPN.

Methods: A randomized double-blind, placebo-controlled clinical trial was conducted between 6/1/2020 and 8/8/2022. Participants with nonmetastatic breast, colorectal, endometrial, and ovarian cancer (all stages) experiencing grade 2-3 CIPN (duration ≤ 2 years) were enrolled after completing taxane or platin-based chemotherapy. The active group received 135 mg/day of CBD full spectrum gelcap generously provided by Ananda Health, or placebo, delivered for 12 weeks, followed by a four-week washout period. Participants were evaluated every two weeks via neurological exam, and questionnaires for CIPN symptoms, QOL, pain, and sleep. Following a washout period, a follow-up open-label observational study was performed for 12 weeks where all participants had the option to take 30 mg CBD daily.

Results: Of 230 participants with CIPN identified, 124 met eligibility, 54 were consented and enrolled, and 46 completed ≥8 of the 12-week treatment phase and were included in the analysis. Mean age was 59.6 years; 89.1% were female. Of those participants, 63% had breast cancer, 19.6% colorectal, 15.2% ovarian, and 2.2% uterine. No differences in outcome measures were seen on primary analysis. On post-hoc analysis, the active CBD group experienced greater improvement in overall sensory symptoms relative to placebo, particularly for symptoms of numbness and tingling. There was no difference in pain or motor function. CBD was well tolerated. N=30 who completed the RCT enrolled in a follow-up open-label observational study for 12 weeks and results showed greater improvement in sensory, motor, and pain symptoms for participants who had received active CBD during both the RCT and observational periods.

Conclusions: This is the first randomized double-blind, placebo-controlled trial of CBD on CIPN that we are aware of. Although primary analysis showed no difference between groups, results on overall sensory symptoms and results from the follow-up observational study provide evidence that CBD may ameliorate sensory CIPN symptoms without serious adverse events.

UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS OF MEDICAL CANNABIS FOR CHRONIC CANCER PAIN

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Introduction: Cancer is one of the most prevalent non-communicable diseases, and its incidence is rising. An estimated 44.5% of cancer patients experience pain. Pre-clinical studies have how cannabinoids and terpenes can affect the transmission of nociceptive signals, as well as the emotional and cognitive manifestations of pain. However, there is a paucity of clinical translation. This is due to the paucity of high-quality clinical trials investigating CBMPs in the setting of chronic cancer pain. This study therefore aimed to examine the reported change in validated patient-reported outcome measures (PROMs) and prevalence of adverse events in individuals prescribed CBMPs for chronic cancer pain.

Methods: This case series was conducted using data from the UK Medical Cannabis Registry. The inclusion criteria included those treated for chronic cancer pain and enrolled on the registry for a minimum of 18 months. Demographic data was captured during initial clinical consultation and data on CBMPs was collected throughout treatment. The primary outcome was change in validated PROMs [Brief Pain Inventory-Short Form (BPI-SF), pain visual analogue scale (Pain-VAS), Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L] from baseline to 1, 3, 6, 12, and 18 months. Missing data was handled using a baseline observation carried forward methods. The common terminology criteria for adverse events v4.0. was used to record adverse events. Statistical significance was defined by a p-value<0.050.

Results: Forty patients met the inclusion criteria for the study, 5 (12.50%) of who were deceased at the end of follow up. The mean age was 52.93 ± 13.62. Twenty-four (60.00%) patients were male. Eighteen (45.00%) patients were already consuming cannabis at baseline, whilst 22 (55.00%) were either cannabis naïve or were ex-users. Improvements in pain severity and interference were observed across the BPI-SF subscales at 1 month only ($p<0.050$). There were also improvements in the GAD-7, EQ-5D-5L, and SQS at 1 month ($p<0.050$), but no other periods beyond this ($p>0.050$). Adverse events were reported by 4 (10.00%) participants.

Conclusions: Contrary to other evaluations of chronic pain conditions from the UK Medical Cannabis Registry, the present analysis only shows improvement in validated measures of pain severity, intereference, anxiety, sleep and general health-related quality of life at 1 month. All subsequent follow up scores until 18 months are not statistically different from baseline. This is, however, in keeping with the findings from an analysis from a cohort of individuals who were prescribed CBMPs as a component of wider palliative care. These results may be counfounded by the natural progression of disease. Conversely, whilst other examinations of chronic pain patients from the UK Medical Cannabis Registry have been largely positive, chronic pain is a heterogenous condition and CBMPs may be less efficacious in addressing chronic cancer pain. Randomised controlled trials incorporating a control arm will ultimately be necessary to determine the true efficacy of the CBMPs in chronic cancer pain.

A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED, WITHIN-SUBJECT CROSSOVER STUDY OF THE EFFECTS OF COMBINATIONS OF CANNABINOIDS AND CAFFEINE

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Introduction: Cannabis and caffeine are two of the most commonly consumed substances in the world, however, data on drug interactions are limited. Preliminary evidence suggests that THC, CBD, and caffeine may interact to alter pharmacodynamic effects. It is unclear if these interactions are observed in low dose ranges often occurring in recreational and clinical use as well as impacts on a diverse battery of pharmacodynamic effects. This double-blind, randomized, placebo-controlled, within-subject crossover study evaluated the effects of combinations of THC, CBD, and caffeine on subjective drug effects, arousal, and cognitive performance. The doses used in this study were selected based on commercial use of comparable products.

Methods: Participants (N=14; 9 female) with infrequent cannabis use (lifetime, but no past month use) completed four outpatient experimental sessions. Drug was administered double-dummy with THC (2.5 mg), caffeine (60 mg), or oral placebo capsules and a syringe with CBD (35 mg) in MCT oil or MCT oil alone. Doses were administered three times each session with 60 minutes separating each administration for total cumulative doses of 7.5 mg THC, 180 mg caffeine, and 105 mg CBD. The four experimental conditions were randomized combinations of THC, CBD, and caffeine (i.e., placebo only; THC only; THC + caffeine; THC + CBD + caffeine). Participants completed measures of subjective drug effects, cognition, and performance during the 7-hour session. Peak change from baseline for measures were analyzed using generalized linear mixed effect models.

Results: A significant effect of condition was observed for overall Drug Effect with all active doses producing higher peak ratings relative to placebo, p values < .001. The THC + CBD + caffeine condition also produced significantly higher peak drug effects relative to THC alone, $p = .02$, $d = 0.74$. These effects were related to greater ratings of Restlessness, $p = .02$, $d = 0.73$, and Jittery, $p = .04$, $d = 0.59$, in the THC + CBD + caffeine condition compared to THC alone. Generalized impairments in psychomotor vigilance were not observed on the Digit Symbol Substitution Task (DSST), however, perceived impairment in task performance was observed with significantly greater perceived impairment in the THC + CBD + caffeine condition compared to THC alone, $p = .02$, $d = 0.69$.

Conclusions: These findings are consistent with data using larger doses (e.g., 20 mg THC + 640 mg CBD) demonstrating stronger drug effects elicited by THC and other medications when combined with CBD. Further data evaluating cannabinoid-drug interactions are needed to inform clinical and regulatory decision-making.

Acknowledgements: Funded by Canopy Growth Corporation.

THE IMPACT OF ORAL CANNABIS ("EDIBLES") INGESTION AND CO-USE OF ALCOHOL ON ACUTE DRIVING AND BEHAVIORAL IMPAIRMENT, SUBJECTIVE DRUG EFFECTS, AND CANNABINOID PHARMACOKINETICS

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Introduction: Oral cannabis products (or "edibles") are increasing in popularity. Cannabis edibles are often co-used with alcohol, but there is limited controlled research to understand how this drug combination acutely impacts driving performance, other pharmacodynamic measures (e.g., subjective drug effects), or cannabinoid pharmacokinetics. This ongoing human laboratory study is evaluating the individual and interactive effects of cannabis edibles and alcohol.

Methods: Healthy adults (n=12) completed seven, Latin-square-ordered, double-blind, double-dummy sessions. In each session, participants ingested a brownie containing whole plant cannabis with 18% THC (doses: 10 or 25mg THC) or a placebo cannabis brownie and, 45 min later, drank an alcoholic beverage (target breath alcohol concentration, BAC: 0.05%) or a placebo beverage with sensory cues of alcohol; a positive control session (0.08% BAC with placebo cannabis) was also completed. Study assessments, which were collected before and for 7.5 hrs after cannabis use, included simulated driving performance, field sobriety tests, subjective drug effects (positive/negative effects; perceived impairment), the DRUID (tablet-based cognitive/psychomotor impairment test), and pharmacokinetics of THC/metabolites in blood. Driving performance was evaluated on several individual tasks and at a global level by integrating outcomes (e.g., lane weaving, speed deviation, reaction time) from these tasks.

Results: When cannabis and alcohol were used alone, dose-dependent increases in subjective impairment (e.g., "confidence to drive" ratings) and decreases in global driving performance were observed. Alcohol (0.05% BAC) with 10mg or 25mg THC produced additive effects on these measures. 25mg THC + 0.05% BAC produced virtually identical driving impairment to alcohol alone at 0.08% BAC. In all active dosing conditions, driving impairment peaked at 3.5 hrs post-cannabis dosing (2.5 hrs post-alcohol) and fully subsided by 7.5 hrs. At 10mg THC alone, participants' perceived level of impairment was low (comparable to placebo), but they still displayed driving impairment (slightly higher than alcohol alone at 0.05% BAC). Impairment on field sobriety tests was consistently detected at 0.08% BAC and 0.05% BAC+25mg THC but was less evident in other conditions, including cannabis alone and 0.05% BAC. DRUID scores differed between placebo and active dosing conditions, but scores were generally lower and less dose-orderly than prior studies. Alcohol did not alter THC or THC metabolite pharmacokinetics. THC concentrations were far lower than those typically seen after cannabis inhalation.

Conclusions: These data demonstrate that cannabis edibles and alcohol have additive effects on impairment and that individuals under the influence of cannabis can be poor judges of their fitness to drive. Results also highlight the need to conduct further research to develop and validate methods for detecting cannabis-impaired drivers and to elucidate possible unique impairment profiles of individuals under the influence of multiple substances.

Acknowledgements: Funded by the National Institute on Drug Abuse (NIDA) R01-DA052295.

PRENATAL OVERACTIVATION OF CB_2 RECEPTOR AFFECTS OFFSPRING GERM CELLS DEVELOPMENT

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Introduction: Cannabis/cannabinoids exert their biological effect by activating two main cannabinoid receptors: CB_1R and CB_2R. We previously demonstrated that CB_2R is expressed at high level in fetal male and female germ cells. *In vitro* overactivation of CB_2R in fetal male and female germ cells by using JWH133, a selective CB_2R agonist, promotes meiotic entry. Gonocytes initiate the meiotic program but become arrested at early stages of prophase I, while oocytes show an increased rate of meiotic entry and progression toward more advanced stage of meiosis. The process of germ-cell development is regulated by both genetic and epigenetic mechanisms. Recent evidence indicates that exposure to cannabinoids induces genome-wide histone modifications and DNA methylation in several cell types including germ cells. Epigenetic modifications in germ cells can be transmitted to the offspring. In this study, we focused on the impact of *in utero* exposure to CB_2R agonist on offspring male and female germ cell development and on the underlying molecular mechanisms.

Methods: Five pregnant CD-1 female mice were intraperitoneally injected with a single dose of JWH-133, at 1.5 mg/kg for 5 days in the time window from E12.5 to E16.5. During this embryonal period male germ cells enter in a quiescence state, while oogonia initiate meiosis. After birth pups were analyzed at post-natal day (PND) 2, 30 and 365. Testes and ovaries from control and *in utero* exposed mice were morphologically analyzed.

Results: Male and female pups from *in utero* exposed mice showed a significant reduction in the body weight at birth.

Histological analysis of the testes at PND 2 showed no differences between control and *in utero* exposed mice, while at PND30, when the first wave of spermatogenesis was already completed, a delay of the differentiative process induced by JWH-133 was found. This result was confirmed by immunofluorescence staining with anti-Acrosin antibody showing the absence of sperm cells in the exposed testes. Interestingly, the delay was associated with an increase of the repressive mark H3K27me3 level in $Plzf^+$ spermatogonia stem cells.

Histological analysis of the ovaries, isolated from newborn females (PND2), showed a significant reduction of about 17% in the primordial and primary follicles in female prenatally exposed to JWH-133 respect to control ovaries. Furthermore, the follicle number declined along lifetime leading to a reduction of the ovarian reserve through apoptotic mechanisms. At adult age, these females *in utero* exposed to JWH133 showed reduced fertility and reproductive lifespan.

Conclusions: Altered CB_2R signalling during fetal life can impair male and female germ cell development. Prenatal exposure to JWH-133, in males, determines a delay of germ cells differentiation associated to an increase of epigenetic repressive mark H3K27me3 in spermatogonia stem cells ($Plzf^+$), while, in females, reduces the ovarian follicles reserve at birth and at adulthood causing a shorter reproductive lifespan.

AN INHALED MODEL OF PRENATAL CANNABIS EXPOSURE IN RODENTS: EFFECTS ON NEURAL-, IMMUNE-, AND ENDOCANNABINOID-SYSTEM DEVELOPMENT, AND LATER-LIFE BEHAVIOUR

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Introduction: Growing evidence suggests that up to 20% of people report use of cannabis during pregnancy, with many individuals and health professionals considering cannabis natural and safe. However, both clinical studies and animal models of prenatal cannabis exposure (PCE) have shown inconsistent results. Some clinical studies have shown low birth weight, growth retardation, increased incidence of autism or social behaviour deficits, immune changes, and cognitive dysfunction. However, other studies have failed to find these or any associations. Clinical studies are confounded by unknown timing and exposure level, as well as exposure to stress and other drugs. Importantly, animal models can control for these variables and some studies have replicated clinical findings. However, many animal models utilize injection administration, which is leads to extremely high exposure levels that are not translatable to humans. Our studies aim to characterize the effects of inhaled cannabis exposure during pregnancy on a wide range of domains. Further, as cannabis exerts its effects through acting on the endocannabinoid (eCB) system, which is involved in many processes of brain development and little is known about the mechanism through which PCE may alter neurodevelopment and subsequent behaviour, our studies also aim to determine if PCE acts through direct modulation of the eCB system and/or indirect modulation of other systems, such as the immune or stress-response systems.

Methods: Utilizing a validated vapor chamber system pregnant rats were exposed to THC-heavy cannabis vapour or vehicle vapour for 15-min a day from gestational day (GD) 1 to G21. Our experiments had three endpoints: 1) fetal development, 2) postnatal development, and 3) adolescence and adulthood. In aim 1 and 2, tissues (maternal blood and spleen, fetal or postnatal brains, placentas) were collected and analyzed for eCB levels, gene expression (eCB-, stress-, and immune-related), and cytokine levels. In aim 3, separate cohorts were tested across the lifespan: a) cytokine levels after an acute immune challenge on P14; b) structural brain development via MRI imaging on ~P28; c) social play behaviours in adolescence; d) stress-reactive behaviour assessed in the elevated plus maze (EPM) in adulthood; and e) acute stress response following 30-min restraint stress in adulthood.

Results and Conclusions: For aim 1 and 2, we found no impact of inhaled PCE on birthweight, fetal eCB levels, fetal CB1R mRNA levels, or maternal behaviour. Analysis of gene expression and cytokine levels are ongoing. For aim 3, we found no effects of inhaled PCE on adolescent structural brain development, social play behaviour, or adult stress-reactive behaviour in the EPM. Following acute restraint stress, PCE males did exhibit heightened corticosterone (the main stress hormone in rodents) levels at 60-min. Our findings illustrate subtle effects of inhaled PCE on the stress-response in males but no effects of PCE on any other measurement. Our research has and will continue to contribute to the growing body of literature on the effects of cannabis exposure during pregnancy, and may help formulate a more complete picture of the safety of maternal cannabis consumption.

Acknowledgments: This research was supported by CIHR, NSERC, and HBI funding to MN Hill; a CIHR Vanier Doctoral Scholarship, ACHRI Graduate Award, CCIC Brain Canada Rising Star Award, and several Indigenous Awards to SL Baglot.

CHANGES IN THE ENDOCANNABINOID SYSTEM AFTER INTRAVENTRICULAR HAEMORRHAGE IN IMMATURE RAT BRAIN

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Background: Intraventricular Haemorrhage (IVH) is a frequent complication in extremely low gestational age newborns, increasing the risk of Cerebral Palsy (CP), due to White Matter injury after enhanced neuroinflammation. The endocannabinoid system (ECS), in particular CB_1 and CB_2 receptors (CB1R and CB2R), its ligands 2-AG, AEA, OEA, PEA; and its metabolic machinery (DAGLα, MAGL, NAPE-PLD, FAAH), play an important role modulating neuroinflammation and apoptosis in newborn rat brain after IVH-induced brain damage. However, there is no information regarding ECS responses to acquired brain damage in very immature rat brain. **Aim:** To assess ECS responses to IVH-induced brain damage in immature newborn rats.

Methods: IVH was induced in immature Wistar rats (P1) by injecting *Chlostridium* collagenase into the left Germinal Matrix by stereotactic surgery (GMH). Non-injected animals served as controls (SHAM). Western Blot was performed at PND2 and PND7 on samples from ipsilateral striatum (which is the area adjacent to IVH) and ipsilateral cortex to determine CB2R, DAGLα and, MAGL expression; brain damage (assessed by Caspase 3-Activated expression) and inflammation (assessed by TLR-4 expression). Oxidative stress was assessed by Oxyblot assays, by protein nitrosylation ratio. Brain concentration of endocannabinoids (eCBs) were quantified using mass spectrometry. CB2R cellular localization was determined by immunofluorescence assays, along with microglial and neuronal markers.

Results: IVH led to early striatal and cortical damage as shown by increased active caspase expression 24 h after IVH induction. It also led to augmented inflammation in the striatum at an early stage; together with CB2R, DAGLα, MAGL and FAAH expression, along with decreased 2-AG's concentration, augmented AEA concentration. Cortical CB1R, CB2R, NAPE-PLD and MAGL expression was also increased, but no changes were found in 2-AG concentrations, while AEA levels were reduced. Oxidative stress was increased in both striatum and cortex at PND2. Although striatal damage was still present at PND7; increased inflammation, CB2R or DAGLα augmented expression was no longer observed. However, a higher expression of MAGL along with higher concentrations of 2-AG were found in striatum, No changes were observed in AEA levels in striatum. Cortically, at PND7, only MAGL expression was still increased. No changes were seen in AEA levels. Oxidative stress was still increased in both striatum and cortex at PND7.Striatal immunofluorescence at PND2 showed Iba-1 and CB2R positive cells increase, alongside with a higher co-localization of both markers in IVH group, this expression was not maintained at PND7. No CB2R and Iba-1 increased co-localization was found in cortex neither at PND2 nor at PND7. Colocalization of CB2R with NeuN marker showed same NeuN fluorescent area in both groups, both ages and both tissues; but a higher $NeuN^+$ and $CB2R^+$ colocalization, at PND7 in cortex of IVH animals.

Conclusions: Endocannabinoid system, and specially CB2R, its ligand 2-AG and its metabolic mechanism play a role in the immediate response to IVH-induced brain damage in preterm newborns, through its particular role in microglia and neurons. Thus, some of these elements, specifically CB2R and MAGL appear as a therapeutic target for early treatments after IVH**.** Further studies are needed in this regard.

ADOLESCENT EXPOSURE TO AB-FUBINACA, A SYNTHETIC CANNABINOID FOUND IN SPICE/K2 PREPARATIONS, INDUCES NEUROBIOLOGICAL ALTERATIONS IN A SEX DEPENDENT MANNER

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Introduction: Spice/K2 herbal mixtures, containing synthetic cannabinoids such as AB-FUBINACA, have been marketed as marijuana surrogates. The consumption of synthetic cannabinoids during adolescence is reported to be a risk factor for the appearance of psychiatric disorders later in life. This study aims to analyze the long-term effects of the exposure to AB-FUBINACA during adolescence, in both male and female mice. This work is mainly focused on studding different behavioral alterations (anxiety, depression, sociability, emotional and non-emotional memory, and psychotic-like behaviors), as well as possible biochemical modifications underlying the behavioral consequences previously found.

Methods: C57BL/6J mice were treated from post-natal day 35 to 49 with increasing doses of AB-FUBINACA (PND 35-39: 1 mg/kg, PND 40-44: 1.5 mg/kg, and PND 45-49: 2 mg/kg). Behavioral long-term effects were analyzed 20 days after the end of the treatment in both sexes. To carry out biochemical studies, brain samples were collected as required on each technique, 24 hours after the last behavioral test.

Results: AB-FUBINACA treated-male mice showed a significant anxiolytic-like behavior compared with controls in the elevated plus maze test. However, the administration of AB-FUBINACA induced an anxiogenic-like effect in female mice. The forced swimming test revealed an antidepressant-like behavior in males, but not female mice. On the other hand, females but not males, showed a significant reduction in the prepulse inhibition of the startle reflex and in the novel object recognition test. No changes were observed in sociability and in fear extinction memory paradigm neither in males nor in females. In addition, RNAseq studies revealed an increase in mRNA levels of PLEKHG2 and SH3TC1 in the mPFC in AB-FUBINCA treated female mice. As PLEKHG2 is a RhoGEF which plays an essential role in the correct dendritic growth and spine morphology, Golgi staining quantification in mPFC is being carried out at present in female animals.

Conclusions: To conclude, the consumption during adolescence of AB-FUBINACA leads to the appearance of different behavioral and biochemical alterations in a sex-dependent manner.

Acknowledgments: Funded by Plan Nacional Sobre Drogas (#2019I024) and MICINN (PID2020-116579RB-100).

THE EFFECTS OF VAPORIZED CANNABIS ON ADOLESCENT NEURODEVELOPMENT

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Introduction: Roughly one third of Canadian youth consume cannabis. This population is particularly vulnerable to the potential harms associated with cannabis use, as neurodevelopment occurs until ~25 years of age. However, not all youth are using cannabis with the same frequency. Further, different patterns of use may be associated with different relative risks. At present, clinical research in adolescent populations has found a possible relationship between cannabis-induced neuroanatomical changes and cognitive-emotional changes. However, much of this research has been difficult to replicate as clinical adolescent research is often retrospective and lacks control. Pre-clinical models allow for control over dosage, timing, and frequency of exposure. However, past animal research has predominantly administered high doses of synthetic cannabinoids via injection. Given that inhalation is the most common method of cannabis consumption, and that there are vast pharmacokinetic differences between injected and inhaled cannabis, there is a need to use a more translationally relevant pre-clinical model. This project aims to investigate the effects of varied patterns of vaporized cannabis inhalation on adolescent rodent neurodevelopment and behaviour.

Methods: Adolescent (P34) male and female Sprague-Dawley rats were exposed to vapour via a validated system for 23 days. Rats were split into four usage cohorts: 1) control non-users (exposure to vehicle vapor), 2) infrequent users (exposure to cannabis once per week), 3) frequent users with plateaued use (exposure to cannabis once daily), and 4) high frequency (HF) users with escalating use (exposure to cannabis every day, up to 3 times/day). Cannabis (100 mg/ml tetrahydrocannabinol [THC]) or vehicle (polyethylene glycol [PEG]) was vaped in single 15-min sessions to achieve a blood-THC level comparable to humans. Magnetic resonance imaging (MRI) scans were taken within five days pre- and post-vapour exposure, to quantify individual volumetric differences in brain volume in corticolimbic regions of interest (ROI). Weeks after vapour exposure, rats were subject to three behavioral tests: 1) light-dark box (LDB) and 2) fear conditioning, both to study emotional dysregulation, and 3) novel-object-context-mismatch (NOCM) to study cognitive dysregulation.

Results: *MRI:* In half of the corticolimbic ROIs analysed, including the hippocampus, thalamus, and neocortex, HF-exposed rats had significantly more volumetric growth than control rats. In a select few ROIs, the daily and weekly exposed rats had significantly less growth than controls. *Behaviour:* During LDB, the weekly- and daily-exposed rats showed significantly less anxiety-like behaviour than controls and HF, whereas the HF-exposed rats did not differ significantly from controls. During FC, there were no effects of treatment on conditioning or extinction behaviour. However, during extinction retrieval, the daily- and weekly-exposed rats had a stronger association with the fear memory than controls. During NOCM, there were no significant effects of treatment on novelty preference or contextual discrimination. There were no sex effects in any behaviours.

Conclusions: In both imaging and behavioural analyses, we saw a frequency-dependent effect of adolescent cannabis exposure on neurodevelopment. Particularly, we saw that HF-exposed rats were most vulnerable to increased corticolimbic growth, which could be due to defective pruning. We also saw that at lower frequencies of exposure, cannabis had an anxiolytic effect, whereas at higher frequencies anxiety-like behaviour was comparable to baseline. Lastly, we saw that daily and weekly rats had a stronger association with fear memory, which supports the idea that cannabis use during adolescence is correlated with emotional dysregulation. In all cases, these effects were subtle and frequency-dependent, which is why it is imperative to continue modeling different patterns of use in future studies, so that we can promote accurate harm reduction policies.

Acknowledgements: Funded by the Canadian Institute of Health Research and the University of Calgary.

ANANDAMIDE IS AN EARLY BLOOD BIOMARKER OF HERMANSKY-PUDLAK SYNDROME PULMONARY FIBROSIS: ARE WERE THERE YET FOR PULMONARY FIBROSIS PREVENTION CLINICAL TRIALS?

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Introduction: Hermansky-Pudlak syndrome (HPS) is a rare genetic disorder which, in its most common and severe form, HPS-1, leads to fatal adult-onset pulmonary fibrosis (PF) with no effective treatment. Unlike idiopathic pulmonary fibrosis (IPF), the occurrence of PF in HPS-1 is highly predictable based on the natural history of the disease; hence, identification of a clinical biomarker could allow therapeutic intervention at the beginning of disease and possibly before the onset of symptoms associated with PF. Accordingly, a rational therapeutic intervention could save lives in this deadly disease. We have previously shown that endocannabinoid anandamide (AEA) levels are increased in the bronchoalveolar lavage fluid (BALF) of patients with IPF or HPSPF and negatively correlated with pulmonary function parameters in both human and mouse. Furthermore, we demonstrated that endocannabinoid/cannabinoid receptor 1 (CB_1R) overactivity promotes fibrogenesis in lungs, and peripheral CB_1R antagonism provided therapeutic benefit in bleomycin induced-PF mouse models of IPF and HPSPF. We investigated whether endocannabinoids could serve as blood biomarkers of PF in HPS.

Method: We measured endocannabinoids in the serum of HPS, IPF, and healthy human subjects and in a mouse model of HPSPF. Pulmonary function tests (PFT) were correlated with endocannabinoid measurements. In a pale ear mouse model of bleomycin-induced HPSPF, serum endocannabinoid levels were measured with and without treatment with zevaquenabant (MRI-1867), a peripheral CB_1R and iNOS antagonist.

Results: In three separate cohorts, circulating AEA levels were increased in HPS-1 patients with or without PF, compared to healthy volunteers. Circulating AEA levels were negatively correlated with PFT, suggesting its link to disease progression. Furthermore, a longitudinal study over the course of 5-14 years with HPS-1 patients indicated that circulating AEA levels begin to increase with the fibrotic lung process even at the subclinical stages of HPSPF. Similar to the HPS-1 and HPSPF patients, serum AEA levels were significantly increased in the mouse model of bleomycin-induced HPSPF in the earliest stages of PF and remained elevated at a later fibrotic stage. Zevaquenabant (MRI-1867) was identified as a candidate drug to treat patients with HPSPF with its superior anti-fibrotic efficacy and is being tested in phase 1 clinical trial. Importantly, Zevaquenabant administration starting at day 8 post bleomycin completely reversed elevated circulating AEA levels in *pale ear* mice and halted progression of fibrosis, suggesting its target engagement, inhibition of the endocannabinoid/ CB_1R system and therapeutic efficacy.

Conclusion: Both human and animal data support a role of AEA in PF initiation in HPS-1 and suggest the use of serum AEA levels as potential blood biomarkers for disease progression in patients with HPS-1 for sub-clinical and overt HPSPF. Furthermore, the current study demonstrates that circulating AEA could also serve as a surrogate marker for a potential clinical intervention with Zevaquenabant in HPSPF.

Acknowledgements: Funded by the intramural research program of the NIAAA (to R.C.).

DISTINCT GENE REGULATION PATTERNS UNDERLIE CANNABIDIOL BENEFIT IN A MODEL OF MITOCHONDRIAL EPILEPSY

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Introduction: Inherited defects in mitochondria-related genes cause mitochondrial disease (MD), that often involves treatment resistant, severe epilepsy (mitochondrial epilepsy, ME). Mice with conditional knockout of the mitochondrial respiratory chain protein NDUFS4 in *Gad2*-positive GABAergic neurons (GABA-cKO) exhibit a severe epileptic phenotype and effectively model ME. We have previously shown that cannabidiol (CBD) extends lifespan, fully prevents fatal spontaneous seizures and ameliorates the behavioural deficits in GABA-cKO animals. Although seizures in GABA-cKO are causally related to neuroinflammation in the external globus pallidus (GPe), molecular pathogenesis and CBD mechanism of action in this area – specifically its GABAergic neuronal population - appear to be multimodal and are not yet fully known. The present study aimed to elucidate the gene/protein expression patterns in both whole GPe and ribosome-associated RNA derived from GPe GABAergic neurons using different RNA Sequencing and immunochemistry approaches.

Methods: GABA-cKO mice and age/sex-matched controls were chronically treated with CBD or vehicle until the established endpoints. Excised GPe were subjected to RiboTag ribosomal isolation based on Gad2-Cre-driven expression of a HA tag. RNA was extracted for sequencing from both the input and GABAergic immunoprecipitate (IP), followed by determination of differentially expressed genes (DEGs) by DESeq2. Gene ontology (GO) analysis was performed using categorical overrepresentation (ORA) of the DEGs via the online WebGestalt tool. Immunohistochemistry (IHC) and western blotting (WB) against neuroinflammatory markers Iba1, GFAP, p-NFkB, p-STAT3 and posttranslational lysine lactoylation (KLac) was performed, analysed by 2-way ANOVA and multiple t-tests with Tukey's multiple testing correction. Genes/proteins with a corrected p-value < 0.05 were considered as significant, as well as GO categories with an enrichment ratio (ER) ≥ 10.

Results: We report a total of 245 significant DEGs in the whole GPe input fractions and only 47 in the GABAergic IP. ORA of the DEGs in both the GPe input and GABAergic IP showed a clear upregulation of genes related to the innate immune response which is known to be exacerbated in MD. CBD partially attenuated overexpression in these categories, but more prominently affected membrane and ion flux processes in the input, contrasted to synaptic transmission and cell cycle category enrichment in the GABAergic neurons. These results were corroborated by IHC/WB, showing that CBD attenuates the increases in Iba1, GFAP, p-NFkB & p-STAT3 induced by GABA-cKO. We also observe genotype-driven levels of KLac increases across the sample groups, which CBD attenuated in some proteins.

Conclusions: Here we provide the first molecular evidence of CBD beneficial effects in a highly affected brain region of GABA-cKO mice, in line with its previously reported anti-epileptic and pro-survival function in this ME model. Our results support recent findings that CBD directly affects membrane lipid processes as well as protein receptors and ion channels driving its anti-inflammatory action, supporting its multimodal benefit against various MD-affected parameters. Taken together, this study provides novel insights into the molecular mechanisms underlying both the detrimental effects of mitochondria-related mutations and the therapeutic potential of CBD in the context of MD and ME.

Acknowledgements: This work was supported by the MINECO Ramon y Cajal fellowship (RYC2020-029596-I) and funds from MICINN (PID2021-125079OA-I00).

A NOVEL CANNABIDIOL:TETRAMETHYLPYRAZINE (ART12.11, CBD:TMP) COCRYSTAL IMPROVES THE EFFICACY AND BIOAVAILABILITY OF CANNABIDIOL TO INDUCE ANXIOLYTIC AND ANTI-DEPRESSANT EFFECTS

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Introduction: Cannabidiol (CBD), a principal constituent of the *Cannabis sativa* plant, is a drug gaining traction as a treatment option for neuropsychiatric disorders. Specifically, CBD displays anxiolytic (Onaivi et al., 1990) and anti-depressant properties (Xu et al., 2019) with little to no psychotropic effects (Alves et al., 2020). However, the therapeutic utility of CBD is limited by its pharmacokinetic properties, including poor oral bioavailability and solubility (Xu et al., 2019). Therefore, a novel cocrystal of CBD with the co-former tetramethylpyrazine (TMP) was developed to modify the pharmacokinetic properties of CBD whilst retaining the pharmacological drug activity. TMP is a constituent of the *Ligusticum* plant species commonly used in Asian traditional medicine (Lin et al., 2022). This cocrystal is called CBD:TMP (or ART12.11). We sought to investigate the pharmacotherapeutic potential of ART12.11 in anxiety and depressive phenotypes.

Methods: For this integrative research project, we investigated the dose-dependent effects of oral administration of ART12.11 (5 mg/kg, 15 mg/kg) compared to vehicle, CBD (10 mg/kg), TMP (1.5 mg/kg), and a CBD + TMP mixture (3.5 mg/kg, 1.5 mg/kg) on anxiety, depression, social, and cognition behaviours in adult male Sprague Dawley rats following a 2-week chronic unpredictable stress protocol. We then ran molecular analyses of anxiety and depression biomarkers in specific brain regions involved in anxiety processing. Further, we ran electrophysiological analyses in the mesolimbic dopamine circuit following administration of ART12.11 to evaluate addictive potential and possible neuronal mechanisms. We also performed pharmacokinetic studies with ART12.11 which will be featured in another presentation at ICRS 2024.

Results: In our behaviour studies, we report that ART12.11 reduced anxiety in stressed animals and induced anti-depressant behavioural effects such that stressed animals that received ART12.11 demonstrated reduced anhedonia, increased active coping behaviours, reduced learned helplessness, and increased pro-social behaviour. ART12.11 significantly outperformed vehicle, CBD, TMP, and CBD + TMP mixture treatment groups. Molecular and electrophysiological analyses are currently ongoing. These results suggest that ART12.11 improves the efficacy of CBD.

Conclusions: Overall, this research supports the development of ART12.11 as a promising and superior alternative to CBD in the pharmacotherapeutic treatment of anxiety and depressive symptoms and phenotypes.

Acknowledgements: This work is supported by Natural Sciences and Engineering Research Council of Canada (NSERC) and Mitacs Canada.

A RANDOMIZED PLACEBO-CONTROLLED TRIAL ASSESSING THE SAFETY AND EFFICACY OF CANNABIDIOL IN RHEUMATOID ARTHRITIS PATIENTS

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Introduction: Rheumatoid arthritis (RA) is an autoimmune inflammatory arthritis that can lead to joint destruction and decreased quality of life. There is no cure for RA and remission rates are <50%, thus there is a strong push to investigate safe alternative agents to decrease inflammation and improve RA outcomes. The use of cannabidiol (CBD) in preclinical RA models have been promising in reducing pain, disease severity, and inflammatory cellular infiltrate. Disease activity score (DAS28) is a validated clinical outcome used in RA clinical trials and musculoskeletal ultrasound (MSUS) is an objective imaging outcome used to quantify synovitis, inflammation within the joint, by measuring power doppler (PDUS) and grey scale synovial hypertrophy (GSUS). The objective of this pilot study was to examine the dose-dependent safety and efficacy CBD relative to placebo (PBO) in RA patients with moderate to severe disease activity.

Methods: We conducted a randomized, double-blind, PBO-controlled (RCT), 12-week trial of oral CBD 200mg b.i.d, CBD 400mg b.i.d, vs placebo (1:1:1) in non-cannabis using RA patients with moderate to severe RA disease activity defined as DAS28 $\geq$ 3.2 and PDUS $\geq$ 5. The primary outcome was change in DAS28 over 12 weeks and secondary outcomes included pain visual analogue scale (VAS), PDUS, and GSUS over 12 weeks. During the 12-week trial, detailed account of adverse events and serious adverse events were captured. Linear regression models were used to compare DAS28 and pain VAS each CBD arm versus placebo. Wilcoxon rank sum tests were used to compare the changes in MSUS measures over time between each CBD arm and placebo.

Results: RA patients were randomized as follows: 15 to PBO, 16 to CBD 200mg b.i.d, and 16 to CBD 400mg b.i.d. Participants were 60.6 years (SD 14.0), 96% female, disease duration 15.9 years (SD 13.7), 62% Caucasian, and 47% seropositive (either rheumatoid factor or anti cyclic citrullinated peptide antibody positive). After 12 weeks of treatment, DAS28 was not significantly different between CBD arms and PBO. For secondary outcomes PDUS, GSUS, and pain VAS, there were no significant differences between CBD arms and PBO. However, there was numerically higher response of pain VAS reduction within the CBD 200mg b.i.d. versus PBO (-0.95 vs -0.31, p=0.08). More patients withdrew from the CBD 400mg b.i.d. group (N=6) than CBD 200mg b.i.d. (N=4) and PBO (N=1). CBD 400mg b.i.d. group had numerically higher number of GI side effects (63%) than CBD 200mg b.i.d. (43.8%) or PBO (33.3%).

Conclusions: This is the first RCT study examining the safety and efficacy of CBD in RA patients. Findings indicate that high doses of CBD may not be well tolerated in RA patients with moderate to severe disease activity. Generally, CBD was not effective in reducing RA disease activity measures, by clinical and imaging outcomes. However, numerical improvement in subjective pain ratings between CBD 200mg bid and PBO groups suggests future research is warranted.

Acknowledgements/Funding: Funded by the Center for Medicinal Cannabis Research (CMCR) and the Semel Charitable Foundation. Study drug provided by Canopy Growth Corporation.

THE EFFECT OF CANNABIDIOL ON SUBJECTIVE RESPONSES TO AEROBIC EXERCISE: A RANDOMISED CONTROLLED TRIAL

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Introduction: Exercise is known to improve health. However, it can be unpleasant, often inducing negative feelings, or 'affect'. We have previously reported that cannabidiol (CBD), taken at the moderate oral dose of 300 mg, increases positive affect in trained individuals performing fixed-intensity aerobic activity (treadmill running exercise) (*Sahinovic et al., 2022 Sports Med Open*). Here, we investigated the effects of CBD on subjective responses to exercise under more ecologically valid conditions; namely, at the lower oral dose of 150 mg and in recreationally active individuals performing self-paced aerobic activity.

Methods: A randomised, double-blind, placebo-controlled, crossover trial was conducted at Griffith University. Students studying sports nutrition were invited to take part, with eligible volunteers ≥18 years of age and able to perform aerobic exercise. Participants ingested a placebo or 150 mg CBD in two soft-gel capsules 90 minutes before completing a self-paced 25-lap (10 km) run around an outdoor athletics track. The primary outcomes were affective valence, assessed during exercise using the 'Feelings Scale', and positive and negative affect, assessed at baseline, pre-run and post-run using the 'Positive and Negative Affect Schedule'. Exercise enjoyment, motivation and self-efficacy, the core features of the 'runner's high' (i.e., euphoria, pain, anxiety, sedation), perceived exertion, and run time were also assessed.

Results: Fifty-two participants were randomised and 51 were included in the final sample (n = 22 female; age: 21.9 [21.0–25.3] years). Exercise induced negative affect (at the time of undertaking) and increased pain (Fig 1A & 1B). CBD did not counteract either response. In fact, CBD had no significant effects on any of the outcomes measured. In contrast, exercise, *once completed*, increased positive affect, and decreased negative affect and anxiety (Fig 1C).

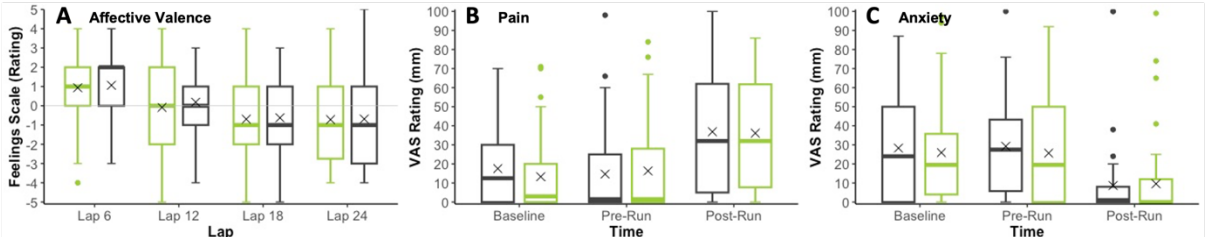

Fig. 1. Subjective responses on Placebo (**Grey**) and 150 mg CBD (**Green**).

Conclusions: CBD, taken at the low oral dose of 150 mg, does not appear to enhance the subjective experience of self-paced aerobic exercise in recreationally active individuals. Nor, however, does it appear to compromise it (or exercise performance). These findings suggest that CBD use is safe under exercise conditions and unlikely to impede physical activity participation. Our study also reaffirms the powerful mood-enhancing effects of exercise.

Acknowledgements: Funded by the Lambert Initiative for Cannabinoid Therapeutics.

ENDOCANNABINOID SIGNALING DEFICITS IN THE CEREBELLUM DISRUPT SOCIAL PREFERENCE

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Introduction: Autism spectrum disorder (ASD) is an incurable lifelong neurodevelopmental condition currently diagnosed in 1 out of 44 children in the USA. The core ASD symptoms include impairments in social interactions and increased social anxiety, behaviors notably regulated by the cannabinoid (CB) signaling system. Mutations in the components of the endogenous CB signaling system (ECS) are strongly linked to ASD, and in clinical trials, CB augmentation alleviates social anxiety in ASD patients.

The cerebellum is strongly implicated in ASD pathology: perinatal cerebellar trauma and congenital cerebellar malformations are associated with a dramatically higher incidence of ASD, and cerebellar Purkinje cell (PC) loss is commonly found in ASD patients. Notably, the CB synthesizing enzyme Diacylglycerol Lipase Alpha (DAGL-alpha) is prominently expressed in PCs, where ECS signaling regulates neuronal activity. Yet very little is known about the role of CB signaling in cerebellar regulation of social and emotional behaviors or ASD pathology. The lack of data on CB-dependent cerebellar roles in ASD pathology creates a barrier to the comprehensive evaluation of the benefits and risks associated with the development of novel CB-based pharmacological treatments for ASD.

Methods: To bridge this gap, we generated a mouse model where DAGL-alpha expression is specifically reduced in PCs. These mice exhibit decreased social preference, confirming the role of cerebellar PC-derived ECS signaling in ASD-associated behaviors.

Results: We used this KO mouse model to assess a panel of motor and social behaviors and to characterize alterations in the molecular profile and activity of DAGL-alpha-null PCs. Our results show that reduced cerebellar ECS signaling negatively affects social preference. Furthermore, our results identify the deregulated excitability of PCs as the neurological substrate for these behavioral changes.

Conclusions: Attenuation of cerebellar ECS signaling is associated with reduced social preference and altered activity of cerebellar PCs. This study offers insights into neurological mechanisms and the therapeutic potential of CB signaling augmentation in ASD.

Acknowledgments: This work was supported by the National Institutes of Health (R21-DA044000); Indiana University Bloomington College of Arts and Sciences, OVPR, and Psychological and Brain Sciences Department (FRSP-SEED); and CTSI (Core Pilot).

THE EFFECTS OF ENDOCANNABINOID MODULATION ON AN ACUTE MOUSE MODEL OF PARKINSON'S DISEASE

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Introduction: Parkinson's Disease (PD) is a neurological disorder marked by Lewy body formation and loss of dopamine neurons projecting to the striatum, resulting in symptoms like resting tremor, bradykinesia, and postural instability. L-DOPA, the precursor to dopamine, is the gold standard treatment for PD, but long-term use or high chronic doses may result in a debilitating condition named L-DOPA Induced Dyskinesia (LID), characterized by involuntary movements. The endocannabinoid system (ECS) modulates dopamine in the striatum by releasing endocannabinoids like 2-arachidonoyl-glycerol (2-AG) and activating pre-synaptic CB1 receptors, altering neurotransmitter release. Dysfunction of the ECS in PD is increasingly recognized, with alterations in CB1 receptor expression and endocannabinoid levels observed in patients. This study aims to identify therapies targeting the ECS to prolong L-DOPA efficacy without inducing dyskinesias, given its role in modulating dopamine transmission in the striatum.

Methods: We employed an acute Parkinson's mouse model by inhibiting tyrosine hydroxylase with alpha-methyl-p-tyrosine (aMPT) in dopamine transporter knockout (DAT-KO) mice, resulting in full dopamine depletion and extreme PD-like symptoms. L-DOPA administration bypasses TH inhibition and restores locomotor activity. This model is known as the Dopamine Deficient Dopamine Transporter Knockout (DDD) model. Adult DAT-KO mice were administered with aMPT (250 or 125 mg/kg i.p.), endocannabinoid modulators (see below, i.p.), and L-DOPA (25 or 12.5 mg/kg s.c.). Locomotor activity was assessed acutely, or chronically where injections were repeated for 14 days with locomotor testing done every other day. MAGL (monoacylglycerol lipase, 2-AG metabolizing enzyme) inhibitors MJN110 (5 mg/kg i.p.) and ABX-1431 (5 mg/kg i.p.) have been tested acutely, with ABX-1431 tested chronically as well. DAGL (diacylglycerol lipase, 2-AG synthesizing enzyme) inhibitor DO34 (30 mg/kg i.p.) and CB1 receptor inverse agonist rimonabant (3 mg/kg i.p.) were also tested in the acute paradigm. Data were analyzed with 3-way R-ANOVA (drug, sex, time) with post-hoc Sidak's test.

Results: Acutely, MAGL inhibitors potentiated L-DOPA induced locomotor effects. A combination of MAGL (5 mg/kg) and L-DOPA (12.5 mg/kg) results in comparable locomotor effects to a higher L-DOPA dose alone (25 mg/kg). This enhanced response with MAGL inhibitors was blocked by the CB1 receptor inverse agonist rimonabant. DO34 treatment alone reduced L-DOPA locomotor response. Our results show that increases in 2-AG enhance L-DOPA responses, while reductions in 2-AG reduce L-DOPA responses. Chronic administration of L-DOPA in mice resulted in reduced horizontal locomotor activity and increased vertical activity. Vertical activity is a proxy measure of dyskinesia, and drugs that reduce dyskinesia in humans are shown to reduce vertical activity in chronically L-DOPA treated mice. Mice treated with a combined dose of ABX-1431 (5 mg/kg) and L-DOPA (12.5 mg/kg) had a delayed onset of vertical activity compared to mice treated with L-DOPA alone, implying delayed onset of LID.

Conclusions: Acute enhancements of L-DOPA locomotor effects via increased 2-AG with MAGL inhibitors is likely mediated via CB1. Conversely, reducing 2-AG levels with DAGL inhibition reduces the L-DOPA induced locomotor effects. This highlights the potential for MAGL inhibition as a concomitant therapeutic used in conjunction with L-DOPA to delay, or prevent, LID.

Acknowledgements: Funded by the Parkinson's Society Canada (Project Grant).

THE ENDOCANNABINOID SYSTEM IN BIPOLAR DISORDER AND ITS EFFECTS ON MITOCHONDRIAL FUNCTION

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Introduction: Bipolar disorder (BD) is a mood disorder with a complex etiology, presenting in patients as episodes of mania, hypomania or depression. While there are many theories regarding the underlying mechanisms that cause BD, the pathophysiology of BD is not fully understood. The dysfunction of mitochondria, the organelles in eukaryotic cells responsible for producing cellular energy, may be one important component of BD. Multiple lines of evidence demonstrate that mitochondrial dysfunction and altered energy production are characteristics observed in BD patients *(Gimenez-Palomo et al., 2021)*. As recent research demonstrates, the endocannabinoid system may be an important regulator of mitochondrial energy production (*Jimenez-Blasco et al., 2020*). A recent PGC-BD GWAS study identified DAGLA as part of a genetic locus significantly associated with the BD phenotype (rs174592; *Mullins et al. 2021).* In the present study, we use a combination of genetic approaches and inducible pluripotent stem cell derived neurons from BD patients to understand what role the endocannabinoid system plays in BD.

Methods: Whole genome sequencing collected from age and sex-matched patient samples (3 healthy controls and 3 BD) were used to screen for variants in the endocannabinoid system common to all BD patients which were not found in healthy controls. Summary statistics from the PGC-BD wave 3 genome-wide association study (n=41,917 BD cases and N=371,549 controls) were used to perform gene-based analyses using MAGMA software to assess the contribution of endocannabinoid-related genes in BD subtypes [BD type I (BD-I) and BD type II (BD-II)]. Small-molecule protocols were used to derive cortical neurons from patient-derived inducible pluripotent stem cells (3 healthy controls and 3 BD). Cortical neurons were loaded with the mitochondrial calcium indicator dye Rhod2-AM and subjected to 2-photon microscopy to measure calcium alterations in response to 200 nM 2-AG as compared to vehicle (0.1% DMSO). Calcium imaging data was analyzed using a two-tailed T test ($p < 0.05$ considered significant).

Results: Within our patient samples, genetic analysis revealed that there were many intronic variants (SNPs/indels/deletions) in genes related to 2-AG metabolism common to all BD patients. Stratifying PGC-BD GWAS summary statistics by BD-I and BD-II revealed that DAGLA variants are related to BD-I, although DAGLA is not significant in BD type I ($p = 8.56e-6$, p-value of significance is 2.597e-6). Calcium imaging experiments demonstrate that 200 nM of 2-AG increases mitochondrial calcium content, which is blocked by 2.5 μM rimonabant. This increase in mitochondrial calcium was not observed in BD cortical neurons in response to 2-AG.

Conclusions: Genetic evidence suggests that DAGLA, and consequently 2-AG regulation, may play a role in BD- I. Our findings in cortical neurons suggest that 2-AG can impact mitochondrial physiology through altered calcium content in a system which is disrupted in BD. Further studies using cortical other neuronal types generated from patient stem cells are ongoing.

THE CB_2 RECEPTOR IS AN ANTIPRURITIC TARGET IN MICE

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Introduction: Pruritus is the experience – akin to pain – that induces one's desire to scratch. Current therapeutic strategies for reducing pruritus are limited by ineffectiveness and adverse side effects, prompting a need for novel treatments. Despite evidence of the anti-pruriceptive effects of various cannabinoids, clinical translation is hindered by their CB_1 receptor mediated cannabimimetic effects, particularly in studies that used higher doses of cannabinoids seen to also cause locomotor deficits. The goal of the present study was to test the hypothesis that the cannabinoid receptor full agonist, WIN 55,212-2 (WIN), reduces compound 48/80-induced scratching via a mechanism requiring the CB_2 receptor.

Methods: Adult male and female C57BL/6J mice were administered WIN 55,212-2 (0.1, 0.3, 1, or 3 mg/kg, i.p.) 50 min prior to compound 48/80 (50 µg in 100 µL, s.c.), and immediately placed in sound-attenuating chambers and video recorded for 30 min. Hind paw scratching time and bouts were quantified by a blinded observer. To probe potential receptor mechanism, a separate cohort of naïve mice was administered the CB_1 receptor-selective antagonist rimonabant (3 mg/kg, i.p.), the CB_2 receptor-selective antagonist SR144528 (3 mg/kg, i.p.), or vehicle 10 min prior to WIN 55,212-2 (0.3 mg/kg, i.p.). The antipruritic effects of the CB_1 receptor positive allosteric modulator ZCZ011 (10 or 40 mg/kg, i.p.) or the CB_2 receptor selective agonist JWH-133 (10 or 20 mg/kg, i.p.) were determined. Finally, the established antipruritic effects of WIN 55,212-2 were tested in male and female CB_2 (-/-) and CB_2 (+/+) littermates.

Results: WIN 55,212-2 (≥0.3 mg/kg, i.p.) reduced compound 48/80-induced scratching without inducing locomotor deficits. The antipruritus achieved by WIN 55,212-2 administration (1 mg/kg) was seen to be attenuated by either rimonabant or SR144528 pretreatment. Because rimonabant is a pruritogen, the CB_1 receptor positive allosteric modulator ZCZ011 was also tested versus compound 48/80-induced scratching. ZCZ011 had no effect, but JWH-133 reduced pruritus without altering locomotor output. Finally, WIN 55,212-2 reduced scratching in CB_2 (+/+) wildtype mice, but this antipruritic effect was absent in CB_2 (-/-) mice.

Conclusions: WIN 55,212-2 reduced experimentally induced scratching, and this anti-pruritis was blocked by either chemical antagonism or genetic deletion of CB_2 receptor. Similarly, CB_2 receptor selective agonism reduced scratching, further supporting a CB_2 receptor mediated mechanism of cannabinoid-induced antipruritus.

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.

REGULATION OF THE CB1 RECEPTOR BY NEURENSIN-2 IN DEPRESSION

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Introduction: Major depressive disorder (MDD) is a prevalent psychiatric condition with a significant global impact, leading to alarmingly increasing morbidity and mortality rates. Inhibition of cannabinoid receptor 1 (CBR1) has been long associated with both depression and anxiety disorders in yet unknown mechanisms. Recently, we identified a novel depression-mediating protein, Neurensin-2, expressed specifically in CCK inhibitory interneurons in the hippocampus, the same neurons that selectively express CB1R. Neurensin-2 is a stress-responsive vesicular protein with a putative role in protein trafficking. Nonetheless, the cellular mechanisms by which Neurensin-2 regulates depression and anxiety remain unexplored. Here, we demonstrate for the first time that Neurensin-2 regulates CB1R expression, providing a molecular mechanism that explains how Neurensin-2 may mediate depression and anxiety.

Methods: To identify the cellular pathways regulated by Neurensin-2, we overexpressed Neurensin-2 in a neuroblastoma cell line followed by RNA sequencing (RNA-seq). Differentially expressed genes (DEGs) were identified, and pathway analysis was applied. The identified genes were further validated using Western blot and quantitative polymerase chain reaction (qPCR). Chronic stress was applied in mice using the chronic social defeat model. CB1R downstream signaling pathways were detected using western blot.

Results: We found that Neurensin-2 overexpression downregulates synapse-related genes. Specifically, we observed that the retrograde endocannabinoid signaling was downregulated and that the *Cnr1* gene expression (encoding CBR1) was significantly attenuated. Biochemical analysis confirmed that Neurensin-2 overexpression resulted in a reduction of CBR1 mRNA and protein levels. Additionally, Neurensin-2 knockdown using small interfering RNA (siRNA) triggered increased gene expression of *Cnr1*. Furthermore, in hippocampi harvested from chronically stressed mice that show a dramatic increase in Neurensin-2 levels, the *Cnr1* expression was reduced. Conversely, *Cnr1* expression was elevated in hippocampi harvested following chronic treatment with the antidepressant fluoxetine, a treatment that reduces Neurensin-2 levels. Finally, we validate that Neurensin-2 overexpression downregulates the CB1R downstream signaling pathways, including Akt, Erk1/2, and mTOR phosphorylation.

Conclusion: Our results strongly suggest that the stress-induced dynamic induction of Neurensin-2 directly downregulates CB1R expression. Thus, we propose a novel cell-type-specific mechanism linking CB1R inhibition with depression and anxiety. The newly identified interplay between Neurensin-2 and CB1R may offer valuable cell-type-specific insights for developing targeted therapies for depression and anxiety.

THE EFFECT OF FAAH INACTIVATION IN PRP-TDP43^{A315T} TRANSGENIC MICE: RELEVANCE FOR POSSIBLE NEW ENDOCANNABINOID-BASED THERAPIES IN ALS

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Introduction: Amyotrophic lateral sclerosis (ALS) is a chronic disease characterized by the selective and progressive death of upper and lower motor neurons leading to muscle impairment. Several studies highlighted the relation of the endocannabinoid system with the pathogenesis of ALS, suggesting that changes in endocannabinoid levels could play an endogenous protective role in early stages of the disease. In particular, studies in mutant SOD1 mice showed elevated levels of endocannabinoids as a compensatory response to disease progression, while genetic deletion of FAAH enzyme slowed this progression, highlighting FAAH as a promising therapeutic target. To confirm this and assuming the broad spectrum of ALS-related genes, we wanted to explore the impact of FAAH inhibition in the PrP-TDP43^{A315T} mice, an alternative murine model to classic mutant SOD1 mice. Previous work in PrP-TDP43^{A315T} mice proved a reduction of FAAH levels in parallel to increased levels of anandamide and 2-AG in presymptomatic stages.

Methods: We used two experimental approaches in PrP-TDP43^{A315T} mice: (i) genetic inactivation of FAAH enzyme by crossing FAAH knockout mice (FAAH$^{-/-}$) with transgenic PrP-TDP43^{A315T} mice to generate double mutants (TDP43^{A315T}/FAAH$^{-/-}$); and (ii) pharmacological inhibition of FAAH enzyme by chronically administering the selective FAAH inhibitor URB597 i.p. at 0.2 mg/kg or vehicle from presymptomatic (PND65) to symptomatic stages (PN9D5) in TDP43^{A315T} mice. In both cases, disease progression was monitored weekly using behavioral tests (rotarod and clasping tests), whereas their spinal cords were collected at the end of treatment and used for histological and biochemical analyses to assess neuronal survival and glial reactivity.

Results: TDP43^{A315T}- FAAH$^{-/-}$ mice showed a delay in motor decline and increased survival compared with TDP-43^{A315T} mice with normal FAAH. These improvements were associated with a higher preservation of motor neurons and a significant decrease in Iba-1 immunoreactivity. By contrast, no differences in behavioral responses were observed after the URB597 administration, although this treatment was associated with reduced neuronal death and GFAP immunoreactivity, and increased P2RY12 expression, a microglial homeostatic marker. Interestingly, URB597 administration had no effect on FAAH mRNA levels, which confirms that there is no compensatory FAAH expression in response to the treatment.

Conclusions: Our results suggest the neuroprotective potential of FAAH inactivation demonstrated using genetic or pharmacological tools. This inactivation increases neuronal survival and attenuates glial reactivity, supporting the idea that the pharmacological management of this enzyme may have therapeutic value in ALS.

UNLOCKING THE THERAPEUTIC POTENTIAL OF THE DUAL INOS AND PERIPHERAL CB1R BLOCKER S-MRI-1867 FOR PRESERVING BETA CELL FUNCTION AND PREVENTING TYPE 1 DIABETES

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Introduction: The blockade of the cannabinoid type 1 receptor (CB1R) protects insulin-producing beta cells from stress factors such as hyperglycemia, fatty acids, and proinflammatory cytokines while enhancing their secretory function. In mice, genetic ablation of *CNR1* (gene encoding CB1R) in beta cells prevents immune cell infiltration into the islets, a distinctive form of inflammation named insulitis that precedes the onset of autoimmune type 1 diabetes (T1D) and delays the onset of the disease. We have shown that the peripheral inverse agonist JD-5037 prevented insulitis and preserved beta cell function in mice and humans, concomitant with the prevention of cytokine-mediated nitric oxide (NO) production, a mediator of endoplasmic reticulum stress, cell death and dysfunction. Herein we investigated the therapeutic potential of S-MRI-1867, a dual inhibitor of iNOS and peripheral blocker of CB1R, for the treatment of T1D.

Methods: Human insulitis *ex vivo* model: human islets and peripheral blood mononuclear cells were isolated from cadaveric donors, and 3D-cultured in solubilized extracellular matrix gel with or without cytokines (IL-1β, TNF-α, IFN-γ). *CNR1* was ablated via CRISPR/Cas9 (dCas9 for control). CB1R was blocked using S-MRI-1867 (vehicle for control). NO production was determined using DAF-FM staining and imaged by microscopy. The islet function was measured as glucose-stimulated insulin secretion (GSIS) in a perifusion system. Infiltration of immune cells into the islets was monitored by microscopy. Pre-T1D (dysglycemic) NOD/ShiLtJ female mice were treated for 4 weeks with S-MRI-1867, and blood glucose and beta cell function were monitored to assess progression to T1D.

Results: Genetic ablation of *CNR1* in islets prevented cytokine-mediated NO production and delayed insulitis *ex vivo* in humans. Dual blockade of CB1R and iNOS fully prevented insulitis in human *ex vivo* even at lower concentrations than JD-5037. S-MRI-1867 not only protected beta cell function upon cytokine treatment but also strongly enhanced it by 3.7-fold increase in GSIS. *In vivo*, treatment with S-MRI-1867 protected beta cell functional mass, which was 1.8-fold more responsive to glucose to secrete insulin. S-MRI-1867, when given for 4 weeks, significantly delayed the onset of T1D, with only 1 mouse reported as diabetic at the age when 90% of NOD/ShiLtJ mice are diabetic ($p < 0.0001$ by Mantel-Cox).

Conclusions: These results suggest that dual peripheral CB1R/iNOS inhibition is a potent therapeutic strategy for T1D with strong potential for translation to humans.

PERIPHERALLY-ACTING CANNABINOID PREVENTS MIGRAINE-LIKE PAIN AND SENSITIZATION OF DURAL NOCICEPTORS

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Introduction: Migraine is a debilitating neurological disorder that affects approximately 15% of the population globally with greater prevalence in females. Those afflicted can experience severe headaches and hypersensitivity to light, smell, and sound, in addition to nausea and fatigue. These attacks may be episodic or chronic, often triggered by various extrinsic and intrinsic stimuli such as stress, diet, weather fluctuations, and menses. Cannabinoids acting on Gi/o-coupled cannabinoid 1 and 2 receptors (CB1Rs and CB2Rs) alleviate migraine symptoms in humans and in animal models. However, side effects mediated by CB1Rs in the central nervous system (CNS) limit their widespread use. We developed synthetic peripherally restricted cannabinoid (PRCB) agonists, targeting the CB1R, that do not cross the blood-brain barrier. The prototype compound, 4-{2-[(1E)-1-[(4-propylnaphthalen-1-yl) methylidene]-1H-inden-3-yl] ethyl} morpholine (PrNMI) effectively suppressed chronic pain symptoms in preclinical models of cancer, chemotherapy- and traumatic nerve injury-induced neuropathies, all with minimal CNS-mediated side effects or tolerance development.

Methods: Here, we used non-invasive supradural injection of pH 6.0 saline in 5-week old female C57BL/6J mice to induce migraine-like pain symptoms of periorbital cutaneous allodynia, which causes priming to normally subthreshold pH 7.0 stimulation of the dura (at 3 days) following resolution of the initial pain symptoms. This model allowed us to address the mechanisms of sensitization of trigeminal dural afferents and the utility of PRCBs in suppressing behavioral symptoms of this sensitization.

Results: Supradural co-administration of pH 6.0 saline with the PRCB, PrNMI (5 μM), prevented both the initial and latent symptoms of cutaneous allodynia. Co-administration of pH 6.0/PrNMI with the peripherally-restricted selective CB1R inverse agonist, 18A (20 μM), or the brain-permeant selective CB2R inverse agonist, SR144528 (20 μM), abolished the preventative effects of PrNMI. Patch clamp recordings from fluorogold-labeled dural afferent trigeminal ganglion neurons (dTGs) acutely isolated 3 days after fluorogold co-injection with pH 7.4 or pH 6.0 saline revealed altered membrane properties and increased excitability of dTGs from pH 6.0-treated animals. Fluorogold labeled dTGs were small (< 25 μm, n = 51) to medium (25-45 μm diameter, n = 15) size, respectively, with majority (52/14) positive for binding the isolectin B4, which predominantly labels non-peptidergic nociceptors. Experiments examining changes in responses of acutely isolated dTGs to application of acidic solutions are ongoing.

Conclusions: These data demonstrate increased excitability of dural nociceptors 3 days after administration of acidic solutions and that PrNMI effectively prevents behavioral symptoms of acid-induced hyperalgesic priming and latent sensitization.

Acknowledgments: NIH grants NS128148, DA005010, UL1TR001881, CA196263, and U54HL119893.

PRELIMINARY EVIDENCE OF PALMITOYLETHANOLAMIDE SUPPLEMENTATION IN CLINICAL HIGH-RISK FOR PSYCHOSIS: A 12-WEEK OPEN-LABEL TRIAL

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Introduction: Aberrant endocannabinoid (eCB) signaling has been implicated in psychosis pathophysiological mechanisms, suggesting that molecules targeting the eCB system may act as antipsychotic agents. A recent systematic review from our group indicates that palmitoylethanolamide (PEA), a naturally occurring eCB-like compound with neuroprotective and anti-inflammatory properties, is overproduced in early psychosis, possibly to control the disorder, while being reduced as the disorder progresses, reflecting exhausting productive capacity in the longer-term. A few studies of PEA supplementation in established psychosis suggest that it may be useful to reduce both psychotic and affective symptoms. However, no study has been conducted in patients at clinical high-risk of psychosis (CHR), a potentially modifiable prodromal phase.

Methods: We started a 12-week, open-label, investigator-initiated proof of concept study (Phase-2 Pilot Study) of oral Ultramicronized PEA supplementation (600 mg/day) for the treatment of attenuated psychotic symptoms (APS) in CHR patients. Data were also collected on PEA safety and tolerability as well as biological mechanisms underlying its potential therapeutic effect. Assessments were performed at baseline, 4 weeks (T1), and 12 weeks (T2).

Results: So far, 8 CHR patients (sex: 4 males; age: 23.25 ±2.88 years) have enrolled into the trial. Administering the Comprehensive Assessment of at Risk Mental States (CAARMS) interview revealed a progressive reduction of the total positive symptoms overtime ($F(2,12)=4.37$, $p=0.037$), with significant improvements from baseline observed at T1 already ($t(7.0)=+2.65$, $p=0.033$) and maintained at T2 ($t(6.0)=+2.83$, $p=0.030$; Figure 1), particularly unusual thought content (UTC) overtime ($F(2,12)=6.85$, $p=0.010$), with significant improvements from baseline observed at T1 already ($t(7.0)=+3.42$, $p=0.011$) and maintained at T2 ($t(6.0)=+3.27$, $p=0.017$). Similarly, aggression/dangerous behavior (ADB) reduced overtime ($F(2,10)=5.00$, $p=0.031$), with significant improvements from baseline observed at T1 already ($t(6.0)=+2.71$, $p=0.035$) and maintained at T2 ($t(5.0)=+2.70$, $p=0.043$). At T2, reductions in alogia severity ($t(5.0)=+3.16$, $p=0.025$) and anxiety symptoms ($t(5.0)=+2.91$, $p=0.034$) were also detected. Preliminary evidence seemed to indicate that such effects are underpinned by modulation of iron homeostasis and blood viscosity (i.e., iron, ferritin, and hematocrit) as well as immunometabolism and protein synthesis (i.e., TNF-α, neutrophils, albumin, and globulins; all $p \leq 0.048$). Of interest, by using the Udvalg for Kliniske Undersøgelser (UKU) Side Effects Rating Scale for Patients (K-UKU-SERS-Pat), no side effects were reported over the 12-week period, while patients self-reported a reduction of psychic, neurological, and autonomic symptoms (all $p \leq 0.031$), likely inherent to the CHR state. Finally, no patient transitioned to full-blown psychosis during the study period.

Conclusions: To the best of our knowledge, this is the first interventional study addressing the potential therapeutic effect of PEA in the context of CHR for psychosis. Evidence indicates that PEA administration is exempt from side effects and reduces attenuated psychotic symptoms (APS) and aggressiveness after just 4 weeks of treatment and that such improvement remains stable for the entire period of PEA supplementation. Potential immunometabolic modulations, deserving further investigations, seem to account for PEA antipsychotic effects.

Acknowledgments: Epitech Group SPA supplied the investigational product.

PART I, OPEN LABEL RESULTS OF THE SAFETY AND EFFICACY OF MEDICANE'S MEDICAL CANNABIS OIL FOR TREATMENT OF AGITATION AND DISRUPTIVE BEHAVIORS IN SUBJECTS WITH DEMENTIA

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Introduction: Agitation and disruptive behaviors occur in over half of the patients suffering from dementia during the course of their illness. These are the most challenging behaviors to treat, associated with increased patient suffering, caregiver burden, treatment costs and institutionalization rates. The interventions currently used for treatment include off-label use of antipsychotics, sedatives/hypnotics, anxiolytics, and antidepressants, some of these are associated with limited efficacy, increased risk of side effects, morbidity, and mortality. Our study investigated the safety and efficacy of MediCane's cannabis oil as an add-on therapy to standard of care, in reducing agitation and disruptive behaviors in subjects with dementia.

Methods: The trial was approved by the Israel Ministry of Health, and the Ethics Committees of the three participating sites. The investigational product is a Medical Cannabis oil extracted from a proprietary single cannabis strain. The open label part presented here included up to four weeks of screening, eight weeks of dose titration (up to 8 drops* 3 times a day), four weeks of treatment on an optimal stable dose, up to two weeks of down titration, and up to four weeks of follow-up. During the trial, the patients attended five site visits, five home nurse visits, and six phone call visits. Safety endpoints included overall incidence, frequency, and severity of adverse events, physical examination, electrocardiogram, and laboratory analysis. Efficacy endpoints included the Cohen-Mansfield Agitation Inventory, the Neuropsychiatric Inventory (NPI-12), the Mini Mental State Exam, the Clinical Global Impression Scale, and the C-DEMQOL and DEMQOL-proxy quality of life questionnaires.

Results: The open label phase began recruiting in October 2022. The last patient out is expected by the end of March 2024. To date, 29 patients were screened, five of them were screen failures, and 24 enrolled. Fifteen out of the 24 patients enrolled, had already completed the trial, 5/24 underwent early withdrawal, 4/24 are ongoing. Of the patients completed, 11/15 opted to acquire a medical cannabis license to continue treatment for agitation and disruptive behaviors in dementia with MediCane's cannabis oil.

Conclusions: Our preliminary analysis shows promising safety and efficacy outcomes. Results are expected be available after database lock, ready for presentation at the ICRS conference in June 2024.

CANNABIDIOL TREATMENT OF PEDIATRIC EPILEPSY AND COMORBID ANXIETY: INTERIM RESULTS FROM A CLINICAL TRIAL

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Introduction: Despite anxiety and mood disorders commonly co-occurring with epilepsy, few studies have evaluated a treatment response for both conditions simultaneously. A pharmaceutical cannabidiol product, Epidiolex®, is USFDA-indicated for treating complex epilepsy in Lennox Gastaut, Dravet's and Tuberous Sclerosis syndromes, but may also effectively treat a wider range of epilepsy subtypes. Similar to other broad spectrum anti-seizure medications, cannabidiol may further serve a "dual role" in treating both seizures and mood/anxiety disorders. This open label, adjunctive, flexible dose clinical trial is geared to assessing outcomes in simultaneously treating anxiety and epilepsy. Recruitment is active and to date has reached approximately 80% of the intended recruitment goal. Interim analysis has been performed to identify trends. The purpose of this study is to assess whether pharmaceutical grade cannabidiol improves seizures as well as comorbid anxiety in children and adolescents. A single treatment with dual benefit would significantly impact clinical care in pediatric epilepsy with neuropsychiatric comorbidity.

Methods: This study recruits participants ages 6-17, with active epilepsy requiring medication treatment, and with impairing anxiety symptoms. Titration from 5 mg/kg/day to a target dose of 20 mg/kg/day occurred over a 4-week period, with flexible dosing as needed to more closely mimic clinical practice. Standardized behavior rating scales targeted to anxiety are done every four weeks and clinical outcomes including seizure control and subjective anxiety ratings are also done. Given this study's observational nature, ratings from completers will be used to evaluate primary outcome measures such as changes from baseline to end-of-study (EOS) scores in anxiety, as measured by Clinical Global Impression Improvement (CGI-I) ratings, where ratings of a 1 or 2 on a 7-point scale will be considered positive responses.

Results: 17 participants have enrolled (mean age = 12.9 years; 58.8% female), and 10 have completed the study, while four terminated early. 88% of the enrolled participants presented with baseline CGI-Severity ratings of markedly ill (5) or higher in the overall subcategory (mean = 5.2, SD = 0.7), and 82% in the anxiety subcategory (mean = 5.1, SD = 0.7). Across the 10 completers, the mean CGI-I scores at EOS were 1.7 (SD = 0.8) and 1.6 (SD = 0.8) for the overall and anxiety subcategories, respectively; eight completers (80%) demonstrated a final score of 1 or 2, indicating a robust positive response. Furthermore, nearly all completers (90%) showed improvement in anxiety from baseline to EOS visits based on parent-reported anxiety measures such as the Screen for Child Anxiety Related Emotional Disorders (mean decrease of 14.5 points). This improvement in anxiety was similarly reflected in the Behavioral Assessment Score for Children anxiety subcategory (baseline mean T-score = 68.0 [at-risk range from 60-69, clinically significant 70+], SD = 10.1; EOS mean = 56.3, SD = 7.4).

Conclusions: Overall, interim data suggest that Epidiolex® may offer a dual benefit of seizure control and anxiety reduction. Flexible dosing has proven to be useful for mitigating side effects as well as for optimizing outcomes. Clinicians may reasonably consider cannabidiol in cases of pediatric epilepsy with comorbid anxiety.

Acknowledgement: Funded by Jazz Pharmaceuticals via an investigator initiated and managed protocol.

CB1 RECEPTOR NEGATIVE ALLOSTERIC MODULATORS FOR REVERSAL OF CANNABIS TOXICITY

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Introduction: While the opiate crisis has justifiably occupied news headlines, emergency rooms are seeing many thousands of visits for another cause: cannabinoid overdose. This is partly due to the spread of cheap and extremely potent synthetic cannabinoids that can cause serious neurological and cardiovascular complications, and roughly a dozen deaths per year. But to the surprise of many who assume that cannabis is benign, states are seeing a surge in cannabis-related emergency room visits due to cannabis toxicity. For opiate overdose there is naloxone, but no such tool is available for cannabis overdose despite hundreds of thousands of cannabis-related emergency room visits each year. Without an antidote, doctors rely on sedatives, with their own risks, to 'wait out' the overdose. As more states legalize cannabis, this unaddressed need will only increase. Because the newer Spice cannabinoids are often high affinity CB1 agonists, competitive antagonists may struggle to rapidly reverse Spice cannabinoid overdose. Negative allosteric modulators (NAMs) have the potential to reverse the effects of synthetic cannabinoids without having to compete for binding with these potent compounds at the orthosteric site.

Methods: We tested a panel of CB1 negative allosteric modulators for their ability reverse the effects the canonical spice compound JWH018 *in vitro* in a neuronal model of endogenous cannabinoid signaling and *in vivo*.

Results: We tested ABD1085, RTI189, RTI329, MOST338 and PSNCBAM1 in autaptic hippocampal neurons, which endogenously express a retrograde CB1 dependent circuit that inhibits neurotransmission. We found that all of these compounds blocked/reversed JWH018 (40nM) though some proved more potent than others. We then tested whether four of these compounds could block the effects of JWH018 (1mg/kg, IP) *in vivo*, using a test of nociception in mice. We found that two of these compounds – RTI189 and PSNCBAM1 – blocked JWH018 when applied in advance. Notably, the potency of a compound in the *in vitro* assay was not a predictor of *in vivo* potency. PSNBCAM1 proved to be the most potent of the compounds and also reversed the effects of JWH018 when applied afterward, a condition that more closely mimics an overdose situation. Lastly we determined that PSNCBAM1 did not elicit withdrawal after chronic JWH018 exposure.

Conclusions: In summary, CB1 NAMs can, in principle, reverse the effects of the canonical Spice compound JWH018 both *in vitro* and *in vivo*, without inducing withdrawal. These findings suggest a novel pharmacological approach to at last provide first-responders with a tool to counter cannabis toxicity.

HIGH CANNABIGEROL HEMP EXTRACT MODERATES COLITIS AND MODULATES THE MICROBIOME IN AN INFLAMMATORY BOWEL DISEASE MODEL

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Introduction: Ulcerative colitis (UC) is one of the two major forms of inflammatory bowel disease (IBD) in which inflammation and ulceration are restricted to the colon. Current therapies to treat UC (steroids, immunosuppressants, and 5-aminosalicylic acid) have limited effectiveness and frequently have undesirable side-effects. Recent surveys of patients with IBD suggest that between 10-12% use cannabis or cannabis products to treat the symptoms of their disease, despite the lack of evidence to support the use of cannabis for UC.

Methods: The widely utilized dextran sodium sulfate (DSS) model of colitis was employed in this study. Mice received 2% DSS in their drinking water for 5 days and then were returned to normal water. Animals were treated daily with either vehicle (coconut oil) or CBG/CBD hemp extract (20 mg/kg CBG; 20.7 mg/kg CBD) via intraperitoneal injection. Tissue samples were collected at days 5 and 7 of the procedure and stool was collected at these two timepoints and prior to the start of DSS (day 0) for histological, metabolomic, and microbiome analysis.

Results: We found that treatment with high CBG hemp extract largely reduced disease symptoms, as measured by disease activity index (greater than 60% reduction). This effect was confirmed histologically through hematoxylin and eosin staining of colonic sections, that revealed normal colonic architecture was largely maintained in mice treated with hemp extract. In contrast, animals that received vehicle treatment showed large areas of ulceration. Additionally, our data show that CBG/CBD oil minimized the changes in the microbiome associated with colitis. Finally, metabolome analysis performed on stool samples collected pre- and post-colitis indicate that CBG/CBD oil treatment reduces changes in the metabolome associated with colitis.

Conclusion: We show here, for the first time, that hemp extract (CBG/CBD oil) is effective at reducing the symptoms of colitis in a murine model. There is a reduction in ulceration which coincides with reduced changes in the microbiome and metabolome. These data indicate that CBG/CBD oil may be a novel therapeutic for reducing disease flare-ups in patients with UC.

Acknowledgements: Funded by a sponsored research agreement between Penn State College of Medicine and PA Options for Wellness.

TOWARDS A MECHANISTIC UNDERSTANDING OF CBD AND CBG IN THE REVERSAL OF OBESITY-INDUCED FATTY LIVER DISEASE

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Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) poses a significant global health challenge, with a dearth of effective pharmacological interventions. The escalating obesity epidemic compounds this condition, highlighting the pressing need for innovative treatments. Previous research from our lab has demonstrated that administrating the non-psychoactive phytocannabinoids cannabidiol (CBD) and cannabigerol (CBG) can significantly reverse phenotypic manifestations associated with obesity and MASLD, including hepatic steatosis, alterations in body composition, hyperglycemia, glucose intolerance, and dyslipidemia, independent of overall body weight changes. Building upon these foundational findings, this study aims to delve into the molecular mechanisms underlying the therapeutic effects of CBD and CBG on MASLD and related metabolic abnormalities.

Methods: This study utilized a high-fat diet-induced obese mouse model treated with CBD (5 mg/kg/day, IP) or CBG (12.5 mg/kg/day, IP). The research focused on elucidating the mechanistic pathways behind previous reported phenotypic improvements through comprehensive metabolomics (analyzing 550 metabolites) and lipidomics (evaluating 82 fatty acids) assessments. Additionally, protein analysis (via Western blotting) and gene transcription analysis (via qRT-PCR) were integrated to uncover the molecular mechanisms driving the observed therapeutic benefits.

Results: Hepatic lipid profiling revealed a remarkable overall reduction in fatty acid levels, encompassing both free fatty acids and those derived from triacylglycerols, indicative of the compounds' efficacy in mitigating MASLD-associated lipid accumulation. Metabolomic analysis further identified significant alterations in 93 metabolites following CBD and CBG treatment, highlighting their broad impact on metabolic pathways. Notably, the creatine–phosphocreatine system showed marked upregulation, consistent with its potential to ameliorate hepatic steatosis. Additionally, while the fatty acid oxidation pathway remained unchanged, as demonstrated by the absence of any changes in the levels of carnitines, acetyl-CoA, and TCA cycle metabolites, reduced fatty acid uptake, demonstrated by decreased *Cd36* expression, coupled with a decrease in *de novo* lipogenesis, as evidenced by reductions in *Fasn* and *Srebp-1c* expression, provided further insight into the molecular mechanisms of action of these phytocannabinoids in reversing fatty liver disease.

Conclusion: Our study underscores the significant potential of CBD and CBG in reversing MASLD, emphasizing their impact on hepatic steatosis and metabolic regulation. Key molecular insights gleaned from this research offer a deeper understanding of their mechanisms of action. These results not only advance our understanding of phytocannabinoid therapy but also pave the way for novel approaches to treating MASLD and related disorders, representing a significant step forward in metabolic disease management.

Acknowledgments: Funded by the University of Toronto – Hebrew University of Jerusalem Research & Training Alliance Grant to J.T.

CB1 RECEPTORS INVOLVED IN ANTIALLODINIC EFFECTS OF MYRCENE IN RODENT MODELS OF CHRONIC PAIN

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Introduction: Chronic pain affects one in five US adults and many report unmet pain symptoms. Cannabis has been found effective for treating some types of chronic pain in adults, and up to 65% of patients use medicinal cannabis to treat chronic pain. While cannabis has been widely studied for its analgesic potential, cannabis constituents other than the most studied cannabinoid, delta-9-tetrahydrocannabinol, may have analgesic potential. Myrcene is one of the most abundant terpenes in cannabis and can decrease heat algesia and inflammatory hyperalgesia in mice and rats, respectively. The present study investigates the antiallodynic effects of myrcene in a mouse mode of neuropathic pain and to what extent its effects are mediated via the endocannabinoid system.

Methods: To model chronic pain, we used the Chronic Constriction Injury (CCI) model of neuropathic pain in adult male and female cohorts of C57Bl/6J mice. Two weeks after surgery, mice were given myrcene (100 mg/kg) or vehicle following pretreatment with a CB1 antagonist (1 mg/kg SR141716) or vehicle. Mice were tested with an electronic von Frey (eVF), with lower thresholds indicating increased mechanical hypersensitivity. Separate cohorts of mice were used to test the dose dependent effects of myrcene (1-200 mg/kg). Statistical analyses were performed in GraphPad Prism using a one-way ANOVA or two-way testing sex and eVF scores in the following conditions: vehicle + vehicle, vehicle + myrcene, and SR141716 + myrcene.

Results: Myrcene increased eVF thresholds compared to vehicle at 10, 100, and 200 mg/kg ($p < 0.01$) but not 1 mg/kg ($p > 0.05$). A two-way ANOVA evaluating the effects of sex and treatment condition revealed a significant main effect of sex ($p < 0.01$) and treatment ($p < 0.0001$) but not an interaction. Males had a higher withdrawal threshold compared to females when given vehicle + myrcene and when given SR141716 + myrcene (ps < 0.05). In both males and females, withdrawal thresholds were increased for vehicle + myrcene compared to vehicle + vehicle as well as for vehicle + myrcene compared to SR141716 + myrcene (ps < 0.05). There were no differences between vehicle + vehicle and SR141716 + myrcene (ps > 0.05).

Conclusions: In both males and females, myrcene dose dependently increased grams of force compared to vehicle in neuropathic pain but not sham mice. SR141716 pretreatment decreased these effects, suggesting that effects are mediated by the CB1 receptor and establishes a novel mechanism of action for myrcene-induced analgesia.

Acknowledgement: This study was funded by the Center for Medicinal Cannabis Research (CMCR) and drug was provided by True Terpenes.

CHARACTERIZING THE ANALGESIC POTENTIAL OF CANNABIDIOL AND SELECT TERPENES

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Introduction: Chronic pain affects nearly 100 million individuals in the US, and current analgesics have significant drawbacks. The use of opiates for pain management has contributed to an increase in opioid use disorder and overdose deaths, highlighting the need for alternative therapeutics with lower abuse potential that can effectively treat chronic pain. Cannabidiol (CBD) and terpenes are both constituents of *Cannabis*, act within the endocannabinoid (eCB) signaling system, and hold promise as alternative analgesics. Recent studies suggest that CBD and terpenes may act together, where their combined effect is greater than each individually. It is also known that eCB signaling within the basolateral amygdala (BLA) mediates pain and that it contains glutamatergic nocifensive ensembles that are sensitive to chronic pain. It remains unclear whether terpenes can be used as analgesics individually, how their interaction with CBD affects pain states, and whether these compounds act via glutamatergic BLA (BLA^{glu}) ensembles to regulate nociception.

Methods: We have employed a voluntary oral gelatin consumption model in combination with classical pain behavioral paradigms (i.e. von Frey and hot plate) and modern machine-learning (SLEAP) using a linear track to classify novel neuropathic chronic pain phenotypes. To investigate whether CBD and terpenes act locally within the BLA, we performed bilateral cannulations and microinjected CBD and terpenes prior to formalin-induced pain and measured nocifensive responses. Lastly, to uncover whether these compounds modulate BLA^{glu} ensemble activity during chronic pain, we utilized *in vivo* 2-photon imaging paired with infrared (IR) laser or von Frey filament delivery to the tail base of head-fixed mice to measure thermal hyperalgesia and mechanical allodynia respectively.

Results: Mice consistently consumed gelatin containing a mixture of four terpenes (α-pinene, β-myrcene, linalool, β-caryophyllene) and/or CBD ad libitum. We found that the combination of CBD and terpenes significantly alleviated allodynia for weeks following chronic pain induction. During naturalistic behavior, we identified alternations in the behavioral patterns of mice in chronic pain that were attenuated by the combination of CBD and terpenes. Within the BLA, we observed blunted nocifensive responses in mice given local CBD and terpenes. Lastly, we found that BLA^{glu} neuron responses scale with von Frey fiber weight and are sensitive to chronic pain. Systemic delivery of the combination of CBD and terpenes after chronic pain reduces both the recruitment of IR laser-activated BLA^{glu} neurons and the amplitude of their activity, supporting the role of the BLA in the action of CBD and terpenes in pain regulation.

Conclusions: With the growing use of *Cannabis* and the lack of non-opioid pain treatments, these results provide important information regarding CBD and terpenes in the context of pain and suggests that the BLA may be important for mediating this effect.

Acknowledgments: Funded by NCCIH R01AT011524, T32DA007278, and ADAI Cannabis Small Grants.

A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED, SINGLE-DOSE, CROSSOVER, PILOT INVESTIGATION OF CANNABINOL (CBN) 30 MG AND 300 MG EFFECTS ON SLEEP AND NEXT-DAY FUNCTION IN INSOMNIA DISORDER ('CUPID').

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Introduction: Insomnia is a pervasive and debilitating sleep disorder with few safe and effective pharmacotherapies. Cannabinol (CBN) has been proposed as a potential intervention, but its isolated effects on objective sleep measures have never been examined. This randomised, double-blind, cross-over study investigated CBN efficacy and safety in adults aged 25-65 years with clinician-diagnosed insomnia disorder.

Methods: Participants self-administered an oral liquid CBN preparation (30mg [1.5%] and 300mg [15%] suspended in medium-chain triglycerides oil) and matched placebo across three treatment arms with ≥2-weeks washout at the Woolcock Institute of Medical Research. The primary outcome was wake-after-sleep-onset (WASO) minutes via overnight in-laboratory polysomnography. Secondary outcomes included sleep stages, sleep onset latency (SOL), and absolute spectral power during non-rapid eye movement (NREM) sleep. Ancillary outcomes assessed subjective and objective sleep and next-day neurobehavioral, including driving, function (Clinical Trials.gov Identifier: NCT05344170).

Results: All randomised participants (n=17/20 female; age 42±13years) completed the protocol and were statistically analysed (AUG2022-SEP2023). Compared to placebo, WASO did not significantly reduce with 300mg or 30mg CBN (-6.3mins [95%CI: +18.2+5.5], p=0.29 and -4.0mins [-15.9+7.9], p=0.50, respectively). 300mg and 30mg did not respectively change N1 (-0.6% [-1.7+0.5], p=0.27 and -0.3% [-1.4+0.8], p=0.62), N3 (-0.1% [-2.3+2.1] p=0.94 and -0.7% [-2.9+1.5] p=0.51), REM (-0.4% [-2.8+2.0], p=0.76 and -0.6% [-3.1+1.8], p=0.59), or wake (-2.8% [-6.6+1.1], p=0.16 and 0.9% [-2.9+4.8], p=0.62) stages. 300mg but not 30mg, respectively, increased N2 (3.8% [+0.5+7.1], p=0.03 and 0.7% [-2.6+4.0], p=0.67); and decreased SOL (-7.0mins [-11.6-2.3,], p=.004 and -0.8mins [-5.4+3.9], p=0.74) and absolute NREM delta power (-140.2μV^2 [-246.7-33.7,], p=0.01 and -74.7μV^2 [-185.7+36.4], p=0.18). No other NREM spectral power changes occurred. Regarding tertiary outcomes, 300mg CBN reduced the arousal index (p=0.02) and increased subjective sleep quality assessed via the Leeds Sleep Evaluation Questionnaire (p=0.01) and Richard Campbell Sleep Questionnaire (p=0.01). 300mg and 30mg CBN respectively reduced REM sleep absolute theta (p=0.04 and p=0.02) and beta (p<.001 and p=0.01) power. 30mg reduced REM sigma power (p=.02). No other tertiary outcomes changed. 300mg CBN increased subjective drug effects subscales of 'feel effects' at 1-hour post (p=.03) and 'like the effects' at next-day (p=.01) timepoints. Next-day mood disturbance reduced in 300mg and 30mg arms (p=.02 and p=.03). Next-day driving simulator measures of headway distance (p=0.04) and speed variability (p=.01) increased with 300mg, but not lateral position measures. A total of189 non-serious adverse events were reported (57 in 30mg and 68 in 300mg arms).

Conclusions: We observed changes to sleep with an acute 300mg dose of CBN in insomnia disorder patients; however, not the primary outcome of WASO. CBN (300mg) improved SOL, arousal indices, and subjective sleep. Next-day mood improved with both doses. The SOL effects may present a potentially application for sleep onset insomnia phenotypes. Future studies might assess whether positive effects are maintained with repeated CBN dosing regimens in larger samples.

Support: The study was funded by the Lambert Initiative for Cannabinoid Therapeutics, University of Sydney.

CBD ALTERS SENSITIVITY TO THC IN PEOPLE WITH PSYCHOSIS AND COMORBID CANNABIS USE DISORDER

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Introduction: Cannabidiol (CBD) is a candidate novel treatment for both psychotic disorders and cannabis use disorder. Experimental studies in healthy volunteers suggest that pre-treatment with CBD can reduce the acute adverse effects of delta-9-tetrahydrocannabinol (THC). We investigated whether oral CBD can reduce the effect of THC in people with psychosis and a comorbid cannabis use disorder.

Methods: We recruited people with schizophrenia or schizoaffective disorder (mean age 40 years) who also had a cannabis use disorder (n=30). All participants were regular and heavy cannabis users (mean use = 5 days/week; 1.1 grams/day). Participants were studied on two occasions, in a double-blind, randomised, placebo-controlled, within-subjects crossover trial. In one session, participants received cannabidiol (1000 mg, oral) 3 hours before administration of 20 mg THC via a vaporiser. In the other session, pre-treatment was with a placebo. If participants did not show a significant response to the drug challenge, they were invited to complete additional pairs of experimental sessions with higher doses of THC (40 mg and then 60 mg)

Primary outcomes were i) change in Positive and Negative Syndrome Scale-Positive subscale (PANSS-P) and ii) delayed verbal recall on the Hopkins Verbal Learning Task-Revised. Blood samples were collected for measurement of plasma THC, CBD and their metabolites at six timepoints. The statistical analysis plan was pre-registered. Analyses were per-protocol and included data from the pair of experiments where participants received the highest dose of THC. Linear mixed models were used to assess the effect of CBD on continuous outcomes, including arm and visit order as fixed effects and participant as a random effect. McNemar's test was used to compare categorical outcomes.

Results: Following pre-treatment with CBD, the mean change in PANSS-P after administration of THC was +5.0, compared to +2.9 following placebo (mean difference [MD] = 2.1 [95% CI: 0.60 to 3.7]; p = 0.011). A PANSS-P increase of ≥9 was evident in seven experiments following CBD, but not in any following placebo (p = 0.0000045). PANSS scores on conceptual disorganisation (MD = 0.65 [95% CI: 0.17 to 1.1]; p = 0.012) and suspiciousness/persecution (MD = 0.55 [95% CI: 0.15 to 0.95]; p = 0.012) were higher following CBD than after placebo.

Delayed verbal recall following CBD pre-treatment was 3.5 words, compared to 4.8 words following placebo (MD = 1.3 [95% CI: 0.61 to 2.0]; p = 0.0011). Pharmacokinetic analyses indicated that CBD pre-treatment had no effect on plasma THC levels (C_{max}: +6.6 ng/ml; p=0.43; $AUC_{0\text{-}90}$ = +481 ng*min/ml, p=0.22).

Conclusions: In people with psychosis and a cannabis use disorder, pre-treatment with CBD increased sensitivity to the effects of THC on psychotic symptoms and cognition. This effect was not attributable to a pharmacokinetic interaction between CBD and THC. Cannabis use disorder is associated with alterations in brain CB1 receptor density, which might account for the results. Further studies are needed to clarify whether this effect of CBD is evident in people with psychosis that are occasional cannabis users.

DIFFERENCES IN STRESS RESPONSE IN INDIVIDUALS WITH CANNABIS USE DISORDER ALONE AND COMORBID CANNABIS USE DISORDER AND MAJOR DEPRESSIVE DISORDER

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Introduction: Cannabis use disorder (CUD) is highly comorbid with Major Depressive Disorder (MDD). Stress is a known contributor to relapse in CUD, and stress responding is dysregulated in individuals with CUD or MDD alone. This study sought to assess differences in stress response between individuals with CUD alone and those with comorbid MDD/CUD, with the goal of informing development of pharmacotherapies for stress-induced relapse.

Methods: Seventeen participants (CUD=11, MDD/CUD=6) completed the 6-day study. Participants could use cannabis as usual for the first 3 days and were asked to abstain from cannabis use for the latter 3 days. On Day 6, participants completed the Trier Social Stress Test (TSST). Immediately prior to and for 4 time points following completion of the test (0, 10, 30, 60 minutes), heart rate and blood pressure were measured, blood was collected to assess peripheral plasma cortisol and lipid tone, and participants completed subjective assessments of stress, anxiety, and desire to use cannabis. Data were analyzed using mixed-effects models.

Results: Stress was associated with significant increases in cortisol, systolic, and diastolic blood pressure immediately following the TSST in individuals with CUD (all p's<.01), but not MDD/CUD (all p's>.05). Individuals with CUD alone also reported increased need to use cannabis (p<.01), and both groups reported increased anxiety and stress (all p's<.05). The endocannabinoid 2-AG was elevated immediately following the TSST in individuals with MDD/CUD (p=.02), but not CUD (p=.85), whereas concentrations of the related lipid SEA were decreased at the 10-, 30-, and 60-minute time points in participants with CUD (all p's<.01), but not MDD/CUD (all p's>.05). Levels of the lipids AEA, OEA, PEA, SEA, LEA, and DEA, but not 2-AG or cortisol, were consistently negatively associated with subjective measures of desire to use cannabis, stress, and anxiety across groups.

Conclusions: Stress response is highly dysregulated in individuals with comorbid MDD/CUD, but, unlike in individuals with CUD alone, appears uncoupled from desire to use cannabis. Concentrations of lipids primarily degraded by fatty acid amide hydrolase (FAAH) are negatively associated with stress, anxiety, and desire to use cannabis following a stressor. Pharmacotherapies that inhibit FAAH may have potential in stress-induced relapse to cannabis use.

TOWARD OBJECTIVE BIOMARKERS OF CANNABIS USE DISORDER

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Introduction: Cannabis use disorder (CUD) is a growing public health concern. Current diagnostic criteria of CUD are based on self-reported costs, from dependence to psychosocial issues. However, CUD is marked by impaired insight into these costs. Thus, objective markers of CUD are needed. Cannabis use is associated with reduced resting state brain activity and an accumulation of delta-9-tetrahydrocannabinol (THC) in blood. However, whether objective measures of brain activity or THC blood levels are meaningful determinants of abuse liability is unknown. Thus, the aim of the current study was to examine associations between resting state electroencephalography (EEG) and THC levels, and cannabis abuse potential operationalized by the Cannabis Use Disorder Identification Test (CUDIT), cannabis self-administration, and subjective cannabis effects.

Methods: In this double-blind, placebo-controlled, within-subjects study, male volunteers that use cannabis monthly to daily (n = 17) participated in 3 laboratory sessions. Smoked cannabis (0%, 4%, and 10% THC) was administered in a randomized order (one dose per session). EEG activity and blood draws to measure THC levels were collected prior to drug administration. Reinforcing effects were assessed with a self-administration task wherein participants chose to smoke 0-3 puffs of the same cannabis smoked earlier that session at a monetary cost ($1 per puff). Subjective drug effects related to abuse liability and intoxication were assessed with visual analog scales (VAS), including the smoked cannabis rating form (SC-RF). The 17 participants were median split into two groups within the continuous variables of resting state EEG spectral power, specifically under the anterior-frontal 8 (AF8) electrode, baseline THC plasma levels, and CUDIT scores.

Results: Participants reported a mean of 4.2 days of cannabis use per week. The high baseline THC group showed a mean plasma concentration of 33.9 ng/mL THC, whereas the low THC group mean was 1.20 ng/mL THC. We found that the high baseline THC group resulted in dose-dependent increases in self-administration, which was not observed in the low baseline THC group (linear repeated measures ANOVA dose*concentration; $p = 0.017$). In addition, low baseline THC predicted both greater "Bad effect" ($R = -0.5$, $p = 0.036$) and drug "Liking" ($R = -0.6$, $p = 0.011$), suggesting greater sensitivity to subjective cannabis effects. In examinations of EEG power, low resting state brain activity similarly trended toward dose-dependent increases in self-administration, whereas high resting state brain activity did not, however this interaction was not significant (linear repeated measures ANOVA dose*EEG power; $p = 0.067$). Nonetheless, higher brain activity predicted higher drug "Liking" similar to low baseline THC ($R = -0.5$, $p = 0.029$). Finally, similar to baseline THC, high CUDIT scores resulted in dose-dependent increases in self-administration (linear repeated measures ANOVA dose*CUDIT; $p = 0.017$). CUDIT scores did not correlate with baseline THC, EEG power, or subjective cannabis effects.

Conclusions: Increases in self-administration as a function of THC dose occurred in individuals that have high baseline THC and CUDIT scores. Individuals with low baseline THC were more sensitive to drug liking, as were individuals with high brain activity, indexed by low EEG spectral power. These data point toward objective biomarkers of CUD that may be refined to identify abuse potential of cannabis for at-risk individuals.

Acknowledgements: NIH/NIDA (R01DA047296 and P50DA044118) and the Semel Charitable Foundation.

SEXUAL IDENTITY, SEXUAL MINORITY STRESS, AND CANNABIS USE MOTIVES AND HARMS IN PATIENTS ACCESSING ADDICTION TREATMENT IN ONTARIO, CANADA

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Introduction: Sexual minority identity is associated with greater prevalence of cannabis use and potentially greater risk of cannabis-attributable harms (e.g., cannabis use disorder [CUD]). Data from general community samples suggests that sexual minority stress (e.g., experiences of discrimination, harassment, and social exclusion related to sexual identity) may be associated with cannabis use and risk of CUD. Our goal was to examine relationships between sexual identity, sexual minority stress, cannabis use motives, and cannabis-related harms among patients accessing addiction treatment in Ontario, Canada.

Methods: We conducted an online anonymous survey using REDCap (Research Electronic Data Capture) that recruited patients aged 18+ years accessing addiction treatment services in Ontario, Canada. Our final sample size was 545 (n=434 heterosexual, n=111 sexual minority). The Sexual Minority Stress Scale (SMSS) included five subscales: Internalized Homophobia (IH), Expectations of Rejection (ExR), Satisfaction with Outness (SO) and Concealment (Clm), and Sexual Minority Negative Events (SMNE). The Marijuana Motives Measure (MMM) included five domains: Coping, Conformity, Social, Enhancement, and Expansion. Presence and severity of cannabis-related harms were captured by the Cannabis Use Disorder Identification Test - Revised (CUDIT-R) and the Marijuana Problems Scale (MPS; including a Number score that captures total number of cannabis-related problems endorsed and a Severity score that captures the mean severity of problems endorsed). Unadjusted linear regression models were used to test the relationship between SMSS subscales and cannabis use motives and harms.

Results: The sexual minority group reported significantly lower Conformity motives for using cannabis compared to the heterosexual group (1.34 vs. 1.95, $p<0.001$), though there were no group differences in Coping, Social, Enhancement, or Expansion motives. There was no difference in CUDIT-R or MPS Number scores, but the sexual minority group had significantly lower MPS Severity score (9.17 vs. 11.80, $p=0.024$). The IH subscale of the SMSS significantly predicted CUDIT-R score, though in an unexpected direction ($\beta=-0.024$, $p=0.035$) and it was not significantly related to other cannabis outcomes. The ExR subscale significantly predicted CUDIT-R score ($\beta=0.31$, $p=0.007$), MPS Number score ($\beta=0.32$, $p=0.002$), MPS Severity score ($\beta=0.264$, $p=0.011$), and Expansion motives ($\beta=0.22$, $p=0.032$), but not other motives. SO, Clm, and SMNE subscales were not significantly related to any cannabis outcomes.

Conclusions: In a sample of patients accessing addiction treatment, expectation of social rejection was a significant predictor of cannabis-related harms among sexual minorities, despite there being few overall differences in cannabis use outcomes between the sexual minority and heterosexual groups. Our results extend previous findings from community samples demonstrating relationships between sexual minority stress and cannabis-related harms, and suggest that specific dimensions of sexual minority stress (e.g., fear of social exclusion) may play a larger role in increasing risk of harms.

Acknowledgements: Funding was received from the Mental Health Commission of Canada, whose work is supported by a grant from Health Canada. The views represented herein solely represent the views of the researchers and do not represent the views of the Mental Health Commission of Canada and Health Canada.

EVALUATION OF ANTIDEPRESSANT AND ANXIOLYTIC EFFECTS OF MEDICINAL CANNABIS USE VIA ECOLOGICAL MOMENTARY ASSESSMENTS

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Introduction: Anxiety and depressive disorders are two of the most devastating psychiatric illnesses globally, and available treatments have limited benefits. Medicinal cannabis has mixed evidence for efficacy as a treatment for anxiety or depression and most studies are short in duration, yet patients frequently use medicinal cannabis for these purposes. The aim of this study was to evaluate the effect of medicinal cannabis use on mood in patient with anxiety and/or depression.

Methods: Adults (N=33; 20 women) with clinically significant anxiety or depression [Hospital Anxiety and Depression Scale (HADS) score > 8] were enrolled in this observational cohort study. Participants were newly initiating medicinal cannabis use with no more than 5 instances of cannabis use in the prior six months. HADS assessments were completed at baseline and 1, 3, and 6 months after medicinal cannabis initiation. Ecological Momentary Assessment (EMA) measures were completed four times daily for 8 weeks after cannabis initiation with event-level measures (0-10 visual analog scale) for depression, anxiety, "high" feeling, and perceived driving ability, collected prior to each episode of cannabis use and at the time of expected peak effect. Changes in these measures were evaluated using linear mixed effect models.

Results: Statistically significant decreases from baseline HADS anxiety and depression scores were observed after initiating medicinal cannabis ($p<.05$; $d=0.67$-1.16). Mean HADS anxiety and depression scores dropped below clinically significant levels (<8) by the three-month timepoint and one-month timepoint, respectively. These changes were maintained through 6 months for both outcomes. Acute reductions in anxiety (mean reduction=2.0; $p<.001$), depression (mean reduction=1.3, $p<.001$) and perceived driving ability (mean reduction=2.2, $p<0.001$), as well as acute increases in feeling "high" (mean increase=4.1, $p<0.001$) were reported after cannabis administration on EMA assessments. The greatest acute reductions in anxiety were observed after acute oral doses of 5-15 mg THC and inhaled doses of 3 or more "puffs." The greatest reductions in depression were observed after oral doses of 10-15mg THC. Ratings of feeling "high" and perceived impairment of driving ability after acute dosing decreased over time, but the acute effects of cannabis on ratings of anxiety or depression did not change during the period of observation.

Conclusions: Initiation of medicinal cannabis was associated with statistically and clinically significant decreases in anxiety and depression that were sustained over a 6-month period of observation. Acutely, doses that reduced symptoms also resulted in feeling "high" and decreased perceived driving ability. Tolerance to adverse effects, but not efficacy was observed over time. Replication in controlled clinical trials and with well-characterized cannabis products is needed.

ASTROCYTIC FABP5 MEDIATES SYNAPTIC ENDOCANNABINOID TRANSPORT IN THE HIPPOCAMPUS

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Introduction: Endocannabinoids are lipophilic retrograde messengers that regulate an array of behavioral outputs via modulation of synaptic transmission and plasticity. While the mechanisms underlying endocannabinoid mobilization in postsynaptic neurons and signaling at presynaptic cannabinoid receptor 1 (CB1R) are well characterized, endocannabinoid trafficking at the synaptic level remains poorly understood. Fatty acid binding protein 5 (FABP5) is an intracellular carrier of the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG). We previously demonstrated that FABP5 localizes to the synapse and serves a key role in controlling retrograde 2-AG mediated synaptic plasticity at central synapses. However, the precise cellular source and mechanism by which FABP5 regulates retrograde 2-AG signaling remains undefined. In the present study, we combine pharmacological and genetic approaches including cell-type specific FABP5 deletion as well as engineered FABP5 variants to test the hypothesis that FABP5 secreted from astrocytes mediates retrograde 2-AG transport.

Methods: *Ex vivo* whole cell recordings were performed from the hippocampal CA1 region of WT, constitutive, and inducible FABP5 KO mice. Depolarization-induced suppression of inhibition (DSI) was induced in voltage clamp by membrane depolarization to 0 mV for 5-10 sec. The amplitude of GABAA-mediated IPSCs was measured before and after membrane depolarization. Conditional and cell-specific knockdown of FABP5 in the hippocampus of $FABP5^{Flox}$ mice was accomplished by injecting AAVs expressing Cre or by crossing with Cre-driver lines. The efficacy of knockdown was confirmed by immunohistochemistry and qPCR.

Results: 2-AG mediated DSI was profoundly impaired following pharmacological FABP5 inhibition by SBFI-103 and in global FABP5 KO mice. DSI was rescued by AAV-mediated expression of FABP5 in the hippocampus of FABP5 KO mice but not by an FABP5 mutant that lacks affinity for 2-AG. Importantly, expression of an engineered secreted variant of FABP5 in FABP5 KO mice readily rescued the DSI. In contrast, overexpression of FABP7, an FABP subtype that exhibits high affinity for 2-AG but does not undergo cellular secretion, failed to do so. To determine the cellular source of FABP5, we selectively deleted FABP5 from neurons or astrocytes using a Cre-mediated approach in $FABP5^{Flox}$ mice. While control $FABP5^{Flox}$ mice exhibited robust DSI and normal CB1R function, DSI was abolished following deletion of FABP5 using AAVs expressing CMV-Cre-GFP. Strikingly, selective deletion of FABP5 in astrocytes but not neurons resulted in profound inhibition of DSI. Consistent with its role in mediating 2-AG signaling, selective AAV-mediated rescue of FABP5 expression in astrocytes of FABP5 KO mice fully restored the DSI.

Conclusions: These results demonstrate that astrocytic FABP5 controls synaptic 2-AG transport at hippocampal GABA synapses.

Acknowledgements: This work was funded by NIMH grant MH122461.

SYNERGISTIC ROLES OF 2-AG AND ANANDAMIDE IN HIPPOCAMPAL LONG-TERM DEPRESSION

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Introduction: Exogenous cannabinoids can impair short-term memory and cognition in humans and other animals, which is likely related to the disruption of synaptic plasticity by the global and sustained activation of CB1 cannabinoid receptors by exogenous agonists. Conversely, the temporally and spatially restricted release of endogenous cannabinoids may mediate or enhance synaptic plasticity, including long-term potentiation and long-term depression (LTD), in a synapse-specific manner. The functional roles of endocannabinoids are complex because they can modulate synaptic transmission via suppression of GABA and glutamate release, with opposing effects on postsynaptic excitability. In addition, the relative contributions of 2-AG and anandamide to synaptic plasticity are unclear.

Methods: All experiments were performed on postnatal day 15-30 CD-1 mice using protocols approved by the University of Connecticut Institutional Animal Care and Use Committee. Coronal slices containing the hippocampus were cut with a vibratome and transferred to a recording chamber continuously perfused with carboxygenated artificial cerebrospinal fluid at room temperature. Field excitatory postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum of the hippocampus in response to Schaffer collateral stimulation. LTD was induced by a 10-minute exposure to the metabotropic glutamate receptor agonist (S)-3,5-dihydroxyphenylglycine (DHPG).

Results: We examined the role of endocannabinoid signaling in DHPG-induced LTD by recording fEPSPs in the CA1 stratum radiatum in hippocampal slices from juvenile mice. Significant LTD (~40% decrease from baseline) could be induced by 10 min of exposure to DHPG and lasted at least sixty minutes. LTD was completely blocked by combined exposure to the mGluR1 antagonists MPEP and CPCCOEt. DHPG-induced LTD, in contrast to electrical stimulation-induced LTD, did not require NMDA receptor activation, as it was not affected by the NMDA antagonist CPP. The magnitude of LTD was significantly reduced by blocking cannabinoid receptor activation with either the CB1 receptor antagonist NESS-0327 or the VR1 receptor antagonist capsazepine, and LTD was completely blocked by their combination. The roles of the endogenous ligands 2-AG and anandamide (AEA) were examined by using selective inhibitors of DAG-lipase and NAPE-PLD, respectively. DHPG-induced LTD was significantly reduced by the NAPE-PLD inhibitor LEI-401 as well as the DAG-lipase inhibitor DO34.

Conclusions: These results indicate that DHPG-induced LTD is mediated by activation of both CB1 and VR1 receptors. Interestingly, the endogenous cannabinoids 2-AG and AEA each contribute to full expression of this form of LTD. Understanding the specificity of endocannabinoid signaling and the synergistic effects of AEA and 2-AG mobilization will be critical in unraveling their contributions to synaptic plasticity, learning, and memory.

Acknowledgments: Funded by the National Institutes of Health (Grant no. NS111986).

CANNABINOID RECEPTOR 2 SIGNALLING CONSTITUTES A METABOLIC REORGANISATION IN T CELLS, AWAY FROM GLYCOLYSIS TOWARDS THE PENTOSE PHOSPHATE PATHWAY

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Introduction: Inflammatory Bowel Disease (IBD) is characterised by cyclical immune cell recruitment into the gut, driving chronic intestinal inflammation. Inappropriate T cell activation plays a pivotal role in IBD pathogenesis. The cardinal feature of these cells once activated, is the ability to expand 40-100 fold within days, making them highly reliant on cellular metabolism, particularly glucose metabolism, to support the massive proliferative demands. Redox-sensitive signalling pathways have been implicated in controlling a number of T cell functions including cell cycle progression, effector T cell differentiation, tissue invasion and inflammatory behaviour. Our studies have highlighted a link between cannabinoid receptor 2 (CB_2R) signalling and metabolic reorganisation in such cells, away from glycolysis towards the proinflammatory pentose phosphate pathway (PPP).

Methods: Using Jurkat T cells, we activated CB_2R with the synthetic receptor agonist JWH-133, assessing cellular glucose uptake, glycolytic rates and key enzymatic responses via fluorescent glucose analogue assays, Seahorse XF Pro Analyser, and ELISA. Metabolomic analysis of cellular lysates was performed using Quant500 liquid chromatography with tandem mass spectrometry. Multivariate statistical analysis of acquired dataset were performed using MetaboAnalyst.

Results: CB_2R activation significantly increased cellular glucose uptake, reduced glycolytic flux, and elevated PPP-associated enzyme activity, indicating metabolic reorganisation and shunting towards the PPP. Metabolomic analysis confirmed this, indicating significant alterations in both glycolytic- and PPP-associated amino acids following receptor activation.

Conclusions: CB_2R activation promotes metabolic reprogramming of T cells, redirecting them away from glycolysis, pyruvate and ATP production towards the PPP, where they generate NADPH and consume cellular ROS. This metabolic reorganisation has previously been associated with the development of hypermigratory, hyperinflammatory memory T cells due to imbalances in redox-sensitive signalling. This likely contributes to worsening disease outcomes in people with chronic intestinal disease. Our research challenges the dogma of cannabis, a CB_2R activator, as a potential IBD therapy.

Acknowledgments: Funded by the Crohn's and Colitis Foundation and UCD Conway Institute Director Fund.

CANNABINOID SIGNALING MACHINERY REGULATES CEREBELLAR MOSSY FIBER AXON GROWTH

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Introduction: The increasing prevalence of cannabis use during pregnancy highlights the urgent need to elucidate cellular and molecular mechanisms of endocannabinoid (eCB) signaling in neurodevelopment and how plant-derived cannabinoids interfere with it. Prior work from our lab showed robust and developmentally dynamic expression of the eCB signaling system in the developing cerebellum. The cerebellum contains over half of all neurons in the human brain. Its role is to sequence and coordinate movements, thoughts, and emotions. Mossy fiber (MF) axons are the major source of excitatory inputs to the cerebellum. MFs grow long distances from the spinal cord and the brainstem to the cerebellar cortex, where they elaborate synaptic contacts – a process that takes place throughout the third trimester and the first few postnatal months in humans. Dysregulation of MF growth and targeting due to either environmental or genetic insults affects cerebellar function and causes behavioral pathology.

Methods: We utilized synthetic and plant-derived agonists and antagonists of eCB signaling *in vivo* and *in vitro* to assess the growth and development of MFs, in wildtype and cannabinoid receptor 1 (CB1) knockout (KO) mice. We employed proteomics analysis to elucidate molecular pathways involved in cannabinoid-dependent regulation of MF growth. To analyze rapid and local effects of eCB agonists and antagonists on MF growth we utilized timelapse videomicroscopy. Image analysis was performed using Fiji and Imaris, and statistical analysis was done in Prism.

Results: Our analysis demonstrates that perinatal exposure to plant-derived cannabinoid, Δ9-tetrahydrocannabinol (THC), disrupts the morphology of MF axon tracts of mice *in vivo*. Our proteomic analysis reveals significant changes in the regulation of small GTPases, suggesting a molecular effector cascade through which THC-agonism of CB1 regulates cytoskeletal dynamics in developing MFs. We show co-expression of CB1 and the eCB synthesizing enzyme, DAGLα, in MFs during the axon elongation developmental stage, suggesting an autocrine mode of signaling. Both CB1-KO and CB1 agonism with either THC or a synthetic agonist, WIN, results in a similar phenotype of stunted MF axon growth. 48-hour exposure to DAGLα inhibitor, RHC80267, dramatically reduces MF axon length. We also demonstrate that short exposure to RHC80267 induces growth cone retraction within minutes.

Conclusions: We demonstrate that eCB signaling regulates cerebellar MF growth through the regulation of the activity of small GTPases, rapidly affecting growth cone dynamics. Exposure to exogenous cannabinoids affects MF growth and distribution both *in vivo* and *in vitro*.

Acknowledgements: This work was supported by the National Institutes of Health (R21-DA044000); Indiana University Bloomington College of Arts and Sciences, OVPR, and Psychological and Brain Sciences Department (FRSP-SEED); and CTSI (Core Pilot).

REVISITING THE CANNABINOID-OPIOID INTERACTION HYPOTHESIS USING CONDITIONAL CB1 AND μ OPIOID RECEPTOR KNOCKOUT MICE

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Introduction: The roles of the CB1 receptor (CB1R) in cannabinoid effects and the μ opioid receptor (MOR) in opioid effects are well characterized. Growing evidence indicates that co-administration of cannabinoids and opioids produces an enhanced analgesic effect, suggesting a possible interaction between these two receptors. One hypothesis is that a direct interaction between membrane CB1R and MOR may underlie this phenomenon; although numerous studies support this hypothesis, many others do not. The current study aims to address this discrepancy using conditional CB1R- or MOR-knockout mice. Our hypothesis is that if CB1R and MOR directly interact, then 1) both receptors should be co-localized on the same GABA or glutamate neurons and 2) selective deletion of one receptor should alter pharmacological or behavioral responses to activation of the other.

Methods: Wild-type mouse brain sections were processed by RNAscope *in situ* hybridization to visualize CB1R and MOR mRNA distribution in GABA and glutamate neurons. CB1R and MOR mRNA co-localization was quantified in the nucleus accumbens (NAc), paraventricular area of the thalamus (PVT), substantia nigra pars reticulata (SNr), and ventral tegmental area (VTA) (n = 3 brains per region). Behavioral tetrad (analgesia, hypothermia, catalepsy, and immobility) effects were assessed following Δ^9-THC administration in Oprm1-flox x Vgat-Cre (GABA neuron-MOR-KO) and Oprm1-flox x Vglut2-Cre (glutamate neuron-MOR-KO) mice or following oxycodone administration in CB1-flox x Vgat-Cre (GABA neuron-CB1-KO) mice. Measures of Δ^9-THC (5 mg/kg) conditioned place aversion (CPA) or oxycodone (3 mg/kg) conditioned place preference (CPP) were determined following a seven-day conditioning period wherein drug and vehicle injections were alternated daily. n = 8 per group for all behavioral experiments.

Results: CB1R and MOR mRNA displayed distinct regional distributions in mouse brain sections. CB1R-MOR co-localization was observed in ~50% of glutamate neurons in the PVT, ~35% of GABA neurons in the SNr, ~25% of GABA neurons in the VTA, and <10% of GABA neurons in the NAc. Using conditional knockout mice, we found that MOR deletion from GABA or glutamate neurons failed to alter Δ^9-THC-induced tetrad effects, and CB1R deletion from GABA neurons also failed to alter oxycodone-induced analgesia and hypothermia. Additionally, deletion of CB1R or MOR from GABA or glutamate neurons failed to alter conditioned place preference or aversion to oxycodone or Δ^9-THC, respectively.

Conclusions: Together with the overall distinct regional distributions of CB1R and MOR mRNA, our tetrad and conditioned place preference/aversion experiments do not support the CB1R-MOR interaction hypothesis.

Acknowledgements: Funded by the National Institute on Drug Abuse Intramural Research Program.

EFFECTS OF Δ^9-THC IN HUMANIZED SICKLE CELL DISEASE MICE

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Background: Sickle cell disease (SCD) is caused by a mutation in the hemoglobin gene which lowers the protein's solubility resulting in formation of fibrous precipitates that distort red blood cell shape. Deformed cells adhere to the vascular endothelium and plug microcapillaries, leading to occlusion and infarction. These events recruit complex neurovascular and neuroimmune processes that result in a chronic pain state punctuated by debilitating episodes of acute pain. Current opioid-based treatments have limitations, prompting exploration of alternative strategies targeting the endocannabinoid system, a critical regulator of nociception. Preclinical studies demonstrated the analgesic potential of cannabinoids in SCD, yet clinical trials yielded mixed results. We aim to address these discrepancies by evaluating the antinociceptive efficacy of THC, CBD, and their combinations in humanized SCD mice, a well-established model.

Methods: We used transgenic male mice expressing human HbS ('SCD' mice) or normal HbA ('HbAA' mice). To establish baseline measures, animals were subjected to a battery of behavioral tests to evaluate anxiety (EPM), cognitive function (NOR) and pain-related behaviors. Subsequently, mice received acute or subchronic intraperitoneal administrations of THC (0.1, 0.3, 1, and 3 mg/kg) or its vehicle. Following THC injection, mechanical allodynia (dynamic plantar esthesiometer), heat hyperalgesia (Hargreaves test), and cold hypersensitivity (dry ice pellet) were measured at various time points, along with body temperature, motor activity, and catalepsy.

Results: As expected, SCD resulted in substantial mechanical (HbSS vs HbAA: Δmean withdrawal threshold = -1.440 ± 0.4641; $P = 0.0073$) and cold allodynia (Δmean withdrawal latency = -9.360 ± 1.385; $P < 0.0001$), while heat hyperalgesia was notably absent (Δmean withdrawal latency = 1.333 ± 1.056; $P = 0.2240$). Acute THC administration time- and dose-dependently alleviated these pain-related behaviors. ED_{50} values were 0.43 mg kg^{-1} for mechanical and 0.45 mg kg^{-1} for cold allodynia. Subacute THC administration led to the emergence of tolerance primarily in cold allodynia, while mechanical allodynia remained relatively unaffected. Remarkably, acute THC did not cause catalepsy or hypothermia, though it did result in moderate rotarod performance impairment, especially in SCD mice. Of significance, SCD mice exhibited marked anxiety (Δmean anxiety index = 0.3660 ± 0.09046; $P = 0.0009$) and cognitive deficits (Δmean discrimination index = 0.3660 ± 0.09046; $P = 0.0435$), which were not altered by acute THC (1 mg/kg) administration.

Conclusion: Presently, our findings indicate that SCD induces anxiety, cognitive deficits, and persistent pain-related behaviors in mice. While THC shows promise in mitigating SCD pain, it exhibits limited efficacy against anxiety and cognitive impairments. Caution is warranted in prolonged THC usage, considering the potential development of tolerance, particularly in addressing cold allodynia.

Acknowledgements: Supported by CMCR grant awarded to Daniele Piomelli.

STRESS-INDUCED ANTINOCICEPTION IN THE RAT SPARED NERVE INJURY MODEL OF NEUROPATHIC PAIN IS MEDIATED BY CANNABINOID CB_1 RECEPTORS

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Introduction: Neuropathic pain is a major unmet clinical need. Stress modulates pain, and the endocannabinoid system plays a key role in pain, stress, and their interaction. However, whether the endocannabinoid system plays a role in stress-induced modulation of neuropathic pain is unknown. The aims of the present study were to (1) investigate the effect of acute restraint stress on nociceptive behaviour in the rat spared nerve injury (SNI) model of neuropathic pain, and associated alternations in the endocannabinoid system and (2) investigate the effect of cannabinoid receptor-1 (CB_1R) antagonism on restraint stress-induced modulation of nociceptive behaviour in the rat SNI model of neuropathic pain.

Methods: In experiment 1, male and female 11-12-week-old Sprague-Dawley rats underwent SNI or sham surgery and in experiment 2, all rats underwent SNI surgery. In both experiments, mechanical and cold hypersensitivity (electronic von Frey and acetone drop test, respectively) were assessed weekly until post-surgery day (PSD) 21. On PSD21, rats were assigned to acute restraint stress (30 minutes) or non-restraint control (experiment 1; n=11 per group, 8 groups and experiment 2; n=12 per group, 4 groups [male and female combined]). In experiment 2, immediately after the restraint stress or control, rats received an acute intraperitoneal injection of the CB_1R antagonist AM251 (3mg/kg) or vehicle. Pain-related testing was carried out 1-hour post-restraint in both experiments. Tissue levels of endocannabinoids or related *N*-acylethanolamines in discrete CNS region were measured by LC-MS/MS. Parametric data were analysed by ANOVA and Tukey's post-hoc test and non-parametric data by Kruskal-Wallis and Dunn's post-hoc test ($p<0.05$ considered significant).

Results: Restraint stress attenuated SNI-induced nociceptive behaviour, with effects being more pronounced on mechanical hypersensitivity than on cold hypersensitivity, and in males compared with females. Associated alterations in levels of endocannabinoids or related *N*-acylethanolamines were observed in discrete CNS regions. The CB_1R antagonist AM251 significantly attenuated the restraint stress-induced reduction in mechanical hypersensitivity in SNI rats.

Conclusions: Acute restraint stress attenuates mechanical hypersensitivity in the rat SNI model of neuropathic pain via a CB_1R-dependent mechanism.

Acknowledgements: This work was supported by grants from the Irish Research Council (GOIPG/2019/3945, GOIPG/2020/1496 and GOIPG/2021/1434), Science Foundation Ireland (13/RC/2073-P2), Hardiman Scholarship University of Galway, Marie Skłodowska-Curie Actions (955684).

MONOACYLGLYCEROL LIPASE INHIBITION ALLEVIATES POST-INCISIONAL PAIN VIA SUPPRESSION OF DOWNSTREAM EICOSANOID BIOSYNTHESIS

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Introduction: Surgical incision induces acute postoperative pain that is typically treated with opioids and non-opioid analgesics including cyclooxygenase (COX) inhibitors and acetaminophen. Despite these interventions, a large proportion of patients undergoing invasive surgical procedures report significant postoperative pain. Consequently, identification of new targets for the development of non-opioid analgesics remains a major focus area. Monoacylglycerol lipase (MAGL) is the major enzyme that hydrolyzes the endocannabinoid 2-arachidonoylglycerol (2-AG) to arachidonic acid (AA) to terminate its signaling. AA serves as a major precursor to a host of proalgesic eicosanoids (EICs) including cyclooxygenase-1/2 (COX-1/2) and 5-lipoxygenase (5-LOX) derived EICs. Consequently, MAGL inhibition elevates 2-AG levels to promote activation of cannabinoid receptors 1 and 2 (CB1R and CB2R, respectively) while concurrently suppressing the biosynthesis of diverse sets of EICs. Here, we employed the plantar incision model to characterize the contribution of MAGL to acute postoperative pain.

Methods: WT and MAGL KO mice were subjected to a unilateral incision of the hind paw. Pain-related behaviors were assessed via von Frey testing, home cage locomotion during the dark phase of the light-dark cycle, and dynamic weight bearing on the hind paws. Mice were treated with the MAGL inhibitor MJN110 (5 mg/kg, i.p.) in the presence or absence of the CB1R antagonist AM251 (3 mg/kg, i.p.) and the CB2R antagonist AM630 (3 mg/kg, i.p.). Mice also received ketorolac (10 mg/kg, i.p.) and CJ-13610 (10 mg/kg, i.p.) to inhibit COX-1/2 and 5-LOX, respectively. Levels of 2-AG, AA, and EICs were quantified via mass spectrometry.

Results: Plantar incision produces pain-like responses characterized by a decrease in mechanical thresholds, home cage locomotion, and a weight bearing imbalance. Treatment with MJN110 produced antinociceptive effects as evidenced by increased mechanical thresholds, locomotion and weight bearing. Administration of AM251 or AM630 did not significantly alter mechanical thresholds, suggesting the involvement of downstream EICs. Mass spectrometry revealed elevated levels of the COX metabolite prostaglandin E2 and the 5-LOX metabolite 5-hydroxyeicosatetraenoic acid after incision, which were largely suppressed by MJN110. Accordingly, while ketorolac and CJ-13610 produced antinociceptive effects in vehicle treated mice, they failed to exert additional effects when administered in conjunction with MJN110. MAGL KO mice exhibited a similar post-surgical EIC profile as WT mice treated with MJN110. Unexpectedly, MAGL KO mice displayed comparable mechanical withdrawal thresholds to WT mice, while antinociceptive effects were observed in the locomotion and weight bearing assays. Additionally, administration of ketorolac, CJ-13610, or MJN110 did not alter thresholds in MAGL KO mice, consistent with a role of downstream EICs in post-incisional pain.

Conclusions: These results indicate that MAGL inhibition mitigates postoperative pain through the modulation of multiple EIC pathways. MAGL inhibitors may constitute promising analgesics to treat postoperative pain.

Acknowledgements: This work was funded by NIDA grant DA048002.

BLOCKAGE OF CB1 RECEPTORS POTENTIALIZES RENAL ANTI-FIBROTIC EFFECTS INDUCED BY SGLT2 INHIBITION IN DIABETIC MICE

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Introduction: Diabetic nephropathy (DN) is one of the most common complications of diabetes. While pharmacological approaches exist, including renin angiotensin aldosterone system (RAAS) blockers and sodium-glucose cotransporter type 2 inhibitors (SGLT2i), the cannabinoid 1 receptor (CB1R) has emerged as a new potential therapeutic target. Pharmacological blockade of CB1R leads to an improvement of many DN features in rodents. Since SGLT2i has become the new standard of care for DN, we evaluated if combining CB1R antagonism with SGLT2i leads to better reno-protection than SGLT2i or CB1R blockade alone.

Methods: For this, 40 C57BLKS-Leprdb/db mice and 6 control non-diabetic mice were fed a high protein diet for 9 weeks. After 5 weeks, diabetic mice were exposed to 4 different treatments: placebo, empagliflozin (SGLT2i), INV-202 (CB1R blocker), or a combination of the two compounds by oral gavage for 28 days. Blood and urine biochemistry, histological analyses, as well as quantifications of gene and protein expressions were carried out in order to study the different parameters involved in DN including fibrosis.

Results: Both SGLT2i and INV-202 alone significantly improved albuminuria and the urinary albumin-creatinine ratio while the combo was significantly more effective. This observation is important as these parameters are frequently used for DN diagnostic. Interestingly, similar observations were noted for inflammatory markers expression and for oxidative stress markers. Furthermore, we observed an additive protective effect of the combo regarding the glomerular morphology, prevention of podocyte loss and proximal tubular epithelial cells (PTEC) morphology. PTEC injury was also significantly lowered by the combo as established by a marked decrease in urinary KIM1 and DKK3 levels. Moreover, in Sirius red stained slides, we observed a significant reduction in tubulointerstitial fibrosis after combo treatment compared with monotherapy and vehicle-treated mice. In parallel, a greater repression of pro-fibrotic gene expression was noted for the combination compared with single treatments. Following these observations, we carried out a transcriptomic analysis which made it possible to identify different signaling pathways involved in the anti-fibrotic effect observed with the combo.

Conclusion: Our results suggest that a poly-pharmacological approach combining both SGLT2i and CB1R antagonism might represent a promising therapeutic strategy for the management of DN.

Acknowledgements: Funded by the French National Research Agency (ANR-22-CE14-0055-01) and Inversago Pharma, Inc.

ENDOCANNABINOIDS: A LINK BETWEEN CIRCADIAN DISRUPTION AND WEIGHT GAIN

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Introduction: Circadian (daily) rhythms govern all physiology and behavior and are a fundamental aspect of biology. Disruption of circadian rhythms is associated with significant increases in risks for psychiatric disorders and cardiometabolic disease. We have demonstrated that a model of environmental circadian disruption (exposure to a 20-hr day (T20)) leads to metabolic effects including weight gain, increased adiposity, and elevated plasma insulin and leptin. Given the role of endocannabinoids (eCBs) in regulation of metabolism, we asked if the metabolic effects of T20 are in part driven by eCBs.

Methods: Wild-type male and female C57/BL6N mice, global cannabinoid-type 1 receptor G-CB1r) and liver (hepatocyte) specific (L-CB1r) knockout (KO) mice were housed under normal T24 (LD12:12) conditions followed by 5 weeks of T20 (LD10:10) or T24 (control) conditions. Weight, feeding, drinking, locomotion, and gas exchange were continuously monitored using a behavioral and metabolic phenotyping system (TSE Systems). Plasma and hepatic endocannabinoid levels were assessed using mass spectrometry. Data were analyzed by ANOVA (repeated measures, when appropriate) followed by post-hoc tests. Circadian rhythm specific measures, including phase-angle computations and periodogram analyses, were used to quantify properties of rhythmic physiology and behavior.

Results: Our data show T20 causes weight gain in WT male mice *without* an increase in feeding. We report a significant disruption of diurnal rhythms for all measured behaviors following exposure to T20. Remarkably, WT female mice were resistant to T20 induced metabolic dysregulation, though behavioral changes were similar to those observed in males. We next asked if eCB signaling may be involved in the T20-induced metabolic phenotype. We found that plasma and liver AEA and 2-AG levels were rhythmic but had altered daily patterns in T20. To test the role of eCB signaling, we exposed G-CB1r KO mice to T20 while monitoring behavior, as above. We found that G-CB1r KO mice were clearly protected from T20-induced weight gain, despite exhibiting similar changes in behavior as WT mice following T20. Given CB1r KO mice were protected from T20-induced weight gain in a manner independent of behavior, we posited this could be peripherally mediated. Since the liver regulates macronutrient metabolism and has important eCB signaling activity, we asked if hepatic eCB signaling could be a critical node in the effects of T20. Liver-specific CB1r KO mice were exposed to T20, and we report that L-CB1r KO mice gained significantly less weight than WTs mice in T20.

Conclusions: We conclude that circadian disruption through exposure to a 20-hour day leads to weight gain in male mice, which is in part mediated through the eCB system. Using global and hepatocyte-specific CB1r KO mice, we demonstrate that the liver is an important locus of eCB action mediating T20-induced weight gain.

Acknowledgements: Funded by NIH R01DK110811 to I.K. and NSF GRFP to B.F.

MOLECULAR INSIGHTS INTO MITIGATING DIET-INDUCED OBESITY AND METABOLIC DYSREGULATIONS BY A PERIPHERALLY RESTRICTED CB1R ANTAGONIST

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Introduction: The efficacy of cannabinoid-1 receptor (CB_1R) antagonists in mitigating diet-induced obesity (DIO) and associated metabolic abnormalities has been established both in preclinical models and human studies. However, due to the CNS-related neuropsychiatric adverse effects of brain-penetrating CB_1R blockers, there is a growing interest in developing peripherally restricted compounds for clinical use. Nevertheless, the precise molecular mechanisms underlying the metabolic benefits of these compounds remain largely unexplored. In this study, we describe the synthesis and physiochemical evaluation of a novel peripherally restricted CB_1R blocker, BNS822, and investigate its metabolic efficacy and mechanism of action in liver and adipose tissue.

Methods: A library comprising 28 compounds (designated BNS801 through BNS828) was synthesized through the chemical conjugation of various functional groups to the core scaffold of 5,6,7,8-tetrahydrooxepino[3,2-c]pyrazol-8-amine, with an anticipated reduction in CNS exposure. The binding affinity to the CB_1R, its activity, and selectivity against CB_2R were evaluated. Furthermore, assessments were conducted to determine the potential for brain penetration, CNS-mediated adverse effects, and the therapeutic efficacy of BNS822 in mitigating DIO and its metabolic dysregulations including hyperglycemia, dyslipidemia, hepatic injury, and steatosis in mice. Additionally, to elucidate the underlying mechanism of action of BNS822, multicolor flow cytometry and *ex vivo* three-dimensional (3D) human hepatic spheroid cultures were employed to model the pathophysiological conditions of liver steatosis, oxidative stress, and insulin resistance typically observed in obesity and fatty liver disease.

Results: Among the library of 28 compounds, BNS822 emerged as a remarkably potent and selective CB_1R antagonist, demonstrating *Ki* values of 1.3 nM. Importantly, BNS822 exhibited diminished brain penetration and minimal CNS-mediated adverse effects, including no hyperactivity, inability to reverse CB_1R-induced catalepsy, and absence of anxiogenic effects. Upon chronic oral administration of BNS822 (at doses of 5, 20, 60 mg/kg/day for 21 days) to DIO mice, a significant and dose-dependent reduction in body weight, food intake, and fat mass was observed. Furthermore, BNS822 treatment led to improvement in glycemic control, amelioration of hypercholesterolemia, and reduction in triglyceridemia. Notably, BNS822 administration also resulted in the attenuation of hepatic injury and steatosis. At the molecular level, we found that BNS822 influenced the presence of CD11c+ adipose tissue macrophages (ATMs), known to contribute to ATM bioenergetics and lipid-associated phenotype. In addition, BNS822 modulated hepatic fatty acid oxidation via the AMPK-CPT1A molecular signaling pathway.

Conclusions: Our study highlights the promising potential of peripherally restricted CB_1R antagonists, exemplified by BNS822, in effectively addressing DIO and its associated metabolic aberrations, while exhibiting reduced CNS-mediated side effects. Insights into hepatic and immune modulation by BNS822 shed light on its unique molecular mechanism of action. These findings underscore the therapeutic promise of such compounds for the management of obesity and its related metabolic complications, warranting further investigation and clinical exploration.

Acknowledgment: This study was funded in part by the Israel Innovation Authority Grant via BioNanoSim Ltd to S.B. and J.T., as well as the Lise Meitner Grant for Israeli-Swedish Research Collaboration for N.K. and J.T.

CANNABIS HAS REMARKABLE SELF-REPORTED EFFICACY IN PEOPLE WITH EATING DISORDERS: FINDINGS FROM THE INTERNATIONAL MED-FED SURVEY

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Introduction: Eating disorders (EDs) are serious and potentially life-threatening mental health conditions, acknowledged as amongst the most difficult psychiatric conditions to manage and treat. Most EDs do not have an approved pharmacotherapy with demonstrable efficacy. The MED-FED survey is the largest international survey to investigate the self-perceived efficacy and tolerability of all drugs (prescription psychotropics, legal/non-prescription, and illicit substances) in individuals with an eating disorder/disordered eating. The survey was developed to address the pressing need to identify alternative treatments for EDs.

Methods: The MED-FED survey was open between November 2022 to May 2023 and open to individuals with an eating disorder/disordered eating, who were 18 years or older and confident in English. The survey asked their current use of prescription medications (prescribed by a doctor), caffeine, alcohol, tobacco, nicotine, cannabis, stimulants, psychedelics, opioids, and other drugs. Respondents were asked to rate their agreement or disagreement (5-point Likert scale: strongly disagree [-2], disagree [-1], neutral [0], agree [+1], strongly agree [+2]) with several statements relating to drug effects on eating disorder symptoms, general mental health, and side effects, e.g. *This medication/drug makes my eating disorder symptoms better.* Statistical analyses were conducted using R version 4.3.2 and total average response to each statement for all drugs was normalised between -2 and 2, to assess self-perceived efficacy on ED symptoms, mental health, and side effects. Logistic regression analysis was also conducted to determine possible predictors of benefits.

Results: The final sample included 7046 participants from 89 different countries of whom 5125 completed the entire survey. The mean participant age was 24 and the sample predominantly identified as female (94.0%). Most respondents were from Australia (30.0%), the United Kingdom (21.3%) and the United States (18.1%). Cannabis was the third most used drug among respondents (3018 of 5386 [56.0%]) after caffeine (98.0%) and alcohol (83.1%). The majority were using cannabis products containing THC with only 103 (3.4%) respondents using CBD-only products. Cannabis was rated more highly than all commonly prescribed psychotropics for making ED symptoms better, in individuals with avoidant/restrictive food intake disorder (ARFID) (+0.96), other specified feeding or eating disorders (OFSED) (+0.72), and anorexia nervosa (AN) (+0.63). A diagnosis of ARFID [OR = 2.24, B = 0.361, SE = 0.0714, $p < 0.001$] or AN [OR = 1.44, B = 0.708, SE = 0.115, $p < 0.001$] was also a significant predictor of positive response to cannabis. In contrast, having a binge-type ED (e.g. bulimia nervosa, binge eating disorder) was a significant predictor of a negative response to cannabis.

Conclusions: These findings suggest that persons with AN, ARFID, and OFSED find use of cannabis helpful for their ED symptoms. Future clinical trials could usefully examine this efficacy empirically relative to placebo as a novel treatment for restrictive type EDs. However, cannabis may be contraindicated in those with binge-type EDs presumably due to appetite stimulatory effects.

Acknowledgements: Funded by the Lambert Initiative and InsideOut Institute.

SAFETY, TOLERABILITY, AND CLINICAL EFFECTS OF HEMP-DERIVED CANNABIDIOL IN ALCOHOL USE DISORDER

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Introduction: Cannabidiol (CBD) has recently garnered attention as a potential pharmacotherapy for AUD with emerging evidence from animal studies suggesting one of its pharmacological effects is the amelioration of drug and alcohol-motivated behaviors. In preclinical studies, CBD reduces the reinforcing properties of alcohol and decreases alcohol consumption and drinking motivation. Although empirical human research is scant, recent studies found that CBD attenuates cue-elicited craving for opiates in humans (Hurd *et al.*, 2019), suggesting that CBD might have broad benefits with respect to cue-elicited craving and addiction. Hemp-derived CBD can be found in two primary formulations: one with the legal limit of THC (<.3%), often known as full-spectrum CBD, and one without THC, often known as broad-spectrum CBD. The objective of the present study was to conduct a Phase II randomized clinical trial (RCT) to examine the clinical effects of full-spectrum CBD (fsCBD, contains less than 0.3% THC) vs. broad-spectrum CBD (bsCBD, which does not contain THC) vs. a matching placebo in a population of subjects with moderate to severe AUD in an 8-week-long treatment trial. We hypothesized that both the fsCBD and bsCBD conditions would be associated with reduced craving and AUD symptoms at the end of treatment compared to the placebo group.

Methods: Individuals with mild to moderate AUD (n=43) were randomly assigned to one of three conditions: bsCBD 150 mg daily, fsCBD 150 mg of CBD ~5 mg of THC, or a matching placebo control for 8 weeks of treatment. At weeks 1 through 3, and 5 through 7, participants completed safety measures. At weeks 4 and 8, participants completed an in-person visit that included a battery of self-report assessments, blood draw and vitals, breathalyzer, urinalysis, and cue-reactivity tasks. An online week 16 post-treatment visit concluded participation in the trial. Differences at baseline were assessed with analyses of variance (ANOVAs). No differences in the three conditions were observed at baseline. The analyses then proceeded with a 3 (4, 8, and 16 weeks) by 3 (placebo, bsCBD, fsCBD) repeated measures analysis of covariance (ANCOVA) to assess changes and group-level differences in alcohol-related outcomes (i.e., AUDIT, PACS, ICS-FC). All ANCOVA models covaried baseline scores.

Results: There were no significant treatment effects on fatigue, sleep, dizziness, or liver function tests during the trial ($p > .05$). Individuals were not able to guess the treatment condition at greater than chance levels ($p > .05$). As expected, the analyses indicated significant differences in THC-COOH and CBD-COOH across conditions ($p < .01$). The analyses indicated a treatment x time interaction ($p < .05$) suggesting that individuals in the fsCBD demonstrated reductions in craving (PACS, $p < .05$), AUD severity (AUDIT and ICS-FC scores, $p < .05$), and cue-elicited craving ($p<.05$), while individuals in the BS-CBD and placebo conditions did not change over time.

Conclusions: The results of this preliminary study suggest that daily doses of both bsCBD (150 mg) and fsCBD (150 CBD) were well tolerated and safe. Side effects were minimal with no differences across conditions in liver enzymes, somnolence or dizziness. In addition, it is worth noting that the participants were unable to distinguish between placebo, bsCBD, and fsCBD at statistically greater than chance levels. Finally, the results indicated that fsCBD was superior to bsCBD and placebo over the study period with respect to craving and AUD measures. The results are consistent studies suggesting that CBD may have a beneficial effect on AUD models in mice (Viudez-Martínez *et al.*, 2018) and rats (Gonzalez-Cuevas *et al.*, 2018) and consistent with clinical studies suggesting that CBD may decrease craving more broadly for opioids (Hurd *et al.*, 2019). Because the study was a small proof of concept study, larger studies are needed to verify and extend these results to better understand the effects of CBD with and without THC in an AUD population

ASSESSING THE IMPACT OF DIFFERENT SMOKING METHODOLOGIES: SECONDARY ANALYSIS OF A DOSE-VARYING STUDY OF SMOKED CANNABIS

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Introduction: Determining the optimal method for administering cannabis in human laboratory research remains a challenge. Fixed or cued-smoking paradigms aim to standardize cannabis administration and delta-9-tetrahydrocannabinol (THC) dosing by controlling inhalation depth and smoke retention times. However, these methods do not accurately reflect cannabis consumption in naturalistic settings, and may increase the risk of adverse reactions if participants are required to consume more cannabis or in a manner different from what they are accustomed. In contrast, *ad libitum* self-administration allows participants to consume cannabis in a manner mirroring more closely their real-world consumption patterns and potentially reducing the risk for adverse events. This approach, however, introduces variability in dosing and cannabinoid blood concentrations, due to differences in participant smoking topographies. Our objective was to compare the impact of a fixed and *ad libitum* smoking paradigm on cannabis smoking topography and adverse event profiles within the context of a human laboratory experiment exploring the impact of varying potencies of smoked cannabis on simulated driving performance.

Methods: We conducted a randomized, double-blind, placebo-controlled, within-subjects trial of adults (ages 19-45 years) who regularly use cannabis (1-5 days/week). Participants attended four sessions, spaced at least 72 hours apart, at the Center for Addiction and Mental Health (CAMH). At baseline, an indwelling catheter was inserted in participants' forearms to facilitate serial blood draws, and participants underwent baseline driving simulation testing. They then smoked cannabis cigarettes of varying potencies (0.0%, 6.25%, 12.5%, 22.0% THC), each containing 750mg of plant material. Participants drove the simulator again at 30 and 90 minutes after smoking. Blood, oral fluid, and vital signs were collected and participants completed subjective effects questionnaires and cognitive testing. The study began with participants smoking cannabis according to a fixed paced procedure. Due to the occurrence of adverse events at higher THC doses in this paradigm, and after consulting with the Drug Safety Monitoring Board, we transitioned to an *ad libitum* smoking paradigm, allowing participants to smoke as they typically would. Smoking topography data (number of puffs, smoking duration, amount smoked, and estimated THC dose) were collected by research personnel observing the participants through a one-way window. Cannabis cigarettes were weighed before and after smoking to estimate the amount of cannabis consumed and the THC dose. Any adverse events that arose during the sessions were documented using a modified version of the SAFTEE questionnaire, which recorded the type, onset, duration, severity, relationship to the study, and action taken of the adverse events. We employed mixed models to assess changes in smoking topography and the frequency of adverse events across conditions.

Results: There was a significant effect of cannabis potency on the amount smoked, smoking duration, and estimated THC dose (all $p<0.001$), but not on the number of puffs ($p=0.052$). Participants consumed less and smoked for shorter durations as THC potency increased, with significant reductions in the high-dose condition ($p<0.001$). The incidence of adverse events varied with smoking methodology; 15 events were reported during the 43 sessions conducted using the fixed smoking paradigm, compared to 9 events during the 101 sessions that utilized the *ad libitum* smoking procedure. Intense cannabis intoxication was notably higher in the high-dose condition, and incidents of fainting and nausea/vomiting were more common during fixed smoking paradigm.

Conclusion: Different doses of THC may affect cannabis smoking behaviour/topographies, with higher doses leading to less consumption, smoking duration and amount smoked. *Ad libitum* smoking paradigms may potentially mitigate the incidence of adverse events at higher doses of THC. These findings are pertinent in the context of the high potency of cannabis products available across both regulated and unregulated markets. These findings highlight the need for careful consideration of smoking procedures in human cannabis research, especially when investigating high potency cannabis products, to ensure a balance between accurate dosing and ensuring participant safety.

Acknowledgements: Funded by Public Safety Canada.

FATTY ACID AMIDE HYDROLASE SINGLE NUCLEOTIDE POLYMORPHISM rs324420A/C ASSOCIATIONS WITH ALZHEIMER'S DISEASE

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Introduction: The endocannabinoid system has demonstrated roles in Alzheimer's Disease (AD), such as modulation of inflammation. Fatty Acid Amide Hydrolase (FAAH) is the enzyme responsible for the rapid inactivation of the endocannabinoid anandamide into arachidonic acid and ethanolamine. In doing so, FAAH modulates the concentration of anandamide and influences neurobehavioral functions and physiological conditions such as nociception and inflammatory responses. A missense single nucleotide polymorphism in the *FAAH* gene, rs324420-C>A is associated with higher levels of anandamide, reduced susceptibility to PTSD, and increased risk of substance use disorders. However, the relationship between rs324420 and AD progression has not been explored. FAAH inhibition is suggested to drive microglial polarisation towards anti-inflammatory phenotypes, but in AD, FAAH methylation is lowered, resulting in increased activity, rs324420 increases proteolytic degradation, thus reducing the functionality of FAAH. Due to lowered FAAH activity, we hypothesise that rs324420 minor allele carriers will have slower neurodegeneration and slower cognitive decline in AD.

Methods: This retrospective, longitudinal, observational study used data from the Alzheimer's Disease Neuroimaging Initiative (2/GO/3 cohorts). Patients diagnosed with PET amyloid-beta (Aβ) positive cognitively normal or mild cognitive impairment or mild AD (MCI or AD) were selected. Linear regression models evaluated baseline differences between rs324420 minor allele carriers (CA/AA) and major allele homozygotes (CC), controlling for sex, age, baseline cognitive status, APOE-e4 status and baseline intracranial volume. Longitudinal associations were assessed as interactions between SNP and time (baseline to 48 months) in mixed effects models.

Results: In Aβ-positive patients with AD or MCI, rs324420 minor C-allele carriers (minor allele frequency=26.4%) have smaller nucleus accumbens volume ($F_{(1,311)}$=15.7,p=0.018) at baseline. Cognitively normal Aβ-positive C-allele carriers at baseline had significantly larger caudate nucleus volume ($F_{(1,93)}$=4.01,p=0.047). Longitudinally, several brain regions showed less volumetric decline in C-allele carriers over 48 months: caudal anterior cortex ($F_{(1,163)}$=5.18,p=0.024), fusiform gyrus ($F_{(1,85.6)}$=5.28,p=0.024), rostral anterior cortex ($F_{(1,59.9)}$=6.41,p=0.014) and nucleus accumbens ($F_{(1,84.7)}$=4.75,p=0.032). Cognitively normal Aβ-positive C-allele carriers also showed significantly slower rates in whole-brain degradation ($F_{(1,84.7)}$=4.75,p=0.032). These protective effects were not observed in Aβ-negative patients.

Conclusions: Baseline data for rs324420 Aβ-positive minor allele carriers did not directly support our hypothesis in MCI or AD patients, but Aβ-positive cognitively normal carriers showed a larger baseline caudate nucleus volume which may imply protective effects through decreased FAAH activity. Slower rates of cognitive and neuroanatomical decline in Aβ-positive patients with MCI or AD, as well as cognitively normal carriers, suggest a potential protective role of the rs324420 minor allele in AD. Findings from this study support our hypothesis that the decreased FAAH activity serves as a protective role in AD only when carriers are predisposed to increased Aβ levels, as this slowing of degradation was not observed in Aβ-negative carriers. Further investigation is warranted, including cognitive, anatomical, fluid biomarker and neuropsychiatric symptom profiles.

CANNABIDIOL POTENTIATES SOME ANALGESIC EFFECTS OF MORPHINE

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Introduction: Cannabidiol (CBD), a minimally psychoactive constituent of cannabis, has been shown in preclinical studies to have analgesic effects. The use of CBD as an adjunct to opioids for pain treatment could reduce risks of opioid use, but the effects of CBD on morphine abuse liability are unclear. We assess the effects of CBD alone and combined with morphine on responses to laboratory pain tasks and examine whether CBD alters morphine abuse liability in humans.

Methods: In this placebo-controlled, double-blind, crossover study, healthy individuals (18-55 yrs) complete three laboratory sessions in which they receive oral morphine (0mg, 15mg, and 30mg; one dose per session) and vaporize CBD (0.5g cannabis containing 0.003% THC and 0.00% cannabidiol [smoking bout 1] vs 9.7% cannabidiol [smoking bout 2]). Pre- and post-drug administration, antinociceptive responses to thermal and pressure stimuli (Medoc Q-Sense) and Cold Pressor Test (CPT) are assessed, and self-reported subjective drug effects (e.g., drug liking, drug strength, etc.) are collected. Scores on the pain tasks were converted to change from baseline values.

Results: Participants completed to date include seventeen (10 female) healthy adults (28.4±5.9 years) with $\geq$1 lifetime use of smoked cannabis and $\geq$3 episodes of opioid use, and no diagnosis of pain or substance use disorder. Repeated measures ANOVAs conducted on pain task data revealed main effects for morphine dose on CPT pain threshold ($F(2,30)=5.63$, $p=.03$, $\eta_p^2=.27$), with longer latency to report cold-induced pain following 30mg morphine relative to 15mg and placebo. A main effect for CBD was found on CPT pain threshold ($F(1,15)=23.82$, $\eta_p^2=.61$) and pain tolerance ($F(1,16)=13.21$, $p=.002$, $\eta_p^2=.45$), with longer latencies to report and escape cold stimulation after CBD relative to placebo. A morphine X CBD interaction on pain tolerance ($F(2,32)=5.71$, $p=.008$, $\eta_p^2=.26$) indicated greater pain tolerance after CBD when combined with 30mg morphine compared to 15mg morphine, and after 30mg morphine after CBD compared to placebo. Linear regression indicated CBD is potentiating "drug strength" of 30mg morphine ($F(1,6)=6.806$, $p=.04$, $\eta_p^2=.531$) but not 15mg.

Conclusions: Morphine increased CPT pain threshold, and CBD increased both CPT pain threshold and pain tolerance, but did not have effects on thermal or pressure stimuli. The highest level of pain tolerance was observed with high dose morphine combined with CBD. These results indicate that CBD may potentiate opioid analgesic effects, and self-reported subjective effects, especially at higher doses. It appears that CBD may not have opioid sparing effects.

Acknowledgements: Funded by NIH R21 DA047662 (LHL), Gertrude Levin Endowed Chair in Addiction and Pain Biology (MKG), Michigan Department of Health and Human Services (Lycaki/Young Funds).

GROWING UP: HOMEGROWN CANNABIS IN THE AUSTRALIAN CAPITAL TERRITORY FOLLOWING DECRIMINALISATION OF CULTIVATION

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Introduction: In December 2020, the state government of the Australian Capital Territory (ACT), which includes the nation's capital Canberra, legislated to allow the small-scale cultivation and possession of cannabis by individuals. This marked a shift away from conservative drug laws within Australia where cannabis possession and cultivation has been traditionally illegal and legal pathways to access prescription medical cannabis has only been recently introduced.

Methods: The CAN-ACT survey (open from September 2022 to August 2023) was an observational, non-interventional online survey which examined cannabis cultivation and use by ACT residents relative to the new legislative changes. Respondents were queried on their medical and non-medical cannabis-use, cultivation behaviours, adverse events arising from cannabis-use and/or cultivation, and their understanding of, attitudes towards and future preferences for the legislation. Cultivators were encouraged to provide an anonymous sample of their home-cultivated cannabis for analysis of phytocannabinoids and contaminants, and results were provided back to them.

Results: *Survey:* A total of 434 respondents completed or part-completed the survey and were mostly cultivators (66%): 38% were using cannabis only non-medicinally, 11% only medicinally, and 38% for both purposes. Reasons for cultivation included preference for homegrown cannabis, enjoyment of cultivation, and affordability in relation to prescription products. Many respondents admitted being technically in breach of current laws by (i) growing more plants and harvesting more dry cannabis plant material than was legally allowed (e.g. > 50 g) (45% and 63% respectively), (ii) illegally obtaining or supplying seeds and cuttings to enable cultivation, (iii) employing indoor hydroponic cultivation (11%), and (iv) supplying family and friends with homegrown cannabis (59%). Despite decriminalisation, many cultivators (45%) remained anxious about arrest and 50% were dissatisfied with the legislation. *Chemical analyses:* A total of 72 cannabis samples were obtained for analysis. THC was the main cannabinoid present in most samples (mean 8.97 ± 0.51 % [w/v]) with only 5 samples showing CBD content >1% (mean 0.54 ± 0.22 % [w/v]). After THC and CBD, the next three highest total concentrations were CBG, CBC, and CBN. A higher proportion of cultivators had expected high CBD content than was present in submitted samples. The majority of cannabis samples adhered to regulatory limits for concentrations of heavy metals, pesticides and mycotoxins, making them comparable to prescription cannabis products. Four of the samples that exceeded regulatory safety limits were due to the pesticide fluvalinate, and one sample for the pesticide methamidophos. While most samples had trace levels of heavy metals, only one sample exceeded regulatory limits for arsenic.

Conclusions: Decriminalisation of cannabis use and cultivation in the ACT appears to generally support the needs of the community with few obvious drawbacks. Legislative change could be further refined by clarifying cultivation restrictions, permitting community-based cannabis testing facilities, and providing guidance on cultivation practices.

Acknowledgements: Funded by the Lambert Initiative for Cannabinoid Therapeutics.

CANNABIDIOL (CBD) BLOCKS MARKERS OF EXERCISE-INDUCED MUSCLE DAMAGE FOLLOWING DOWNHILL RUNNING IN A DOUBLE-BLIND RANDOMISED CROSSOVER TRIAL

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Introduction: Cannabidiol (CBD) has demonstrated anti-inflammatory, analgesic, anxiolytic and neuroprotective effects, which have attracted the attention of athletes. CBD is available commercially in the UK with a daily limit of 70mg advised by the Food Standards Agency at the time of the study design. This study investigated the effects of CBD following muscle-damaging exercise.

Methods: Twenty healthy volunteers (mean ± SD) age 22 ± 3 years (10 males and 10 females), $\dot{V}O_2$max: 46.5 ± 6.7 mL.min-1.kg-1, participated in this randomised, double-blind, crossover trial. Participants completed two experimental sessions, separated by a ≥7-day washout. On the first study visit, participants were administered a single oral dose of CBD (70 mg in medium-chain triglyceride (MCT) oil) or placebo (MCT oil) (randomised), co-administered with consumption of a standardized snack bar (257 kcal, 41g CHO, 8g fat, 3g protein), 1 hour before undertaking a submaximal -10% downhill run for 30 minutes at 70% $\dot{V}O_2$max. Venous blood was drawn pre- and post-run, then at 24- and 48-hours post-run. Data were analysed using mixed-model ANOVA with a Šidák post-hoc test. Significance was defined as $p<0.05$.

Results: Downhill running caused a significant decrease in pain tolerance (algometer pressure) that was blunted in the CBD treatment (post-run: -19.6 ± 15.6% in placebo, -2.4 ± 17.3% in CBD, $p <0.01$). This was associated with an increase in plasma creatine kinase activity 24h after running in the placebo treatment that was similarly blunted by CBD (24h: +25.3 ± 23.4% in placebo, +3.1 ± 14.2% in CBD, $p <0.01$). CBD caused a change in substrate utilisation through a 38.6% increase in fat oxidation (RER, $p <0.01$). Downhill running increased plasma TNF-α ($p <0.05$) across the 48h following downhill running, but there was no effect of CBD ($p >0.05$). Lipidomic analysis of plasma samples pre- and post- downhill running revealed an increase in N-acylethanolamines AEA ($p <0.001$), OEA ($p <0.001$), PEA ($p <0.001$), SEA ($p <0.001$), and LEA ($p <0.001$) but there was no effect of CBD ($p >0.05$). CBD and its metabolites were evident in the expected plasma samples.

Conclusion: A single ingested dose of 70mg CBD blunts markers of exercise-induced muscle damage within the 48h following exercise. The mechanism of this benefit is not reflected in plasma-derived inflammatory cytokines nor changes in fatty acid amide metabolites. Further lipidomic and metabolic analyses (ongoing) could reveal a potential mechanism.

OPEN LABEL CLINICAL TRIAL OF SELONABANT AS AN ANTIDOTE FOR HIGH-DOSE ACUTE CANNABINOID INTOXICATION IN HEALTHY ADULTS

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Introduction: Emergency department visits due to acute cannabinoid intoxication (ACI) and unintentional cannabis poisoning have increased in the US as more US states have liberalized cannabis policy. Clinical effects of ACI include anxiety, panic attacks, tachycardia and psychosis and are mediated through cannabinoid type 1 receptor (CB1) primarily by the CB1 agonist delta-9-tetrahydrocannabinol (THC). Parts A and B (six cohorts) of a Phase 2 study previously showed single doses of CB1 antagonist selonabant (formerly ANEB-001) were safe, well tolerated and rapidly reversed THC-induced symptoms in healthy adults. Part C of the study presented here, assessed the potential of selonabant to prevent effects induced by very high doses of THC in healthy adults.

Methods: Part C was an open label extension to the randomized controlled trial of selonabant (NCT05282797) and comprised two cohorts of 10 cannabis-experienced adult participants each: cohorts 7 (C7) and 8 (C8). Participants received single oral doses of selonabant (C7: 10 mg, C8: 20 mg) simultaneously with high oral doses of THC (C7: 40 mg, C8: 60 mg). Primary pharmacodynamic (PD) measures included visual analogue scales (VAS) for "Feeling High" and "Alertness", body sway and heart rate. Using mixed model ANCOVA, changes from baseline were analyzed and PD outcomes of Part C were also compared to pooled placebo participants (N=10) of two cohorts of Part B (dosed with 21 or 30 mg THC and placebo selonabant). Adverse events (AEs) were assessed.

Results: Only C8 PD results are reported here for brevity; C7 PD results are similar. When administered with selonabant, a 60 mg dose of THC had minimal effects on PD outcomes. For VAS "Feeling High", Estimated Difference (ED) from baseline was 0.2 log(mm) (95% Confidence Interval (CI): 0.1, 0.2 log(mm), **p<.0001**). VAS "Alertness" did not differ significantly from baseline (ED -0.5 mm, 95% CI -2.0, 0.9 mm, p=.45). Body sway (ED 71.0 mm, 95% CI: 47.9, 94.0 mm, **p<.0001**) and heart rate (ED 5.8 bpm, 95% CI: 4.3, 7.4 bpm, **p<.0001**) were also minimally increased from baseline. When compared to previous THC doses without selonabant in Part B, participants in C8 had improved PD outcomes: significantly lower VAS "Feeling High" (ED -1.0 log(mm), 95% CI: -1.4, -0.7 log(mm), **p<.0001**), increased VAS "Alertness" (ED 12.6 mm, 95% CI: 6.8, 18.4 mm, **p=.0001**), reduced body sway (ED -97.2 mm, 95% CI: -187.5, -6.9 mm, **p=.04**) and reduced heart rate (ED -11.2 bpm, 95% CI: -17.7, -4.7 bpm, **p=.002**). All AEs were transient and mild, except one event of vomiting of moderate severity in C7.

Conclusion: Selonabant effectively mitigated the effects of high oral doses of THC, as evidenced by very limited THC effects and the absence of moderately severe symptoms of ACI that would typically be induced by such high doses of THC.

Acknowledgements: This research was funded by Anebulo Pharmaceuticals.

SELF-REPORTED EFFECTS OF TETRAHYDROCANNABIVARIN ON ACTIVITY, ENERGY LEVEL, MOTIVATION, AND APPETITE: A DOUBLE-BLIND, PLACEBO-CONTROLLED, DOUBLE CROSSOVER TRIAL

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Introduction: Tetrahydrocannabivarin (THCV) is a rare cannabinoid with potential impacts on energy, activity level, motivation, focus, and appetite. Anecdotal evidence suggests that ingesting THCV results in the perception of increased energy and activity levels and may mitigate the increase in appetite associated with THC.

Methods: We evaluated 78 adult participants in a double-blind, placebo-controlled trial with a within-individual crossover design. Each participant was randomly assigned to either ingest a placebo, THCV+THC (6.5 mg THCV, 3.4 mg THC), or THC (5 mg THC) gummy on 3 days during one week. In two subsequent weeks, participants then crossed over into each of the other treatment arms. Participants reported on daily and weekly subjective metrics of energy, activity, motivation, focus, appetite, and adverse events using the CHLOE platform, (Consumer Health Learning & Organizing Ecosystem) (People Science Inc., Venice, CA). Kruskal Wallis (non-parametric ANOVA) tests were used to evaluate potential group differences. Dunn's correction was used to evaluate p-values for individual comparisons among groups. Bonferroni correction was used to account for comparisons. This study and all procedures were approved by Advarra IRB (Pro00071993).

Results: Both THCV+THC and THC increased subjective energy, activity, exercise performance, and well-being compared to placebo ($p<0.05$). Participants taking THCV+THC reported significantly fewer fatigue adverse events than both placebo and THC only, and THC resulted in significantly more fatigue adverse events than placebo ($p<0.05$ for all 3 comparisons). Participant-reported THC consumption habits prior to the study determined the impact of the interventions on hunger, with the THC-only intervention significantly increasing hunger compared to placebo ($p<0.05$).

Conclusions: THCV+THC, as well as THC alone, increased subjective energy, activity, motivation, and well-being compared to placebo. However, THCV may alleviate symptoms of fatigue associated with taking THC alone. In addition, THCV ameliorated the increase in appetite associated with THC consumption.

EFFECTS OF PHYTOCANNABINOIDS ON BLOOD PRESSURE AND PREFRONTAL CORTEX OXYGENATION IN FEMALE POST-CONCUSSION SYNDROME PATIENTS: CASE SERIES

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Introduction: Post-concussion syndrome (PCS) can alter neurophysiological and cerebral hemodynamic activity. Cannabidiol (CBD) has the potential to be a therapeutic option for recovery from PCS, although more human evidence is needed. While improvements in PCS symptoms and anxiety have been associated with CBD administration, there is still a need to study how CBD influences cerebral oxygenation activity in response to fluctuations in heart rate and blood pressure. This study assessed the cardiac and respiratory contribution to cerebral oxygenation following twice-daily administration of CBD in three female PCS participants. We have previously shown that the baroreflex and blood pressure dynamics improve with CBD intake when using a slow-paced breathing maneuver.

Methods: Oxyhemoglobin (HbO_2), deoxyhemoglobin (HHb), total hemoglobin (tHb = HbO_2 + HHb), and hemoglobin difference (HbD = HbO_2 – HHb) were monitored at the right prefrontal cortex using near-infrared spectroscopy (NIRS). Three participants completed a 5-min seated rest, and then were guided through a 5-min controlled breathing protocol (consisted of a 5-sec inhale, followed by a 5-sec exhale; 0.1 Hz). A wavelet transformation separated the NIRS signals into interval I (0.6–2 Hz; cardiac activity) and interval II (0.1–0.6 Hz; respiratory function). Participants self-administered the CBD under the guidance of their physician, with dosages ranging from 25mg to 400mg per day. One participant self-administered 20mg CBD: 1mg tetrahydrocannabinol (THC) per day, with a maximum dose of 40mg CBD: 2mg THC per day. Participants were assessed before any CBD administration (baseline), and every 17 ± 6 days (up to 70 days of CBD administration) after beginning CBD intake. Blood samples were collected to analyse venous plasma CBD concentrations. Changes in median wavelet amplitudes (from the highest measured plasma CBD concentration) in comparison to baseline are presented.

Results: At a CBD concentration of 3.27ng/mL, Participant 1 (40mg CBD:2mg THC dose) had a 40% decrease at both intervals I and II for the HbO_2 and HbD amplitudes, respectively. At 5.7ng/mL, Participant 2 (200mg CBD dose) showed a 23% increase at interval I for HbD amplitude, and a 60% increase at interval II for tHb amplitude. At a concentration of 12.2ng/mL Participant 3 (50mg CBD dose) showed a 28% increase at interval I and a 62% increase at interval II , both for tHb amplitude.

Conclusions: CBD administration can alter the respiratory and cardiac contribution to cerebral oxygenation during a paced breathing maneuver, with the combination of THC and CBD appearing to have contrasting results to CBD alone. These findings suggest that CBD can modulate cerebral hemodynamic activity in PCS, although a standardized dosing regimen is required in further research.

Acknowledgments: Mitacs Canada Accelerate Grant funding supported a post-doctoral fellowship, and funding from the National Football League (NFL) and NFL Players Association (NFLPA) Joint Pain Management Committee (New York, USA) supported this research related to the methodology.

DIFFERENCES IN IMPULSIVITY AND RISK TAKING AMONG PEOPLE WHO USE COCAINE AND CANNABIS

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Introduction: Data suggest that drug use is associated with impulsivity and risk-taking, including increased gambling risks, greater gambling loss sensitivity, steeper delay discounting across multiple domains, increased risky sexual behavior, and increased thrill seeking (e.g., Berry & Johnson, 2018; Reynolds, 2006). Data also suggest that impulsivity and risk-taking may also be related to polysubstance use (e.g., Liu et al., 2018); however, little work has examined associations between and among impulsivity, risk-taking, and polysubstance use. No work to date has examined the potential impact of concurrent cannabis and cocaine use on risk-taking and impulsivity. The current study aimed to examine differences between and among individuals with Cocaine Use Disorder (CUD) who tested positive for cocaine only (+coc/-thc), those who tested positive for both cocaine and cannabis (+coc/+thc), and those who tested negative for both cocaine and cannabis (-coc/-thc) with respect to drug use, risk-taking, and impulsive behaviors.

Methods: Data for the current study were derived from baseline measures of a randomized controlled trial examining effects of reduced cocaine use on indices of cardiovascular, immune, and psychosocial health (R01DA043938). Participants (N = 122) completed a Timeline Followback for cocaine, cigarette, alcohol, and other drug use, a DSM-V CUD assessment, the Risk Aversion Task, the HIV Risk-Taking Behavior Scale, the Balloon Analog Risk Task, and the Barratt Impulsiveness Scale-11. Participants were categorized based on urine tests result for cocaine and cannabis.

Results: One-way ANOVAs revealed that endorsed symptoms of CUD were similar across groups; however, those who tested -coc/-thc reported the lowest past 7, 30, 90 day cocaine use, and those who tested +coc/+thc reported the highest cocaine use. Other drug use in the past 7, 30, 90 days followed a similar trend to cocaine. Findings suggest individuals who tested -coc/-thc gambled more (i.e., number of gambles) than those who tested +coc/-thc, but not those who tested +coc/+thc; co-use or lack of use did not differ with respect to highest gamble placed. Further, those who tested +coc/+thc reported greater risky sexual behavior compared to others. Groups did not differ with respect to impulsivity, intravenous drug use or risk taking.

Conclusions: Co-use of cocaine and cannabis among those with CUD may impact outcomes of risk-taking and impulsivity but further data are needed to fully elucidate these phenomena.

Acknowledgements: This research was supported by a grant from the National Institute on Drug Abuse (R01DA043938; NCT03224546). The funding agency had no role in study design, data collection or analysis, or preparation and submission of the presentation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

CANNABIS USE AND NON-HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AMONG YOUNG ADULTS WITH A POSITIVE COVID-19 TEST: PRELIMINARY FINDINGS FROM THE HERBAL HEART STUDY COHORT

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Introduction: Elevated non–high-density lipoprotein cholesterol (non-HDL-C) levels are strongly associated with long-term cardiovascular disease (CVD) risk and is a more powerful predictor of CVD risk versus low density lipoprotein (LDL) cholesterol alone. It has been reported that pre-COVID-19 disease non-HDL-C levels are a prognostic biomarker for disease severity. Studies have also reported an increase in HDL-C and partial reductions in LDL-C and total cholesterol after cannabis ingestion. Yet, no studies have explored the association between elevated non-HDL-C and cannabis use by COVID-19 disease status.

Methods: Data are from the ongoing Herbal Heart Study cohort of 18-to-35-year-old cannabis users and non-users in the Southeastern United States that investigates the effect of cannabinoids and various routes of cannabis consumption on subclinical cardiovascular risk. Cannabis was self-reported and confirmed by urine and blood toxicology. Route of use included joint, blunt (cannabis rolled in tobacco leaf), and vape. Blood samples were obtained after 8 hours of fasting. Study participants were relatively healthy and excluded from the study if they had a history of taking any medications for blood pressure, glucose, or cholesterol. Elevated non-HDLC level was defined according to the American Heart Association criteria (>130 mg/dL). COVID-19 test results were self-reported by study participants. Chi-squared tests/Fisher's exact test, where appropriate, and multivariable logistic regression analysis were conducted to examine associations of interest.

Results: Of the total sample (N=150, 50.7% cannabis users), 70 (46.7%) self-reported testing positive for COVID-19. Among those who tested positive, 67.1% were female; 58.6% were cannabis users. In the overall sample, no significant differences in elevated non-HDL-C levels among cannabis users and non-users (21.3 vs 34.2, p=0.080)Among those who tested positive for COVID-19, more non-cannabis users (41.4%) had elevated non-HDLC levels compared to users (17.5%, p=0.028). After adjusting for age, sex, and race/ethnicity, cannabis users had 71% lower odds (aOR:0.29, 95% CI: 0.10-0.96) of elevated non-HDL-C levels compared to non-users. Among those who tested negative for COVID-19, the majority of vape users (58.3%) followed by non-users (29.5%) had elevated non-HDL-C levels compared to blunt (9.1%) or joint users (9.1%, p=0.029). No significant differences in elevated non-HDL-C levels were found by cannabis administration routes among those positive for COVID-19 (p=0.163), and no significant differences were observed in abnormal non-HDLC levels by cannabis use status among those negative for COVID-19 (p=0.765).

Conclusion: Findings suggest that COVID-19 positive cannabis users had lower odds of elevated non-HDL-C levels than non-users, after adjusting for demographics. Non-users with COVID-19 exhibited a higher prevalence of elevated non-HDL-C. Findings imply a complex interplay between cannabis use, COVID-19, and cardiovascular health, emphasizing need for additional research.

Supported by: NHLBI R01HL153467 (PI: Denise Vidot); NIMHD T37MD008647 (PI: Johis Ortega).

PSILOCYBIN AND CANNABIS USE BY MENTAL HEALTH STATUS IN THE UNITED STATES, CANADA, EUROPE, AND NEW ZEALAND DURING THE COVID-19 PANDEMIC: RESULTS FROM THE COVID-19 CANNABIS HEALTH STUDY

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Introduction: The unforeseen COVID-19 pandemic led to a surge in anxiety and depression globally, influencing change in substance use patterns worldwide. Our objective is to estimate the prevalence of mental health conditions during the pandemic among psilocybin and cannabis consumers by region [United States (US), Canada, Europe, and New Zealand].

Methods: Data are from the COVID-19 Cannabis Health Study, a multisite, global cross-sectional study that administered an online anonymous survey using REDCap open to adults who endorsed cannabis use at least once in their lifetime. Participants self-reported cannabis and psilocybin use. Mental health was assessed using the Generalized Anxiety Disorder-7 (GAD-7) and Centre for Epidemiological Studies Depression Scale (CES-D-10) for anxiety and depression respectively. Presence of minimal to severe anxiety and depression were compared by cannabis and psilocybin use status among regions. Descriptive statistics and Chi-Square/Fisher's Exact tests, where appropriate, were applied using SAS at a two-tailed alpha set to 0.05.

Results: Of 2647 participants, 50.2% were males and 82.7% were non-Hispanic White, with a mean age of 42.3 years (SD=15.7). The majority were from the US (90.3%), followed by Canada (3.6%), Europe (3.5%), and New Zealand (2.6%). Among the overall sample, 94.2% reported cannabis use in the past year, and 96.6% were current cannabis consumers (use in past 30-days); no significant regional differences in current cannabis use were found (p=0.24). Prevalence of psilocybin use during the pandemic varied by region: New Zealand (36.9%), US (25.3%), Europe (19.3%), and Canada (16.1%, p=0.02). Overall, respondents from New Zealand had a mean GAD-7 score of 9.11 (mild anxiety), while those from Canada (12.7, SD=6.7), Europe (10.6, SD=5.7), and the US (11.7, SD=7.0) had mean scores indicating moderate anxiety. Among current cannabis users, 25.6% in Europe had moderate to severe anxiety and 48.8% had depression. In the US, 39.7% had moderate to severe anxiety and 53.1% had depression. New Zealand had 22.4% with moderate to severe anxiety and 43.3% with depression. Canada had the highest prevalence of anxiety (40.9%) and depression (62.5%) than other regions. Notably, among Canadian psilocybin users, 71.5% experienced moderate to severe anxiety, and 85.7% had depression. In Europe, 23.5% of psilocybin users had anxiety, and 41.2% had depression. In the US, 43.6% had anxiety, and 55.1% had depression. In New Zealand, 20.8% had anxiety, and 45.8% had depression.

Conclusion: Findings suggest differential generalized anxiety and depression by cannabis and psilocybin use. Regional differences in psilocybin use and mental health-related symptoms among cannabis and psilocybin users highlight the importance of considering regional variations in understanding substance use patterns and associated mental health implications at times of public health crisis.

STABILITY OF CANNABINOIDS IN CANNABIS: PLANT MATERIAL, EXTRACTS, OIL FORMULATIONS, AND ISOLATES (CBD AND Δ^9-THC) UNDER DIFFERENT STORAGE CONDITIONS

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Introduction: The changes in Cannabis policies, at least on the State level, have generated much interest in clinical investigations using a variety of cannabis derived products (plant material, extracts and isolates). These investigations usually extend over a long period of time and therefore, it is critical to know the shelf life of the products when stored under different conditions. This presentation deals with the stability of different cannabis preparations upon storage at room temperature, refrigerator and freezer conditions over time.

Methods: All products (cannabis biomass of different chemovars, extracts, and oil formulations, pure CBD, and pure Δ^9-THC) were stored under different temperature conditions (room temperature, 4 ^{o}C±2 and -20 ^{o}C±2). CBD extract in an oil formulation (sesame seed oil) was stored at room temperature. All products were periodically subjected to cannabinoid analysis up to 72 months. The analysis is carried out following our previously published and validated GC/FID method. Although the samples were analyzed for all major cannabinoids Δ^9-THC, CBD, CBC, CBG, CBN and THCV), only Δ^9-THC, CBD and CBN content were used to monitor product stability over time.

Results: Preparations with CBD as the major /predominant cannabinoid as well as pure CBD isolate have shown long term stability at all storage condition and for all types of preparations. On the other hand, preparations with Δ^9-THC as the major cannabinoid (THC rich) showed better stability under freezer conditions (-20^{o}C) for longer storage time, than those stored refrigerated, with much shorter stability (± 10% of time zero values) under room temperature storage. It was noticed however, that, for the THC rich extracts, non-decarboxylated extracts had longer shelf life than decarboxylated extracts. CBD extract formulation in sesame seed oil showed stability at room temperature for over 5 years.

Conclusions: Based on the current study, it is recommended to store high THC and THC/CBD cannabis chemovars, extracts and pure Δ^9-THC in freezer (-20 ^{0}C). High CBD cannabis chemovar, and pure CBD should be stored refrigerated (4 ^{0}C). Furthermore, storing THC rich extracts as an ethanolic solution provides much longer shelf life.

Acknowledgement: Supported in part by the National Institute on Drug Abuse, contract #75N95023d00010.

EFFECTS OF PAIN AND PAIN CATASTROPHIZING ON CANNABIS AND OPIOID ANALGESIC USE, AFFECT, SLEEP AND MODERATION BY GENDER AND HIV

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Introduction: The global burden of chronic pain is high and has led to over-prescribing of opioids. Among those with chronic pain, an estimated one-third will express pain catastrophizing. Chronic pain is associated with anxiety, depression, and insomnia. There is increasing evidence that HIV and gender complicate this relationship. Many patients use cannabis for pain. The objective of analysis is to determine whether, in a 18-month longitudinal cohort study, pain and pain catastrophizing were associated with opioid use, medical cannabis use, and symptoms of anxiety, depression, and insomnia. We also examined whether HIV diagnosis or gender moderated these relationships.

Methods: The Medical Marijuana and Opioids (MEMO) Study recruited patients from Montefiore Medical Center (Bronx, NY) and four cannabis dispensaries in New York City. Participants were: adults with chronic pain; taking opioids within 30-days of enrollment (self-report); and recently certified for medical cannabis. It had seven quarterly research visits and web-surveys every two weeks. In web-surveys, participants reported pain (Pain, Enjoyment, and General Activity Scale, 0-10, continuous), medical cannabis use (days of use) and opioid use (name, dose, frequency). Quarterly, we assessed pain catastrophizing (Pain Catastrophizing Scale, 0-52, continuous), anxiety (Generalize Anxiety Disorder 7-item scale, 0-21, continuous), depression (Patient Health Questionnaire 9-item scale, 0-27, continuous), and insomnia (Insomnia Severity Index, 0-28, continuous). We used generalized estimating equations to assess whether pain and pain catastrophizing were associated with medical cannabis use, opioid use, anxiety, depression, and insomnia. We included HIV and gender as moderators.

Results: 223 participants completed the study (M_{age}= 55.8, *SD*=13.0; 54.3% female), 45 of which were people with HIV. Greater pain severity was associated with more opioid use (β=4.68, p=0.017) but not more cannabis use over time. Pain catastrophizing (β=-0.03, p=0.009) and HIV (β=-3.10, p<0.001) were both negatively related to cannabis use but not opioid use over time. Pain severity was positively related to symptoms of depression (β=0.85, p<0.001), anxiety (β=0.72, p<0.001), and insomnia (β=0.92, p<0.001) over time. Similarly, pain catastrophizing was positively related to depression (β=0.21, p<0.001), anxiety (β=0.20, p<0.001), and insomnia (β=0.20, p<0.001) over time. HIV diagnosis attenuated the positive relationship between pain catastrophizing and symptoms of depression (β=-0.06, p=0.029) and anxiety (β=-0.07, p=0.005) over time. Gender was not associated with any outcomes and was not a moderator.

Conclusions: Pain severity and pain catastrophizing were associated with symptoms of depression, anxiety, and insomnia, replicating prior work. Interestingly, pain severity was significantly related to opioid use but not cannabis use, and pain catastrophizing was related to cannabis use but not opioids. Our findings suggest that HIV diagnosis may be a unique determinant of individual pain experiences. More research among economically and ethnically diverse samples such as ours is needed to both understand what drives cannabis versus opioid use, and to further evaluate how affect and HIV diagnosis influence medication-related behaviours.

Acknowledgements: This study was funded by F31AA029632 (PI: Martin-Willett) R01DA044171 (PI: Arnsten); K23DA053997 (PI: Slawek); UL1TR001073; Einstein-Rockefeller-CUNY Center for AIDS Research (P30-AI124414) which is supported by the following NIH Co-Funding and Participating Institutes and Centers: NIAID, NCI, NICHD, NHBL, NIDA, NIMH, NIA, FIC, and OAR.

ANALGESIC, OREXIGENIC, AND SUBJECTIVE EFFECTS OF CO-ADMINISTERED VAPORIZED DELTA-9-TETRAHYDROCANNABINOL AND CANNABIGEROL (CBG): A PREVIEW OF DATA FROM THE CBG STUDY

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Introduction: Pain is a public health burden and there are few effective treatments without adverse effects. Anorexia frequently co-occurs with pain and severity of appetite impairment is positively associated with pain intensity. Delta-9-tetrahydrocannabinol (THC), the primary psychoactive component of cannabis, exhibits promise as an analgesic, and is an FDA-approved therapeutic appetite stimulant. However, THC's analgesic and orexigenic effects can be accompanied by intoxicating and abuse-related subjective effects thus limiting its clinical utility. Preclinical studies report cannabigerol (CBG), a minor cannabinoid, lacks the psychoactive effects of THC, and can have both analgesic and orexigenic effects. We sought to be the first to test the analgesic, orexigenic, and subjective effects of vaporized THC and CBG alone and co-administered in various ratios in healthy human volunteers.

Methods: Preliminary data from healthy male (n = 4, 31.5 ± 3.0 years) and female (n = 4, 31.8 ± 5.4 years) volunteers who use cannabis (males: 1.3 ± 0.6 days/week; females: 1.9 ± 0.7 days/week) were extracted from an on-going placebo-controlled, within-subject study. Participants inhaled vaporized THC (0 mg, 5 mg, 15 mg) and CBG (0, 5 mg, 15 mg) alone, and co-administered in various ratios (5 mg THC + 5 mg CBG, 15 mg THC + 5 mg CBG, 5 mg THC + 15 mg CBG, 15 mg THC + 15 mg CBG), in a randomized order across nine outpatient study sessions. Subjective drug-related effects were assessed with the Mood and Physical Symptoms Visual Analog Scale (MPS-VAS) and the Vaporized Cannabis Rating Form (V-CRF). Pain threshold and tolerance were measured using the Cold Pressor Test (CPT), an experimental pain test with predictive validity for therapeutics used to treat chronic pain. Repeated measures analyses of variance were used to detect the effect of drug condition, and post-hoc pairwise comparisons were used to further compare drug conditions.

Results: THC alone dose dependently increased ratings of "high," "stimulated," "strength," "good drug effect" and decreased pain tolerance when compared to placebo ($p<0.005$). CBG alone did not impact ratings of subjective effects, pain threshold, or pain tolerance when compared to placebo. When co-administered CBG dampened the THC-induced decrease in pain tolerance ($p<0.005$) but did not impact ratings of subjective drug-related effects. Neither THC or CBG alone, nor co-administered, impacted subjective ratings of "hungry" or "want food".

Conclusions: From a preliminary data analysis of this ongoing study, THC reliably produced subjective drug-related effects and decreased pain tolerance, resulting in THC-induced hyperalgesia. CBG did not produce subjective drug-related effects, nor did it impact any of the subjective effects of THC, when co-administered. Although CBG alone did not impact pain sensitivity, when co-administered with THC, it dampened THC-induced hyperalgesia. These preliminary findings suggest CBG can impact some of the acute effects of THC, but additional data are needed to further power these results.

Acknowledgements: Funded by the University of California Center for Medicinal Cannabis Research, the National Institute of General Medical Sciences (K12GM106996), and the Semel Charitable Foundation.

CANNABIS FOR CHRONIC LOW BACK PAIN: ACUTE VERSUS EXTENDED EFFECTS OF EDIBLE PRODUCTS OF DIFFERENT CANNABINOID PROFILES

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Introduction: Nearly two-thirds (62%) of medical cannabis patients in the U.S. and 53% of patients in the U.K. report chronic pain as their reason for using cannabis[1,6]. Some data suggest that cannabis is effective for improving pain[7], yet the research is inconclusive on whether Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), or a combination of the two (THC+CBD) is the more effective cannabinoid profile for pain management[2,5]. The primary aim of this study is to evaluate the use of edible cannabis products that are either CBD-dominant, THC-dominant, or have relatively equal ratios of CBD to THC to assess the acute and extended (2-week) effects of these products in participants with chronic, non-specific low back pain.

Methods: The primary pain measure utilized a single item from the Pain Intensity Short Form 3a[3] and frequency of use was measured by the Online Timeline Followback assessment (O-TLFB)[4]. A total of 250 (Female = 141, Male =104) participants with chronic (at least 12 weeks) low back pain who intended to use cannabis products for pain relief were recruited for the study. Participants self-selected and self-administered an edible cannabis product that was categorized as CBD (n =96), THC+CBD (n =118), or THC (n = 36) over a 2-week period. After two weeks of use, participants were reassessed prior to any cannabis use that day to evaluate the extended effects of cannabis on pain-relevant outcomes. At this same visit, measures were taken pre-use, 1-hour, and 2-hours post-cannabis to assess the acute effects. Mixed effects ANOVA models, controlling for age and cannabis pain-relief expectancy, were used to assess the extended and acute effects of cannabis on pain.

Results. Over the 2-week period, there was a significant effect of time on average pain intensity ($p<.001$), indicating an overall reduction in pain for all product groups over extended exposure period. Further, there was a product group x frequency of use interaction, showing that average ($p=.02$) and high ($p=.01$), but not low ($p>.05$), frequencies of CBD product use were associated with significantly lower average pain levels compared to THC+CBD product use at the 2-week reassessment. Finally, there was a significant acute THC dose x time interaction ($p=.019$), with higher THC doses leading to greater pain reduction at both post-use timepoints (1-hour ($p=.008$) and 2-hour ($p=.001$) post-use).

Conclusions. Participants using a higher THC doses at the acute timepoint reported significantly greater decreases in pain intensity, suggesting THC-dominant products can be effective for acute pain relief. More broadly, over the 2-week period all cannabis users experienced significant decreases in pain, with more frequent use of a CBD product showing the strongest effects. Findings suggest that different cannabinoids may have differential effects for acute versus extended relief from chronic pain.

Acknowledgements. Funded by the National Institute of Health (NIH; 5R01AT009541-02; PI Bidwell).

ASSESSING THE EFFICACY OF CANNABINOID COMPOSITIONS FOR TREATING THREE CLASSES OF CHRONIC PAIN

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Introduction: The U.S. National Academies of Sciences, Engineering, and Medicine has determined that there is substantial evidence that cannabis is effective at treating chronic pain. Research regarding the effects of specific cannabinoids is limited, in part due to the current Schedule I classification for cannabis. There are different mechanisms of chronic pain which may affect treatment. Nociceptive pain results from inflammation or tissue damage, pain is localized and physical activities have consistent effects on pain levels. In contrast, nociplastic pain is a central nervous system or systemic problem, pain is widespread and accompanied by fatigue, sleep, memory, and mood difficulties.

Methods: We conducted the first known study to systematically assess the effect of cannabis products with different cannabinoid compositions on pain symptoms among individuals with different types of chronic pain. Adult (18+ years) California residents with Fibromyalgia (nociplastic pain), Rheumatoid arthritis (nociceptive pain), and Osteoarthritis of the knee and/or hip (nociceptive pain) who had a score of 11 or higher on Patient-Reported Outcomes Measurement Information System (PROMIS) Pain Interference (denoting substantial pain interference) were recruited by MoreBetter. Participants were randomly assigned to receive a 12-week supply of capsules with three different cannabinoid compositions: 12.5mg CBD, 12.5mg THC; 10mg THCa, 10mg CBDa, 5mg CBG, 3mg CBC; and 10mg CBD, 10mg CBDA. Participants completed surveys assessing pain and related symptoms on MoreBetter's Penzai study management software with 12 standard instruments (e.g., PROMIS 29+2) at Baseline, 4,8-, and 12-week timepoints.

Results: MoreBetter recruited 276 individuals, 166 of which (60%) completed all survey measures. Four individuals reported discontinuing product use and were removed from the dataset. There was a large overall effect of improvement across symptoms which did not differ in magnitude across product or type of chronic pain. Improvements were greater for some symptom measures than for others, ranging from small ($d = 0.22$) for Cognitive Function – Abilities to large ($d = 0.91$) for Pain Intensity Interference. The three products had equivalent effects for most symptoms, though there were trends for differential effects on neuropathic pain, anxiety, and sleep disturbance.

Conclusions: The results suggest that cannabinoids are effective in treating three different types of chronic pain. Remarkably, different cannabinoid compositions were equally effective overall. This suggests that non-intoxicating cannabinoids such as CBD and CBDA may provide relief from pain and related symptoms and may be utilized when cannabis intoxication is undesirable or problematic. Future research should continue to explore the differential effects of different cannabinoids.

THC-O-ACETATE: UNDERSTANDING CONSUMER EXPERIENCES WITH A NOVEL SEMI-SYNTHETIC CANNABINOID

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Introduction: There is a growing interest in and consumer demand for semi-synthetic cannabinoids such as THC-O-acetate, also known THC-O. The addition of an acetate group to THC, the primary psychoactive component in cannabis, is thought to increase the potency of this semi-synthetic cannabinoid acetate's interactions with the endocannabinoid system, resulting in stronger and longer-lasting effects. Some cannabis marketers and users have claimed that THC-O produces psychedelic effects. As an acetate ester, THC-O may break down when heated and release toxic ketene gas, which was implicated in e-cigarette or vaping-associated lung injury (EVALI) hospitalizations and deaths, due to cartridge dilution with vitamin E acetate.

Methods: Researchers conducted the first known survey to systematically examine consumer experiences with THC-O. A survey was posted to novel cannabinoid-specific online forums in mid-2022 and included measures from previous studies on the medical use of cannabis and cannabis products and addressed a broad range of issues, including administration methods, use frequency, experiential properties of THC-O, other cannabinoids used, health and medical conditions treated, drug substitution, adverse experiences, and sources of information on THC-O.

Results: Participants (N = 267) primarily consumed THC-O by vaping concentrates or extracts (71%) and edibles (49%), most (66%) used THC-O once a week or more frequently. Although 80% were at risk of ketene exposure, only 12% reported concerns regarding ketene risk. Experiential properties generally resembled those of THC, though with a greater latency of effects. Most participants also used other novel cannabinoids such as HexahydroCannabidiol (HHC, 73%), Cannabinol (CBN, 58%), and Cannabigerol (CBG, 55%), some simultaneously with THC-O. About a third (36%) of participants used THC-O to treat a health or medical condition, most commonly for mental health issues such as anxiety or panic attacks (26%), depression or bipolar disorder (20%), and stress (15%). About a quarter (24%) of participants reported drug substitution. Most (54%) participants were not at all confident in their primary care provider's ability to integrate medical cannabis/marijuana into their treatment and only 14% reported that their primary care provider knew they used THC-O. Internet discussion forums (85%) and websites (82%) were the predominant sources of information on THC-O, only 4% of participants received information on THC-O from their primary care provider. Results did not support claims of psychedelic effects.

Conclusions: As cannabis markets expand and diversify, it is increasingly important to understand the properties and usage of novel cannabinoids. Research is needed to promote consumer education and harm reduction, and health messaging may need to be customized for specific cannabinoids. THC-O-acetate and other cannabinoid acetates pose a specific public health risk due to the potential for toxic ketene gas exposure.

MINOR CANNABINOID USE PREVALENCES, CONSUMER BEHAVIORS, AND MOTIVATIONS FOR USE

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Introduction: Following the passage of the 2018 Farm Bill, manufacturers have developed products featuring "minor" cannabinoids such as delta-8-THC (d8-THC), cannabigerol (CBG), and cannabinol (CBN), in addition to better the known cannabidiol (CBD). Research regarding these compounds has not kept pace with the rapidly growing number of cannabinoids available. Two survey studies examined the prevalence of minor cannabinoid use, motivations for use, administration methods, and experiences.

Methods: The first study utilized the National Opinion Research Center (NORC) AmeriSpeak panel, a nationally representative sample of adults 18 years or older, to estimate the prevalence of minor cannabinoid use. Analyses included sampling weights such that estimates are representative of the US population with respect to gender, age, education, race/ethnicity, and region. The second study utilized a more extensive anonymous and confidential Qualtrics online survey to examine experiences with minor cannabinoids among a large sample of cannabis users. The survey was distributed through social media, press releases, newsletters, and email lists by LeafWell, a telehealth company operating in 35 States that connects potential medical cannabis patients with a qualified healthcare provider.

Results: Nationally representative analyses with AmeriSpeak participants (N = 1169) estimated that 72% of US residents had heard of CBD, compared with 41%, 19%, and 17% for d8-THC, CBG, and CBN, respectively. Similarly, 21% had used CBD in the past year, compared with 12%, 5%, and 5% for d8-THC, CBG, and CBN, respectively. Cannabis users (N = 2941) indicated hearing of and using a wide variety of minor cannabinoids, including nonexistent cannabinoids included by the research team to indicate false positive rates; d8-THC (18%), THCA (Tetrahydrocannabinolic acid; 17%), CBN (13%), and CBG (10%) were the most widely utilized in the past 12 months. Users consumed these products through a wide variety of administration methods and for a wide variety of purposes, including medical use. Participants treated a wide variety of health conditions and frequently reported substitution for pharmaceutical drugs.

Conclusions: Minor cannabinoids are available and being used, necessitating ongoing public health surveillance and other research. The lack of industry standards to protect consumers and the similar pharmacology and subjective effects of some minor cannabinoids to delta-9-THC are of particular concern.

MEDICAL CANNABIS USE IN AUSTRALIA SEVEN YEARS AFTER LEGALISATION: FINDINGS FROM THE ONLINE CANNABIS AS MEDICINE SURVEY 2022 (CAMS-22)

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Introduction: Cannabis was legalised for medical purposes in 2016 in Australia. Uptake was initially slow, but since 2019 there has been a large increase in the number of Australians who have been prescribed cannabis for medical reasons. Yet a significant number of consumers continue to treat their medical conditions via illicitly sourced cannabis. Little is known about how these two groups of medical cannabis consumers differ.

Methods: The anonymous Cannabis-As-Medicine Survey 2022 (CAMS-22) was available for completion online from December 2022 to April 2023 to adult Australians who had used cannabis to treat a medical condition in the previous year. Recruitment occurred through social media, consumer forums, and medical practices. Questions included demographic characteristics, patterns of cannabis use, conditions treated, and self-rated efficacy.

Results: Of the 3323 respondents included in these analyses, 2352 (73%) mainly used prescribed medical cannabis, 871 (27%) mainly used illicit. Prescribed users were significantly more likely than illicit users to have had their health condition diagnosed (OR = 1.7, 95%CI: 1.3, 2.2) and to consume their cannabis via oral (OR = 1.9; CI: 1.5, 2.4) or vaporised (OR = 5.2; CI: 4.0, 6.8) routes, and were significantly less likely to have used cannabis non-medically before medical use (OR = 0.6, CI: 0.5, 0.7) and consume cannabis via smoked routes (OR = 0.2, CI: 0.1, 0.2). The most common conditions among both prescribed and illicit users were pain (37%), mental health (36%), and sleep (15%) conditions. Prescribed users were significantly more likely to use cannabis to mainly treat a pain (OR = 1.3; CI: 1.1, 1.5) or sleep condition (OR = 1.4; CI: 1.1, 1.7) and less likely to treat a mental health condition (OR = 0.8; CI: 0.7, 0.9). There were no between-group differences in efficacy with over 96% saying medical cannabis had improved their symptoms.

Conclusion: From a harm-reduction perspective there is much to recommend about prescribed medical cannabis; it tends to be less harmful and has fewer side-effects than illicit and does not risk consumers being exposed to the criminal justice system. Of concern however is the increased willingness of prescribers to prescribe for indications for which there is no evidence of efficacy, such as mental health and sleep conditions.

ACCESS TO COMMUNITY-BASED CANNABIS DISTRIBUTION MODELS AMONG PEOPLE WHO USE UNREGULATED DRUGS AT HIGHEST RISK OF OVERDOSE

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Introduction: Increasing evidence documents that some people who use drugs (PWUD) at highest risk of overdose use cannabis as a substitute to the toxic unregulated drug supply. However, licit access to cannabis is rare among marginalized PWUD and how cannabis access patterns shape overdose risk is largely unknown. We sought to evaluate access to unregulated community-based models of cannabis distribution and links to overdose risk. Study 1 examined the relationship between PWUD reporting illegal dispensaries as the primary source of cannabis and nonfatal overdose. Study 2 describes PWUD reporting recent use of cannabis substitution programs (CSPs), harm reduction initiatives aimed at providing lowest-barrier cannabis access.

Methods: The studies used data from three open prospective cohorts of community-recruited PWUD in Vancouver, Canada. We used generalized estimating equations (GEE) adjusted for potential confounders to estimate the longitudinal relationship between accessing unregulated dispensaries as a primary source of cannabis and non-fatal overdose. Study 2 used logistic regression modeling (GLM) to analyze the relationship between accessing CSPs and various demographic, health, substance use-related, and socio-structural factors.

Results: Between June 2016 and March 2020, Study 1 followed 751 PWUD who reported cannabis use in the previous six months. In multivariable GEE analysis, reporting dispensaries as their primary cannabis source was associated with reduced likelihood of recent non-fatal overdose in the same period (AOR = 0.82). Study 2 observed 412 cannabis-using PWUD between November 2019 and July 2021, of whom 94 (23%) reported using a CSP. In multivariable GLM analysis, accessing a CSP was significantly and positively associated with using cannabis for pain, sleep, and to reduce symptoms of opioid withdrawal. Participants who accessed CSPs were also more likely to be women, living with extreme pain, and engaging in daily injection drug use.

Conclusions: We observed that community-based models of cannabis distribution serve individuals who face complex challenges to obtaining cannabis from the legal market. Importantly, we found that low-barrier cannabis access via illegal dispensaries had a protective effect on experiencing an overdose. In light of the worsening contamination of the illegal drug supply, our findings echo existing evidence and support calls for lowest-barrier cannabis distribution models to better serve marginalized PWUD seeking cannabis as a harm reduction strategy.

Acknowledgements: We gratefully acknowledge study funders (US NIDA: U01-DA0251525, U01-DA038886; CIHR: MOP-286532, RL2-183257), study participants and staff. This research takes place on the unceded territories of the Coast Salish, including the xʷməθkwəy̓əm, Sḵwx̱wú7mesh, and Sə̓lílwətaɬ.

STUDIES OF CANNABINOID-BASED PRODUCTS IN CLINICALTRIALS.GOV: A SCOPING REVIEW

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Introduction: Given the unique regulatory challenges associated with cannabinoid-based drugs, understanding the state of current research is necessary to inform future research and regulations. Characteristics of ClinicalTrials.gov studies using cannabinoid-based products were surveyed.

Methods: This scoping review was conducted in accordance with PRISMA extension for Scoping Reviews. All studies registered on ClinicalTrials.gov using cannabinoid-based products as an intervention were included. Interventions included FDA-approved drugs, compounds (unapproved drug products and extracts with defined phytocannabinoid content), cannabis (whole cannabis products), and hemp (hemp oil, hemp-derived, and "broad spectrum" products without defined cannabinoid content). Data were extracted using queries, and a relational database was built. Stratification included intervention, sponsor, number of participants enrolled, study length, and disease state (categorized by MeSH terms). Statistical analyses were descriptive.

Results: Of 2428 identified records, 879 were eligible for analysis; of these, 825 were unique (nonduplicate). Study records comprised approved drugs (n=287), specified cannabinoid compounds (n=383), cannabis (n=178), and hemp oil (n=31). In most studies (n=447/825; 54.2%), <50 participants were enrolled. Mean and median enrollment for studies that provided data (n=816) were 104 and 41 participants (IQR, 1–81), respectively. Mean and median lengths of studies with applicable data (n=808) were 837 and 717 days (IQR, 366–1117), respectively. Of 825 unique studies, 582 (70.5%) were sponsored by academic institutions/hospitals, 221 (26.8%) by pharmaceutical companies/commercial entities, 21 (2.5%) by government, and 1 (0.1%) by an individual. There were 521 (63.2%) early phase 1 to phase 2/3, 103 (12.5%) phase 3, and 40 (4.8%) phase 4 studies; 161 studies (19.5%) were not assigned a phase. The top 5 most common MeSH categories were mental disorders (20.6%), pathological conditions—signs and symptoms (18.5%), nervous system disease (15.4%), chemically-induced disorders (11.7%), and studies in healthy volunteers (9.3%). Over time, the number of registered studies per period increased (2013 or earlier, n=163; 2014–2018, n=199; 2019 and after, n=450).

Conclusions: ClinicalTrials.gov-registered studies using a cannabinoid-based intervention were typically randomized, phase 1–2, and small (<50 participants). The results of this review highlight the diverse nature of clinical studies across disease states and reinforce the need for larger, placebo-controlled studies of cannabinoid-based interventions.

Acknowledgments: Jazz Pharmaceuticals, Inc, funded this study, as well as medical writing and editorial support from Sachi Yim, PhD and Dena McWain of Ashfield MedComms, an Inizio company.

CHARACTERIZING CANNABIS AND ALCOHOL CO-USE AND ITS ASSOCIATIONS WITH MENTAL HEALTH SYMPTOMS AMONG CANADIAN TRANSGENDER AND GENDER DIVERSE YOUTH WHO DRINK

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Introduction: Cannabis and alcohol are commonly co-used among youth, a behaviour associated with increased substance use-related harms, including mental health concerns. Growing evidence suggests that transgender and gender diverse (TGD) youth are disproportionately affected by substance use and related harms. This disparity may be driven by the minority stress and marginalization that TGD youth face in their daily social environment. To date, nearly all studies of TGD youth's substance use have been cross-sectional or descriptive. Consequently, very little is known about associations between mental health symptoms and real-time use and co-use of these substances. The current study employed ecological momentary assessment (EMA) to better characterize TGD youth's co-use patterns and their associations with cannabis use disorder (CUD), alcohol use disorder (AUD), anxiety, and depression symptoms.

Methods: Thirty-eight Canadian TGD youth who typically consume alcohol at least twice per week and who reported consuming cannabis or alcohol at least once during the EMA period were included in this analysis. Of these, 13 participants (34.2%) identified as transmasculine, 8 (21.1%) as transfeminine, and 17 (44.7%) as gender diverse (e.g., nonbinary, gender fluid). Participants first completed baseline measures on CUD, AUD, anxiety, and depression symptoms. Then, they completed daily EMA surveys which assessed their substance use for 21 days. To examine associations between type of substance use (i.e., co-use versus single-substance use) and CUD, AUD, anxiety, and depression symptoms, independent samples t-tests were conducted.

Results: Across all participants, 104 cannabis-only days, 180 alcohol-only days, and 41 co-use days were reported. On cannabis use days, cannabis flower, edibles, concentrates, and beverages were consumed on 52.4%, 23.4%, 22.8%, and 1.4% of days, respectively. On drinking days, the mean number of standard drinks consumed was 0.99 (SD = 1.81). T-test results revealed that participants who engaged in cannabis and alcohol co-use during the EMA period (M = 10.83) experienced significantly greater CUD symptoms than participants who engaged in single-substance use (M = 5.47; $t(20.16) = -2.06$, $p < .05$). Although t-test results for AUD, anxiety, and depression symptoms showed no significant differences between use groups, mean scores collapsed across groups were indicative of hazardous alcohol use (M = 9.02, SD = 4.78), moderate anxiety (M = 10.45, SD = 5.24), and major depression (M = 11.18, SD = 4.80), respectively.

Conclusions: Results of this study demonstrate that CUD symptoms are heightened among TGD youth who co-use cannabis and alcohol, which suggests that the co-use of these substances is more detrimental to mental health among TGD youth than the use of either substance alone. Additionally, this study shows that TGD youth who use cannabis and/or alcohol experience high levels of AUD, anxiety, and depression symptoms, regardless of the type of use they engage in (i.e., single-substance use or co-use). The results of this study may ultimately inform tailored harm-reduction policy and intervention efforts for TGD youth.

CANNABIS USE AND MINDFULNESS IN YOUNG ADULTS: PRELIMINARY FINDINGS FROM HERBAL HEART STUDY

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Introduction: Mindfulness, a psychological state characterized by heightened awareness in the current moment, has garnered substantial interest for its potential impact on mental well-being. In the current climate of cannabis legalization and surge in its use among young adults, understanding mindfulness among both cannabis users and non-users is vital as it influences mental well-being, coping strategies, and self-regulation.

Methods: Data are from the ongoing Herbal Heart Study (N=150) of 18-to-35-year-olds. The validated Five Facet Mindfulness Questionnaire (FFMQ-15) was administered to measure. FFMQ-15, measures five subscales of mindfulness: observing, describing, acting in awareness, non-judgement, and non-reactivity. The overall scores of all five subscales were calculated based on standardized instruction. Problematic cannabis use was assessed among cannabis users using Cannabis Use Disorder Test (CUDIT-R); CUDIT-R score ≥8 indicated problematic cannabis use and <8 indicated non-problematic cannabis use. Descriptive statistics, Chi-squared tests/Fisher's exact test or Mann-Whitney U/Wilcoxon Rank-Sum test, where appropriate, were applied using SAS Analytics.

Results: Of the total sample, 50.7% were cannabis users, 68.0% were female. There was not a significant difference in mean age between cannabis users (25.0±4.3) and non-users (24.1±4.6, P=0.25). Most (90.0%) of the sample reported that they heard about mindfulness and 61.5% reported practicing mindfulness (54.5% non-users vs 68.1% users, p=0.10). There was not a significant difference in the total median mindfulness score between cannabis users and non-users (Z= -0.418, p=0.67). Cannabis users had a higher median score for observing mindfulness than non-users (11.0 vs 10.0, Z=-2.2916, p=0.02). Conversely, median score for non-judgement was lower in users than non-users (11.0 vs 12.0, Z=2.7082, p < .01). Among cannabis users only, those with problematic cannabis use had lower median scores for describing mindfulness subscale than non-problematic users (10.0 vs 12.0, Z=1.8147, p=0.03). Non-problematic cannabis users had a higher median score for non-judgement as compared to problematic cannabis users (12.0 vs 11.0, Z=1.7408, p=0.0409).

Conclusion: Findings show that a large proportion of participants despite their age are now aware of mindfulness. A higher median score in the observation aspect among cannabis users suggests increased mindfulness in perceiving both internal and external aspects of the world. A higher median score for non-judgment among non-users indicates a greater non-judgmental approach to inner experiences, reflecting self-acceptance and empathy toward oneself and others compared to cannabis users. Furthermore, problematic cannabis users, reflected in their lower median score on the non-judgmental facet, demonstrate a reduced ability to maintain a non-judgmental stance toward their inner experiences, indicative of challenges in self-acceptance and empathy for oneself and others. While overall mindfulness scores did not statistically differ significantly between users and non-users, specific facets like observing and non-judgment revealed distinct patterns. This nuanced understanding underscores the importance of considering individual components of mindfulness in the context of cannabis behaviors among young adults.

Supported by: NHLBI R01HL153467 (PI: Denise Vidot); NIMHD T37MD008647 (PI: Johis Ortega).

ELUCIDATING NEUROPROTECTIVE SIGNATURES OF CANNABIDIOL IN A VALPROIC ACID MODEL OF AUTISM SPECTRUM DISORDERS

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Introduction: Prenatal brain development is particularly sensitive to chemicals that induce oxidative stress. For example, prenatal exposure to the anti-epileptic drug valproic acid (VPA), induces oxidative stress and synaptic alterations, promoting autism spectrum disorders (ASD) in humans and autism-like behaviors in rodents. There is a need to identify strategies to prevent oxidative stress and protect the developing brain from neural circuit alterations that result in neurodevelopmental disorders, such as ASD. The present research addresses whether cannabidiol (CBD) can prevent oxidative stress through activation of the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2), which strongly promotes expression of detoxification enzymes and antioxidant genes.

Methods: In the following study, we used human neural progenitor cells (hNPCs). To determine whether CBD increases Nrf2 activation, hNPCs were exposed to increasing CBD concentrations and immunostained for the activated phosphorylated form of Nrf2. DAPI counterstain was used to identify all cell nuclei. The nuclear phosphorylated Nrf2 signal was imaged and analyzed by the Cellinsight CX5 high content imaging system. We selected the CBD dose with the greatest increase in the fluorescence intensity for phosphorylated Nrf2. Using this dose, hNPCs were exposed to either DMSO vehicle control, 500μM VPA, CBD or VPA + CBD and unbiased bulk RNA sequencing was performed.

Results: After 24 hours of treatment, 50nM CBD significantly increased pNRF2 intensity from the DMSO vehicle control by ~40%. Intriguingly, 10μM CBD actually reduced pNRF2 intensity. Using 50nM CBD, we addressed whether CBD could alter VPA-induced gene signatures. At this does, CBD alone was almost indistinguishable from the control transcriptome (only 14 transcripts had an adjusted p-value < 0.05). However, comparison of transcriptomic data between VPA+CBD and VPA alone revealed that the addition of CBD significantly upregulated expression of genes associated with mitochondrial respiration, including components of complex I (*NDUFAF4, NDUFA1*) and ATP synthase (*ATP5MK*). These transcripts where not increased by CBD alone when compared to vehicle control conditions.

Conclusions: Our results demonstrate the ability of CBD to engage NRF2 cytoprotective signaling pathways in a dose-dependent fashion. In combination with a model of chemically induced ASD, we were able to elucidate potential neuroprotective signatures of CBD, specifically increased expression of genes associated with mitochondrial function. This is particularly relevant given that VPA is documented to decrease mitochondrial respiration similar to other ASD-associated chemicals, such as organophosphates.

Acknowledgement: Research reported in this publication was supported by seed funding from North Carolina State University Center for Human Health and the Environment through funding from the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Number P30ES025128. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS OF MEDICAL CANNABIS FOR INFLAMMATORY ARTHRITIS

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Introduction: The evidence on the efficacy and safety of prescribed analgesic medications for symptomatic management of inflammatory arthritis is limited, except for non-steroidal anti-inflammatory drugs (NSAIDs). Pre-clinical evaluation of cannabinoids and other active compounds found within the cannabis flower suggest they may possess anti-inflammatory and analgesic properties. There has been limited clinical evaluation of cannabis-based medicinal products (CBMPs) for chronic inflammatory pain. The aim of this study was to assess the changes in patient-reported outcome measures (PROMs) and the prevalence of adverse events in patients prescribed CBMPs inflammatory arthritis-associated chronic pain.

Methods: A case series was conducted using patients enrolled in the UK Medical Cannabis Registry for a minimum of 18 months with inflammatory arthritis-associated chronic pain. Data was collected from clinicians and participants utilising a bespoke digital data collection platform. Primary outcomes were changes the following PROMs from baseline: Brief Pain Inventory-Short Form (BPI-SF), Short Form McGill Pain Questionnaire-2 (SF-MPQ-2), pain visual analogue scale (Pain-VAS), Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L Index Value. The CTCAE version 4.0. was utilised to record adverse event type and severity. Statistical significance was defined as $p<0.050$.

Results: One-hundred and twelve participants with inflammatory arthritis were included in the current study, of which 59 (52.68%) were female and 53 (47.32%) were male. The most common aetiologies of inflammatory arthritis were rheumatoid arthritis (n=36; 32.14%), ankylosing spondylitis (n=32; 28.57%), and psoriatic arthritis (n=22; 19.64%). The mean age was 46.47 ± 13.82 years. Fifty-six (50.00%) participants were current consumers of cannabis as baseline and 41 (36.61%) had never consumed cannabis. There were improvements in both the BPI-SF severity and interference subscales at 1, 3, 6, 12, and 18 months compared to baseline ($p<0.010$). There were also improvements in the SF-MPQ-2 total score and Pain-VAS at each time period ($p<0.050$). Finally, there were improvements in the EQ-5D-5L Index Value and SQS up to 12 months, and the GAD-7 up to 6 months ($p<0.050$). Adverse events (n=292; 260.71%) were reported by 26 (23.21%) patients. The majority of adverse events were mild (n=123; 109.82%) or moderate (n=129; 115.18%).

Conclusions: This study demonstrated an association between prescription of unlicensed CBMPs and improvements in pain-specific outcomes at up to 18 months. Moreover, the study finds that CBMPs are well-tolerated by the majority of participants. This addresses a paucity of literature examining the effects of CBMPs in individuals with chronic pain secondary to inflammatory arthritis. However, the results must be interpreted with caution due to the limitations of study design, with randomised controlled trials required to establish causation.

UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS OF MEDICAL CANNABIS FOR INFLAMMATORY BOWEL DISEASE

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Introduction: Inflammatory bowel disease (IBD) is a chronic disease characterised by inflammation of the gastrointestinal tract and subsequent disruption of the intestinal mucosa. Although pre-clinical studies highlight the potential of medical cannabis for the treatment of inflammatory bowel disease, there is a scarcity of data available from randomised controlled trials. There is therefore significant uncertainty around the role of CBMPs in the management of IBD. Hence, this study primarily aims to evaluate differences in patient-reported outcome measures (PROMs) for patients prescribed CBMPs for IBD over an 18-month period using data collected from the UK Medical Cannabis Registry.

Methods: Patients treated with CBMPs for a primary indication of Crohn's disease and ulcerative colitis were identified. Changes in the Short Inflammatory Bowel Disease Questionnaire (SIBDQ), EQ-5D-5L Index Value, Generalised Anxiety Disorder-7 (GAD-7) and Single-Item Sleep Quality Scale (SQS) at 1, 3, 6, 12, and 18 months after baseline assessment were the primary outcomes. The secondary outcome was reported adverse events according to the common terminology criteria for adverse events version 4.0 (CTCAE v.4.0). Significance was determined as $p<0.050$.

Results: After data extraction, 116 individuals with IBD were included in the analysis, consisting of 78 (67.24%) Crohn's disease and 38 (32.76%) ulcerative colitis patients. The case series comprised of 94 males (81.03%) and 22 females (18.97%), with a mean age of 39.52 ± 9.12 years and BMI of 25.25 ± 5.75kg/m^2. The mean SIBDQ score at baseline was 40.62 ± 10.82. This was improved at 1 (34.98 ± 10.99; $p<0.001$), 3 (34.98 ± 10.99; $p<0.001$), 6 (35.68 ± 11.34; $p<0.001$), 12 (34.89 ± 11.63; $p<0.001$), and 18 (35.83 ± 11.27; $p<0.001$) months respectively. Participants also reported improvements in response to GAD-7, SQS, and EQ-5D-5L Index Value assessments ($p<0.001$). Twenty (17.24%) patients reported 155 (133.62%) adverse events. These were classified according to severity as mild (n=71; 61.21%), moderate (n=66; 56.90%), and severe (n=18; 15.52%). There were no (0.00%) life-threatening or disabling adverse events.

Conclusions: This study builds upon an interim 6-month analysis of patients with IBD from the UK Medical Cannabis Registry, demonstrating that improved symptoms of IBD are sustained at 18 months. This is in addition to improvements in generalised anxiety, sleep quality, and general health-related quality of life. Moreover CBMPs are largely well-tolerated in this cohort. Considering the paucity of available randomised controlled trials, this study may help inform future trials to assess the true efficacy of CBMPs in treating IBD.

UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS OF MEDICAL CANNABIS FOR OSTEOARTHRITIS

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Introduction: Currently available pharmacotherapeutic options for the management of osteoarthritis are limited, with only non-steroidal anti-inflammatory drugs (NSAIDs) and intra-articular corticosteroids supported by international guidelines on non-surgical management of osteoarthritis. Whilst, cannabis-based medicinal products (CBMPs) have been suggested as potential therapies for osteoarthritis, the current evidence is typically of low-quality and is highly heterogenous. This study therefore aims to assess the changes in pain-specific and general patient-reported outcome measures (PROMs), in addition to safety.

Methods: Individuals with a primary indication for treatment with CBMPs were identified from the UK Medical Cannabis Registry. Participants who were enrolled for less than 18 months were excluded. Participants were asked to complete PROMs at baseline, 1, 3, 6, 12, and 18 months. These included: Brief Pain Inventory-Short Form (BPI-SF), Short Form McGill Pain Questionnaire-2 (SF-MPQ-2), pain visual analogue scale (Pain-VAS), Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L questionnaires. Adverse events were captured using the common terminology criteria for adverse events version 4.0.

Results: One hundred and three participants with osteoarthritis met the inclusion criteria for this analysis. There were 57 (55.34%) women and 46 (44.66%) men. The mean age and body mass index were 58.73 ± 13.40 years and 29.67 ± 8.82 kg/m^2 respectively. The majority of patients were users of cannabis at baseline (n=53; 51.46%). The median lifetime cannabis consumption was 20.00 [interquartile range: 5.00-35.00] gram years. Participants reported improvements in all pain specific PROMs at 1, 3, 6, 12, and 18 months from baseline ($p<0.050$). There were also changes identified on applying a repeated measures ANOVA test to GAD-7, SQS, and EQ-5D-5L Index Values ($p<0.001$). Twenty-three (22.33%) participants reported one or more adverse events. The most common adverse events were fatigue (n=23; 22.33%), somnolence (n=19; 18.44%), insomnia (n=18; 17.48%), lethargy (n=18; 17.48%), and headache (n=16; 15.53%). Of the 287 (278.64%) reported adverse events, 114 (110.68%), 134 (130.10%), and 39 (37.86%) were rated as mild, moderate and severe respectively.

Conclusions: The results from this study demonstrated an improvement in pain severity and interference on activities of daily living in individuals prescribed CBMPs for osteoarthritis-associated chronic pain. This was also associated with improved health-related quality of life. However, these findings must be interpreted within the limitations of study design and therefore causality cannot be inferred. Over three-quarters of participants did not report any adverse events and the majority of experienced adverse events were mild or moderate, suggesting that CBMPs are well tolerated in this medium-term analysis. However, future analyses from the UK Medical Cannabis Registry will be important to evaluate any long-term potential risks.

UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS OF MEDICINAL CANNABIS THERAPY FOR ENDOMETRIOSIS

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Introduction: Chronic pain is a cardinal symptom of endometriosis. There is growing evidence that supports the use of cannabis-based medicinal products (CBMPs) for the management of chronic pain, however there is a paucity of data focused on endometriosis. This study aims to assess changes in patient-reported outcome measures (PROMs) and the incidence of adverse effects in endometriosis patients prescribed CBMPs.

Methods: A case series was analysed from the UK Medical Cannabis Registry (UKCMR). Primary outcomes included changes in PROMs from baseline to 1, 3, 6, 12, and 18 months. A repeated measures ANOVA was applied to assess changes in PROMs values at 1-18 months from baseline. Secondary outcomes included adverse event incidence (in accordance with the common terminology for adverse events version 4.0). Statistical significance was defined by a p-value<.050.

Results: After the application of exclusion criteria, 63 patients were included in this study, all of which were female (100%) and had a primary indication of endometriosis for treatment with CBMPs. The mean age and BMI were 33.71 ± 6.48 years and 26.07 ± 5.81 kg/m2 respectively. There was a statistically significant difference in the Brief Pain Inventory-Short Form (BPI-SF) Interference ($p<0.001$) and Severity ($p<0.001$) sub-scales. There was also a difference in the Short Form McGill Pain Questionnaire-2 total score ($p<0.001$), in addition to the affective ($p<0.001$), continuous pain ($p<0.001$), intermittent pain ($p<0.001$), and neuropathic pain ($p=0.019$) sub-scales. One hundred and sixty-two (257.14%) adverse events were reported by 16 (25.40%) patients. The most common severity was mild (n=81; 128.57%). There was 1 (1.59%) reported life-threatening disabling adverse event of urinary tract infection. The most prevalent adverse events were fatigue (n=16; 25.40%), headache (n=13; 20.63%), insomnia (n=12; 19.05%), and dry mouth (n=12; 19.05%).

Conclusions: This study suggests that initiation of CBMPs in individuals with endometriosis is associated with improvements in pain-specific outcomes. This builds upon existing evidence from meta-analyses of chronic pain trials which suggest that CBMPs are associated with a small improvement in pain severity. However, at present there is a paucity of randomised controlled trials which have specifically sought to evaluate the efficacy of CBMPs in endometriosis-associated chronic pain. This study may consequently be utilised to inform future trials in this field.

UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS OF MEDICAL CANNABIS FOR HYPERMOBILITY-ASSOCIATED CHRONIC PAIN

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Introduction: Chronic pain is prevalent among individuals with hypermobility spectrum disorders (HSDs) and hypermobile Ehlers-Danlos syndrome (hEDS). Individuals affected by repeated joint subluxation or dislocation, may develop a combination of nociceptive, neuropathic and nociplastic chronic pain. Although emerging pre-clinical and clinical evidence supports the potential of CBMPs for chronic pain management, most research on the treatment of HSD/hEDS utilises qualitative and anecdotal evidence with modest sample sizes. The primary aim of this study is to report changes in pain-specific and general health-related quality of life patient reported outcomes measures (PROMs) in patients with HSD/hEDS prescribed CBMPs. This study also aimed to assess the prevalence of adverse events in this population.

Methods: Patients enrolled in the UK Medical Cannabis Registry for a minimum of 18 months and prescribed CBMPs for a primary indication of HSD/hEDS were identified. Primary outcomes were changes in Brief Pain Inventory-Short Form (BPI-SF), Short Form McGill Pain Questionnaire-2 (SF-MPQ-2, pain visual analogue scale (Pain-VAS), Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L questionnaires at up to 18 months. Adverse events were reported by clinicians and patients utilising the common terminology criteria for adverse events version 4.0 (CTCAE v.4.0). A p-value<0.050 was determined as being statistically significant.

Results: In total, 161 patients were included in the analysis. Most patients were female (n=130; 80.75%). The mean age was 37.42 ± 10.54 years. The median Charlson comorbidity Index was 1.00 [interquartile range: 1.00-2.00]. Eighty (49.49%) patients were current cannabis users at baseline, whilst 20 (12.42%) and 61 (37.89%) were previous users or cannabis naïve respectively. There were improvements observed in every pain-specific PROM sub-scale including BPI-SF severity, BPI-SF interference, SF-MPQ-2 total, SF-MPQ-2 continuous pain, SF-MPQ-2 intermittent pain, SF-MPQ-2 neuropathic, SF-MPQ-2 affective descriptor, and Pain-VAS scales (p<0.001). Changes were also observed in GAD-7, SQS, and EQ-5D-5L sclaes (p<0.001). Forty-nine (30.43%) patients reported 601 (373.29%) adverse events. The most common adverse events were: headache (n=44; 27.33%), fatigue (n=39; 24.22%), dizziness (n=30; 18.63%), dyspepsia (n=30; 18.63%), dry mouth (n=29; 18.01%).

Conclusions: CBMPs were associated with improved pain-specific and general health-related quality of life improvements in this case series of individuals with HSD/hEDS who had failed to respond to licensed medications. Interestingly, the reported incidence of adverse events is higher in this population, compared to those with other conditions enrolled on the UK Medical Cannabis Registry. This may be secondary to the higher incidence of central sensitisation in HSD/hEDS, which is associated with susceptibility to adverse events. Moreover, it may be reflective of the higher proportion of women in this study, considering prior analysis showed that females were more likely to report adverse events. These findings warrant further assessment in randomised controlled trials to evaluate whether CBMPs are causative of the effects reported in this study.

UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS OF MEDICINAL CANNABIS THERAPY FOR DEPRESSION

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Introduction: At present, there have been no randomised controlled trials evaluating the efficacy of cannabis-based medicinal products (CBMPs) in treating depression. This is despite promising pre-clinical evidence and surrogate evidence from trials in other conditions which include changes in mood as a secondary outcome. The aim of this study was to report an updated analysis of the changes in patient-reported outcome measures (PROMs) and adverse events in a case series of individuals with depression that had failed to respond to licensed therapies.

Methods: A case series of individuals with depression treated with CBMPs was identified from the UK Medical Cannabis Registry. Participants were limited to those enrolled for a minimum of 18 months. Primary outcome measures were changes in Patient Health Questionnaire-9 (PHQ-9), Generalised Anxiety Disorder-7 (GAD-7), Single-Item Sleep Quality Scale (SQS), and EQ-5D-5L questionnaires at 1, 3, 6, 12 and 18 months. Adverse events were recorded using the common terminology criteria for adverse events version 4.0. $p<0.050$ was considered statistically significant.

Results: Three-hundred and ten patients met the inclusion criteria for the present study. The mean age was 36.61 ± 10.60 years. Most patients were male (n=227; 73.23%). The mean PHQ-9 score at baseline was 16.40 ± 6.57. There was a reduction in depression severity at 1 (10.41 ± 6.83; $p<0.001$), 3 (10.57 ± 7.13; $p<0.001$), 6 (11.08 ± 7.08; $p<0.001$), 12 (11.88 ± 7.50; $p<0.001$), and 18 (12.78 ± 7.52; $p<0.001$) months. Improvements in generalised anxiety, sleep quality, and general health-related quality of life were identified utilising a repeated-measures ANOVA test on GAD-7, SQS, and EQ-5D-5L Index values respectively ($p<0.001$). On pairwise analysis with Bonferroni correction, there were improvements at all recorded follow up periods compared to baseline in each of these PROMs ($p<0.001$). Fifty (16.13%) participants reported at least one adverse event. The majority of adverse events were mild (n=175; 56.45%) or moderate (n=175; 56.45%). There were no (0.00%) life-threating / disabling adverse events reported. The most common adverse events were fatigue (n=40; 12.90%), insomnia (n=33; 10.65%) and dry mouth (n=30; 9.68%).

Conclusions: There is an absence of randomised controlled trials assessing the efficacy of CBMPs for treating depression. Consequently there is a need to rely on real-world evidence, including this study, to inform current clinical practice and future research priorities. This study suggests that CBMPs are associated with improvements in reported depression symptoms at up to 18 months of follow-up. Moreover, supplementary benefits are reported in anxiety, sleep-quality and health-related quality of life.CBMPs appear to be well-tolerated. However, high-quality randomised controlled trials are needed to confirm these findings.

UK MEDICAL CANNABIS REGISTRY: A CLINICAL OUTCOMES ANALYSIS OF MEDICAL CANNABIS FOR INSOMNIA

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Introduction: Insomnia disorder affects 10% of people globally. There have been several randomised controlled trials which have sought to evaluate the effectiveness of cannabis-based medicinal products (CBMPs) in the treatment of insomnia disorder. However, these are limited in size, length of follow-up, and hetereogeneity in CBMPs studied. This study therefore aims to assess the changes in sleep quality in individuals with insomnia enrolled in the UK Medical Cannabis Registry. Secondary aims include changes in generalised anxiety and health-related quality of life, in addition to the prevalence of adverse events.

Methods: A case series of individuals treated with CBMPs for insomnia was extracted from the UK Medical Cannabis Registry. Additional inclusion criteria extended to those enrolled for a minimum of 18 months. Participants completed validated patient-reported outcome measures (PROMs) at baseline, 1, 3, 6, 12, and 18 months. Sleep quality was assessed by the single-item sleep quality scale (SQS). Additional measures included the generalised anxiety disorder-7 (GAD-7) and EQ-5D-5L questionnaires. Adverse events were graded in accordance with CTCAE version 4.0.. $p<0.050$ was defined as statistically significant.

Results: 124 participants met inclusion criteria, the majority of which were male (n=87; 70.16%). The mean age was 42.99 ± 13.43 years. Over half of all patients were current cannabis users at baseline (n=72; 58.06%). The SQS at baseline was 2.66 ± 2.41. Sleep quality was improved at 1 month (5.67 ± 2.65), 3 month (5.41 ± 2.69), 6 months (4.80 ± 2.89), 12 months (4.24 ± 3.00), and 18 months (3.81 ± 2.90). There were also improvements in GAD-7 and EQ-5D-5L ($p<0.001$). Eleven (8.87%) participants reported 112 adverse events (mild: n=53, 42.74%; moderate: n=48, 38.71%; severe: n=11, 8.87%).

Conclusions: This results of this study show an associated improvement in self-reported sleep quality in individuals with primary insomnia disorder up to 18 months. In addition, participants reported improvements in anxiety and health-related quality of life. CBMPs were largely well-tolerated with 90% of participants not reporting any adverse events. The results of this study must be interpreted in the context of the limitations of study design. However, it does provide further support for randomised controlled trials to assess the efficacy of CBMPs for insomnia.

DESCRIPTIVE ANALYSES OF MAJOR AND MINOR CANNABINOID USE IN A COLLEGE STUDENT SAMPLE

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Introduction: The majority of prior cannabis use survey studies assessed cannabis use broadly without specifying cannabinoids or assessed for Δ9-THC use only. Measuring cannabis use generally rather than by assessing specific cannabinoid use fails to account for differing psychoactive and pharmacological effects (e.g., anxiety and sleep) of various cannabinoids (Bergeria et al., 2022; Bonn-Miller et al., 2023), as well as the high-inducing/intoxicating effects that are produced by some cannabinoids (e.g., Δ9-THC; Bergeria et al., 2022) and not others (e.g., CBD; Babalonis et al., 2017; Haney et al., 2016; Spindle et al., 2020a). The aim of the current study is to characterize major and minor cannabinoid use patterns among a college student sample as young adults have an elevated prevalence of cannabis use compared to other age groups (Substance Abuse & Mental Health Services Administration [SAMHSA], 2006).

Methods: Participants (N = 299) were undergraduate students who self-reported cannabinoid use in the past 6 months. Participants' cannabinoid use was characterized with regard to frequency of use (i.e., past six-month use), time of day patterns, methods of consumption, methods used to consume cannabinoids (e.g., smoked dried plant/flower, edibles), and formulations of consumed cannabinoids (e.g., isolate, hemp-derived) for 11 different cannabinoids.

Results: Most participants (81.61%, n = 244) reported using more than one cannabinoid, and on average, participants reported using ~3-4 cannabinoids in the past six months. Δ8-THC, Δ9-THC, and CBD were most endorsed for use (at any frequency; 57.86%) in the six months prior to survey, followed by THC-III (32.44%), Δ10-THC (29.77%), and THC-O (23.41%). All other cannabinoid use was endorsed at rates 20%. Participants were most often using either a few times in the past six months or daily. Evening use (i.e., before/just following dinner, after work) and just before sleep use were the most endorsed time of day patterns across most cannabinoids. Trends in methods of consumption show that most individuals reported using smoked flower, vapes, and/or edibles across cannabinoids. Importantly, across cannabinoids, many individuals reported not knowing the formulation of their products (e.g., isolate, full-spectrum).

Conclusions: Findings show that cannabinoid use patterns are variable across type of cannabinoid, frequency of use, pattern of use, and method of consumption among a college sample. Given that cannabinoids impact use-related outcomes differentially (Spindle et al., 2020a, Zamarripa et al., 2022), these findings underscore the need for better characterization of cannabinoid use. Participants' lack of knowledge about product formulation (e.g., cannabinoids that yield positive drug screens) has important implications and highlights the need for improved cannabinoid education among consumers (Dahlgren et al., 2020; Ferretti et al., 2022).

ACUTE CANNABIS AND ALCOHOL EFFECTS ON SIMULATED DRIVING PERFORMANCE AND SUBJECTIVE DRIVING CONFIDENCE IN HUMANS

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Introduction: As cannabis becomes more widely available, determining its effects on driving performance is imperative to public health. The aim of the current study was to compare a range of inhaled cannabis doses (relevant to current medical/recreational products) to the effects of an intoxicating dose of oral alcohol.

Methods: Healthy cannabis users were enrolled in this within-subject, randomized, double-blind, double-dummy, placebo-controlled, outpatient study (n=9). Across 5 experimental sessions the effects of inhaled cannabis (0, 15, 30 mg THC; 15 mg THC+7.5 mg CBD) and oral alcohol (0, 0.8g/kg [15% less for women]) were assessed. Data were collected at baseline and 6 hrs after drug administration. Primary outcomes included standard deviation of lane position (SDLP), variability of speed and steering, and reaction time. Secondary outcomes included subjective ratings driving performance and abuse potential outcomes.

Results: Alcohol produced robust impairments in simulated driving performance (e.g., increased speed, SDLP; p<.05), while cannabis negatively impacted a different array of driving outcomes (e.g., decreased break force, headway distance; p<.05). All active doses of alcohol and cannabis decreased driving confidence (e.g., willingness to operate a real vehicle; p<.05) and increased ratings on abuse potential outcomes (p<.05). Active alcohol and 30mg THC increased ratings of subjective impairment (p<.05).

Conclusions: Although cannabis did not produce profound alcohol-like impairment, it was not without risk. All active cannabis doses decreased participants' willingness to drive and high dose THC increased self-reported impairment. Overall, even in a sample of regular cannabis users, cannabis decreased driving acuity and confidence in safe driving ability.

Acknowledgements: Supported by grant from the National Institute on Drug Abuse (R21DA045101).

ACUTE EFFECT OF CANNABIS VERSUS OXYCODONE ON MEASURES OF IMPAIRMENT: SECONDARY ANALYSIS FROM A RANDOMIZED CONTROLLED TRIAL

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Introduction: Back and neck pain are highly prevalent and disabling musculoskeletal conditions. Although commonly prescribed, opioids are often ineffective and can result in dependency and lethal overdose. An alternative analgesic treatment that has gained increasing recognition is cannabis. However, cannabis containing tetrahydrocannabinol (THC) is also associated with side effects, including the potential for cognitive and motor impairment. It is unclear how the impairing effects of cannabis compare to that of oxycodone, a commonly prescribed opioid for pain. Here we describe a secondary analysis of cognitive and motor impairment from a double-blind, within-participants crossover trial on the efficacy of acute cannabis exposure versus oxycodone for pain analgesia.

Methods: Participants were enrolled after providing informed consent and undergoing a preliminary screening. The study spanned three separate 4-hour sessions, featuring pre- and post-drug evaluations that comprised neurocognitive tests, standardized field sobriety tests, the grooved pegboard test, subjective drug effect assessments, and pain threshold (PTh) determinations using a computerized pressure algometer. Blood samples were collected at baseline, +5 minutes, and +1 hour. Each session involved administration of one of three treatments: active vaporized cannabis containing 5.4% THC (placebo capsule), active oxycodone 5-10mg (placebo cannabis), and placebo/placebo.

Results: 66 participants (34 females, 30 males, and 2 undisclosed) successfully completed the study. Treatment with cannabis resulted in a marginal decline in grooved pegboard test performance at 1-, 2-, and 3-hours post-administration compared to placebo and oxycodone ($p < 0.001$), with no differences observed at the 4-hour mark. In contrast, there were no significant differences across the three groups in standardized field sobriety tests, which are employed by law enforcement to evaluate impaired driving due to intoxication.

Conclusions: Despite a temporary reduction in performance on the grooved pegboard test after cannabis administration, standardized field sobriety tests revealed no significant impairment at the dose of TCH studied. Additional analyses are focused on correlating blood cannabinoid levels with impairment, as well as the extent of prior cannabis use and subjective ratings of analgesia and intoxication with assessments of impairment. Further research is needed to fully understand the long-term effects and efficacy of medical cannabis for pain management, as well as its impact on cognitive and motor functions in a broader population. In conclusion, this study provides initial evidence that certain doses of cannabis may offer a viable analgesic alternative to oxycodone for chronic pain with minimal effects on standard measures of impairment.

Acknowledgements: Funded by the Colorado Department of Public Health and Environment. The CU Clinical & Translational Research Center is supported by NIH/NCATS Colorado CTSA Grant Number UL1 TR002535. Funding for this conference was made possible in part by grant R13DA016280 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

THE EFFECT OF CANNABIS TERPENES ON THE INNATE PULMONARY IMMUNE RESPONSE

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Introduction: *Cannabis sativa* is a flowering plant that produces more than 400 chemical compounds including cannabinoids, flavones, and terpenes. Terpenes represent a broad chemical class containing more than 150 molecules that are responsible for the aroma of cannabis. Little is currently known about the impact of terpenes in innate immune cell populations and whether terpenes exhibit immunomodulatory properties. Further, it is unclear whether terpenes potentiate the immunological effects of the cannabinoids THC and CBD. Cannabis is primarily consumed by smoking or as an aerosol from cannabis vape distillate cartridges. The alveolar macrophage (AM), which patrols the luminal side of the lungs and is important in recycling surfactant and protecting against infectious organisms, is directly exposed to inhaled substances. We recently published that cannabinoids reduce the inflammatory response of alveolar macrophages, but the impact of terpenes on lung macrophage biology is not yet known. We evaluated the effects of common cannabis-derived terpenes on the immune function of alveolar macrophages. Cell viability, inflammatory cytokine production both at the mRNA and protein levels, and phagocytic capability were evaluated.

Methods: We assessed the cytotoxic effects of two commercially available terpene mixes, both alone and in combination with THC and CBD, on MH-S cells (a murine alveolar macrophage cell line) using MTT and Annexin V-PI assays. The effect of these mixtures on inflammation were assessed in MH-S cells with and without exposure to lipopolysaccharide (LPS; a component of the cell wall of gram-negative bacteria). Outcomes included cytokine mRNA quantification by qPCR, as well as cytokine protein quantification via multiplex assay. Macrophage phagocytic capability was assessed using fluorescently labelled latex beads, as well as of mCherry-expressing K12 *E. coli* via flow cytometry and corroborated by confocal microscopy.

Results: At maximal non-toxic doses, we observed no additive cytotoxic effect when both terpene mixtures were applied to cells in conjunction with THC at 24 h timepoint. Further, inflammatory cytokine mRNA and protein production by AMs in response to LPS was not significantly affected by either of the terpene mixtures tested, alone at appropriate concentrations (n=3). In contrast, treatment with terpene mix B caused a significant increase in MH-S phagocytosis of IgG-coated latex beads (n=4, $p<0.05$) and mCherry-expressing *E. coli* (n=2, $p<0.05$).

Conclusions: This study yields important information on the ability of terpenes, both alone and in conjunction with cannabinoids, to modulate alveolar macrophage function. Representative cannabis terpene mixtures do not affect inflammatory cytokine production of AMs *in vitro* nor decrease cell viability additively with cannabinoid THC. Further, one of the terpene mixtures increased AM phagocytic capability against both opsonized particles and *E. coli*, suggesting that terpenes present in cannabis act to stimulate phagocytosis in a pathway-independent manner. These findings raise questions regarding potential utilities of terpenes as immunostimulatory molecules during lung infection and will help elucidate their role in the biological effects of inhaled cannabis.

SEX-SPECIFIC EFFECTS OF EXTENDED CANNABIS ABSTINENCE ON SUBSTANCE USE AND CLINICAL OUTCOMES IN PATIENTS WITH CO-OCCURRING MAJOR DEPRESSION AND CANNABIS USE DISORDER

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Background: Cannabis Use Disorder (CUD) poses a significant challenge for individuals with Major Depressive Disorder (MDD), impacting 15% of patients, a rate markedly higher than the general population (~3%). This co-morbidity is associated with heightened symptom severity, psychosocial impairments, and suboptimal treatment outcomes. Existing studies on cannabis in MDD often rely on cross-sectional designs, which limits conclusions on causal relationships. Furthermore, emerging evidence points to sex-related factors influencing cannabis use and treatment outcomes. Using a controlled design, we investigated the effects of 28-day cannabis abstinence on depressive symptoms as a function of sex differences.

Methods: Using data from an ongoing randomized control trial (N=20), participants with comorbid CUD and MDD were randomly allocated to contingency reinforcement (CR) or non-contingency reinforcement (NCR) groups. Behavioral support sessions provided weekly aimed to foster abstinence and attendance for both groups, with the CR group eligible for a $300 bonus upon biochemically confirmed abstinence on Day 28. Behavioral data were analyzed by repeated measure ANOVAs followed by Bonferroni post-hoc tests ($p<0.05$ considered significant).

Results: Among study completers (n=16) there was a significant reduction in cannabis use for both sexes, evidenced by urine toxicology ($p<0.05$) and self-report ($p<0.05$). Additionally, a significant improvement in depressive symptoms was selectively observed in males ($p<0$ 05).

Conclusions: Selective improvement in depressive symptoms among males following cannabis abstinence suggests a potential sex-specific link between cannabis use and mental health outcomes. These findings underscore the need to better understand interactions between cannabis use, depressive symptoms and sex, to inform more nuanced therapeutic strategies.

Acknowledgements: This study is supported in part by CIHR operating grant PJT-190053 to Dr. George.

NATURALISTIC CANNABIS USE AND CONTEXTUAL FACTORS: LOOKING AT POSSIBLE BENEFITS AND HARMS

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Introduction: As cannabis access expands, more individuals seek clarity on its diverse effects. Clinical research and anecdotal reports are equivocal and inconsistent, with both positive and negative impacts on mental health reported. In studies of other psychoactive drugs used therapeutically (i.e., psychedelics), there is considerable attendance to extra-pharmacological factors during the drug experience, as it is well-known that such contextual factors can significantly impact clinical outcomes. These settings have long been recognized as producing measurable changes in physiology, emotionality, and cognition. Taken together, these factors question the generalizability of extant findings on cannabis and positive and negative outcomes. This apparent disconnect mandates the development of new research paradigms. The Naturalistic Cannabis Assessment Protocol (NCAP) asks cannabis users to self-titrate their cannabis of choice in their home environment. Two potential applications of the NCAP explored the potential for beneficial and harmful outcomes of cannabis use, namely, impacts on cognition and enhanced wellbeing.

Methods: In Study One, participants (N = 28) underwent a cognitive assessment via video conference under two conditions that took place one month apart. Cognitive function was assessed during a no-cannabis control session and compared to cognitive outcomes directly following cannabis use. In Study Two, participants (N = 47) self-administered cannabis under two conditions that took place one week apart. After consuming cannabis, participants completed a 45-minute yoga practice, and in the control session, participated in activities-as-usual for 45 minutes. Outcomes of interest included state mindfulness, mysticality, and positive and negative mood states.

Results: In Study One, within-subjects assessment revealed no differences in immediate verbal recall ($F(1, 27)$ = .28, p = .60), delayed verbal memory ($F(1, 27)$ = 2.73, p = .11), working memory and attention ($F(1, 27)$ = .60, p = .45), processing speed ($F(1, 27)$ = .88, p = .36), or verbal fluency ($F(1, 27)$ = 1.62, p = .22). In Study Two, within-subjects assessment of well-being outcomes indicated significant improvements in mysticality of experience ($F(1,46)$ = 19.82, p <.01, ηp = .30) and state mindfulness ($F(1,46)$ = 34.08, p <.01, ηp = .43) following the yoga condition, and no difference in state affect.

Conclusions: These findings underscore the complexity of the effects of cannabis on cognitive function and well-being. While Study One did not reveal significant differences in cognitive performance immediately following cannabis use, Study Two demonstrated notable enhancements in mysticality of experience and state mindfulness after a yoga session. Results suggest that the effects of cannabis may be nuanced and influenced by various contextual factors, highlighting the importance of employing naturalistic research paradigms.

RESEARCH PHARMACIST EXPERIENCE COMPOUNDING THE NIDA CANNABIS EXTRACTS FOR PRACTICAL USE IN A CLINICAL TRIAL

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Introduction: There is a growing interest in conducting robust clinical research trials to investigate the efficacy and safety of cannabis and its components in various disease states. Currently, the only plant-based cannabis products approved for investigational use at the University of Colorado is either Epidiolex®, cannabis products sourced from the National Institute of Drug Abuse (NIDA) or a few other American companies that have been awarded a production license from the Drug Enforcement Administration (DEA) for federally approved research. At the time of this research study we could only use either Epidiolex® or the NIDA supply. The NIDA cannabis supply is both limited by the crop yield each year and the formulations, concentrations and ratio of cannabinoids. Researchers at the University of Colorado had a need for preparation of the crude cannabis-extract into an oral solution for administration to provide flexibility in dosage concentration, ratio of cannabinoids, and formulation that may be more representative of products used by the public.

Methods: Utilizing clinical research pharmacists' compounding knowledge and expertise, an investigational product was compounded using the NIDA crude plant extract into an oral solution for use in a clinical trial. This product was created using the Epidiolex® package insert as a guide to attempt to create a similar product along with our experience from a previous study. An initial compounded product was sent to a lab for stability and contamination testing to ensure it was of high quality for use in clinical trials.

Results: The clinical research pharmacists at the University of Colorado Anschutz Medical Campus took NIDA crude plant extract and compounded it into a placebo product, a 1:0 tetrahydrocannabinol (THC): cannabidiol (CBD) (0.5%:0) or a 1:10 THC: CBD (0.5:5.0%) cannabis extract oral solution. This compounding process allows for individualized final volumes based on weight of the patient to allow for accurate dosing. At 1 month and 3 months, the accuracy of the initial compounded product was near 95% accurate for CBD and THC stability. However, investigational drug products are compounded and dispensed for patients at the time of their participation in the trial for a dosing timeline of 4 weeks, so the 1-month stability accuracy displays a safe result.

Conclusion: The NIDA crude plant extract was successfully compounded into an oral solution with the desired ratio of CBD and THC for use in a clinical trial. Preparation of the solution provides specialized pharmacists involved in clinical trials with resources to compound the crude plant extract product for use in other clinical research trials. By increasing diversity of formulations and strengths of investigational cannabis products that both meet federal requirements and more similarly mimic what is seen in the real-world use, researchers can begin to bridge the gap in knowledge.

ASSESSMENT OF BLINDING IN A CLINICAL TRIAL COMPARING VAPORIZED CANNABIS TO ORAL OXYCODONE

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Introduction: Clinical trials are typically conducted in a double-blind manner with the intention that participants are not able to discern which intervention they received, and research team members do not inadvertently influence participants' responses. However, blinding can be challenging in clinical trials in which the active treatment is difficult to disguise, particularly in studies where the active treatment has obvious side effects, such as those seen with tetrahydrocannabinol (THC). Further, blinding success/failure is often not reported. Here we describe participants' ability to correctly guess their study drug treatment in a double-blind, within-participants crossover trial on the efficacy of acute cannabis exposure versus oxycodone for pain analgesia.

Methods: After informed consent and screening, participants attended 3 separate 4-hour study visits, at which they received one of the following drug combinations at each visit: active vaporized cannabis containing 5.4% THC (placebo capsule), active oxycodone 5-10mg (placebo cannabis), and placebo/placebo. Participants underwent pre- and post-drug assessments including neurocognitive assessments, standardized field sobriety testing, subjective ratings of drug effects, and pain thresholds (PTh) measured with a computer-controlled pressure algometer. Blood samples were taken at baseline, +5 minutes, and +1 hour. At the conclusion of each study visit, participants were asked to guess which study drug they received that day.

Results: A total of 66 participants completed the study (30 males, 34 females, 2 unspecified). 89% of participants correctly guessed cannabis treatment, 45% correctly guessed oxycodone treatment, and 45% correctly guessed placebo treatment. When comparing males versus females, 97% of males correctly guessed cannabis treatment versus 82% of females.

Conclusions: These data indicate that participants can more readily identify acute treatment with THC than oxycodone or placebo, with males having greater accuracy than females. Participants were less likely to correctly identify treatment with oxycodone or placebo, with more than half incorrectly guessing treatment. Additional analyses are focused on correlating blood cannabinoid levels with blinding, as well as the extent of prior cannabis use and subjective ratings of analgesia and intoxication with the ability to correctly guess treatment. These results support the need for improving blinding in studies of cannabis, perhaps through the incorporation of active control medications that better mimic some psychoactive effects of THC.

Acknowledgements: Funded by the Colorado Department of Public Health and Environment. The CU Clinical & Translational Research Center is supported by NIH/NCATS Colorado CTSA Grant Number UL1 TR002535. Funding for this conference was made possible in part by grant R13DA016280 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

17-YEAR-OLD PATIENT WITH PRADER-WILLI SYNDROME: CASE REPORT OF SYMPTOM MANAGEMENT WITH CANNABIDIOL

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Introduction: Prader-Willi Syndrome (PWS) is a rare genetic disorder that results from loss of gene expression on paternal chromosome 15q11-q13. Its prevalence rate is 1/10-30,000, being characterized by endocrinological abnormalities due to hypothalamic and pituitary insufficiency and complex physical, behavioral and intellectual difficulties (Griggs et al. 2015). Obesity and its complications are the main causes of morbidity and mortality in individuals with PWS (Goldstone, 2004). To date, pharmacological intervention with appetite suppressants (sibutramine), antiabsorption agents (orlistat), topiramate, or glucagon-like peptide 1 (GLP-1) receptor agonists have been ineffective in patients with the syndrome (Salehi et al., 2016). They present high rates of obsessive-compulsive symptoms, And among these, skin-picking appears to be the most common, starting in early childhood (Kates et al., 2009). GH replacement in children with PWS has well-defined benefits and risks, although data are limited to adults with PWS (Butler et al., 2013). Treatment is based on four main pillars: diet, exercise, rhGH therapy and behavioral strategies. However, one of the biggest challenges is treating behavioral symptoms with psychotropic drugs that do not respond well to the symptoms or that further increase appetite as a common side effect.

Case Report: 17-year-old female patient, born at 6 months of pregnancy, by natural birth, weighing 800 g and spent 6 months in the Intensive Care Unit and another month in the Maternity Unit. He had convulsive crises during his stay in the ICU. She walked without support at the age of 4 and spoke his first words at the age of 5. I saw the patient for the first time when she was 6 years old, she was using Phenobarbital 100 mg per day, gradually being replaced by Valproic Acid (500 mg/day) and associated with Flouxetine (10 mg/day) due to compulsions. of self-harm. After 3 months, due to little response, Fluoxetine was replaced by Sertraline up to 100 mg/day with, showing an improvement in the intensity of the picking and eating compulsions. At the age of 8, she was referred for genetic testing, being diagnosed with Prader-Willi Syndrome and started taking somatostanin. Occasionally he had seizures (one crisis every 2 months). At 13 years of age, there was a worsening of psychomotor agitation and compulsions (picking and eating) and Valproic Acid was adjusted up to 1000 mg/day, Sertraline was gradually replaced by Fluoxetine up to 60 mg/day and then Quetiapine was added up to 200 mg at night due to insomnia. There was an initial improvement in the compulsion to self-harm and psychomotor agitation, but the eating compulsion increased. After 2 months, his self-harm worsened. At this point, we decided to introduce a full spectrum extract of cannabis spp rich in CBD (0,3% THC) up to 25 mg/day with good response in relation to psychomotor agitation and the compulsion to poke oneself. And so, Fluoxetine was gradually withdrawn, then Quetiapine and finally Valproic Acid and remaining on full spectrum CBD 25 mg/day. Despite significant behavioral improvement, binge eating persisted. Therefore, we gradually replaced the full spectrum CBD with purified CBD up to 50 mg/ml, and this problem was also resolved. Furthermore, she has never had a seizure again.

CHILDHOOD EXPOSURE TO CANNABIDIOL AND LUNG FUNCTION: THE HEALTHY START STUDY

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Introduction: Cannabidiol (CBD) is an increasingly popular substance in the U.S. In 2022, approximately 7% of parents in the U.S. had given or considered giving their child a CBD product. However, this may underestimate actual exposure in childhood. Concurrently, respiratory health is a growing concern, but little research has explored the impact of CBD exposure on lung function humans, and none in children. Our aims were to objectively quantify childhood exposure to CBD at age five and examine the relationship between biomarker-confirmed exposure and lung function at age five and ten years.

Methods: Mother-child pairs enrolled in a racially and ethnically diverse Colorado-based cohort (Healthy Start) were followed through age ten years. Child urine was collected at five years and analyzed for the presence of CBD (n=744). Lung function was measured via oscillometry, a noninvasive alternative to other pulmonary tests (e.g. spirometry). Separate generalized linear models estimated the association between CBD exposure at age five years with the lung function measures at five years (n=66) and ten years (n=286).

Results: Approximately 19% (141 of 744) of the children had detectable CBD metabolites in their urine. CBD exposure was associated with lower area of reactance (mean z-score difference: -0.7; 95% CI: -1.3, -0.1: p=0.03) and lower resonant frequency (mean z-score difference: -0.9; 95% CI: -1.5, -0.4: p<0.01) at five years (n=66). There were no differences in lung function between exposure groups at ten years (n=286).

Conclusions: CBD exposure may temporarily improve lung function by increasing lung elasticity. As CBD use continues to grow in popularity, more research is needed to validate these findings and clarify the benefits and risks of childhood exposure to CBD.

Acknowledgements: This work was supported by the National Institutes of Health (R01DK076648, UH3OD023248, R00ES028711).

E-CIGARETTE-CANNABIS CO-USE ASSOCIATED WITH GREATER CRAVING FOR CANNABIS AND NICOTINE THAN CIGARETTE-CANNABIS CO-USE

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Introduction: Nicotine and cannabis typically share a route of administration (inhalation), motivations for use, and may mutually enhance reinforcement. Co-use of cannabis and tobacco is related to heightened dependence to both substances and psychiatric comorbidity. It is not known whether a similar pattern exists for individuals who report co-use of cannabis and vaporized nicotine products, which may be informative to the safety of vaping. We assess if co-use of cannabis and nicotine relates to higher psychiatric symptoms than only nicotine use and if this differs by smoking/vaping. Further, among people with problematic cannabis use, do cannabis cravings, nicotine cravings, and cannabis problem severity differ by smoking or vaping?

Methods: As part of a larger study, (n = 73; 34% female) people who smoke cigarettes, (n= 93; 45% female) vape nicotine, or both (n = 30; 17% female) daily were recruited. Participants completed the NIDA-modified ASSIST to assess problematic cannabis use/craving, the Wisconsin Index of Smoking Dependence to assess smoking/vaping dependence and craving, and the Patient Health Questionnaire to assess depression. Results were analyzed by multiple regression and ANOVA.

Results: Participants who smoked were significantly older than those who vaped and dual-users. Cannabis ASSIST scores did not differ by group. Cannabis craving scores were significantly greater in those who vape vs smoke ($t = 2.07$, $p = .04$), but this was not significant after controlling for age ($t = 1.96$, $p = .05$). Vaping dependence was associated with cannabis craving, and this association was greater in dual-users than vaping alone ($t = 3.9$, $p < .001$); smoking dependence was associated with cannabis craving ($t = 2.3$, $p = .03$), but this relation was not moderated by dual-use. Among those who met criteria for moderate-severe risk cannabis use (70% smokers, 71% of vapers, and 67% of dual-users), those who vaped had significantly greater nicotine craving than those who smoke, controlling for age ($t = 2.77$, $p = .007$). Higher scores on the cannabis ASSIST were associated with higher depressive symptoms, controlling for age ($F_{1,167} = 7.94$, $p = .005$) and this did not differ by vaping/smoking ($F_{1,167} = 1.71$, $p = .19$).

Conclusions: Co-use of cannabis and nicotine was associated with greater depressive symptoms than nicotine use alone, regardless of vaping/smoking/dual-use. Among people with problematic cannabis use, there was no difference in cannabis problem severity between people who smoke, vape, or use both daily. However, people who vape daily had significantly greater cannabis and nicotine craving than smokers. These preliminary findings suggest amongst people who co-use cannabis and nicotine, there is a similar risk of depressive symptoms, as well as potentially greater dependence on both nicotine and cannabis among those who vape or vape and smoke relative to those who smoke. Dual-use may confer greater risk to cannabis craving.

Acknowledgements: National Institute Health Grant R01 DA054276-01A1 (to Dr. Hawk), and Canadian Institutes of Health Research (CIHR) operating grant PJT-190053 (to Dr. George).

INVESTIGATING THE NEUROCOGNITIVE EFFECTS OF LOW-DOSE THC IN MEDICAL CANNABIS OIL AMONG NOVICE USERS

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Introduction: As legal access to medical cannabis increases, there is an urgent need to understand its impact on neurocognitive and behavioural functions. This is particularly critical for safety-sensitive behaviours such as driving, where impairment could have serious consequences for drivers and other road users. This study aims to examine the influence of low doses of THC when combined with CBD in 1:1 and 1:20 ratios on neurocognitive performance, with a specific focus on driving-related abilities.

Methods: This study will enroll 31 healthy participants in a five-week, randomized, double-blind, placebo-controlled crossover trial (ACTRN:12622001539729). Participants will orally administer 1mL of full-spectrum THC/CBD oil or placebo consisting of a carrier oil. Neurocognitive performance outcomes will be assessed at approximately 1- and 3-hours post-dosing, using the Cambridge Neuropsychological Test Automated Battery (CANTAB). Domains investigated include multitasking, reaction time, spatial working memory, and rapid visual information processing.

Results: Seven participants have completed the study protocol to date, with plans to report neurocognitive outcomes for all participants who have completed the study by June 2024. Demographic and baseline cognitive performance data will be summarized and analyzed. Linear Mixed Models will be applied to evaluate the differences in neurocognitive performance between different THC dosages, treatment conditions (THC:CBD ratios vs. placebo), and over time. Post-hoc analyses will be conducted following the identification of significant interactions or effects, to assess the impact of time and treatment on neurocognitive functions.

Conclusions: This study's findings will examine the potential neurocognitive effects of low-dose THC in medical cannabis oil, particularly assessing how THC's impact is modified by CBD concentration. Such insights are essential for understanding the nuanced interactions between THC and CBD concentrations, providing insights into the safety profiles of medical cannabis products for novice users.

Acknowledgments: AH is supported by a Rebecca L Cooper Al and Val Rosenstrauss Fellowship (GNT: F2021894). This project is directly supported by a grant awarded to AH by the Office of Road Safety, Department of Infrastructure of the Australian Federal Government.

EVALUATING CANNABINOIDS AS AN ADJUNCT TO OPIOID AGONIST THERAPY: A PHASE II PILOT STUDY AMONG PEOPLE AT HIGHEST RISK OF OVERDOSE

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Introduction: The worsening drug toxicity crisis in the United States and Canada has led to an urgent need for innovative approaches to address opioid use disorder (OUD) and reduce overdose-related morbidity and mortality. While opioid agonist therapies (OAT), such as methadone, have demonstrated efficacy in reducing overdose risk, challenges remain, including low uptake rates and high attrition from care. In response to this crisis, increasing attention has turned to the potential therapeutic role of cannabinoids as an adjunct to OAT. Although cannabis use is common among people on OAT, experimental data on the safety of cannabinoids alongside OAT are entirely lacking. Based on preliminary observational findings showing increasing retention in OAT and reduced exposure to fentanyl among people in OAT using cannabis, we seek to experimentally test the safety and feasibility of co-dispensing cannabinoids alongside OAT for people with OUD in a community setting.

Methods: First, 24 participants with OUD who have recently initiated methadone-based OAT will be randomly assigned to receive either balanced THC:CBD cannabis oil or placebo oil in a blind fashion, while receiving standard OAT management. Participants will self-administer the study medication sublingually once daily, starting at 5 mg/day and titrating in 2.5 mg increments up to a maximum of 40 mg/day, under physician guidance. Phase II is a 12-week single-arm open-label extension that will follow participants who complete Phase I without experiencing adverse events related to the study medication. Primary outcomes include safety, adherence to treatment, treatment acceptability, blinding effectiveness, and dose adequacy. Secondary outcomes include recruitment, enrollment, screening failure and study visit completion rates. Exploratory measures include retention in OAT, evidence of unregulated opioid use, changes in pain, anxiety, depressive symptoms, health-related quality of life, substance use-related problems, and opioid cravings.

Results: Once the study completes regulatory review, the first participant will be recruited later in 2024.

Conclusion: The study aims to determine the feasibility and safety of using cannabis oil as an adjunct therapy to methadone-based OAT for individuals with OUD in a community setting. Findings from this study will provide valuable insights into the potential benefits and challenges of integrating cannabis-based therapy into OUD management protocols, laying the groundwork for future larger-scale efficacy trials and informing clinical practice.

Acknowledgments: Canadian Institutes of Health Research (Grant no. PJT-438428).

PERCEPTIONS OF CANNABIS AND ITS ASSOCIATIONS WITH MENTAL HEALTH AMONGST BUDTENDERS IN SOUTHERN ONTARIO, CANADA

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Background: Legal cannabis dispensary employees in Canada ("Budtenders") play a crucial role in shaping consumer knowledge and use. Given the potential implications for mental health, this study aimed to assess Budtender's knowledge and perceptions of cannabis in relation to mental health disorders, and the extent to which these topics are engaged amongst their customers.

Methods: Participants were Budtenders recruited from legal cannabis dispensaries (Ontario Cannabis Stores) within the Greater Toronto (Ontario, Canada) Area. Participants completed a 15-minute online survey approved by the CAMH Research Ethics Board and the Alcohol and Gaming Commission of Ontario (AGCO). The survey collected non-identifying demographic information and responses to questions pertaining to perceptions, education and engagement surrounding cannabis and mental health. Descriptive statistics and qualitative analysis were employed to characterize findings, while mixed methods modeling was used to understand the relationship between Budtender perceptions, customer interactions, and cannabis and mental health education.

Results: Preliminary findings (N=47 respondents) indicate that majority of Budtender perceptions of cannabis and its influence across mental health disorders diverge from evidence-based knowledge about cannabis and mental illness (e.g., *Lowe, D.J.E. et al., 2019. Eur. Arch. Psychiatry Clin. Neurosci. 269: 107-120*). There was considerable variability in how these perceptions are informed and how Budtenders engage with customers, and in the qualitative discourse around how Budtenders perceive their role and the cannabis retail system in the context of mental health.

Conclusions: Our preliminary results highlight substantial discrepancies between Budtender perceptions and scientific evidence on cannabis use and mental health. Understanding these perceptions is crucial for developing targeted, evidence-based educational interventions and mitigating the risks associated with recreational cannabis use in mental illness.

Supported in part by a CIHR Project Grant (PJT-190053) to Dr. George, and a University of Toronto Excellence Award (UTEA) to Ms. Wang.

QUASI-RANDOMIZATION TO CANNABINOID CONDITION IN A STUDY OF U.S. LEGAL MARKET CANNABIS: CHARACTERISTICS OF ACCEPTORS VERSUS DECLINERS OF RANDOMIZATION

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Introduction: Research on legal market cannabis products is hampered due to federal regulations necessitating innovative research designs. In many cases these regulations prevent clinical trials with randomization to condition. One way to accommodate legal restrictions while also investigating widely available legal market products is to utilize quasi-random assignment of individuals to products varying in cannabinoid content. Specifically, participants are randomly assigned to use of a particular cannabis product condition and are told the condition to which they are assigned but then have the opportunity to either accept their condition assignment or decline and use product from a different condition. To the extent that participants choose a product different from the one to which they were randomly assigned, this method of assignment to condition has the potential for selection threats to internal validity. Thus, investigating whether individuals who accept their assignment differ from those who do not is a crucial research endeavor in projects that are forced to utilize quasi-random assignment methods.

Methods: Data came from a larger project comparing cannabis use versus non-use over the course of four weeks to assess changes in inflammatory biomarkers and insulin sensitivity. Individuals who used cannabis were quasi-randomly assigned via dice roll to purchase and use a cannabis flower product that was either THC-dominant, CBD-dominant, or contained approximately equal parts THC and CBD and were included in the current analyses. Baseline demographic information, cannabis use, general health behavior (e.g., exercise), and anthropomorphic measures (i.e., body mass index [BMI]) were compared via analysis of variance (ANOVA) or chi-square tests across individuals who accepted versus declined their assigned condition.

Results: The majority of participants (N=97, M_{age}=29.87, 35% female, and 77% white) accepted their condition assignment (63%; N=61) while 37% (N=36) declined their assignment and switched to a different product group. Those who accepted did not differ from those who declined on gender, race, age, total days of cannabis use in the past month, frequency per day, typical quantity of cannabis use, positive or negative cannabis use expectancies, cannabis use attitudes, diet or exercise measures, sleep quality, BMI or hazardous alcohol use (ps>0.11).

Conclusions: The majority of individuals quasi-randomly assigned to use of legal market cannabis products differing in cannabinoid ratios accepted their assignment. Accepting versus declining assignment was not associated with any cannabis use variable or behavioral measure relevant to the larger project. While these findings cannot rule out a selection process operating outside the range of variables assessed in this study, the data support the use of this methodology in situations where true random assignment to condition is not possible.

Acknowledgements: Funded by the National Institute on Drug Abuse (1R01DA50515).

ADMINISTRATION OF CANNABIDIOL IN UNIFORMED PERSONNEL WITH PTSD OR ANXIETY DISORDERS BEFORE TREATMENT: STUDY PROTOCOL

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Introduction: Uniformed personnel, such as police-officers, firefighters, healthcare professionals or military personnel display an increased risk of developing trauma and stressor-related disorders or anxiety disorders. Those who seek treatment are often placed on a waiting list, which can last over 4 months. In this period, increases in symptom severity can occur, making any intervention that mitigates symptoms highly desirable. The endocannabinoid system (eCS) is involved in anxiety and stress-related disorders through reduced Anandamide and 2-AG levels. Thus, pharmacological interventions targeting the eCS, such as cannabidiol (CBD), might help to alleviate symptoms due to its potential anxiolytic effects. However, the quality of over-the-counter CBD products is generally rather poor. Moreover, current studies with CBD in clinical populations are limited, often with small sample sizes. Therefore, this study aims to investigate CBD as an intervention to target the endocannabinoid system in reducing anxiety symptoms preceding evidence-based treatment. Moreover, we investigate the effects of CBD on fear conditioning, sleep quality, and stress resilience. Since exposure treatments focus on fear extinction and consolidation, the potential effects of CBD during extinction was also examined.

Methods: We designed a double-blind clinical randomized placebo-controlled trial in Dutch uniformed personnel awaiting treatment, to our knowledge the first ever in this population. In total, 82 participants with anxiety or trauma and stressor-related symptoms will be recruited before they start with treatment. For two weeks, they receive oral capsules containing either CBD (200 mg) or a placebo (0 mg), 3 times daily. Before and after these two weeks of treatment, a fear-conditioning paradigm is conducted to examine the effects of CBD on fear extinction and consolidation. In addition, sleep is measured continuously for two weeks by a Philips Actiwatch. Finally, after two treatment weeks, a stress regulation task is conducted, by performing the Maastricht Acute Stress Test (MAST).

Results/ Conclusions: Data collection is currently ongoing.

Acknowledgements: Funded by the Dutch Ministry of Defence.

ANTIEPILEPTIC EFFECT OF CANNABIS IN A PATIENT WITH 1P36 SYNDROME: A CASE REPORT

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Introduction: 1p36 syndrome is a chromosomal deletion characterized by intellectual disability, developmental delay, and pharmacoresistant epilepsy. Haploinsufficiency of the gene encoding the cannabinoid receptor type 2 (CB2) may compromise its expression in the central nervous system (CNS), leading to epileptiform seizures. Treatment may involve the endocannabinoid system (eCS), with phytocannabinoids being promising molecules. Ultimately, we aim to investigate whether cannabis extract treatment can improve symptoms of refractory epilepsy associated with this clinical condition.

Methods: To conduct our study, we evaluated the clinical history, neuroimaging exams, and electroencephalogram techniques of the patient.

Results: JHSA, 16 years old, male, Brazilian, has been under the care of the pediatric neurology service since infancy due to epileptic seizures. Neuroimaging exams reveal structural brain abnormalities, and the electroencephalogram confirms active epileptogenic activity. Molecular genetic studies showed terminal loss involving the 1p36.33p36.32 band. Over time, the patient presented different types of epileptic seizures, with high frequency, limited interaction with the environment, and repeated episodes of psychomotor agitation. In addition, there was a poor response to antiepileptic drugs at maximum tolerated doses and combinations, maintaining a high number of seizures and worsening the quality of life. Due to the severity of his condition, the patient was given oral treatment with an artisanal extract of Cannabis for 223 days. The extract included various concentrations and doses of isolated CBD (20, 800, 600 or 100 mg per day) and CBD: THC (3.75, 5 or 20 mg per day), as well as maintaining the use of phenobarbital and diazepam in emergency cases. After the patient began treatment with the Cannabis extract full spectrum, his tonic-clonic crises and motor coordination significantly improved, and he experienced only four crises per day. When the patient took Cannabis extract with CBD: THC (1:1), doses of isolated CBD and other anticonvulsant drugs were reduced. Additionally, he only experienced spasms upon awakening and markedly improved his quality of life. However, the patient did experience some side effects from the treatment, including laughter for 24 hours from one of the artisanal extracts of Cannabis; lack of sleep from CBD: THC 5 mg/ml: 3.75mg/ml, respectively and maintenance of seizures with the isolated CBD (20 mg/ml). Our hypothesis is that cannabis extract, acting on the eCS, can reduce epileptic seizures . The gene cnr2 that encoding the CB2 is located on chromosome 1p36 in humans, and its haploinsufficiency can lead to a deficit in the expression of these receptors in the nervous system, increasing neuronal excitability, and the entourage effect to balance this system.

Conclusions: Epileptiform seizures were partially reversed with the use of CBD and THC/CBD due to the entourage effect. Treatment with lower amounts of THC could likely improve spasticity. This study is the first clinical report documenting adjuvant treatment with phytocannabinoids in 1p36 deletion syndrome.

Acknowledgements: The authors thank Dr Paulo César Trevisol Bittencourt and the entire team of the Associação Cannabis Sem Fronteiras for their support of this patient. Financial support provided by the Conselho Nacional de Pesquisa e Desenvolvimento Científico de Brasil (CNPq), the Call N° 105/2020/PRPPG/UNILA and Cannabis Products Revivid Brazil for support in research projects and travel grants.

UPDATE ON NIH RESEARCH ON CANNABIS, CANNABINOIDS, AND RELATED COMPOUNDS

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The US National Institutes of Health supports a broad portfolio of research on cannabis and cannabis constituents and related compounds, as well as the endocannabinoid system. Specific topics of interest vary by Institute, but overall the research portfolio includes studies utilizing the whole marijuana plant (*Cannabis sativa*), cannabinoid compounds, non-cannabinoid constituents of marijuana, synthetic cannabinoids, as well as cannabinoids found in the body (endocannabinoids). This poster will provide an update for the international research community on the growing investment in research, the topics of historical and current interest, opportunities for funding, and the expanding number of NIH's 27 Institutes/Centers that support research in these areas.

There is considerable and growing interest in the study of therapeutic uses of cannabis and its constituent compounds at the NIH. In 2015, the NIH developed three reporting categories to describe the research efforts underway to examine the chemical, physiological, and therapeutic properties of cannabinoids and the physiological systems they affect.

1. Cannabinoid Research reports the *total* NIH investment in *all* cannabinoid research including basic research, animal and human preclinical studies, and clinical research. Studies examining cannabis use disorder and societal/health impacts due to changing marijuana laws and policies are also included. Studies examine all classes of cannabinoids (purified, synthetic, endogenous, phytocannabinoids), molecules that modify their concentration or activity (e.g. FAAH inhibitors), as well as the physiological systems they target (e.g. endocannabinoid system).

2. Cannabidiol Research – subset of the Cannabinoid Research category that reports all NIH projects examining basic, preclinical, and therapeutic properties of CBD.

3. Therapeutic Cannabinoid Research – subset of the Cannabinoid Research category (above) that reports all NIH projects examining the therapeutic properties of *all* classes of cannabinoids (purified, synthetic, endogenous, phytocannabinoids).

A PERIPHERALLY-ACTING CANNABINOID RECEPTOR 1 AGONIST ALLEVIATES NEUROPATHIC PAIN SYMPTOMS WITHOUT ABUSE LIABILITY

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Introduction: Studies show that plant-based and synthetic cannabinoids reduce persistent pain of inflammatory and neuropathic origin in humans and animals. Cannabinoids are also effective in alleviating chronic pain symptoms after repeated treatment, unlike opioids, which have limited long-term effectiveness. However, cannabinoids also have side effects, the most troubling of which are mediated by activation of cannabinoid 1 receptors (CB1Rs) within the central nervous system (CNS). We developed synthetic peripherally restricted cannabinoid (PRCB) agonists, targeting the CB1R, that do not cross the blood-brain barrier. The prototype compound, 4-{2-[(1E)-1-[(4-propylnaphthalen-1-yl) methylidene]-1H-inden-3-yl] ethyl} morpholine (PrNMI) effectively suppressed chronic pain symptoms in preclinical models of cancer, chemotherapy- and traumatic nerve injury-induced neuropathies, as well as migraine and medication overuse headache, all with minimal CNS-mediated side effects or tolerance development.

Methods: Unilateral sciatic nerve entrapment (SNE) injury and an unbiased, counterbalanced design, conditioned place preference (CPP) protocol in adult male and female C57BL/6J mice.

Results: Here we show that administration of PrNMI (0.3 or 0.6 mg/kg, i.p.), dose-dependently alleviated neuropathic pain symptoms induced by SNE injury in mice. We further show that there was no change in CPP score or no difference between vehicle and drug chamber indicating that PrNMI (0.6 mg/kg, i.p.) had no psychoactive effect nor produced negative reinforcement in the SNE-induced chronic pain group.

Conclusions: These data suggest that a PRCB such as PrNMI alleviates neuropathic pain symptoms without abuse liability. We are currently carrying out optimization of PRCBs to facilitate their clinical implementation for the treatment of targeted patient populations.

Acknowledgments: NIH grants NS128148, DA005010, UL1TR001881, CA196263, and U54HL119893.

HIGH-SPECIFICITY FLUORESCENT PROBE TO ENABLE CANNABINOID TYPE 2 RECEPTOR STUDIES

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Introduction: Microglia, as resident immune cells in the central nervous system (CNS), play a role in a wide variety of processes. Its ability to dynamically transition between different polarization states is thought to contribute to the initiation and progression of several neurodegenerative diseases. Alzheimer's disease (AD) is characterized by persistent and intense neuroinflammation derived from the aggregation of amyloid peptides (Aβ) in the brain parenchyma, as well as the formation of neurofibrillary tangles within neurons and microglia play a relevant role in the neuroinflammatory response triggered by amyloid deposition. The Cannabinoid type 2 (CB_2) receptor is primarily expressed by microglial cells under neuroinflammation conditions and is thought to be involved in the modulation of microglial phenotypes. Although the CB_2 receptor holds significant promise, researchers are handicapped by the absence of robust tools, hindering their efforts to understand its expression and complex signaling mechanisms.

Methods: In this study, we employed the $5xFAD^{+/-}$ and $5xFAD^{+/-}/CB_2^{-/-}$ mouse models to study the specificity of the fluorescent probe RO7246360, used successfully across applications and cell types. We evaluated the specificity of the probe RO7246360 for the CB_2 receptor through flow cytometry. Aditionally, we evaluated the ability of the probe RO7246360 to bind to CB_2 receptors in mouse brain tissue sections.

Results: High specificity of RO7246360 for the CB_2 receptor was demonstrated through flow cytometry analysis in microglial cells isolated as $CD11b^{+}/CD45^{lo}$. Additionally, we confirmed a limited *in situ* expression of the CB_2 receptors in the mouse brain cortex.

Conclusions: These findings suggest that the fluorescent probe RO7246360 might be useful in the localization of the CB_2 receptor in tissue sections and could open a path for the precise quantification of its expression in different cell types.

Acknowledgements: supported by Ministerio de Ciencia e Innovación – Agencia Estatal de Investigación and Fondo Europeo de Desarrollo Regional (PID2022-138461OB-I00, supported by MCIN /AEI /10.13039/501100011033 / FEDER, UE).

SHORT EXPOSURE TO NANOMOLAR CANNABINOIDS PROMOTES BREAST CANCER CELL DIFFERENTIATION

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Introduction: Cancer is a highly heterogeneous disease characterized not only by variations in the organ of origin but also by intrinsic tumor heterogeneity. Mutations in cancer cells, along with alterations in the epigenetic landscape, play critical roles in tumorigenesis. Tumor progression, invasiveness, and metastasis are often attributed to these alterations, particularly within a dedifferentiated context. While current therapeutic strategies primarily target tumor cell proliferation and apoptosis induction, dedifferentiated cells often exhibit resistance. Thus, differentiation-based approaches are being explored to overcome resistance and eliminate residual dedifferentiated populations within tumors.
Breast cancer is statistically the most diagnosed cancer worldwide and in fact, it is the second cause of death in women. Most breast cancer-related deaths are associated with relapse or metastasis after surgery. That is directly allied with a partial response to the current therapy and correlates with poorly differentiated tumors. Therefore, in our lab we aim to develop new differentiation-based therapeutic strategies on breast cancer, that would efficiently complement the current therapies in the clinic.

Methods: We are focused on investigating the therapeutic potential of cannabinoids, specifically Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), as pro-differentiation agents in cancer. To assess cancer cell differentiation, we use tumor-derived 3D organoids obtained from both mouse models and patient biopsies. These organoids serve as accurate *in vitro* models that closely mimic tumor emergence *in vivo*. Our experimental approach involves a brief exposure (4-5 days) of breast cancer-derived organoids to nanomolar concentrations of THC or CBD. We then assess their impact on differentiation processes several weeks after treatment withdrawal.

Results: Exposure of breast cancer-derived organoids to THC or CBD resulted in significant long-term tumor cell-differentiation effects. Those included reduction of invasive and self-renewal capacities on cancer cells and global impairment of stemness properties and stem marker expression. Additionally, a basal-to-luminal switch was observed at both morphological and molecular levels. These changes were associated with decreased tumor onset and aggressiveness *in vivo*, suggesting that short exposure to THC or CBD induces lasting alterations in tumor cell differentiation.

Conclusions: Our findings suggest that THC or CBD exposure could serve as a complementary therapeutic strategy in breast cancer treatment. By promoting differentiation, these cannabinoids may enhance the efficacy of current therapies, potentially preventing tumor relapse and metastasis. Further exploration of differentiation-based approaches, particularly in combination with existing treatments, holds promise for improving outcomes in breast cancer patients.

Acknowledgements: Funded by Fundación Científica Asociación Española Contra el Cancer (Grant PRDMA19003GARC)

PHARMACOLOGICAL ABLATION OF MICROGLIA REVEALS SPECIFIC MORPHOLOGICAL CHANGES IN PLAQUE-ASSOCIATED MICROGLIA IN 5XFAD/FAAH$^{-/-}$ MICE

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Introduction: Neuroinflammation is a prevalent condition in various neurodegenerative diseases, including Alzheimer's disease (AD), but its specific role is still controversial. Microglia has been considered as a key regulator of inflammatory responses in the central nervous system and may play a critical role in neurodegenerative processes. It has been described that microglial morphology changes along AD progression, but it is still unclear how these modifications might contribute to the pathological process. Our studies highlight that 5xFAD mice deficient in the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) exhibit an intensified inflammatory profile, which includes increased microglial activation and phagocytosis, that intriguingly coincide with various neuroprotective outcomes. In this work, we aim to evaluate significant changes in microglial morphology in this context by using a model of pharmacological microglial depletion.

Methods: We performed a time course analysis in 5xFAD/Cx3cr1$^{+/GFP}$ and 5xFAD/FAAH$^{-/-}$/Cx3cr1$^{+/GFP}$ mice by *in vivo* multiphoton microscopy. Mice were subjected to a cranial window surgery and were exposed to PLX5622 for 28-days to cause the pharmacological ablation of microglia. Images of amyloid plaques and microglia were obtained once per week. Afterwards, mice were switched to control diet for 9 additional days to allow microglia re-population. Three-dimensional reconstruction of two-photon microscopy images was performed using Imaris Software (V.10.0, Oxford Instruments).

Results: Our results showed that plaque-associated microglia were less responsive to PLX5622 treatment than non-plaque-associated microglia regardless of the activity of the enzyme. Additionally, we found that PLX5622 exposure exacerbated some morphological differences of plaque-associated microglia following FAAH genetic inactivation. 5xFAD/Cx3cr1$^{GFP/+}$ plaque-associated microglia exhibited shorter processes, higher sphericity, and lower ramification index than 5xFAD/FAAH$^{-/-}$/Cx3cr1$^{GFP/+}$ microglia. PLX5622 treatment also revealed an increased phagocytic microglial activity linked to FAAH genetic inactivation.
Conclusions: Pharmacological ablation of microglia revealed a different profile of plaque-associated microglia after the genetic inactivation of FAAH in AD context.

Acknowledgements: supported by Ministerio de Ciencia e Innovación – Agencia Estatal de Investigación and Fondo Europeo de Desarrollo Regional (PID2022-138461OB-I00, supported by MCIN /AEI /10.13039/501100011033 / FEDER, UE).

EXPLORING THE IMPACT OF CANNABIDIOL AND β-CARYOPHYLLENE COMBINATION ON MYELINATION STATUS IN A MURINE MODEL OF DRAVET SYNDROME

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Introduction: Dravet Syndrome (DS) is a rare genetic condition causing epilepsy in children due to mutations in the *Scn1a* gene in 60-80% of patients. Major symptoms in DS are repeated seizures, although patients are often refractory to classic antiepileptic drugs. However, recent studies have demonstrated the anticonvulsant activity of cannabidiol (CBD), which formulated as Epidiolex® is currently approved for alleviating seizures in DS. Epileptic activity in DS has been also associated with long-term behavioural comorbidities, like hyperactivity and autistic traits, derived from events related to oxidative stress, excitotoxicity, and neuroinflammation. These events worsen seizure susceptibility but may also derive in additional damages involving the myelin status and the blood-brain barrier (BBB) permeability. In this study, we investigated possible deficiencies in DS affecting the myelin and the BBB, as well as the possibility that myelin defects may be corrected with treatments with CBD and/or β-caryophyllene (BCP), other compound present in *Cannabis sativa* that, as CBD, may also alleviate seizures and long-term behavioural comorbidities.

Methods: This study was conducted in conditional knock-in *Scn1a*-A1783V mice generated by Cre-LoxP technology, which carry a specific missense mutation (A1783V) in the *Scn1a* gene expressed exclusively in CNS neurons. These mice were used first to analyse the expression of several myelin proteins in some CNS structures at PND25 and PND60, as well as to analyse several BBB proteins, as well as the occurrence of immune cell infiltration into the brain parenchyma at PND25. In a second experiment, mice were treated with a chronic dose of CBD (5 mg/kg) and BCP (10 mg/kg), administered individually or in combination, every two days starting from PND10 until PND24. Once euthanized at PND25, their brains were collected for biochemical and histopathological analysis of myelin defects.

Results: Our data revealed demyelination signs (reduced levels of some myelin proteins) in the prefrontal cortex and the hippocampus at PND25, that persisted at PND60, but only in the prefrontal cortex. As regards to the BBB, our data indicated signs of deterioration confirmed by the occurrence of extravasation of endogenous IgG into the brain parenchyma, as well as by lower levels of some BBB-related proteins (ZO-1, laminin, claudin-5) in these mice. The administration of CBD and BCP, alone or in combination, to DS mice resulted in myelination levels comparable to wildtype mice. Notably, this recovery was prominent in brain regions associated with DS comorbidities, such as the hippocampus and the prefrontal cortex.

Conclusions: These results prove the occurrence of myelin defects and BBB deterioration also in DS, and demonstrate that, at least the demyelination defects, may be treated with those cannabinoids that have shown benefits for seizuring activity and long-term comorbidities.

N-ADAMANTYL-1-ALKYL-4-OXO-1,4-DIHYDROQUINOLINE-3-CARBOXAMIDE DERIVATIVE AS A FLUORESCENT PROBE TO DETECT MICROGLIA ACTIVATION THROUGH THE IMAGING OF CANNABINOID RECEPTOR SUBTYPE 2 (CB2R)

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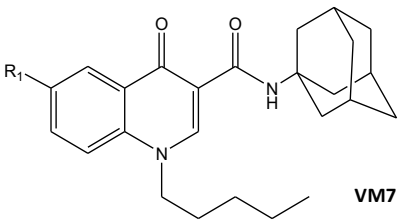
Background: Cannabinoid receptor subtype 2 (CB2R) is emerging as a pivotal biomarker to identify the first steps of several inflammatory-based diseases such as cancer, neurodegeneration, and SARS-COV-2 infection. Thus, there is an urgent need to find specific probes that may result in green and safe alternatives to the commonly used radiative technologies, to deepen the knowledge of the CB2R pathways impacting the onset of all the above-mentioned pathologies. A class of *N*-adamantyl-1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives as CB2R fluorescent ligands was developed, spanning from the green to the Near-Infra Red (NIR) regions of the light spectrum. Among the newly synthesized fluorescent ligands, compound **VM7** exhibited a favorable pharmacodynamic profile characterized by strong CB2R affinity and high selectivity. Notably, this ligand demonstrated its versatility as its use was validated in different experimental settings such as flow cytometry saturation, and competitive fluorescent assays.

Methods: Compound **VM7** was synthesized using organic synthetic procedures (unpublished results). Binding affinity to CBRs was determined as previously described **[1]**. Flow cytometry analysis validated compound **VM7** as a CB2R fluorescent probe in fluorescent-based saturation/competition binding assays. Fluorescent properties were characterized by recording absorption and fluorescence spectra with the Shimadzu UV-1800 spectrophotometer and Tecan Infinite M1000 Pro spectrofluorometer, respectively. Sartorius IncuCyte® system for live-cell imaging was exploited to examine the specificity of **VM7** fluorescent signals in an LPS-stimulated microglial BV2 cell line, whose CB2R expression is reported to be up-regulated under inflammatory stimuli **[2].** Using this system, BV2 cells were cultured in the presence of different concentrations of **VM7** (0.5, 1.0, 2.5 μM) and its signal was monitored every 2h over 24h of incubation. Negative control of fluorescence signal was obtained by incubating cells with LPS 100ng/mL, in the absence of **VM7**. The highest concentration of **VM7** (2.5 μM) was then incubated in the presence of the CB2 antagonist AM630, which was preceded by a 30-minute pre-treatment with 1μM AM630 in the microglial cells.

Results: Compound **VM7** selectively co-localized with BV2 cells. Accordingly, the intensity of the signal positively correlated with the significant increase in concentrations over time. Up to a ~10 and ~4-fold increase was observed after 24h incubation in cells treated with 2.5μM **VM7** compared to 0.5μM and 1μM, respectively. Upon AM630 co-incubation, **VM7** signaling significantly decreased, suggesting **VM7** has a high specificity for CB2R in BV2 cells.

Conclusion: Compound **VM7** shows specific CB2R detection in flow-cytometer analyses and live-cell microscopy. It was able to image microglia activation, induced by LPS inflammation, thus representing a novel and powerful tool for the detection of inflammation processes involving CB2R. Although the efficacy of compound **VM7** in other settings deserves further investigation, the study paves the way for developing novel and powerful fluorescent ligands for the early diagnosis of inflammation-based diseases.



VM7

(R_1= NBD fluorescent tag)

Acknowledgments: NextGenerationEU-PNRR_M4/C2/Investimento.1.1 (Grant: PRIN2022_Project 2022CSYK9L_LS) funded **AL**. NextGenerationEU-PNRR_M4/C2/Investimento.1.1 (Grant: PRIN2022_PNRR_Project Prot. P2022TRR3Y_PE) funded **AL** and **MC**. International PhD program fellowship from UNINA funded **PK**.

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CANNABIGEROL – A USEFUL AGENT RESTORING THE MUSCULAR PHOSPHOLIPIDS MILIEU IN OBESE AND INSULIN-RESISTANT WISTAR RATS?

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Introduction: Recently, numerous strategies have been proposed to minimize obesity-associated health effects, among which phytocannabinoids appear to be effective and safe compounds. In particular, cannabigerol (CBG) emerges as a potent modulator of the composition of membrane phospholipids (PLs), the importance of which we have appreciated in great detail as essential regulators of insulin resistance (IR) state. Therefore, here we consider the potential therapeutic influence of a 2-week CBG application on the intramuscular content of PLs with particular emphasis on distinct phospholipid subclasses (e.g., phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidyl-inositol (PI)) in rats during the high-fat, high-sucrose (HFHS) diet regime. Furthermore, we also assessed the individual fatty acids (FAs) composition, the total content of saturated (SFAs), monounsaturated (MUFAs), and polyunsaturated fatty acids (PUFAs), and the stearoyl-CoA desaturase 1 (SCD1) index in the above-mentioned lipid pools. Additionally, to evaluate the ability of CBG to reduce local inflammation in the skeletal muscle of rats with IR induced by the HFHS diet, we examined the activity of n-3 and n-6 PUFAs pathways as well as the total expression of various proteins involved in the inflammatory process.

Methods: All procedures were performed on male Wistar rats fed the standard rat chow or HFHS diet for 6 weeks. Half of the animals in each experimental group received intragastrically CBG (30 mg/kg of body mass) or its vehicle for the last two weeks of a diet regime. Muscle samples (red gastrocnemius muscle*)* were collected and immediately frozen in liquid nitrogen and stored at −80°C. The content of intramuscular PLs was determined by gas-liquid chromatography (GLC) and based on the composition of individual FAs, we assessed the SCD1 index as well as the activity of n-3 and n-6 PUFAs pathways, whereas expression of various proteins engaged in the inflammatory pathway as well as FAs elongation and desaturation processes was measured using the Western blot technique. Data were analyzed by one-way ANOVA followed by an appropriate post-hoc test ($p < 0.05$ considered significant).

Results: Our research has demonstrated the important association of obesity with alterations in the composition of muscular PLs, which was significantly improved by CBG supplementation. In particular, CBG increased the PE and PI content in both rats fed the standard and HFHS diet. Moreover, CBG enriched the lipid pools in n-3 PUFAs and decreased the content of arachidonic acid (AA), which in turn influenced the activity of PUFAs pathways in various PLs subclasses. Simultaneously, CBG also inhibited the local inflammation development and profoundly reduced the SCD1 activity.

Conclusions: Our results indicate that HFHS diet-induced obesity significantly affects the dynamics of muscular PLs by changing the content of individual PLs subclasses and the composition of forming FAs via modification of the fatty-acyl chain, which is related to the activation of various signaling pathways that can promote pro-inflammatory responses. In addition, we provided new insight into the properties of CBG, demonstrating that it acts as a regulator of PLs metabolism, which is associated with attenuating the local inflammatory response in skeletal muscle. All of these findings revealed the promising potential of CBG therapy, which may contribute to restoring insulin sensitivity of skeletal muscles in obesity, indicating its possible therapeutic usefulness in the treatment of obesity-related metabolic disorders.

Acknowledgments: Funded by the Minister of Education and Science from the state budget under the program "Student science clubs create innovations" (Grant no. SKN/SP/534956/2022).

PERIPHERALLY RESTRICTED CB1 RECEPTOR INVERSE AGONIST JD5037 TREATMENT EXACERBATES LIVER INJURY IN MDR2 DEFICIENT MICE

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Introduction: Previous research highlighted the involvement of the cannabinoid CB1 receptor in regulating the physiology of hepatocytes and hepatic stellate cells. The inhibition of the CB1 receptor via peripherally restricted CB1 receptor inverse agonist, JD5037, has shown promise in inhibiting liver fibrosis in mice treated with CCl_4. However, its efficacy in phospholipid transporter deficiency-induced liver fibrosis remains uncertain. In this study, we investigate the effectiveness of JD3057 in *Mdr2*$^{-/-}$ mice.

Methods: *Mdr2* (*Abcb4*) is a mouse ortholog of human *MDR3* (*ABCB4*) gene encoding for the canalicular phospholipid transporter.11 Genetic disruption of the *Mdr2* gene in mice causes a complete absence of phosphatidylcholine from bile, leading to liver injury and fibrosis. *Mdr2*$^{-/-}$ mice develop spontaneous fibrosis during growth. JD5037 was orally administered to the mice for four weeks starting at eight weeks of age. Liver fibrosis, bile acid levels, inflammation, and injury were assessed. Additionally, JD5037 was administered to three-week-old mice to evaluate its preventive effects on fibrosis development.

Results: Our findings corroborate previous observations regarding global CB1 receptor inverse agonists. Four weeks of JD5037 treatment in eight-week-old *Mdr2*$^{-/-}$ mice with established fibrosis led to reduced body weight gains. However, contrary to expectations, JD5037 significantly exacerbated liver injury, evidenced by elevated serum ALT and ALP levels, and exacerbated liver histology. Notably, JD5037-treated *Mdr2*$^{-/-}$ mice exhibited significantly heightened serum bile acid levels. Furthermore, JD5037 treatment intensified liver fibrosis, increased fibrogenic gene expression, stimulated ductular reaction, and upregulated hepatic pro-inflammatory cytokines. Importantly, JD5037 failed to prevent liver fibrosis formation in three-week-old *Mdr2*$^{-/-}$ mice.

Conclusions: In summary, our study reveals the exacerbating effect of JD5037 on liver fibrosis in genetically MDR2-deficient mice. These findings underscore the need for caution in the use of peripherally restricted CB1R inverse agonists for liver fibrosis treatment, particularly in cases of dysfunctional hepatic phospholipid transporter.

Acknowledgments: Funded by NIH/NIAAA.

ASSESSING CANNABINOID COMBINATIONS FOR SLEEP ENHANCEMENT: FINDINGS FROM *IN VIVO* STUDIES

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Introduction: Sleep disorders present significant challenges, affecting millions globally, especially among older individuals. Cannabis, with its diverse cannabinoids and terpenes, has shown promise in modulating sleep. However, the specific effects of individual cannabinoids and their combinations remain unclear, necessitating further investigation to elucidate their potential therapeutic benefits.

Methods: Using a well-established Loss of Righting Reflex (LORR) *in vivo* mouse model, we investigated the effects of THC, CBD, CBN, and their combinations on sleep latency and duration. All *in vivo* trials were approved by the IACUC and performed following ethical guidelines for the use of animals in research. A total of 193 male Balb/C mice were divided into twenty-four groups and subjected to various treatments. Cannabinoids, either as single test items or in combination, were premixed in and dissolved in an Ethanol: Cremophor: Saline solution (1:1:18). They were administered intraperitoneally (IP) 30 minutes prior to pentobarbital induction, and sleep parameters were recorded, including sleep latency and duration. Diazepam (2 mg/kg) was used as a positive control in all LORR studies. The examined doses for each cannabinoid and their combinations were coded from 1 (low dose), 2 (medium dose), and 3 (high dose) as follows: THC, CBD, CBN [1-0.0.5, 4, 10; 2-0.5, 40, 20; 3-3, 150, 80 mg/kg], respectively.

Results: CBN prolonged sleep duration without affecting latency, while THC negatively impacted latency and CBD showed no significant effect. Combinations of THC, CBD, and CBN exhibited improved sleep latency and a more continuous sleep pattern. Terpene mixtures, alone or with cannabinoids, did not enhance sleep quality. Our screening identified three leading combinations, CRX-121, 122, and 221, with CRX-122 showing the most promise. These combinations were selected based on multiple criteria from the LORR model, aiming to shorten latency, increase duration, provide continuity, and reduce "hangover" effects. CRX-122 demonstrated optimal improvements across all criteria, suggesting its potential for clinical translation. Notably, most test items prolonged sleep duration compared to diazepam, while none shortened latency. In triple cannabinoid combinations, THC primarily influenced sleep onset. Overall, the leading combinations, particularly CRX-122, present promising avenues for further research in sleep disorder management.

Conclusions: Our study highlights the complex interplay between cannabinoids and terpenes in modulating sleep patterns. Although CBN demonstrated promising effects on sleep duration, combinations of THC, CBD, and CBN showed improved latency and continuity of sleep. Interestingly, injection of terpene mixtures did not contribute to enhanced sleep quality. Our findings underscore the importance of a comprehensive understanding of cannabinoid interactions in developing effective therapeutic interventions for sleep disorders. Further research is warranted to elucidate the underlying mechanisms and optimize cannabinoid formulations for improved clinical outcomes in sleep management.

INDAZOLE PARTIAL AGONISTS TARGETING PERIPHERAL CANNABINOID RECEPTORS

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Introduction: Our goal is to develop peripheralized indazole based partial agonists of the cannabinoid receptors CB1 and CB2 that exhibit good drug-like properties without crossing the blood-brain barrier (BBB). Such compounds will not elicit psychoactive effects that are noted with Δ9-tetrahydrocannabinol (THC) and other brain penetrant cannabinergic ligands. While peripheralized full agonists have been reported, there is a paucity of partial agonists. This is important because full agonists rapidly induce tolerance and have limited utility.

Methods: Compounds based on an indazole core were designed and synthesized with a topological polar surface area (TPSA) of 80-140 Å, 2-4 hydrogen bond donors, a cLogP of <5, and a MW of 350-500 Da. These compounds were characterized using functional assays for CB1 and CB2 along with radioligand displacement analyses using radiolabeled CP55,940, which is a full synthetic agonist of both receptors. Stability in human liver microsomes (HLM) and the potential to induce certain cytochrome P450 isoforms were determined. Pharmacokinetic studies were performed in mice for select compounds.

Results: Structure activity relationship (SAR) studies led to the identification of several activators of CB1 and CB2. Of the more promising early leads, an indazole bearing a N-difluorobenzyl and a phenyl carboxamide in the 3-position with adequate potency at CB1 and CB2 along with partial agonism was identified. This compound was stable in HLM but induced CYP3A4. Pharmacokinetic studies showed the compound to be orally absorbed in mice with a half-life of ~7 hr. Brain accumulation of this compound was also limited with a plasma:brain maximum concentration (Cmax) ratio >10.

Conclusions: Structural modifications focusing on tPSA, H-bond donors and strategically placed lipophilic moieties produced early lead partial agonists of CB1 and CB2 with significant peripheral selectivity and good drug-likeness including oral absorption. These analogs are viable leads for optimization in therapies where partial agonism of cannabinoid receptors would be beneficial but without neuropsychiatric liabilities.

Support: We express our gratitude to the NIDA drug supply program for providing radiolabeled probes. This research was funded by grants R01DA040460 and R01DK124615 from NIH.

UNDERSTANDING PHYTOCANNABINOID SIGNALLING PROFILES AT CANNABINOID TYPE 1 AND 2 RECEPTOR

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Cannabis sativa is used therapeutically in a wide range of conditions, yet little is known about the pharmacology of the 120 phytocannabinoids present in the plant, beyond that of Δ^9–tetrahydrocannabinol (Δ^9–THC) and cannabidiol (CBD). These compounds interact with endocannabinoid system (ECS) receptors, including the G protein-coupled receptors cannabinoid type 1 receptor (CB1R) and cannabinoid type 2 receptor (CB2R). Although CB1R and CB2R canonically couple to the inhibitory Gαi/o protein family, there is evidence that these receptors engage other transducers. We sought to characterize the coupling signatures of CB1R and CB2R in the presence of Δ^9–THC, CBD, Δ^9-tetrahydrocannabivarin (Δ^9–THCV), cannabidivarin (CBDV), cannabigerol (CBG), cannabichromene (CBC), Δ^9-9-tetrahydrocannabinolic acid (Δ^9–THCA), and cannabidiolic acid (CBDA). To achieve this goal, bioluminescence resonance energy transfer (BRET)-based biosensors for all families of G protein and both β-arrestin pathways were transiently transfected into human embryonic kidney 293 (HEK293) cells with either human CB1R and CB2R. We hypothesize that each phytocannabinoid exhibits a unique signalling profile at each receptor. Preliminary results suggest that, broadly, there is little difference across Gαi/o family isoforms in the coupling signature of each compound. Additionally, we found that Δ^9-THCV displayed different activity at CB1R and CB2R despite its similar structure to Δ^9-THC, which is a partial agonist at both receptors. Our findings contribute to the characterization of both the phytocannabinoids and their receptor targets. A determination of the signalling signatures elicited by the phytocannabinoids at CB1R and CB2R will yield a better understanding of cannabis pharmacology and may enable us to dissociate the desirable effects from those that are undesirable.

THE INFLUENCE OF CB2 ACTIVATION IN MICROGLIA ON THE REGULATION OF CALCIUM-DEPENDENT CASPASES ASSOCIATED WITH PYROPTOTIC PATHWAY

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Introduction: Microglia, the resident immune cells of the central nervous system (CNS), play a crucial role as the first line of defense in restoring immunological balance during infections or injuries. However, prolonged activation of microglia can lead to the development of neuroinflammation, with microglial pyroptosis emerging as a potential contributor to neuronal inflammation. Despite this, direct studies investigating microglial pyroptosis remain scarce. Therefore, we present data describing the activation of NLRP3 pathways in microglial cells. Currently, there are no therapies available for successfully treating microglia-derived neuroinflammation. However, among the promising strategies proposed, modulation of cannabinoid receptor type 2 (CB2) has garnered attention. The mechanism and molecular pathway underlying the anti-inflammatory effects of CB2 agonists remain unclear. Research in recent decades has emphasized the role of inflammasomes in regulating human disorders. Recent studies have shown that CB2 stimulation reduces NLRP3 inflammasome activity, although the molecular mechanism is yet to be fully revealed. Thus, we focused on delineating the canonical and non-canonical components of the NLRP3 inflammasome pathway as downstream targets of CB2 stimulation.

Methods: In our studies, we utilized a murine cell line (BV2) lacking CB2 receptor expression (CRISPR/Cas9; CR-CB2 BV2) to elucidate the components of molecular pathways involved in decreased pyroptosis in microglia following treatment with CB2 agonists. Cells were exposed to LPS and nigericin or ATP and nigericin to induce inflammatory and stress conditions in an *in vitro* model, with measurements including NO release, ROS detection, expression of various NLRP3 pathway components (qRT-PCR, Western blot), interleukin release (ELISA), and protease activity associated with pyroptosis (kinetic assays).

Results: The administration of a CB2 agonist improved microglial pyroptosis and neuroinflammation in microglial cell cultures. CB2 activation led to a reduction in the expression level of Caspase 4, with no discernible effect on Caspase 1 transcription. Pharmacological activation induced changes in the expression of studied factors; in CR-CB2 BV2 clones, a significant decrease in NLRP3 and ASC expression was observed. However, the absence of CB2 receptor expression resulted in increased expression and release of IL-18, indicating that cells under inflammatory conditions were directed towards the pyroptotic pathway. While accumulating evidence suggests the influence of CB2 activation on NLRP3 activity, most studies focus on NFκB and Caspase 1 involvement. In contrast, our results suggest the involvement of another protease in the NLRP3 inflammasome pathway: Caspase 4. The presence of this protease suggests that microglia undergo non-canonical pyroptosis in the absence of endocannabinoid tone through CB2. Furthermore, CB2 activation reduced the release of IL1β and IL18, which are characteristic of pyroptosis, in macrophages.

Conclusions: Our project hypothesizes that the neuroprotective function of CB2 receptor stimulation in microglia may involve attenuating excessive expression and activity of the NLRP3 inflammasome pathway, thereby protecting cells from pyroptosis.

Acknowledgements: Supported by National Science Center, Poland grants OPUS 2020/37/B/NZ7/03411 and OPUS 2021/43/B/NZ7/01162.

ART12.11, A NOVEL CANNABIDIOL:TETRAMETHYLPYRAZINE CO-CRYSTAL, DEMONSTRATES A UNIQUE PHARMACOKINETIC PROFILE

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Introduction: The therapeutic utility of cannabidiol (CBD) is hampered by its physical and pharmacokinetic properties, including high lipophilicity, polymorphism, poor solubility, and poor oral bioavailability. Co-crystallisation is a useful method for overcoming problematic properties of drugs and is a well-established process in drug development. Co-crystals consist of the drug substance (i.e. CBD) and a co-former molecule which modifies the physicochemical properties, whilst retaining the intrinsic pharmacological drug activity. Artelo Biosciences have developed a patented cocrystal of CBD with the co-former tetramethylpyrazine (TMP; also called ligustrazine), designated ART12.11. We have previously reported ART12.11 offers improvements in, physicochemical, pharmacokinetic (PK; in dogs) and pharmacodynamic properties compared to CBD[1].

Objective: To determine the pharmacokinetic profile of orally administered aqueous suspensions of ART12.11 compared to CBD and to co-administered CBD and TMP in a second species; rats.

Methods: Male Sprague Dawley rats (n=3) were administered a single dose of either CBD (1 mg/kg intravenously (i.v.) or 10 mg/kg oral gavage (PO)), TMP (1 mg/kg i.v. or 4.3 mg/kg PO), a co-administered mixture of CBD and TMP (1 mg/kg & 1 mg/kg i.v. or 10 mg/kg & 4.3 mg/kg PO) or ART12.11 (14.3 mg/kg (containing 10 mg/kg CBD and 4.3 mg/kg TMP) PO). Animals were dosed in the fed and fasted state. Serial blood samples were taken prior to dosing and at 30 minutes and 1, 2, 3, 5, 8 and 24 h. Plasma samples were analysed by LC-MS/MS to provide quantification of the test materials.

Results: Given i.v. CBD, TMP and co-administered CBD and TMP showed similar PK profiles for the parent analytes. In the fed state, orally administered ART12.11 led to increased plasma levels of parent CBD and a major metabolite 7-COOH-CBD when compared to CBD alone or co-administered CBD plus TMP (figure 1). Both the mean C_{max} and mean $AUC_{0\text{-}t}$ for parent CBD analyte from ART12.11(83.7 ng/ml; 355 h.ng/ml) were 10-12 times greater than CBD alone (10.2 ng/ml; 28.5 h.ng/ml) and 4-5 times greater than co-administered CBD plus TMP (17.8 ng/ml; 93.3 h.ng/ml).

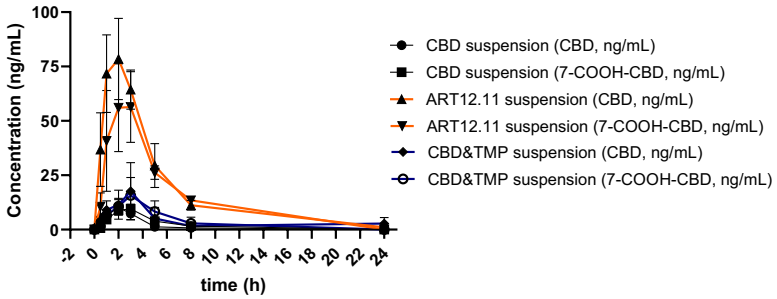

Figure 1. 24-hour PK profiles of CBD and 7-COOH-CBD analytes from PO administration of ART12.11 (10mg/kg CBD/4.3mg/kg TMP) compared with a CBD suspension (10mg/kg) or co-administration of CBD (10mg/kg) and TMP

Conclusions: The unique pharmaceutical properties that co-crystallisation provides translates into greatly increased exposures of CBD and a major metabolite 7-COOH-CBD from ART12.11 compared to CBD alone. The uplift observed is not due to systemic drug-drug interactions between CBD and TMP. Furthermore, the increased CBD and 7-COOH-CBD exposures observed from ART12.11 cannot be replicated by co-administering an equimolar equivalent dose of CBD and TMP. These results are supportive of further development of ART12.11 as an effective approach to dosing CBD in an oral solid dosage form.

Reference: Jones et al. (2023) A novel cannabidiol:tetramethylpyrazine (CBD-TMP, ART12.11) co-crystal improves the efficacy and bioavailability of cannabidiol to induce anxiolytic and anti-depressant effects.

CANNABIDIOL TO MITIGATE FENTANYL INDUCED PERSISTENT APNEA

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Introduction: Opioid therapies are unrivaled in their analgesic efficacy and the cause of a US public health emergency. Opioid induced persistent apnea (OIPA) accounts for more than 100,000 fatalities a year. Naloxone is currently the only available treatment to reverse OIPA events. As the illicit market becomes more adulterated with stronger synthetic fentanyl analogues, more doses of naloxone are necessary to successfully restore breathing. Cannabidiol (CBD), a non-psychoactive cannabinoid that does not alter breathing on its own, has soared in popularity due to its anti-inflammatory and analgesic opioid synergy effects. CBD also mitigated morphine induced respiratory depression at therapeutic doses in mice and increased the efficacy of naloxone in competitive binding assays. In this study, we investigated fentanyl induced effects on breathing following CBD administration and compared to the gold standard treatment, naloxone, in male mice.

Methods: Whole-body plethysmography recordings of awake and freely moving, male C57 mice were collected before and after pretreatment i.p. injection of CBD 250 mg/kg, Naloxone 100 mg/kg, saline, vehicle (10% DMSO, 10% tween80, 80% saline), or a combination of CBD 250 mg/kg + Naloxone 100 mg/kg, and following fentanyl 50mg/kg i.p. Recordings under urethane anesthesia were also collected before and after pretreatment i.p. injection of saline, vehicle, CBD 500 mg/kg, naloxone 100 mg/kg, or CBD 500 mg/kg + Naloxone 100 mg/kg i.p., followed by an intracranial ventricular (ICV) fentanyl infusion at a rate of 100 ng/min till onset of persistent apnea.

Results: Awake behaving male mice pretreated with CBD or Naloxone show no differences in depth of respiratory depression following high fentanyl dose administration. The combination of CBD + Naloxone prevented fentanyl induced changes to breathing in awake mice. Under urethane anesthesia pretreatment with naloxone or CBD similarly increased the fatal fentanyl dose to induce a persistent apnea compared to saline or vehicle pretreated controls.

Conclusions: CBD is equally successful at blunting fentanyl induced respiratory depression as naloxone and together, CBD + Naloxone, are more effective than naloxone by itself at reducing the respiratory depressive effects in awake and anesthetized mice. The addition of CBD may be a unique strategy to help curb the respiratory depressive effects of opioids while also enhancing the efficacy of naloxone in the event a persistent apnea were still to occur, potentially saving thousands of lives.

Acknowledgements: Funded by grant R13DA016280 from the National Institute on Drug Abuse and the Marion Bowen award from the department of Neurobiology (UCLA, DGSOM) (BMW). The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

CANNABIGEROL INFLUENCE ON THE CONTENT OF LIPID AND SPHINGOLIPID IN DIABETIC KIDNEY DISEASE INDUCED BY A HIGH-FAT HIGH-SUCROSE DIET

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Introduction: The consumption of diet rich in fat and sugar leads to excessive lipid deposition and abnormalities in other biologically active lipid pools, namely sphingolipids, contributing to the development of lipotoxicity and inflammatory reactions in the kidney tissue. Sphingolipids are a group of organic compounds that are involved in many biological processes, including inflammation, apoptosis and cell proliferation. Excessive available of fat and sugar in a diet contributes to metabolic diseases development, including diabetes and its complications such as diabetic kidney disease (DKD). Therefore, it is important to search a therapeutic agent that would reduce the deposition of lipid and sphingolipid in the pathogenesis of kidney disorders. Cannabigerol (CBG), a natural derivative from *Cannabis sativa L.*, seems to have a potential regulating effect of lipid and sphingolipid metabolism. Thus, we assessed the impact of cannabigerol on the content of lipid and sphingolipid pools in the kidney tissue of rats subjected to a high-fat diet with 20% sucrose solution.

Methods: The experiment was conducted on male Wistar rats fed a standard diet (Control) or a high-fat high-sucrose diet (HFHS) for 6 weeks. For the last 14 days of experiment, half of rats from Control and HFHS groups received intragastrically CBG supplementation in a dose of 30 mg/kg of body weight. The kidney was isolated, immediately frozen, and stored at -80°C. The concentration of lipid and sphingolipid was measured by the high-performance liquid chromatography (HPLC) and gas-liquid chromatography (GLC), respectively. Obtained data were analyzed by two-way ANOVA followed by post-hoc test. $p<0.05$ was set as a significant difference.

Results: The content of sphinganine (SFA), sphingosine (SFO), sphingosine-1-phosphate (S1P) was significantly decreased in the kidney tissue of rats from the HFHS group in comparison with the Control group. In the same experimental group, we noticed a vital increase in the content dihydroceramide (DH-CER), free fatty acid (FFA) and triacylglycerol (TAG) fractions. Importantly, CBG treatment of rats with DKD increased the content of SFA, SFO and sphinganine-1-phosphate (SA1P) in relation with the HFHS group. Moreover, CBG supplementation to rats with the diabetic kidney disease induced by HFHS diet caused a reduction in the level of DH-CER, S1P and TAG compared to the HFHS group.

Conclusions: The herein data suggest that cannabigerol considerably affects the concentration of lipid and sphingolipid pools. We suppose that cannabigerol by the impairment especially in the triacylglycerol and dihydroceramide may limit the development of lipotoxicity in the kidney tissue. CBG may also provoke an activation of salvage pathway, which was reflected in the increment of SFO and SA1P content, and simultaneously may reduce the intracellular transport of fatty acid (FA) and ceramide *de novo* synthesis pathway.

Acknowledgments: Funded by the Minister of Education and Science from state budget under the program ”Student science clubs create innovations” (Grant no. SKN/SP/534956/2022) and the Medical University of Bialystok (Grant no. B.SUB.24.128).

INVOLVEMENT OF CB_2 AND 5-HT_{1A} RECEPTORS IN CANNABIDIOL NEUROPROTECTION AFTER INTRAVENTRICULAR HEMORRHAGE IN IMMATURE RATS

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Background: despite Cannabidiol (CBD) not being traditionally recognized as a CB_2 receptor (CB_2R) agonist, previous investigations demonstrated that CB_2R antagonism abolishes CBD neuroprotection following hypoxic-ischemic brain injury in neonatal rats and piglets. In this particular model, there is an overexpression of CB_2 - $5HT_{1A}$ heteromers and the beneficial effects of CBD are mediated by these complexes. This study aims to test the potential involvement of CB_2R and $5HT_{1A}$ in CBD-mediated neuroprotection in a different model of acute acquired brain damage in immature brain, utilizing a rat model of intraventricular hemorrhage (IVH), a frequent complication in extremely low gestational age newborns that heightens the risk of Cerebral palsy development.

Methods: IVH was induced in 1-day-old Wistar rats via paraventricular injection of 0.2 U of *Chlostridium collagenase* into the left Germinal Matrix. Animals exposed to anaesthesia but not to surgery remained as controls (SHAM). Vehicle (VEH) or CBD or were administered prenatally (10 mg/kg i.p. to pregnant rats at E21) and post-insult (5 mg/kg i.p. 1, 24 and 48h post-insult). Some rats received CB_2 antagonist AM630 (5 mg/kg) or 5-HT_{1A} antagonist WAY100635 (5 mg/kg) i.p. 1h pre-insult and 1h prior to VEH or CBD post-insult administration. Brain injury was assessed at PND6 using magnetic resonance image (MRI) and at PND14 exploring motor coordination (negative geotaxis) and grasp reflex. Aditionally, inflammation and Blood Brain Barrier (BBB) impairment were analysed at PND6 and PND14 using Western Blot studies to determine the expression of TLR4, indicative of the main biochemical pathway of neuroinflammation in immature brain IVH-induced brain damage; and Mfsd2a, a membrane transport protein expressed in the endothelium of the BBB.

Results: IVH induction resulted in brain damage, as observed in MRI studies, along with functional impairment demonstrated by poorer performance in the geotaxis and grasp reflex tests. These effects were associated with exacerbated neuroinflammation and BBB disruption, evidenced by increased TLR4 and decreased Mfsd2a expression. CBD treatment reduced both anatomical and functional brain damage in a manner linked to the modulation of inflammation and BBB integrity. Co-administration of CBD with the CB_2R antagonist led to the loss of CBD protective effects on damage volume, neurobehavioral tests, TLR4 and Mfsd2a expression. Moreover, co-administration of CBD and the 5-HT_{1A} antagonist at both 5 and 10 mg/kg doses, abolished CBD effects on negative geotaxis and TLR4 expression but not on grasp reflex and Mfsd2a expression.

Conclusions: CBD treatment attenuated IVH-induced brain damage in immature rats in a manner linked to the modulation of inflammation and BBB permeability. The blunting of CBD-mediated neuroprotection by CB_2R antagonism suggests the involvement of CB_2R in CBD mechanisms of action in this IVH rat model. Conversely, the $5HT_{1A}$ receptor appears to play a role in CBD´s effects on preserving BBB.

CANNABIDIOL METABOLIC INTERACTIONS WITH PROPOFOL IN MICE

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Introduction: Previous studies from our lab in mice found that cannabidiol (CBD) produced synergistic enhancement of the hypothermic and sedative effect of propofol, but the mechanism of this drug-drug interaction is not known. Similar to propofol, CBD has also been shown to be a positive allosteric modulator of GABA-A. So the most likely explanations for this interaction are a pharmacologic interaction at GABA-A or a metabolic interaction via inhibition of propofol metabolism.

Methods: C57BL/6 male and female mice were administered 200 mg/kg cannabidiol (CBD), or vehicle (20% sesame oil: 8% Tween-80: 82% ddH_2O), and 1 hour later 125 mg/kg propofol (in 10% Tween-80: 90% ddH_2O) i.p. at 0.01 ml/g. Trunk blood was collected after 10 minutes and plasma was isolated using centrifugation. Drugs and metabolites were extracted from plasma using acetonitrile and quantified via High-Performance Liquid Chromatography-Mass Spectroscopy (HPLC-MS). All data were analyzed by 2-way ANOVA (sex vs treatment) at $\alpha = 0.05$.

Results: Propofol, propofol glucuronide and propofol sulfate were detected above the LOQ. Significant sex difference was found in that female mice exhibited higher levels of both propofol metabolites compared to males. Also, CBD increased the level of propofol in males (but not females), and decreased the amount of propofol glucuronide in females (but not males).

Conclusions: Female mice metabolized propofol to a greater extend than males and CBD appeared to inhibit the glucuronidation of propofol in females at 10 minutes after administration. This may at least partially explain why males showed greater effects of propofol than females. However, the levels of propofol (at least at 10 minutes after administration) were not altered by CBD implying that additional mechanisms of interaction(s) between CBD and propofol may exist.

HEALTH CANADA'S VIGILANCE FRAMEWORK FOR CANNABIS: A SUMMARY OF ADVERSE REACTIONS ASSOCIATED WITH CANNABIS PRODUCTS SINCE THE COMING INTO FORCE OF THE CANNABIS ACT AND ITS REGULATIONS IN 2018

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Introduction: The main objective of Canada's *Cannabis Act* is to protect public health and public safety with respect to cannabis. Health Canada's (HC) Vigilance Framework for Cannabis Products addresses this objective by allowing for the detection, collection, monitoring, and assessment of adverse reactions (ARs) to support decision-making, knowledge translation and communication of the risks of cannabis products to the public. With more than a quarter of Canadians aged 16 years and older reporting past-year use of cannabis for non-medical purposes, it is important to continue to monitor any potential ARs arising from the use of cannabis and cannabis products particularly given that consumers have differing risk profiles that may increase the risk of ARs.The aim of this presentation is to introduce HC's Vigilance Framework for Cannabis Products and summarize ARs suspected of being associated with cannabis products received by HC since the coming into force of the Cannabis Act and its Regulations in 2018.

Methods: Adverse reaction reports are submitted to HC by consumers and healthcare professionals on a voluntary basis, and by licence holders who are obligated to report serious ARs according to the Cannabis Regulations. Reports are received and coded in the Canada Vigilance Database, and then screened, triaged and assessed in near time. Screening of new reports is conducted to ensure proper coding and quality assurance/quality control using the Medical Dictionary for Regulatory Activities. Case reports involving a new and unexpected ARs of interest undergo preliminary assessment to determine if they should be further evaluated (signal assessment). A descriptive analysis of all cases involving legal cannabis products in a suspected role and received from October 17, 2018, to December 31, 2023, was conducted to better understand case patterns by demographic and use characteristics since the coming into force of the *Cannabis Act* and its Regulations.

Results: A total of 634 AR cases involving legal cannabis products were reported to HC during the reference period. Most cases were serious (63%) and involved females (47%); males (35%); not reported (17%); cannabis use for self-reported medical purposes (68%) and cannabis extracts (71%). The most frequently reported events were hallucination, headache, nausea, dizziness, malaise and dyspnoea. Of the cases assessed, most were assigned a causality of 'Possible' meaning the product may have contributed to the AR but that the contribution of other factors could not be ruled out.

Conclusions: This analysis has identified several patterns in AR cases reported to HC involving legal cannabis products. Health Canada will continue to monitor and analyse trends in cannabis ARs and publish public-facing surveillance reports of ARs annually. These data will help inform educational and outreach resources for consumers, healthcare professionals and other reporters of ARs, in addition to informing other activities, such as potential risk communications.

EXPRESSION AND FUNCTION OF DAGLa IN CEREBELLAR PURKINJE CELLS

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Introduction: The cerebellum plays a key role in the regulation of implicit behaviors, ranging from motor coordination and balance to emotional processing. Cerebellar outputs are controlled by Purkinje cell (PC) activity, which, in turn, is influenced by endocannabinoid (eCB) signaling. eCB signaling machinery is prominently expressed in the cerebellum. Diacylglycerol lipase alpha (DAGLα), the primary synthesizing enzyme of 2-Arachidonoylglycerol (2-AG), a major neuronal eCB, is highly expressed in cerebellar PCs; while cannabinoid receptor 1 (CB1) is expressed in the axons and the presynaptic terminals of both excitatory and inhibitory cerebellar afferents. eCB signaling plays a key role in cerebellar learning and short-term synaptic plasticity, i.e., long-term synaptic depression (LTD) and depolarization-induced suppression of excitation and inhibition (DSE and DSI). Furthermore, mutations in DAGLα are associated with neurodevelopmental disorders and cerebellar ataxias in humans. However, anatomical and functional consequences of cerebellar-specific mutations in DAGLα have not been previously characterized.

Methods: PC-specific DAGLα conditional knockout mice were generated to explore the function of DAGLα in the synaptic development and plasticity of cerebellar PCs. Immunohistochemistry and 3D volumetric reconstruction were used to elucidate differences in synaptic morphology in DAGLα-null PCs, and electrophysiology in cerebellar slices was used to evaluate short-term synaptic plasticity.

Results: DAGLα-null PCs exhibit reduced CB1 expression in vGluT1-positive presynaptic terminals, while presynaptic terminals' overall distribution and density on PC dendrites and soma are normal. Both DSE and DSI are dramatically reduced in DAGLa-null PCs.

Conclusions: PC expression of DAGLα regulates PC excitability and synaptic plasticity.

Acknowledgements: This work was supported by the National Institutes of Health (R21-DA044000); Indiana University Bloomington College of Arts and Sciences, OVPR, and Psychological and Brain Sciences Department (FRSP-SEED); and CTSI (Core Pilot).

ROLE OF Δ^9-TETRAHYDROCANNABINOL IN GPR119-MEDIATED METABOLIC REGULATION AND OBESITY TREATMENT: AN *IN VITRO* AND *IN VIVO* STUDY

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Introduction: GPR119, known for its role in regulating glucose homeostasis and energy metabolism, acts by stimulating the release of incretin hormones such as GLP-1, which in turn promotes insulin secretion in a glucose-dependent manner. This study aims to examine the impact of THC on GPR119 activation through both *in vitro* assays and tissue analysis in diet-induced obese (DIO) mice, offering insights into the metabolic outcomes of THC interaction with this receptor.

Methods: Our research employed a two-pronged approach: *in vitro* assays to assess the biochemical responses to GPR119 activation by THC and it's metabolites; and *in vivo* analyses, focusing on THC's effects in DIO male mice. Mice were placed on a high-fat diet starting at P35 and treated via gavage with THC at a dosage of 10 mg/kg for 12 weeks. At the end of this period liver and visceral fat content, lipid droplet size in the liver, and markers of lipogenesis were measured. The study also compared the effects of THC treatment on wild type (wt) vs GPR119 knockout (KO) DIO mice to delineate the role of GPR119 in mediating THC's metabolic effects.

Results: THC activation of GPR119 produced changes in cAMP and IP-1 levels, receptor internalization, and β-arrestin recruitment *in vitro*. *In vivo*, DIO mice had reduced liver and visceral fat. Livers also displayed smaller lipid droplets, and changes in lipogenesis markers. THC-treated wt and GPR119 KO mice displayed reduced weight gain with THC treatment. Interestingly, while THC treatment resulted in weight loss among mature DIO wild-type mice, comparable GPR119 KO mice lost significantly less weight, indicating a critical role of GPR119 in mediating THC's effect on weight in mature obese mice.

Conclusion: Our findings underscore the complex influence of GPR119 on metabolic regulation, suggesting variable effectiveness of GPR119-targeted treatments based on the organism's metabolic state.

ART12.11, A NOVEL CANNABIDIOL:TETRAMETHYLPYRAZINE CO-CRYSTAL, DEMONSTRATES A PHARMACOKINETIC PROFILE COMPARABLE WITH EPIDIOLEX® IN RATS

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Introduction: Cannabidiol (CBD) is available as an approved medicine Epidiolex, an oral solution of CBD in ethanol and sesame oil used for controlling seizures in rare childhood disorders. Ongoing clinical research suggests CBD may be useful in treating a larger range of conditions; however, the wider therapeutic utility of CBD is hampered by its physical and pharmacokinetic properties, including high lipophilicity, solid polymorphism, low solubility, and poor oral bioavailability. For broader use, especially in the adult population, an oral solid formulation may ultimately be preferred. Artelo Biosciences have developed a patented cocrystal of CBD with the co-former tetramethylpyrazine (TMP; also called ligustrazine), designated ART12.11 intended for development as an oral solid dosage form. Artelo previously reported ART12.11 offers improvements in, physicochemical, pharmacokinetic (PK; in dogs) and pharmacodynamic properties compared to CBD[1].

Objective: To determine the pharmacokinetic profile of orally administered aqueous suspensions of ART12.11 compared to an oral solution of CBD in ethanol and sesame oil (Epidiolex-like formulation) in rats.

Methods: Male Sprague Dawley rats (n=3) were administered a single dose of either aqueous suspension of CBD (10mg/kg PO), CBD Epidiolex-like formulation (10mg/kg PO) or an aqueous suspension ART12.11 (14.3mg/kg (containing 10mg/kg CBD and 4.3mg/kg TMP) PO) in the fed and fasted state. Serial blood samples were taken prior to dosing and at 30 minutes and 1, 2, 3, 5, 8 and 24 h. Plasma samples were analysed by LC-MS/MS.

Results: In the fasted state, orally administered ART12.11 had a lower C_{max} of parent CBD compared to the Epidiolex-like formulation, but similar 7-COOH-CBD levels (Fig 1A), and similar overall exposure (AUC_{0-t}) to either parent (C) or a major metabolite (D). In the fed state, orally administered ART12.11 demonstrated similar plasma levels of parent CBD and 7-COOH-CBD as the Epidiolex-like formulation (Fig 1B), and similar AUC_{0-t} (Fig 1C, 1D). When comparing the ratio of 7-COOH-CBD metabolite to CBD parent (M:P ratio), in the fasted state, the CBD aqueous solution had a ratio of approximately 1.8 (1.8 x metabolite vs parent), ART12.11 has a ratio of 0.8 and CBD in the Epidiolex-like formulation has a ratio of 0.4 (with a reference value of 0.2 for CBD delivered intravenously (i.v)). In the fed state, these ratios were 1.8 (CBD aqueous solution), 0.7 (ART12.11) and 0.5 (Epidiolex-like formulation). Differences in M:P ratios are likely to be driven by differences in CBD absorption (gut versus lympathic system; affected by feeding) and first pass metabolism.

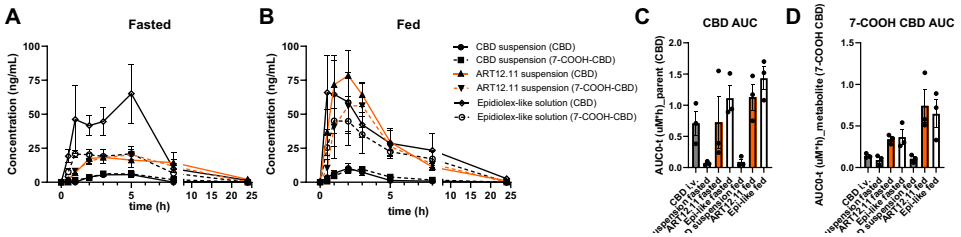

Figure 1. 24-hour PK profiles of CBD and 7-COOH-CBD analytes from PO administration of ART12.11 (10mg/kg CBD/4.3mg/kg TMP) compared with an Epidolex-like formulation (10mg/kg) in fasted and fed rats.

Conclusions: The unique pharmaceutical properties of ART12.11 translates into increased exposures of CBD and a major metabolite 7-COOH-CBD comparable to an Epidiolex-like formulated CBD. These results are particularly noteworthy as ART12.11 was delivered as an un-optimised aqueous suspension that appeared comparable to an optimized liquid mimic of Epidiolex. These results, together with the improved physiochemical properties, including higher melting point, support further solid-dosage form development of ART12.11. The data highlight the importance of the formulation and drug substance used in relation to metabolite formation. Ongoing research with ART12.11 is progressing toward development of an optimised oral solid dosage formulation for future clinical studies.

STIMULUS-SPECIFIC REGULATION OF NEURONAL 2-AG PRODUCTION BY THE INFLAMMATORY MEDIATORS ATP AND BRADYKININ

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Introduction: 2-arachidonoyl glycerol (2-AG) is the most abundant endocannabinoid (eCB) in the brain and plays a role in multiple physiological functions, including pain perception. Evidence suggests that pharmacological increase in 2-AG levels in neuropathic and inflammatory pain preclinical models induces CB_1 receptor-dependent analgesia. Although 2-AG synthesis is increased by the inflammatory mediators ATP and bradykinin (BK), which act on neuronal cells to mediate pain processing, the underlying mechanisms and physiological role of this stimuli-induced increase in 2-AG signaling remains unknown. Here, we leveraged a genetically encoded eCB sensor ($GRAB_{eCB2.0}$) to measure changes in 2-AG levels in neural cells in culture to elucidate the molecular mechanism of ATP and BK-stimulated 2-AG production.

Methods: $GRAB_{eCB2.0}$ was expressed in N2a cells in culture. Live-cell microscopy or a 96-well plate reader were used to detect changes in $GRAB_{eCB2.0}$ fluorescent signal. LC-MS/MS was used to measure 2-AG levels.

Results: ATP (300 μM) and BK (1 μM) increased 2-AG levels in N2a cells by 45% and 95%, respectively, as compared to vehicle when measured by LC-MS. ATP and BK also triggered increases in $GRAB_{eCB2.0}$ fluorescent signal within seconds and with distinct dynamics (e.g. time to peak signal). The ATP-induced increase in $GRAB_{eCB2.0}$ signal was blocked by A740003 (30 μM), an ionotropic $P2X_7$ receptor ($P2X_7R$) antagonist; while the BK-induced increase in $GRAB_{eCB2.0}$ signal was blocked by HOE 140 (1 μM), a B2 receptor (B2R) antagonist. Despite acting at ionotropic and metabotropic receptors, both ATP and BK-stimulated 2-AG production were dependent on increases in intracellular calcium and diacylglycerol lipase activity as shown by BAPTA-AM (30 μM) and the DAGL inhibitor DO34 (10 nM) reducing these responses.

Conclusion: The inflammatory mediators ATP and bradykinin stimulate production of 2-AG in calcium- and DAGL-dependent mechanisms. Thus, this study identifies molecular mechanisms linking eCB signaling with mediators of pain processing in neurons and helps characterize a model system that can be used to test therapeutics that enhance 2-AG signaling for the treatment of conditions such as migraine and inflammatory pain.

Acknowledgements: Funded by NIH (NS118130 and DA047626 to N.S., DA055448 to AE) and the Pharmacological Sciences Training Program of the University of Washington.

PSYCHOSIS ASSOCIATED WITH CANNABIS WITHDRAWAL: CASE SERIES AND SYSTEMATIC REVIEW

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Introduction: Cannabis use is associated with an increased risk of developing psychosis, especially with daily use of high-potency cannabis. Persistent cannabis use after illness onset is associated with relapse. Cessation of heavy cannabis use can cause a withdrawal syndrome characterised by irritability, anxiety, insomnia, reduced appetite and restlessness. Symptoms typically peak after 2 to 6 days. Recent case reports have described cases in which cannabis withdrawal has been associated with the acute onset of psychosis.

Methods: Our objective was to identify cases of psychosis associated with cannabis withdrawal. We searched a large electronic database of secondary mental healthcare records in South London (UK) for clinical entries with terms related to both psychosis and cannabis withdrawal. All demographic and clinical data were either extracted or reviewed by a psychiatrist. We also completed a systematic review of previously published case reports, case series and other studies.

Results: In the health record study, we identified 69 cases in which cannabis withdrawal was associated with acute psychosis. Fifty-seven (83%) cases were male, and the mean age was 27.3 years. Forty-eight cases involved a first presentation of psychosis while 21 were relapses of an existing psychotic disorder. Only 17% of cases had a co-morbid alcohol or (non-cannabis) substance use disorder. Almost all cases were daily cannabis users who stopped using cannabis abruptly. Psychosis emerged within 1 week of cessation in 48 (70%) of cases, with the highest incidence at four days post-cessation. Clinicians noted the presence of cannabis withdrawal syndrome in a minority of cases (24; 35%). The symptoms with the highest prevalence were persecutory delusions (77%), poor appetite (71%), initial insomnia (70%), loss of insight (68%), and agitated activity (67%). A sleep disturbance of any type was identified in 62 (90%) of participants. Cases who continued to use cannabis after the acute episode had a much higher risk of subsequent relapse than those who did not (Odds ratio = 14.7 [95% CI: 4.3 to 60.0]; X^2 = 21.1, p<0.00001). At follow-up, 40 (58%) individuals had a primary diagnosis of a schizophrenia-spectrum disorder, 10 (14%) a substance use disorder, and 9 (13%) an affective disorder. The systematic review identified a total of 33 cases from 17 studies. The findings were consistent with those from the health record study.

Conclusions: Collectively, these studies comprise the largest set of cases of cannabis withdrawal associated with psychosis collected to date (n=102). They suggest that this syndrome is more common than previously recognised. It is important that clinicians are aware that psychotic symptoms can emerge after the cessation of cannabis use, as well as following cannabis use.

ENHANCES SYSTEMIC ENERGY PRESERVATION THROUGH TAURINE AND ANANDAMIDE MODULATION VIA GLUT2 NULLIFICATION IN THE KIDNEY

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Introduction: Selective inactivation of glucose transporter 2 (GLUT2) in kidney proximal tubular cells (KPTCs) has emerged as a promising strategy for ameliorating diabetic kidney disease in mice (Hinden et al., Nat.Commun., 2022). In normoglycemic conditions, KPTC-GLUT2 knockout (KO) mice exhibit notable enhancements in whole-body carbohydrate oxidation and food consumption, alongside alterations in glucose uptake across various tissues. Moreover, KPTC-GLUT2 KO mice manifest improvements in hepatic homeostasis and circulating lipid profile. However, the precise mechanisms underlying these metabolic changes resulting from GLUT2 ablation in KPTCs remain incompletely understood.

Methods: Employing a multidisciplinary approach integrating semi-targeted metabolomics profiling and molecular biology techniques, we investigated the principal metabolic shifts within the kidneys of KPTC-GLUT2 KO mice, elucidating their impact on kidney endocannabinoid 'tone' and signaling.

Results: Our investigations revealed profound metabolic adaptations within the kidney characterized by increased energy metabolism, concomitant with elevated taurine levels and upregulation of the taurine transporter (SLC6A6). Notably, elevated KPTC-SLC6A6 levels were found to be mTORC1-dependent, with marked mTORC1 activation observed in the kidneys of KPTC-GLUT2 KO mice. Building upon our previous published work demonstrating the activation of KPTC-mTORC1 by the cannabinoid-1 receptor (CB1R), we observed elevated levels of *N*-acylethanolamines (NAEs), including anandamide (AEA), *N*-palmitoylethanolamine (PEA), and *N*-oleoylethanolamine (OEA), alongside reduced expression and activity of the NAE-degrading enzyme fatty acid amide hydrolase (FAAH) and unaltered levels of their metabolizing enzyme, *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD). Additionally, a positive correlation was noted between AEA and *N*-arachidonoyl taurine levels in the kidneys of KPTC-GLUT2 KO mice, aligning with the significantly diminished kidney FAAH activity in these animals. Furthermore, elevated taurine and AEA levels were detected in the circulation of KPTC-GLUT2 KO mice, suggesting their potential contribution to the systemic metabolic alterations observed in these mice.

Conclusions: Our findings shed light on the systemic metabolic benefits arising from GLUT2 ablation in the kidney, particularly through the elevation of taurine and AEA levels both locally and systemically. These results offer novel perspectives into the therapeutic potential of modulating KPTC metabolism to address diabetes and obesity, thereby identifying avenues for future therapeutic interventions.

Acknowledgments: This work was supported by grants from the Israel Science Foundation (#158/18) and JDRF (1-INO-2022-1128-A-N) to J.T.

A NOVEL FATTY ACID-BINDING PROTEIN 5 INHIBITOR SHOWS EFFICACY IN PRECLINICAL MODELS OF PSORIASIS

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Introduction: Fatty acid-binding protein (FABP) 5, also known as epidermal FABP, was first discovered in psoriatic lesions (Madsen et al., 1992). FABP5 regulates keratinocyte homeostasis, and is upregulated in psoriasis tissue (Takahashi-Shishido et al., 2021). Knock-out of FABP5 is beneficial in preclinical psoriasis models (Dallaglio et al., 2013), and our aim was to assess whether Artelo's novel oral FABP5 inhibitor ART26.12 (Warren et al., 2024) is efficacious in psoriasis.

Methods: *In vitro,* recombinant human epidermis was stressed with a cytokine mix (IL-17, IL-22, and TNF-alpha). The mix was co-administered with either vehicle, JAK1 inhibitor I (CAS-No 457081-03-7; 10 µM), or ART26.12 (1, 3, or 10 µM) for 48 hrs. Change in the mRNA levels of 64 relevant genes were compared against two housekeeping genes. *In vivo*, male Balb/C mice were given either vehicle, BMS-986165 (Deucravacitinib, tyrosine kinase 2 inhibitor) (10 mg/kg p.o. QD), or ART26.12 (25 or 100 mg/kg p.o. BID) for two days prior to application of imiquimod (IMQ, 62.5 mg of a 5% cream for 7 days) on their backs, and throughout IMQ treatment.

Results: *In vitro*, the cytokine mix upregulated genes related to innate immunity (eg DEFB4A, S100A7) and cytokine markers (especially IL8), as well as reducing differentiation markers (KRT10 and LOR)(Fig1A). The JAK1 inhibitor I (10 µM) largely reversed this effect. 3 and 10 µM ART26.12 reduced genes related to the JAK/STAT pathway (PIAS3 and SOCS3; $p<0.001$), keratinocyte proliferation (KRT14, TP63; $p<0.001$), and chemokines and cytokines (CXCl10, TNF, IL1R1; $p<0.001$). 10 µM ART26.12 also upregulated genes related to anti-microbial peptides and innate immunity (eg CAMP, DEFB4A, TLR2, RNA SE7, SLP1, PI3; $p<0.01$). *In vivo*, the psoriasis area severity index (PASI) scores in the IMQ-vehicle group were near maximum by day 7 (Fig1B and C). Oral treatment with BMS-986165 attenuated PASI scores on day 6 and 7. Oral treatment with ART26.12 (25 mg/kg) also reduced PASI scores on day 6 ($p<0.05$) and 7 ($p<0.001$). The higher dose of ART26.12 (100 mg/kg) reduced scores on day 7 ($p<0.05$). All drugs worked by attenuating skin scaling and thickness, with no effect on erythema. ART26.12 also reduced histopathological signs of damage (reduced hyperkeratosis, parakeratosis, inflammatory infiltrates, and epidermal acanthosis).

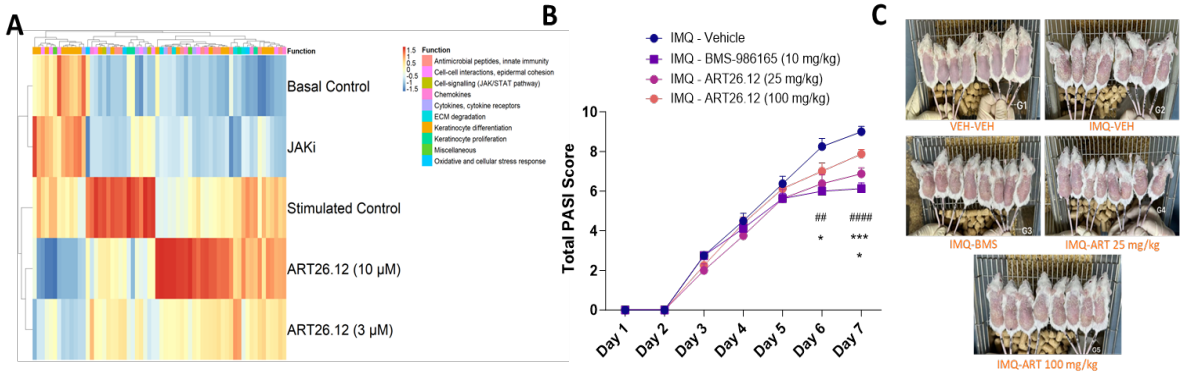

Figure 1. The in vitro (A) and in vivo (B,C) effects of ART26.12 in preclinical models of psoriasis.

Conclusions: In models of skin inflammation, ART26.12 had a positive effect on *in vitro* gene profiling and attenuated the effects of IMQ *in vivo*. These data suggest that ART26.12 shows promise as a novel oral treatment for psoriasis, and possibly other dermatological conditions where FABP5 is elevated such as atopic dermatitis. This is an area of continued research for Artelo Biosciences.

MATERNAL OBESITY INCREASES ENDOCANNABINOID SIGNALING AND TRIGLYCERIDE CONTENT IN THE LIVER OF WEANLING RAT OFFSPRING

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Introduction: Maternal obesity is an important public health problem worldwide, with deleterious metabolic outcomes for mothers and descendants, such as obesity, diabetes, and non-alcoholic fatty liver disease (NAFLD). NAFLD has been associated with increased activity of the endocannabinoid system (ECS) that induces lipogenesis, inflammation, oxidative stress and fibrogenesis in the liver. However, it is unknown whether perinatal maternal obesity can modulate the ECS and fatty acid profile in the liver of weanling rat offspring, contributing to the development of NAFLD.

Methods: Female Wistar rats received a control diet (9% fat; CT group) or an obesogenic diet (40% fat and 9.5% sugar; OD group) for 9 weeks prior mating and throughout gestation and lactation. At weaning, male and female offspring were euthanized to collect blood and liver samples. Serum levels of leptin and insulin were evaluated by immunoassay and serum triglyceride (TG) and cholesterol by colorimetric kits. The liver content of the endocannabinoids AEA, 2-AG, PEA and OEA was evaluated by HPLC-MS and the fatty acid profile by GC-FID. Liver TG content was quantified by a commercial kit based on an enzymatic-fluorimetric method. Data were analyzed by two-way ANOVA followed by Tukey post-hoc test. Pearson correlations were also performed between liver 2-AG and leptinemia and polyunsaturated fatty acid (PUFA) liver content, considering the total animal sample stratified by offspring sex. $p<0.05$ was considered significant.

Results: Maternal obesity increased body weight and adiposity of weanling rats regardless of offspring sex ($p<0.05$). This profile was associated with high serum levels of leptin, insulin and glycemia ($p<0.05$). OD offspring presented increased liver weight, liver TG content and hypertriglyceridemia, compared with CT offspring ($p<0.05$). Maternal obesity increased the liver content of 2-AG ($p<0.05$) but did not change the content of AEA, PEA and OEA in the weanling offspring. In addition, maternal obesity increased the liver content of saturated fatty acids, monounsaturated fatty acids and omega 6 (n6) PUFA ($p<0.05$). In contrast, maternal obesity decreased the liver content of total omega 3(n3) PUFA as well as the amount of the eicosapentaenoic acid (EPA, n3) and docoxahexaenoic acid (DHA, n3) ($p<0.05$). Liver 2-AG content was positively correlated with leptinemia only in males ($p<0.05$) and with n6 PUFA only in females ($p<0.05$). The strongest correlation was found between liver 2-AG and the n6/n3 ratio (r: 0.63 in males and r: 0.86 in females, $p<0.001$).

Conclusions: Maternal obesity induced several endocrine and metabolic deleterious outcomes in the offspring already in early life. We have previously shown that maternal high-fat diet programs the development of obesity, NAFLD and hypertriglyceridemia in the adult offspring associated with increased ECS signaling in the liver. The present results showed that NAFLD in the OD offspring may be associated with an increase of liver 2-AG in early life, and possibly, increased ECS signaling. Because 2-AG is synthesized from the arachidonic acid (n6 PUFA), it is expected a positive correlation between these bioactive lipids as well as with the n6/n3 ratio. Increased n6/n3 ratio is a common profile of obesogenic diets but here, interestingly, we showed this association with the maternal diet. Collectively, our results suggest that maternal nutrition at critical stages of development can modulate the offspring's ECS, predisposing to the onset of metabolic diseases.

Acknowledgements: Foundation for Research Support in Rio de Janeiro (E-26/200.366/2023).

EXPLORING CANNABINOID MODULATION OF PRIMARY MICROGLIA AND ASTROCYTES FROM A TDP-43 RELATED FRONTOTEMPORAL DEMENTIA MOUSE MODEL

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Introduction: Cannabinoids exert neuroprotective effects through the modulation of inflammatory responses, a common feature in neurodegenerative disorders such as frontotemporal dementia (FTD), but poorly explored in this disorder. FTD is characterized by degeneration in the frontal and temporal brain lobes, resulting in impairments in behavior, cognition, and language. As there is no effective treatment for this disease, cannabinoids may represent a novel therapeutic strategy due to their anti-inflammatory potential among other options. Therefore, to further investigate the contribution of neuroinflammation to FTD pathology and the role of the endocannabinoid system, we propose to characterize microglial and astrocytic populations and explore the role of cannabinoids in these glial cell responses in a TDP43-FTD mouse model.

Methods: Primary cortical microglia and astrocytes from CaMKII-TDP43-FTD mice were cultured to assess their response to lipopolysaccharide (LPS), an inflammatory insult. Additionally, glial cells were pre-treated with a non-selective CB_1/CB_2 receptors agonist, WIN 55212-2, to investigate the pathways involved in the anti-inflammatory potential of this compound. Furthermore, microglia and astrocyte secretomes were used to treat NSC34 motor neuron cell line to evaluate whether the anti-inflammatory effect of cannabinoids has any impact on their survival.

Results: Under basal conditions, FTD-microglia displayed reduced CB_2 receptor expression and alterations in some disease-associated microglia (DAM) markers compared to wildtype (WT)-microglia. Upon LPS stimulation, FTD-microglia showed heightened reactivity, evidenced by increased cytokines, inflammasome components, and Toll-like receptor 4 (TLR-4) expression. On the other hand, FTD-astrocytes exhibited reduced expression of the CB_1 receptor under basal conditions, with diminished reactivity to LPS stimulation, shown by a down-regulated expression of pro-inflammatory cytokines, TLR-4, and glutamate transporter-1. Pre-treatment of these glial cells with WIN 55212-2 reduced the expression of pro-inflammatory cytokines and the production of nitrites following LPS stimulation, indicating the anti-inflammatory potential of this compound in FTD glial cells. However, administration of CB_1 and CB_2 antagonists did not completely reverse the observed effects, suggesting the involvement of both cannabinoid receptors and/or alternative targets. Notably, the secretome of microglia and astrocytes treated with WIN 55212-2 and LPS prevented the NSC-34 cell death.

Conclusions: FTD microglia and astrocytes display altered responses to inflammatory stimulation, potentially contributing to FTD pathology. Modulating these responses with cannabinoids appears to be a promising approach for treating this disorder.

INHIBITION OF ANGIOTENSIN-CONVERTING ENZYME (ACE) ACTIVITY BY CANNABINOIDS FROM *CANNABIS SATIVA* L.

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Introduction: Though the cannabinoids of *cannabis sativa* L. (hemp) are reported to affect blood pressure, the mechanistic effects/interactions of these cannabinoids on blood pressure regulation have yet to be described. In this study, using an *in vitro* assay we have investigated the inhibitory activity of hemp extracts on the essential enzyme, angiotensin-converting enzyme-I (ACE), of the blood regulating system renin-angiotensin system (RAS). Analytical techniques of flash chromatography and mass spectrometry were applied to identify potential ACE-inhibiting cannabinoids and terpenes that were isolated, tested, and compared to known ACE inhibitor, lisinopril.

Methods: Three cultivars of locally grown hemp (R5, C4, and PO) were evaluated for their inhibitory activity using a colorimetric enzymatic reaction assay. Hemp inflorescences of the hemp cultivars were extracted utilizing a solvent-based (96% ethanol) extraction technique. Crude hemp extracts were then reconstituted to a fixed concentration of 2.5 mg/mL before being evaluated for their ACE inhibitory. The R5 crude extract was then chromatographically separated and purified via a flash chromatography system producing chemically diverse fraction extracts which were then evaluated for their effectiveness against ACE activity at a concentration of 4 mg/mL. ACE inhibitory activity of the fractions was then paired with mass spectrometry data consisting of known hemp cannabinoid and terpene standards aiding in the identification of the potential bioactive chemical constituents. The identified cannabinoids, terpenes, and lisinopril were then tested at a fixed concertation of 10 µM. Compounds that displayed high ACE inhibition were then evaluated for their potency by determining the half-maximal inhibitory concentration (IC_{50}).

Results: R5 and C4 crude extracts lowered ACE activity to 50%. The R5 crude extract was chromatically separated generating a total of 32 fraction extracts. The 32 fraction extracts displayed varying ACE inhibition with a select few limiting ACE activity to 20%. Targeted mass spectrometry revealed that the high-performing fractions contained varying amounts of CBD, CBG, CBN, CBC, CBDA, and CBGA as well as terpenes valencene, farnesene, and β-caryophyllene. When testing the identified cannabinoids and terpenes at a fixed concentration of 10 µM, CBC lowered ACE activity to less than 20% while terpenes displayed no ACE inhibitory activity. CBC generated an IC_{50} of 2.361 µM compared to lisinopril with an IC_{50} of 0.058 µM.

Conclusion: It is concluded that *in vitro,* cannabinoids from hemp can inhibit ACE activity at varying concentrations. This suggests that ACE could be a potential pharmaceutical target of cannabis cannabinoids. However, future studies in cells/animal models are required to assess the ACE inhibitory activity of cannabinoids from hemp.

COMPUTATIONAL STRATEGY FOR IDENTIFYING MOLECULAR TARGETS OF PLANT-DERIVED COMPOUNDS WITH APPLICATIONS IN CANNABIS-BASED DRUG DISCOVERY

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Introduction: Plants represent a vast reservoir of potentially therapeutic compounds, evidenced by their increasing utilization in modern medicine. However, a significant hurdle with their development as drugs lies in the elucidation of their molecular targets, especially for those of traditionally used plants like cannabis. While cannabis has a long history in traditional medicine for various ailments, isolating and utilizing its specific compounds for modern medicine presents an additional challenge of target identification. To address this issue, we propose a computational strategy that bridges the gap between compound characterization and target identification.

Methods: We present a novel computational pipeline, DRIFT, to identify potential drug candidates. Integrating molecular docking, cheminformatics methods, and artificial neural networks, DRIFT predicts protein-ligand binding affinity and prioritizes potential targets with unprecedented accuracy. Additionally, we have devised an algorithm to compare drugs and evaluate their similarities based on shared target profiles. Further, we have formulated a heuristic function that predicts targets from the composition of cannabis compounds.

Results: Our findings demonstrate the ability of DRIFT to predict potential targets for the cannabis compounds with high accuracy. The algorithm for comparing and evaluating their similarities based on shared target profiles revealed intricate relationships between compounds. The heuristic function for predicting targets from the composition of cannabis compounds provided clear identification of targets for known cannabis plant variants. Thus, we have curated this information into CANDI, a user-friendly database that not only aids in identifying potential off-targets but also unveils intricate relationships between drugs, leading to the discovery of novel therapeutic candidates with similar target profiles.

Conclusion: Our findings promise to reshape drug discovery by offering a comprehensive framework for assessing drug similarity and facilitating the development of more precise and tailored therapeutic interventions.

Acknowledgements: This project was supported by the Pennsylvania Department of Health using Tobacco CURE Funds (NMG), and R01AT012053-01 (NIH/NCCIH) to KEV and NVD. KEV (and the Penn State College of Medicine) receives research support from PA Options for Wellness (a state-approved medical marijuana clinical registrant).

CORNEAL PENETRATION OF CANNABIDIOL AND ITS POTENTIAL THERAPEUTIC EFFECTS

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Introduction: Cannabidiol (CBD), the non-psychoactive component of cannabis, has poor aqueous solubility so ocular administration is difficult. The objective of the current study was to measure CBD concentration in aqueous humor and cornea following topical applications and to determine the effects of topical applications of CBD on intraocular pressure (IOP) and corneal pain.

Methods: A cyclic oligosaccharide carrier methyl-β-cyclodextrin (mβCD) and a semifluorinated alkane, 1-(perfluorobutyl)pentane (PFBP), were tested for their ability to aid corneal penetration of CBD following topical applications to porcine eyes *ex vivo*. CBD concentrations were determined using an HPLC-UV method. C57/B6 mice were used to assess the effects of topical applications of CBD on IOP and corneal pain. IOP was measured by rebound tonometry with an ICARE TONOLAB tonometer and corneal pain was determined using the eye wipe assay following topical application of hypertonic saline.

Results: The mβCD formulation enabled CBD penetration through the cornea, reaching an aqueous humor concentration of 1061±389 ng/mL after topical application of 0.1 mg/mL of CBD. In contrast, CBD was not detected in aqueous humor following 1 mg/mL CBD topical instillation in PFBP but was measured in the cornea at a concentration of 5.78±1.88 ng/mg post topical application. *In vivo*, 0.1 mg/mL CBD in mβCD produced a reduction in IOP which lasted for 5 hours post topical application. On the other hand, hypertonic saline-induced eye wipe counts were reduced when CBD was applied at 1 mg/mL in PFBP, indicative of a corneal analgesic effect.

Conclusions: This study demonstrates that mβCD enabled delivery of CBD to aqueous humor, whereas PFBP localized delivery of CBD to the cornea. Therefore, appropriate formulations of CBD should be used for different potential ocular therapeutic purposes, such as reducing IOP or relieving corneal pain.

Acknowledgement: This work is supported in part by NIH grant EY030186.

SYNTHESIS AND *IN VITRO* EVALUATION OF NOVEL OXIZID SYNTHETIC CANNABINOID RECEPTOR AGONISTS

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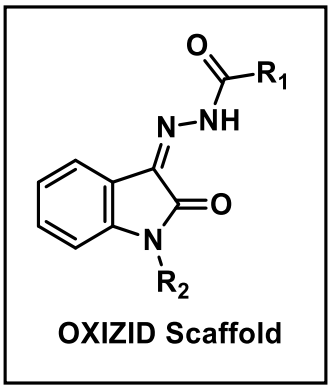
Introduction: Synthetic cannabinoid receptor agonists (SCRAs) were first detected in the recreation drug market in 2008, marketed as "legal" alternatives to cannabis to skirt legislative controls. Following a 2021 ban of 7 generic scaffolds in China, clandestine labs have regularly synthesized and distributed novel compounds not covered by these control measures, including the OXIZID class, named for their common 2-oxoindolin-3-ylidene core and hydrazide linker. First developed in 2008 as potential therapeutic tools, OXIZID compounds were detected in the recreational market in Spain in 2016 but did not attain wide circulation until 2021 where 4 analogues were detected for the first time in the US, China, and Europe. In late 2023, the number of detections of OXIZIDs began to eclipse more established indole and indazole-based SCRAs. Previous work by this group has sought to synthesise and characterise compounds before they appear on the recreational market, equipping health and forensic communities with valuable resources to identify these compounds. This project aims to continue this effort for the emerging generation of SCRAs.



OXIZID Scaffold

Methods: Through the application of common medicinal chemistry techniques and previously observed trends in the SCRA market, a systematic library of 48 compounds was synthesized. *In vitro* functional activity of these compounds was evaluated using a fluorescence-based membrane potential assay in AtT20 cells expressing either CB_1 or CB_2. Induced-fit docking (IFD) was performed at both CB_1 and CB_2 using Maestro software to model drug-receptor interactions and help rationalise observed structure-activity relationships (SARs).

Results: Preliminary data from a representative set of compounds showed activity at CB_1 and CB_2, albeit to varying degrees. Eleven compounds were selected for evaluation of potency and efficacy. In general, these compounds were less potent than CP55,940 (CP; pEC_{50} 7.23±0.09 at CB_1, 7.06±0.12 at CB_2), with potencies ranging from 5.57±0.23 – 6.97±0.23 and 6.14±0.08 – 7.62±0.25 at CB_1 and CB_2, respectively. Efficacy ranged from 66±12% – 123±7% at CB_1, 83±3% – 107±7% at CB_2 compared with CP (108±4% at CB_1, 113±5% at CB_2). Overall, the compounds were determined to be mildly CB_2 selective (mean CB_2 EC_{50}/CB_1 EC_{50} = 0.46±0.13). Enhanced potency and efficacy were associated with compounds bearing *para*-methoxyphenyl, *para*-fluorophenyl and *tert*-butyl carboxylate R_1 substituents. Modelling via IFD provided convincing rationalisations for these observed trends.

Conclusions: The low CB_1 potency of most of these compounds relative to previously detected OXIZID compounds suggest that they are unlikely to gain traction in a recreational market geared toward maximising potency. However, the tert-butyl carboxylate derivatives do have comparable potency to emerging SCRAs. Combined with their relative ease of synthesis, the low cost of their precursors and the current lack of legislative controls around this class of compounds, these compounds should be made available to the medical and toxicological researchers for ongoing new psychoactive substance monitoring. Additionally, these findings contribute to furthering our understanding of SARs at CB_1 and CB_2.

Acknowledgements: Supported by the Lambert Initiative for Cannabinoid Therapeutics.

(*S*)-MRI-1867, A PERIPHERALLY RESTRICTED HYBRID CB_1R AND iNOS ANTAGONIST, MODULATES β-ADRENERGIC STIMULATION IN RAT ATRIA AND VASOREACTIVITY OF RAT AND HUMAN PULMONARY ARTERIES – A PRELIMINARY *IN VITRO* STUDY

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Introduction: (*S*)-MRI-1867 (zevaquenabant), a peripheral hybrid dual cannabinoid CB_1 receptors antagonist and inducible nitric oxide synthase inhibitor, showed a significant promise in preclinical studies on pulmonary fibrosis. The aim of our study was to determine the influence of (*S*)-MRI-1867 on cardiostimulatory effects of isoprenaline (ISO) in rat atria and to investigate its vasoactive properties in pulmonary arteries (PAs) isolated from rats, also with pulmonary hypertension, and/or humans.

Methods: Atria and PAs were isolated from male Wistar rats. PAs were additionally taken from monocrotaline (MCT; 60 mg/kg, *s.c.*)-induced pulmonary hypertensive rats. Human PAs were obtained from patients undergoing lobectomy or pneumonectomy during the resection of lung carcinoma. Tissues were placed in organ baths. Then, we determined the influence of (*S*)-MRI-1867 (10 μM) on concentration-response curves for (1) chronotropic and inotropic effects of ISO in right and left rat atria, respectively; (2) thromboxane A_2 analog U46619 in rat and human PAs; (3) acetylcholine (Ach) in rat PAs. Data were analyzed by one-way ANOVA, followed by Bonferroni post-hoc tests ($p<0.05$ [*] was considered significant). pEC_{50} of (*S*)-MRI-1867 vs respective controls are given in parentheses.

Results: (*S*)-MRI-1867 diminished cardiostimulatory effect of ISO on atrial rate (pEC_{50} 7.9±0.1* vs 8.5±0.2, n=5), but enhanced its force of contraction (pEC_{50} 8.1±0.1* vs 7.8±0.1, n=5-6). MCT enhanced vasoconstrictor response to U46619 in rat PAs (pEC_{50} 6.6±0.1* vs 6.1±0.1; n=4-6) but failed to modify the vasodilatory effect of Ach. In PAs from MCT-treated rats (*S*)-MRI-1867 attenuated the effect of U46619 (pEC_{50} 6.1±0.1* vs 6.6±0.1; n=3-4) and enhanced the vasodilatory influence of Ach (pEC_{50} 7.0±0.1* vs 6.3±0.1, n=3-4). In human PAs (*S*)-MRI-1867 diminished vasoconstriction to U46619 (pEC_{50} 8.9±0.1* vs 9.3±0.1; n=3).

Conclusions: Our results showed that (*S*)-MRI-1867 differentially modified the positive chronotropic and inotropic effects of ISO in rat atria and favored vasodilatation in rat and human PAs. Nevertheless, further studies are required to fully establish mechanisms underlying its cardiovascular effects.

Acknowledgements: This project has been supported by the National Science Centre, Poland, under the agreement No. UMO-2021/41/B/NZ7/03757 and Medical University of Białystok, Poland (B.SUB.24.236).

SHORT-TERM THC EXPOSURE ABOLISHES CB1-DEPENDENT SYNAPTIC PLASTICITY IN THE VENTRAL TEGMENTAL AREA OF YOUNG, BUT NOT ADULT MICE

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Introduction: The ventral tegmental area (VTA) plays a key role in drug-dependence as it mediates incentive salience and reward behavior through dopamine (DA) cells, which are regulated by inhibitory VTA GABA cells. We previously identified the excitatory synapses onto the VTA GABA cells of adolescent mice exhibit a cannabinoid 1 receptor (CB1R)-dependent form of long-term depression (LTD) synaptic plasticity that is dependent on 2-AG production (J Neuroscience, Friend et al., 2017), which we later identified in adults was qualitatively identical to adolescent LTD, though quantitatively different (Frontiers in Neuro., Ostlund et al, 2023). Drug-induced occlusion of synaptic plasticity correlates strongly in many studies to dependence and withdrawal creation, and as chronic (10 days) Δ9-tetrahydrocannabinol (THC) occluded LTD in both adult and adolescent mice, it suggested this circuit could be involved in the induction of marijuana dependence or cannabis use disorder. In addition, because adolescent mice are more susceptible than adult mice to the cognitive and addictive effects of THC, we sought to understand age-dependent differences in VTA GABA cell plasticity altered by THC.

Methods: We examined the impact of short-term (3 day) versus chronic THC intraperitoneal injections, on LTD synaptic plasticity in adult versus adolescent male and female mice using *ex vivo* whole cell electrophysiology in VTA transverse brain slices with extracellular stimulating electrodes. Excitatory postsynaptic currents were recorded in VTA GABA cells identified by GFP florescence in a GAD67-GFP mouse model.

Results: As long-term outcomes of THC use are more dramatic in adolescent versus adult ages, we employed VTA plasticity as a model to examine this difference, building off our prior findings. Therefore, we examined whether the number of *in vivo* THC exposures required to eliminate LTD is effected by age. Interestingly, LTD was eliminated after 3 days of THC exposure in adolescent mice ($n = 10$, $p > 0.1$ compared to baseline), but LTD continues to be present in adult mice ($n = 6$, $p < .001$, compared to baseline), which were also significantly different from each other ($p < .001$). This is the first determination that age-dependent differences in drug-induced plasticity occur in the VTA reward circuit. Data also confirming RNA changes noted after chronic THC exposure in adolescents, begin after short-term THC exposure, in targets such as CB1, DAGLα, , FAAH, and GluA1, etc. using quantitative reverse-transcription PCR (qRT-PCR) of flow sorted VTA GABA cells.

Conclusions: These findings suggest that age-dependent differences in THC impact on VTA GABA cell plasticity may contribute to the increased vulnerability of adolescents to the negative effects of THC. Further research is warranted to explore age-dependent differences in CB1-dependent plasticity/function in other brain circuits as well. Collectively, our data demonstrate the first age-dependent GABA neuron plasticity in the brain, which could have implications for decreased THC dependence capacity in adults.

Acknowledgements: This work was funded by the NIH (grants R15DA038092 and R15DA049260).

PRODUCTION OF HEMP-DERIVED HIGH-PURITY CBD ISOLATE GROWN IN A SMART-FARM, ANTI-ACNE ACTIVITY AND SAFETY TESTS

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Introduction: Cannabidiol (CBD) is known to relieve pain, stress and inflammation. Acne is one of the most common skin diseases. The pathophysiology of acne involves not only genetic and environmental factors, but also other factors including hyperseborrhea, over-keratinization of follicular keratinocytes and overgrowth of *C. acnes*. Side effect of topical acne therapeutic include skin irritation, erythema and dry skin. Therefore, there are actively making an attempt for less side effects, and effective treatment in acne therapy. The objective of this study, in order to confirm the applicability of CBD as an acne OTC drug, a research on the mechanism of acne was conducted using a standard CBD and CBD derived from the grown hemp in smart farms. And then we confirmed the safety of the CBD in animal replacement tests.

Materials and Method: Hemp were grown from smart-farm, it were dried for 12 h. The dried hemp were extracted with 99 % EtOH for 2 days, concentrated by the filtration. It were proceed decarboxylation by microwaves, after fractionated with Hexane:DW. We obtain full-spectrum cannabinoid, using the distillation. It was further purified by crystallization to produce a purity > 99 %. We investigated the inhibitory effect of standard CBD and high-purity CBD on lipid synthesis in sebocytes was examined. And then, we performed that the effect of regulating the expression of key transcription factors related to sebum synthesis. Also we confirm the effect of CBD on inflammation caused by hyperkeratosis in HaCaT. Furthermore, we evaluate local toxicity of CBD isolate, using reconstructed human epidermis (EpiDerm™, SkinEthic™ RHE, LabCyte EPI-MODEL24 and KeraSkin™) and cornea-like epithelium models (MCTT HCE™) according to OECD TG 439 and 492.

Results: The high-purity CBD was more than 99% pure against a standard (Cayman chemical), and cannabinoids containing THC were not detected. It treated cells with CBD and found that Nil red O staining showed that inhibited by CBD treatment at all doses. These data suggest that CBD inhibited cell proliferation, and decreased sebaceous gland size. CBD increases p-AMPK and decrease p-Akt, suggesting that CBD inhibits adipogenesis by regulating the Akt/AMPK-SREBP-1 signaling pathway. The levels of CXCL8 and IL-1α cytokines in HaCaT were decreased after CBD treatment. For local toxicity test, all test materials were classified as non-irritant in terms of skin and eye irritancy.

Conclusion: Common side effect of acne therapeutic include skin irritation, erythema and dry skin. Therefore, there are actively making an attempt for no apparent side effects, and effective treatment in acne therapy. CBD are therefore some potential for its use in active pharmaceutical ingredient, functional food and cosmetic materials. Our studies demonstrate that CBD inhibits adipogenesis by regulating the Akt/AMPK-SREBP-1 signaling pathway and inflammation caused by hyperkeratosis, providing potential for use as a therapeutic agent for acne. Also, For local toxicity test, CBD was classified as non-irritant in terms of skin and eye irritancy. CBD is an active ingredient possibility of using for acne treatment, and diversity of commercial products can be made from CBD.

CANNABINOID RECEPTOR-MEDIATED ANTI-TUMOR EFFECTS IN GASTROINTESTINAL CANCER ARE SUSTAINED BY INFLAMMATION RESOLUTION RESPONSE: A USEFUL BIOACTIVE LIPIDS CROSSTALK

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Introduction: Bioactive lipids, such as specialized pro-resolving mediators (SPMs) and endocannabinoids (eCBs), are key players in regulating inflammatory response and tissue homeostasis. Characterized by a chronic low-grade inflammatory state, gastrointestinal (GI) cancers exhibit a high incidence and poor patient survival statistics. SPMs are potent anti-angiogenic mediators in GI tract cancers[1] and CBR activation exhibits different anticancer activities in the GI tract (i.e. cell proliferation, migration, invasion, stemness)[2-5]. Since bioactive lipids seem to be good candidates to control GI cancer progression, in gastric (AGS) and colorectal (HCT116) human cancer cell lines, we looked to possible interconnections of the two systems by defining the dependence of one system on the another to achieve specific anticancer effects.

Methods: Cells were treated with SPMs (RvD1 and LxB4) or CBs (ACEA and JWH133). We tested effects on proliferation (cell count, cell cycle analysis), survival (SRB, Trypan blu, Anx V/PI staining, Casp3/7 activation), epithelial to mesenchymal transition (EMT) (marker expression, Boyden chamber, and scratch assay), angiogenesis (VEGF expression and tubule formation on matrix), stemness (marker expression and spheroids formation), cell metabolomics (NMR). LC-MS or EIA assays were used to detect the production of eCBs and SPMs, respectively. Cells silenced for ALOX15 crucial in SPM synthesis were used to define the dependence of one system on the other. **Stats**: T-test with Welch's correction or One-way ANOVA followed by Dunnet's post hoc test.

Results: In AGS and HCT116 cells, both ACEA (100nM) and JHW-133 (1μM) induced RvD1 and LXB4 release, while no effects on eCB production were detected upon SPM treatment suggesting that a sustained induction of resolution may support some of the CB-mediated anti-tumor effects. CBR activation via ACEA or JWH-133 significantly reduced AGS ($p<0.01$) and HCT116 ($p<0.05$) cell proliferation in an SPM-independent manner as assessed by the results observed in the parental shALOX15 cells, which are unable to produce SPM. Treatments with the two CBs significantly ($p<0.01$, $p<0.001$) inhibit EMT and stemness programs in both cancer cell lines as assessed by the reduced RNA and protein expression of the master regulators Slug or Snail (EMT) and SOX2 or OCT4 (stemness). Moreover, ACEA and JWH-133 treatments also inhibit migration and invasion processes as well as the number and diameter of spheroids formed in both GI cancer types. However, the same treatments in AGS or HCT116 shALOX15 cells indicated that these latter actions were not sustained by a CB-mediated SPM production. Both ACEA and JWH-133 reduced VEGF expression and VEGF-A release in AGS and HCT116 cells in a CBR-mediated manner dependent on SRC and ERK pathway activation, as demonstrated by the abolishment of the effect by specific MAPK/ERK inhibitors and CBR antagonists. Interestingly, the antiangiogenic effect mediated by CBR activation was strictly dependent on SPM synthesis. Both treatments in AGS and HCT116_shALOX15 cells failed in reducing VEGF expression and VEGF-A release. Intriguingly, in support of the idea that CBs/SPMs can share molecular pathways in each of the cancer types (gastric and colorectal), NMR-based pathway analysis showed that both classes of lipids significantly modulated identical metabolic pathways.

Conclusion: These findings indicate a beneficial overlap between SPMs and CBs in selected actions of GI cancer suggesting that these molecules, only for specific molecular mechanisms, may interact and augment the synthesis and action of each other. The possible cross-talk between the two biolipid systems on cell metabolism is currently under investigation.

Acknowledgments: AL and **NP** are founded by NextGenerationEU-PNRR_M4/C2/Investimento.1.1 (Grant: PRIN2022_Project 2022CSYK9L_LS). PK is a recipient of an International PhD program fellowship from UNINA.

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CELL TYPE-SPECIFIC MOLECULAR MECHANISMS ALTERED BY REPEATED THC EXPOSURE IN THE HIPPOCAMPUS

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Introduction: The hippocampus plays a pivotal role in memory formation, and cannabinoids, including delta9-tetrahydrocannabinol (THC), the primary psychoactive compound in marijuana, are known to modulate hippocampus-dependent memories and various forms of synaptic and structural plasticity. In this study, we aimed to elucidate the cell type-specific molecular mechanisms that may underlie some of these effects following repeated THC exposure.

Methods: We utilized Wfs1-Cre:RiboTag mice expressing hemagglutinin (HA)-tagged ribosomes specifically in CA1 pyramidal neurons. These mice were subjected to daily treatment with THC (10 mg/kg, i.p.) or its vehicle for six consecutive days. We then performed cell type-specific isolation of HA-ribosome-associated mRNAs, followed by RNAseq analysis, which unveiled 215 downregulated and 167 upregulated genes induced by THC exposure.

Results: Subsequent Gene Ontology and KEGG pathway analyses of these differentially expressed genes revealed key biological terms associated with memory, structural plasticity (e.g., dendritic spine organization, dendritic development), and synaptic plasticity (e.g., regulation of neurotransmitter receptor activity, regulation of postsynaptic membrane potential, chemical synaptic transmission, regulation of synaptic plasticity). Intriguingly, among the latter terms, numerous categories related to glutamatergic transmission emerged (e.g., glutamate receptor signaling pathway, glutamatergic synapse, glutamatergic synaptic transmission, ionotropic glutamate receptor activity), which were correlated with decreased expression of glutamate receptor subunits in THC-treated mice.

Conversely, our bioinformatic analyses uncovered terms associated with the negative regulation of transcription, consistent with the observed reduced expression levels of immediate early genes in mice chronically exposed to THC.

Conclusions: In summary, our results reveal novel molecular pathways dysregulated by repeated cannabis exposure, offering potential targets for improving the medicinal use of cannabis.

MINOR PHYTOCANNABINOID MODULATION OF CB1 SIGNALLING IN TWO NEURONAL MODELS, PART II

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Introduction: Δ^9-THC and CBD are the two phytocannabinoids that are most abundant in cannabis, but there are more than 100 phytocannabinoids that are present in the plant at lower concentrations. The pharmacology of Δ^9-THC, the main psychoactive constituent of cannabis, has been extensively studied and it is well understood that it acts via the endogenous cannabinoid signaling system that includes receptors such as CB1 and CB2, lipid messengers (endocannabinoids), and the enzymes that produce and degrade these messengers. The changing legal status of cannabis has spurred interest in the 'minor' phytocannabinoids, but they remain poorly characterized. We have previously characterized the neuronal signaling of various phytocannabinoids but more work remains to be done.

Methods: We tested the interaction of three 'minor' phytocannabinoids using whole cell patch clamp recording in a well characterize neuronal model with endogenous neuronal CB1- and 2-AG-based cannabinoid signaling. Autaptic hippocampal neurons express CB1 receptors, the cellular machinery to produce and metabolize the endocannabinoid 2-AG, as well as three forms of retrograde CB1-mediated neuronal plasticity. We additionally tested these compounds in cultured dorsal root ganglion neurons. Cannabinol (CBN), cannabigerol (CBG), and cannagerolic acid (CBGA), were chosen based on their range of proposed effects.

Results: We found that at 1μM, CBN substantially reduces cannabinoid signaling likely via inhibition of CB1 receptor activation. CBG also inhibits neurotransmission but appears to do so in a CB1-independent manner. CBGA does not affect neurotransmission in this neuronal model. None of these compounds produced reliable effects on calcium responses in dorsal root ganglion neurons.

Conclusion: In testing three additional 'minor' phytocannabinoids in a complex endogenous neuronal CB1-based signaling system we continue to see a diversity of effects. CBN appears to act as a CB1 antagonist, CBG inhibits neurotransmission via an as-yet undetermined presynaptic target, and CBGA is without effect.

THE STABILITY OF MINOR CANNABINOIDS

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Introduction: Increasing interest in the study of minor cannabinoids as well as the proliferation of consumer products that contain these cannabinoids necessitates a better understanding of their stability. While stable compounds allow for longer shelf life and assurance that effects associated with their use are reliably obtained over time, unstable compounds degrade into other compounds that may be associated with unknown or unintended effects. Identifying cannabinoids that are unstable in common formulations will guide efforts to develop improved formulation methods or highlight the need for modifying the structure of those cannabinoids to improve their stability.

Methods: Cannabidiol (CBD), Cannabigerol (CBG), Cannabinol (CBN), Cannabichromene (CBC), Cannabidivarin (CBDV), Cannabitriol (CBT), Δ8/9 Tetrahydrocannabivarin (THCV), as well as the acid forms of CBD (CBD-A) and CBG (CBG-A) were obtained as isolates and formulated into both a medium chain triglyceride (MCT) oil tincture and pectin-based gummy. Tincture formulation involved dissolving cannabinoids at room temperature for up to 2 days before aliquoting samples. Gummies were formulated in a pectin matrix (pH 3.5) with citric acid and were subjected to some heat during the manufacturing process. Both tinctures and gummies were placed in real time (25°C, 60%RH) and accelerated (40°C, 75% RH) stability chambers for monitoring of cannabinoid concentration over time. Cannabinoid potency testing was completed by a third-party laboratory using a validated HPLC-DAD method.

Results: The cannabinoids CBD, CBG, CBN, CBC, CBDV, CBT, and THCV were all found to be stable in oil and gummy formats. Indeed, very little degradation was observed in both real-time and accelerated conditions, with CBC and CBT being slightly less stable than the other non-acid cannabinoids while showing less than 5% degradation after 6 months in real-time. Both CBG-A and CBD-A were significantly less stable than their decarboxylated forms, though CBG-A was far more stable than CBD-A. In MCT oil, 1.4% and 12.3% of CBG-A was decarboxylated to CBG in real-time and accelerated conditions after 6 months, respectively. In the same matrix and under similar conditions, 9.8% and 85.5% of CBD-A was decarboxylated to CBD in real-time and accelerated conditions, respectively. While it was expected that increased decarboxylation of the acids would be observed in accelerated conditions, the observed differences between the two acids were noteworthy.

Conclusions: Data from the present study provide valuable information regarding the viability of many minor cannabinoids for pre-clinical and clinical studies as well as for consumer and pharmaceutical product development and commercialization. CBD-A was particularly unstable in both oil and gummy matrices, highlighting the need for further research into methods of stabilizing this acid prior to its use. Future work should determine the stability of other minor cannabinoids, with particular emphasis on less stable acidic forms, including whether the presence of other phytochemicals (e.g., within a botanical extract) can affect cannabinoid stability.

KINETIC MODELING OF HEMP CANNABINOID DEGRADATION IN STORAGE CONDITIONS: SHELF-LIFE MODELING USING NON-DESTRUCTIVE FT-NIR

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Introduction: Currently, there is no approved shelf-life testing of hemp or cannabis inflorescence. Food products are required to have an expiration date that will ensure consumer liking and safety. In food products, equations useful for determining the "end of life" of a product include the Arrhenius and Eyring equations and the Weibull model. The Arrhenius and Eyring equations are used to determine the kinetic parameters of a chemical reaction, which can provide mechanistic insight into the degradation of cannabinoids in hemp matrix.

Methods: Hemp samples were aliquoted into tubes and incubated at 4 different temperatures (25°C, 35°C, 45°C, 55°C) for one-week increments for 5 weeks. A portion of the hemp mixture was placed into a -40°C freezer for the initial cannabinoid concentrations. After sample incubation, all samples were scanned using a handheld Fourier Transform- Near Infrared (FT-NIR) sensor. Samples were then extracted for cannabinoid quantification by liquid chromatography-mass spectrometry (LC-MS/MS) by a validated method. After the data was collected, partial least squares regression (PLSR) was performed on the spectral and chromatographic datasets using Pirouette® to obtain regression vectors for quantification of unknown samples. For the Weibull model, cannabinoid concentrations were used to determine the 20% loss, which is related to the time to product failure. Weibull models were generated using cannabinoid concentrations and time using the OriginPro software.

Results: PLSR models show the quantification of major and minor cannabinoids from the inflorescence with low detection limits (0.0021% w/w CBNA) and high quantification limits (20.7% CBD acid). Regression statistics ($R_{cv}>0.95$) show that the PLSR models show a tendency towards reproducibility and sensitivity similar to LC-MS/MS reference values. The Arrhenius and Eyring equations produced kinetic parameters, showing that a first-order reaction or pseudo-first-order reaction governs cannabinoid degradation. The Weibull distribution shows that increasing the temperature of storage will decrease the product failure time, meaning a shorter shelf life.

Conclusions: Cannabis shelf life and cannabinoid degradation are both complex mechanisms that can be affected by many factors. In this study, the effect of temperature and time were analyzed to determine a definitive shelf life for hemp inflorescence based on the degradation of different cannabinoid compounds. FT-NIR served as a novel technique to quantify major and minor cannabinoids of hemp, with specificity between acidic and neutral forms. FT-NIR is also a field deployable technology, which can aid in the quality control of hemp and cannabis by determining compliance (<0.3% Δ9-THC) and if degradation of cannabinoids has started. This technology can also be implemented by hemp growers to have a cannabinoid analysis prior to sending the sample to analytical laboratories.

FULL SPECTRUM CBGA/CBDA, CBDA INDUCE DIFFERENTIAL GENE EXPRESSION IN DIFFERENTIATED SHSY-5Y CELLS *IN-VITRO* COMPARED TO CBD ISOLATE

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Introduction: There is a plethora of information regarding the utilization of CBD *in vitro* to alter cellular gene expression, mostly utilizing supraphysiological concentrations. To date, the investigation into other minor cannabinoids found in hemp are minimal; particularly the acidic forms due to prior literature regarding instability making them unsuitable to utilize therapeutically. Recent publications have shown that oral application of CBGA and CBDA lead to far greater absorption and retention of the acids when compared to their neutral counterparts renewing interest in their therapeutic potential. (Amstutz et al., J Vet Pharm Ther., 22: 245-254;Wakshlag et al., Frontiers Vet Sci., 7:505) The aim of this study was to investigate the differential gene expression of neuronally differentiated SHSY-5Y cells using whole hemp extracts of CBDA and CBGA/CBDA compared to CBD isolate after 48 hours of treatment in cell culture at Cmax concentrations observed in animal models (Schwark et al, Am J Vet Res., 84:ajvr.23.02.0031).

Methods: SHSY-5Y cells in DMEM medium with 15% FBS were treated with retinoic acid for 72 hours to induce differentiation. Cells were then treated with 3 uM total cannabinoid from CBDA, CBGA/CBDA predominant whole hemp extracts (CBDA [85%], CBGA/CBDA [44, 27%] compared to 1 uM CBD isolate or DMSO vehicle for 48 hours. Cells were then harvested utilizing Qiagen RNeasy Mini extraction kit in triplicate and tested for quality of RNA using the 2100 Agilent Bioanalyzer before undergoing RNA seq utilizing the Novoseq 6000 to assess differentially expressed genes (DEG). The following bioinformatics pipeline was utilized: Hisat2 (mapping), featureCounts (quantification), DEseq2 (differential analysis), clusterProfiler (KEGG enrichment analysis). KEGG analysis was performed utilizing two different DEG cut-off thresholds; stringent and less stringent (log2fold change > 0.58, padj < 0.05 and log2fold change > 0.2, padj < 0.05; respectively) for pathway identification and a q value $< .05$ cut-off was used to identify significant pathways.

Results: When comparing DEG results there were 124 differentially expressed genes with CBDA extract compared to CBD isolate; and 118 with CBGA/CBDA whole plant extract when compared to CBD isolate. KEGG analysis revealed 104 pathways of enrichment for CBGA/CBDA treatment when adjusted for false discovery (FDR $<.05$) leading to 15 pathways of interest; while CBDA extract and CBD isolate showed only one pathway of interest compared to DMSO which was retinol metabolism.

Conclusions: Although the acidic forms of the cannabinoids have been largely ignored there appear to be significantly affected pathways when utilizing stringent KEGG analytics. CBD isolate and CBDA extract are far less active than the whole plant CBGA predominant extract, suggesting that CBGA may have more bioactivity than other acidic cannabinoids. Interestingly, the CBGA/CBDA predominant strain appear to affect pathways involved in infectious disease and immunological regulation more so than neuronal regulation. CBDA with less stringent analytics reveals potential effects on metabolic pathways which is different from the other two extracts. These data suggest that further research regarding CBGA and CBDA isolate is needed to fully understand the potential utility of CBGA and/or CBDA in comparison to these results which may be part of an "entourage" effect due to the whole plant extract nature of this dataset.

Acknowledgment: This study was funded by Cultivate Biologics.

PRECLINICAL ASSESSMENTS OF CANNABINOID CB_2 RECEPTOR AGONISTS AND CANNABIDIOL IN CARDIAC ISCHEMIA-REPERFUSION INJURY NEEDS URGENT IMPROVEMENT IN ORDER TO BE CONSIDERED FOR THERAPEUTIC APPLICATION

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Pre-clinical studies suggest that agonists of CB_2 cannabinoid receptors (CB_2R) and cannabidiol (CBD) might be potential cardioprotective strategies against ischemia-reperfusion injury. Indeed, systematic review analysis of all publications from PubMed, Medline and EMBASE demonstrates that CB_2R activation and CBD exert cardioprotective effects (decrease in infarct size, improvement of cardiac function, anti-inflammatory or anti-oxidative actions) in animals exposed to temporary or permanent occlusion of the left coronary artery (LAD) and in experiments on isolated cardiomyocytes undergoing hypoxia and on isolated hearts subjected to LAD occlusion. The potential cardioprotective role of the (endo)cannabinoids under hypoxia, ischemia and reperfusion has also been demonstrated in patients with coronary heart disease. However, cannabinoids are still not even considered as part of the most recent analysis of multitarget strategies proposed in order to reduce myocardial ischemia-reperfusion injury [1].

The aim of our study was to re-evaluate the cardioprotective effects of cannabinoids against ischemia-reperfusion injury according to the IMproving Preclinical Assessment of Cardioprotective Therapies (IMPACT) criteria published by the European Union (EU) CARDIOPROTECTION COST ACTION [2]. Unfortunately, in our evaluation of the papers regarding the cardioprotective effects of CB_2R activation or CBD in the context of various protocols and models none of the publications so far met all IMPACT criteria. Thus, additional experiments are needed to confirm the cardioprotective activities of cannabinoids on small animals with comorbidities (e.g. age, diabetes mellitus, hypertension) and co-medications and on large animals. Additionally, the proper moment of administration of drugs (so far generally administered before ischemia, which is the most difficult to predict for the patient) should be identified and the new generation of CB_2Rs agonists (characterized e.g. by better solubility) should be examined.

In conclusion, the assessment of the potential cardioprotective efficacy of CB_2R agonists and CBD in preclinical studies needs urgent re-evaluation in further experiments performed in accordance with IMPACT criteria.

Acknowledgements: Supported by grants from the Medical University of Bialystok, Poland (B.SUB.24.340).

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ENDOCANNABINOID BASIS OF PERSONALITY – INSIGHTS FROM ANIMAL MODEL OF SOCIAL BEHAVIOR

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Introduction: The endocannabinoid system is known to be involved in learning, memory, emotional processing and regulation of personality patterns. Here we assessed the endocannabinoid profile in the brains of mice with strong characteristics of social dominance and submissiveness, as a model for different personality types.

Methods: A lipidomics approach was employed to assess the endocannabinoidome in the brains of Dominant (Dom) and Submissive (Sub) mice. The endocannabinoid showing the greatest difference in concentration in the brain between the groups, docosatetraenoyl ethanolamine (DEA), was synthesized, and its effects on the physiological and behavioral responses of Dom and Sub mice were evaluated. mRNA expression of the endocannabinoid receptors and enzymes involved in polyunsaturated fatty acids biosynthesis was assessed using qRT-PCR.

Results: Targeted LC/MS analysis revealed that long-chain polyunsaturated ethanolamides including arachidonoyl ethanolamide (AEA), DEA, docosatrienoyl ethanolamide (DTEA), eicosatrienoyl ethanolamide (ETEA), eicosapentaenoyl ethanolamide (EPEA) and docosahexaenoyl ethanolamide (DHEA) were higher in the Sub compared with the Dom mice brain extracts, unlike the endocannabinoids which contain shorter chain fatty acids or saturated/monounsaturated fatty acids or other types of endocannabinoid-like molecules (i.e. 2-acyl glycerols, acyl-amino acids). Untargeted LC/MS analysis showed that the parent fatty acids, docosatetraenoic and eicosapentaenoic, were higher in Sub vs. Dom. Gene expression analysis revealed increased mRNA expression of genes encoding the desaturase FADS2 and the elongase ELOVL5 in Sub mice compared with Dom mice. Acute DEA administration at the dose of 15 mg/kg produced antinociceptive and locomotion-inducing effects in Sub mice, but not in Dom mice. Subchronic treatment with DEA at the dose of 5 mg/kg augmented dominant behavior in wild-type ICR and Dom mice but not in Sub mice.

Conclusion: This study suggests that the endocannabinoid system may play a role in the regulation of dominance and submissiveness, functional elements of social behavior and personality. While currently we have only scratched the surface, understanding the role of the endocannabinoid system in personality may help in revealing the mechanisms underlying the etiopathology of psychiatric disorders.

EFFICACY OF ART26.12, A NOVEL FATTY ACID BINDING PROTEIN 5 INHIBITOR, IN AN ORTHOTOPIC HCT-116-LUC HUMAN COLON CANCER MODEL

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Introduction: Fatty acid binding protein 5 (FABP5) is overexpressed in a number of cancers including colorectal cancer, implicated in growth and metastasis. Pharmacological inhibitors of FABP5 have shown efficacy in preclinical models of prostate and lung cancer (Warren *et al*., 2023). ART26.12, a potent and selective FABP5 inhibitor, has previously shown efficacy in oxaliplatin-induced peripheral neuropathy (Warren *et al.,* 2024). The aim of this study was to test the potential direct anti-tumour effect of ART26.12 in an orthotopic HCT-116-Luc colon cancer model.

Methods: BALB/c nude mice were inoculated with $1x10^6$ HCT-116-luc cells, injected under the serosa membrane of the colon. On day 14 mice were randomly assigned to groups based on the bioluminescent signal of the tumour, using a computer generated randomisation procedure. Treatment with ART26.12 (100 mg/kg p.o. BID), oxaliplatin (OXA, 2 mg/kg i.v. twice weekly) and vehicle control was begun on day 15 (denoted day 1 in results), and continued for 34 days. Tumour growth was tracked weekly using bioluminescent imaging. Pain behaviour was assessed weekly using Von Frey (VF) measurements. Bodyweight was measured twice weekly.

Results: *In vitro*, ART26.12 killed HCT116 cells with an IC_{50} of 39.4 µM. *In vivo*, ART26.12 (100 mg/kg p.o. BID) attenuated tumour growth by day 20 ($p<0.001$) compared to vehicle, with 3 animals tumour free at the end of study (Figure 1A). OXA (2 mg/kg i.v. , biw) did not have a significant effect on tumour burden (2 animals in particular did not respond to OXA). ART26.12 ameliorated weight loss compared to vehicle control from day 27 ($p<0.05$, Figure 1B). Tumour-bearing animals had reduced VF values compared to naïve mice, indicating induction of pain. Pain behaviour was improved in animals treated with ART26.12 compared with vehicle on days 6 ($p<0.05$), 20 ($p<0.001$) and 27 ($p<0.0001$)(Figure 1C).

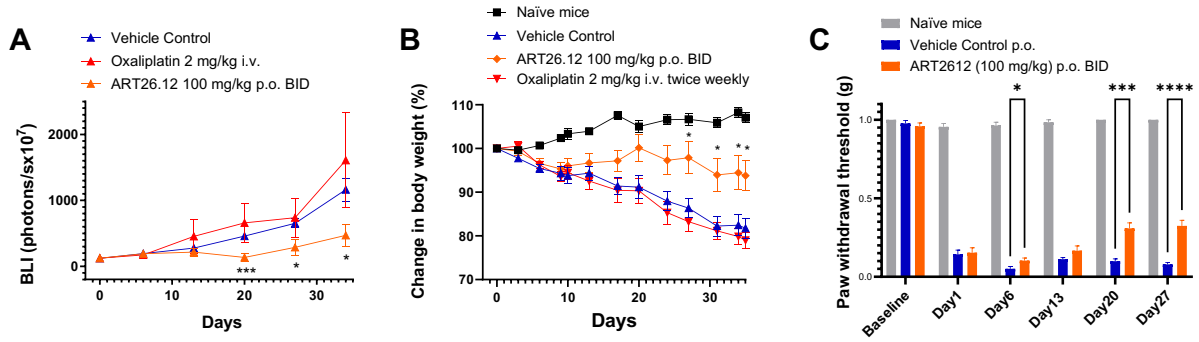

*Figure 1. The effects of the FABP5 inhibitor ART26.12 in HCT-116-luc orthotopic xenograft. A) Tumour growth measured by bioluminescence imaging (BLI). B) Effect of tumour burden and treatments on body weight. C) Effect of tumour burden and treatments on paw withdrawal threshold, measured by Von Frey filaments. *<0.05 ***<0.001, ****<0.0001, 2 way ANOVA.*

Conclusions: In an *in vivo* orthotopic model of colorectal cancer, ART26.12 attenuated tumour growth, and ameliorated weight loss and cancer induced pain. This data supports the development of ART26.12 as a novel analgesic in oncology settings, and establishes a direct anti-tumoral effect of FABP5 inhibition in colorectal cancer.

References: Warren *et al.* (2023). The emerging role of fatty acid binding protein 5 (FABP5) in cancers. *Drug discovery today*, *28*(7), 103628, doi: 10.1016/j.drudis.2023.103628; Warren et al. (2024). Discovery and preclinical evaluation of a novel inhibitor of FABP5, ART26.12, effective in Oxaliplatin-induced Peripheral Neuropathy. *Journal of Pain*, doi: 10.1016/j.jpain.2024.01.335.

PHARMACOKINETICS AND ORAL BIOAVAILABILITY OF CANNABIGEROL IN HORSES AFTER INTRAVENOUS AND ORAL ADMINISTRATION WITH OIL AND MICELLAR FORMULATIONS

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Introduction: Intravenous (IV) pharmacokinetics (PK) and oral bioavailability of cannabigerol (CBG) have not been investigated in horses and may represent a starting point for clinical studies, especially due to the potential use of oral route for veterinarians and owners. This issue is of great importance since there is a lack of knowledge of the PK properties of CBG and their applications. As a consequence, the current research aims: a) to characterize PK after IV and oral administration of CBG in healthy horses; b) to determine oral bioavailability; c) to evaluate the absorption with different oral formulations and d) to simulate different treatments and plasma levels as points of reference for further clinical assays.

Methods: A single IV experiment and two-way randomized PO assays Latin-square design were used. Eight healthy horses received IV CBG at dose 1 mg/Kg, in addition to PO CBG in sesame and micellar formulations, both at 10 mg/Kg. CBG concentrations were measured using LC-QTOF MS/MS and subsequently analyzed by non-linear mixed effect models. The obtained parameters were used to simulate a 14-day treatment and calculate the maximum and minimum concentrations at steady state.

Results: CBG kinetics was described by two compartment model with Weibull absorption, with different absorption constant rate (ka) of 0.48 and 0.28 1/h for micellar and sesame formulations. In this way, maximum plasma concentration (C_{max}) was similar between formulations but the time to reach C_{max} (T_{max}) was shorter for the micellar formulation. The oral bioavailability ranged from 25 to 29% and was not influenced by the formulation used. Regarding plasma disposition, a high volume of distribution of 65 L/Kg and a high plasma clearance of 1.52 L/h/Kg were observed, resulting in longer half-lives of 27-36 h for IV and micellar formulation and 52 h for oil formulation. Simulated PK profiles showed differences between steady state concentrations.

Conclusions: This as a first report to describe IV and oral PK of CBG in horses. Oral bioavailability in horses is low but it could be suitable for long-term treatments. The micellar formulation showed faster absorption than the sesame oil formulation, suggesting that higher concentrations could be achieved in multiple dose treatments. These finding indicated that CBG could be of interest in equine clinical pharmacology, although further studies are necessary in this context to evaluate its use in horses.

CANNABINOID CONTENT IN VAPING PRODUCTS CONFISCATED FROM STUDENTS OF WESTERN NEW YORK SECONDARY SCHOOLS

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Introduction: Electronic vaping products (EVPs) popularity has risen sharply among US middle and high school students. While previous studies have examined the product and chemical characteristics of EVP devices designed for nicotine use, few studies have examined these characteristics among EVPs used for cannabis. This study evaluated product characteristics and cannabinoid content in EVPs confiscated from secondary school students in Western New York (WNY).

Methods: Confiscated EVPs (n=555) were collected from 10 secondary schools in WNY from 2020-2023. Active ingredients were extracted from EVPs and diluted with methanol. Gas chromatography/mass spectrometry (GC/MS) was used to simultaneously detect nicotine and nine cannabinoids (Δ9-THC, Δ8-THC, THCV, total-CBD, CBDV, CBC, CBL, CBN, and total-CBG).

Results: Most EVPs (n=492, 89%) exclusively contained nicotine, while 10% (n=57) contained one or more cannabinoids only. Among the cannabinoid-containing EVPs, the most abundant (8.8%, n=5) was Torch, a non-refillable rechargeable-disposable device. Six products (1%) had detectable nicotine levels and at least one cannabinoid. Concentrations of Δ9-THC, Δ8-THC, and total-CBD averaged 338.0±202.1, 199.8±258.5 and 10.9±6.5 mg/g, respectively. Among cannabinoid-containing EVPs, Δ9-THC was the most abundantly identified cannabinoid (n=41, 65%) and constituted, on average, 75.8±11.4% of all cannabinoids present in a product. Δ8-THC was detected in 21% (n=13) of EVPs and constituted, on average, 90.6±7.4% of all cannabinoids. Three EVPs contained at least 50% CBN per product. We also found high concentrations of unknown THC derivatives in 3 different EVPs.

Conclusion: Our data suggests that the predominant EVPs used by secondary school youth in WNY continue to be nicotine devices. However, the study also found other psychoactive ingredients, including Δ9- and Δ8-THC, in EVPs used by students. Importantly, CBD-based EVPs appear less popular despite fewer accessibility restrictions. Several EVPs contained both nicotine and cannabinoids or other unknown chemicals, suggesting that youth may be modifying nicotine-containing EVPs with cannabinoids.

A 90-DAY REPEATED DOSE ORAL TOXICITY STUDY OF CANNABIDIOL IN SPRAGUE DAWLEY RATS ACCORDING TO OECD TG408

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Introduction: The use of cannabidiol (CBD) for human health and wellness has gained increasing interest. However, only sparse toxicological studies conducted in accordance with pertinent guidelines are currently available to the public. Therefore, it is crucial to continue generating data on the exposure and safety of CBD to enable its safe use.

Methods: The toxicity and toxicokinetics of a hemp-derived CBD isolate was assessed in a 90-day repeated oral toxicity study. Low (5 mg/kg), mid (15 mg/kg) and high (150 mg/kg) doses of CBD together with a vehicle control (olive oil) were administered in rats once daily for 13 weeks. Select satellite groups were allowed to recover for 28 days after the discontinuation of the treatment to assess the reversibility of the potential toxicity.

Results: Evidence of systemic plasma exposure to CBD and its metabolites 6-hydroxy (OH)-CBD, 7-OH-CBD, and 7-carboxy (COOH)-CBD was observed in all CBD-treated rats following single and 13-week repeated oral gavage administration with a half-life between 5 to 8 hours. Following the 90-day exposure period, higher food consumption, not correlated with body weight change, was observed in rats treated with the high dose of CBD. Higher levels of cholesterol, high-density lipoprotein and low-density lipoprotein were observed in female, but not male rats in the CBD high group, returning to vehicle control level at the end of the recovery period. Other changes in hematology, clotting potential and urinalysis were either very minor or considered sporadic due to the lack of dose dependency and were not of toxicological concerns. Hepatocyte hypertrophy was observed in the CBD high group (both sexes), correlating with higher liver weight in this group. This was not accompanied by other microscopic lesions and was resolved in both sexes at the end of the 28-day recovery period. Dose dependent adrenal cortical vacuolation (minimal to moderate) was observed in both sexes in CBD high dose groups accompanied by increased adrenal weight which trended towards normal at the end the recovery period. Other test item-related histopathological changes included thyroid follicular hypertrophy and pituitary gland pars distalis hypertrophy, which were fully reversible after discontinuation of treatment.

Conclusions: Taken together, CBD was well tolerated at the tested doses and the treatment related effects reflected adaptive changes to the xenobiotic treatment, with no significant toxicological relevance. Based on the observations of histopathological changes in adrenal gland and thyroid in all dose groups, the no observed effect level (NOEL) was lower than the current low dose (5 mg/kg/day). Furthermore, as the currently observed effects were non-adverse, the no observed adverse effect level (NOAEL) was considered to be the current high dose (150 mg/kg/day).

EVALUATING THE EFFECT OF CB2 AGONIST JWH-133 ON TUMOR GROWTH IN THE ID-8 ECTOPIC OVARIAN TUMOR MODEL IN WILD-TYPE MICE

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Introduction: Approximately 115 out of every 10,000 women will receive a diagnosis of ovarian cancer in their lifetime. Ovarian cancer represents the leading cause of gynecological cancer death and is the fifth most common cause of cancer death in women. The five-year survival rate, with treatment, of ovarian cancer depends on the stage at which it is diagnosed and varies from 93.1% (at an early stage) to 30.8% (at a late stage). Cannabinoid-based compounds are frequently used for the treatment of chemotherapy-induced nausea and vomiting and there is increasing interest in their potential effects on cancer growth. As a number of preclinical studies have revealed the utility of cannabinoids in reducing the size of breast cancer tumors, there has been a particular interest in investigating their potential utility for ovarian cancer. Previously, we have identified that the Cannabinoid Receptor 2 (CB_2) selective agonist JWH-133 causes an increase in ectopic ovarian tumor growth, using the SKOV-3 and OVCAR-5 models, in immunocompromised SCID-SHO mice. In this study, we assessed the effects of different concentrations of ID-8 cancer cells on tumor growth in C57BL/6j wild-type mice. After determining the most effective concentration of cancer cells, we evaluated the effects of JWH-133 on ectopic ovarian tumor growth, using the ID-8 model, in C57BL/6j wild-type mice.

Methods: This study was performed using adult female C57BL/6j wild-type mice. ID-8 ovarian cancer cells were injected subcutaneously in the right flank of randomly assigned mice at a concentration of 1, 3, or 10 million cells in 0.2 mL. Tumor growth was assessed daily via measurement using a digital caliper for 41 days. The most effective concentration was then determined, and those mice were then randomly assigned to receive daily intraperitoneal injections of either vehicle (a 1:1:1:17 ratio solution of ethanol, dimethylsulfoxide, Tween80, and physiological saline) or JWH-133 (at 1 mg/kg) for 31 days while tumor growth continued to be assessed daily. Mice were euthanized after 72 total days of tumor growth and both tumor and ovarian tissue were collected. Data was analyzed using ANOVA with repeated measures with Greenhouse-Geisser correction ($p < 0.05$ was considered significant).

Results: The concentration of 10 million ID-8 cells in 0.2 mL significantly increased tumor growth compared to the concentrations of 1 million and 3 million and this effect persisted when controlling for days of tumor growth. Chronic daily injection of JWH-133 for 31 days did not significantly increase tumor growth relative to vehicle solution. Western Blot analysis for changes in protein levels of cannabinoid receptors, estrogen receptors, and inflammatory markers will also be conducted.

Conclusions: This study demonstrates a concentration-dependent effect on tumor growth in the ID-8 ectopic ovarian cancer model. Further, this study emphasizes the importance of evaluating the effects of cannabinoid compounds on established tumor growth in order to better understand the potential utility of cannabinoid-based therapies. Finally, this study shows the need to study the effects of JWH-133 in wild-type mice using a variety of different ectopic ovarian cancer models.

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

INSIGHTS INTO THE GPR18 ACTIVITY OF THE PYRAZOLYLRESORCINOL SCAFFOLD

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Introduction: GPR18 is an orphan G-protein coupled receptor involved in a myriad of physiological functions, mainly related with the immune system and inflammation. It is considered as a putative cannabinoid receptor, as various phytocannabinoid and cannabinoid-like ligands are able to activate GPR18. Very few potent and selective ligands have been developed for the modulation of this receptor, thus impeding the study of its mechanism of action and its specific physiopathological functions. In this context, a series of novel GPR18 modulators based on the pyrazolylresorcinol scaffold have been designed, synthesised and tested at GPR18 and the CBRs, determining a structure activity relationship.

Methods: The chromenones were prepared from the corresponding alkylresorcinol and submitted to reaction with different hydrazines leading to the pyrazolilresocinols. These were tested using PathHunter® β-arrestin recruitment assays in CHO-K1/GPR18 cells. Radioligand binding studies were used to determine their affinity towards CB1R and CB2R. Active and inactive state homology models were performed analizing the sequence similarity of GPR18 with the class A GPCRs and selecting the best templates using different criteria. These templates were mutated using SwissModel and minimized with Maestro (integrated in the Schrödinger Suite). The best compounds were used for docking studies in a tridimensional homology model of GPR18.

Results: A family of over 40 compounds was obtained and tested *in vitro* for their functionality at GPR18.[1] Their ability to bind CB1R and CB2R was also determined and further *off target* evaluation has been done for the most promising compounds. A preliminary picture of the binding mode of these compounds was obtained by performing docking studies supported with the biological data.

Conclusions: Among this series of compounds not only agonists and antagonist of GPR18 were discovered, but also dual ligands CB2-GPR18, further functional assays and potential therapeutic effects are currently under evaluation.

[1] Patent: EP20382324; US2021323927A1

CANNABINOIDS PROFILE AND YIELD PARAMETERS OF TWENTY REGISTERED EU HEMP CULTIVARS GROWN IN SOUTHWEST GERMANY OVER THREE CONSECUTIVE YEARS

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Introduction: *Cannabis Sativa L.* is an ancient plant with a broad range of applications to the pharmaceutical, nutritional and industrial fields. Hemp is the nomenclature used for the industrial varieties selected over the centuries for the production of fibers and grains while containing minimum levels of Δ9-THC (tetrahydrocannabinol), which is still a controlled substance in the majority of countries. Nonetheless, besides fibers and grains, hemp varieties can also yield other cannabinoids (CBD, CBG, etc.) once the process parameters and cultivation systems are adapted. This study presents the performance of twenty EU-registered hemp cultivars grown over three consecultive years in southwest Germany.

Methods: A trial field with 20 industrial hemp varieties (Finola, Fédora 17, Santhica 27, Santhica 70, Eletta Campana, USO 31, Futura 75, Futura 85 etc.) was conducted in 2021 and 2022 at the experimental station of the University of Hohenheim, in Renningen, Germany. In adittion, in 2023, the five most prominent genotypes were cultivated to check for yield stability. For a statistical comparison of the growth parameters and biomass sections, the trial was set up as an alpha design with four true replicates and a plot size of 8 square meters. The sowing density was 120 plants per square meter with a row spacing of 15 cm. Ten representative plants per variety and replicate were harvested according to the variety-specific seed maturity, the plants were fractionated into stems, leaves, grains and threshing residues and the dry weight of the individual fractions was recorded for yield. At seed maturity, flowers of representative plants of all varieties were harvested for cannabinoid analysis. The dried leaf and whole flower fractions were ground to 1 mm particle size, extracted and the content of phytocannabinoids in the extract was measured by HPLC analysis. The statistical analysis were conducted in SAS using a mixed model approach.

Results: Across all years, stalk yields of the industrial hemp varieties varied between 6.1 – 48.5 g plant^{-1} dry matter. The leaf yields varied between 1.5 g – 15.3 g plant^{-1} dry matter. The grain yields varied between 2.1 g – 19.1 g plant^{-1} dry matter. On average, a threshing residue yield of 4.5 – 12.6 g plant^{-1} was achieved. The cannabinoid analysis showed that the hemp leaves had a total cannabidiol (CBD) content between 0.07 - 1.3 %, while flowers (threshing redisues) had a CBD content between 0.04 – 2.2 % and a CBG content between 0.03 – 1.4 %. Some varieties are characterized either by a high stalk or grain yields, while others presents high leaf and threshing residues yields, being also the ones with the highest CBD and CBG content.

Conclusion: Based on the results, selected industrial hemp varieties are suitable for possible cascade use. Varieties such as CS, Kompolti, Futura 83 and Tibrorszallasi, which produced the highest stalk biomass, may be suitable as feedstock for fiber production including a cascade for the production of grains (dual purpose varieties). In addition, waste products such as leaves and threshing residues can be used to extract phytocannabinoids without penalizing the stalk or grain yields.

CANNABIS POTENCY, CANNABIS DEPENDENCE, AND ANXIETY: WHERE DOES SEX FIT IN?

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Over the past 20 years, levels of Δ^9-tetrahydrocannabinol (THC) in cannabis have significantly increased while levels of cannabidiol (CBD) have lowered to THC:CBD ratios as high as 80:1. Cannabis with higher THC potency may lead to exacerbation of symptoms related to previous trauma exposure (e.g. anxiety, psychosis). However, studies of cannabis potency effects on anxiety and cannabis dependence have not examined sex moderation. N=l99 regular cannabis users (> lg/week in past month) with trauma histories (55.8% female/women) completed an online survey which included a measure of self-reported THC and CBD levels in participants' typjcally used cannabis. The validated Generalized Anxiety Disorder-7 (GAD-7) assessed anxiety levels while the Cannabis Use Disorder Identification Test-Revised (CUDIT-R) assessed cannabis dependence levels. Consistent with previous research, THC proportions were significantly positively correlated with CUDIT-Rs cores (r(193)=.206, P=.002). Unexpectedly, GAD-7 scores and CUDIT-R cores were not higher in the males/men nor were THC proportions in cannabis used. Moreover, the positive relationship between THC:CBD ratio and cannabis dependence did not differ significantly by sex (z=.278, p=.78). Results are consistent with a sex convergence of previously reported differences in cannabis dependence levels among male vs. female regular cannabis users. Findings also point to the importance of considering relative THC potency as a risk for cannabis dependence in both males and females.

THE INTERPLAY OF CANNABIDIOL AND PROBIOTIC IN THE TREATMENT OF DIABETES

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Introduction: According to world health organization, almost half a billion people live with diabetes globally. Importantly, the majority of diabetics belong to low income countries. Among individuals with diabetes, it is about 90% live with type two diabetes, caused by multiple factors. Despite of many medical and pharmaceutical advancements in the field, it is a dire need to find new therapeutic mechanisms and modalities to treat diabetes. Therefore, it is plausible to test whether cannabidiol and microbiome can improve the symptoms and alleviate the adversarial effects of diabetes.

Materials and Methods: We employed db/db mice, a model for type 2 diabetes. We used a combination of cannabidiol and probiotic culture through oral route by drinking. We monitored the subjects in a daily fashion, measuring weight, blood sugar and A1c protein. We further analyzed the samples using histology as well as flow cytometry and imaging techniques.

Results: Our interventional method reduced the A1c significantly in cannabidiol /probiotic treated subjects compared to placebo group ($p<0.05$). Although the weight and blood sugar did not show any significant difference between treatment versus placebo, however, the treated subjects showed a trent of reduction in blood sugar compared to placebo group

Conclusion: Our novel findings indicate that a combination of cannabidiol and probiotic can be used as a relatively safe, affordable, accessible, and effective modality in the treatment of type 2 diabetes.

PSILOCYBIN DRIVES CHANGES IN THE LIPIDOME INCLUDING THE ENDOCANNABINOID 2-AG

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Psilocybin is a psychedelic prodrug natural product compound produced by more than 200 species of fungi. A leading hypothesis of its mechanism of action is through the activation of serotonergic receptors in the CNS. There are also reports that it can modulate both dopamine and glutamate activity. Though the list of cognitive and behavioral effects of both acute and chronic administration of psilocybin suggest that like other small molecule lipid natural products (e.g. cannabinoids), these mechanisms of action are likely only part of a larger picture of global modulations of CNS function.

Recently, a study with a similar, but synthetic and highly potent psychedelic, lysergic acid diethylamide (LSD) was shown to reduce hippocampal levels of the endocannabinoid, Anandamide, and its N-acyl ethanolamine (NEA) congeners after 7 days of repeated treatment with little to no changes in 5-HT levels. Suggesting that another mechanism of action of this class of psychedelic compounds may be through the endogenous cannabinoid system.
While these classes of psychedelics are undergoing a resurgence in both recreational and medicinal applications, it is the lower potency, shorter acting, natural product, psilocybin, that has emerged as the specific drug of its class with the most wide-spread use. To date, however, there are no published reports on the effects of psilocybin on systemic and CNS changes of the lipidome. Here, we have undertaken a survey of over 100 endogenous lipids in the plasma and CNS. There are modulations of different classes of lipids; however, of importance here in the meeting is the significant, dramatic decrease observed in the endogenous cannabinoid, 2-arachidonoyl glycerol (2-AG) and select congeners that likely holds additional answers for how this drug is acting on the CNS. Additionally, more moderate changes in NEAs were observed as well as more significant changes many lipo amino acids such as *N*-acyl glycines and *N*-acyl alanines. Data will be presented to illustrate that psilocybin drives changes in endocannabinoids and related lipids differentially and this is an alternative and complementary mechanism of action for the wide range of cognitive and behavioral effects observed with drug treatment.

USING A RODENT VAPOR SELF-ADMINISTRATION PARADIGM TO STUDY THE REINFORCING EFFECTS OF VAPORIZED DELTA-8 TETRAHYDROCANNABINOL

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Introduction: Delta-8 Tetrahydrocannabinol (THC) had the largest growth in sales in the 2021 cannabinoid market. This, combined with an increased interest in delta-9 THC due to its legalization and decreasing perceptions of harm associated with use, makes the need to better understand the reinforcing effects of cannabis and its derivatives paramount. While an intravenous (I.V.) self-administration paradigm is the gold standard for studying reinforcing effects in a rodent model, there are limitations when looking at I.V. cannabis drugs. The development of a vapor self-administration paradigm may overcome some of these limitations and be more relevant to humans. The current study was designed to determine if response-contingent vapor deliveries of delta-8 THC results in reinforcing effects using a rodent self-administration procedure.

Methods: Eight male Sprague Dawley rats (PND 21) had daily 75-minute sessions to lever press for vaporized concentrations of delta-8 THC (2.5, 5, 10, 15, 30mg/300ml) and vehicle (ethanol). Standard operant conditioning chambers were retrofitted with a custom vapor nozzle on the front wall, which attached to Volcano vaporizers that aerosolized the drug and pushed a 3.6-second "puff" of vapor into the chamber following completion of a Fixed-Ratio 2 on the active lever. Following food-training animals were placed on an acquisition dose of 10mg/300ml for 12 sessions. The dose of delta-8 THC was then varied every four days to establish a dose-effect curve.

Results: Across the study rats' discrimination between the active and inactive levers improved. Rats had a discrimination greater than 2:1 active to inactive lever presses prior to establishing the dose effect curve. A two-way repeated measures ANOVA of the dose effect curve found there was a significant interaction of lever by dose. Post hoc analysis indicated that relative to vehicle substitution, the 5mg/300ml dose of delta-8 THC resulted in significantly greater active lever responding. At all active doses tested rats responded significantly more on the active lever compared to the inactive lever.

Conclusions: The current results indicate that vaporized administration of delta-8 THC can result in mild reinforcing effects. The study also illustrates that under the current contingencies, 5mg/300ml dose of delta-8 THC appears to be the peak of the dose effect curve and maintain levels significantly above vehicle substitution. Ongoing studies are investigating whether delta-8 THC differs in female rats. Future studies look to further understand the important variables in maintaining vaporized cannabis self-administration in rodents, as well as comparing delta-8 THC to delta-9 THC.

CANNABINOID 2 RECEPTOR DELETION AMELIORATES L-DOPA-INDUCED DYSKINESIA IN PARKINSON'S DISEASE MOUSE MODEL

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Introduction: A major hallmark of Parkinson's disease (PD) is the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) resulting in motor problems. Although levodopa (L-dopa) is a drug of choice for symptom management in PD, nearly 50% of those treated experience L-dopa induced dyskinesia (LID), a problem of excessive movement that limits L-dopa utility. Recent preclinical studies reveal that LID can be mitigated by CB2 cannabinoid receptor modulation. We have expanded these studies to explore the hypothesis that microglial CB2 receptors specifically modulate LID in a 6-hydroxy-dopamine (6-OHDA) mouse model of Parkinson's Disease.

Methods: Male C57BL/6 mice with different genotypes (WT, $CB_2^{eGFP/eGFP}$, $CB_2^{-/-}$, $CX3CR1Cre^{+/-}$ and $CX3CR1Cre^{+/-}$ $CB_2^{eGFP/eGFP}$) were anaesthetized and 6-OHDA·HBr (in 0.02% ascorbate) or vehicle were injected stereotaxically into the right striatum (+0.4mm AP; +1.8mm ML and -3.5 DV). After 3 weeks of recovery, mice performed a battery of assays (cylinder test, pole test and balance beam) to evaluate motor function and started receiving intraperitoneal injections of L-DOPA at 20 mg/kg daily for 5 weeks. LID was evaluated by measuring abnormal involuntary movements (AIMs) on days 42, 46, 49, 52 and 55 after surgery.

Results: Independent of genotype, 6-OHDA-lesioned mice showed motor deficiencies in balance beam, pole test and cylinder test, compared to mice receiving sham surgeries, confirming that Parkinson's disease-like symptoms are present. Dyskinesia was evaluated after 3 weeks of L-DOPA treatment, showing very severe and frequent AIMs in all the genotypes. Moreover, animals with CB_2 deleted (either globally as $CB_2^{-/-}$ or specifically on microglia as $CX3CR1Cre^{+/-}$ $CB_2^{eGFP/eGFP}$) showed lower LID scores compared to their controls or WT littermates.

Conclusions: In contrast to earlier studies, these data indicate that genetic deletion of the CB2 receptor either globally or specifically in CX3CR1-expressing cells reduces AIMs frequency and severity. These studies suggest that reducing activity of microglial CB_2 cannabinoid receptors, perhaps through antagonism, is a promising target to treat LID and thereby increase the beneficial effects of L-DOPA treatment in PD.

Acknowledgements: Funded by the Advancing a Healthier Wisconsin Endowment at the Medical College of Wisconsin and the G. Frederick Kasten Jr. Research Chair in Parkinson's Disease.

IN VIVO EFFICACY OF NOVEL NEGATIVE BITOPIC/ALLOSTERIC CANNABINOID RECEPTOR 1 MODULATORS IN ALCOHOL DRINKING

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Introduction: Cannabinoid receptor 1 (CB_1R) antagonism holds therapeutic potential in the treatment of several disorders. Recent studies have exhibited clinical promise for functionally selective blockade of CB_1R to improve safety in metabolic and addictive disorders. Novel pharmacological tools to investigate allosteric/dualsteric strategy could aid mechanistic understanding of functional selectivity in CB_1R that eventually help developing effective and safer therapies. Accordingly, we designed, synthesized, and evaluated a series of novel four-arm diarylpyrazoline compounds in the *in vitro* and *in vivo* assays.

Methods: Selective and potent compounds for CB_1R with allosteric potential and/or bitopic nature were screened. The pharmacokinetics, tissue distributions were assessed by LC-MS/MS after intraperitoneal and oral administration in C57BL6/J mice. *In vivo* efficacy of CB_1R antagonism was assessed by upper gastrointestinal (GI) motility assay in mice. Potential anxiogenic activities were assessed using ambulatory activity assay in mice. Then *in vivo* efficacy of the compounds in alcohol drinking behavior was tested using drinking in the dark (DID) experimental paradigm in mice.

Results: Novel chiral compounds with high affinity and selectivity for CB_1R in the sub- and low nanomolar range were tested in functional assays using [^{35}S]-GTPγS binding. The tested compounds retained high potency for CB_1R antagonism. Six compounds behaved as non-competitive CB_1R antagonist in GTPγS binding with Schild plot analysis indicating negative allosterism. In pharmacokinetic studies, the tested non-competitive antagonists provided good systemic exposures with Cmax at 200-300 nM using 3 mg/kg intraperitoneal injections. Acute treatments with enantiomerically pure MRI-2265 or MRI-2479 at 3 mg/kg dose provided maximum *in vivo* efficacy for CB_1R antagonism with fully attenuating CB_1R agonist effect in upper GI motility assay. MRI-2265 was peripherally restricted with 8% brain/plasma ratio whereas MRI-2479 was moderately brain penetrant with 34% brain/plasma ratio. Unlike rimonabant (10 mg/kg), neither of the two tested compounds (10 mg/kg) induced hyperambulatory activity. Additionally, both compounds dose (1, 3, 10 mg/kg) dependently and equipotently reduced alcohol drinking in DID experimental paradigm.

Conclusions: We generated peripherally restricted or moderately brain-penetrant bitopic modulators for inhibiting CB_1R function with favorable pharmacokinetic properties and potent *in vivo* efficacy without inducing anxiogenic behavior. Future studies warrant to further characterize these compounds in different experimental models and behavioral paradigms to demonstrate *in vivo* functional selectivity and improved CNS safety.

Acknowledgements: This work was supported by the funds from Intramural Research Program of the NIAAA (to R.C., M.R.I).

CB1 RECEPTOR NEGATIVE ALLOSTERIC MOLECULES: *IN VITRO* PHARMACOLOGY AND *IN VIVO* EFFICACY IN PRECLINICAL MOUSE MODELS OF HYPERDOPAMINERGIA

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Introduction: Drug discovery programs for novel therapeutics rely on *in vitro* screening using relevant functional assays and *in vitro* DMPK before testing *in vivo*. Targeting the CB_1 receptor by negative allosteric modulation serves as a viable therapeutic avenue to normalize endocannabinoid signalling that is dysregulated in several pathological conditions. We reported that a CB_1 receptor allosteric modulator (ABM300) ameliorated psychosis-type behaviours in the dopamine transporter knockout (DATKO) genetic mouse model of hyperdopaminergia (Mielnik *et al.*, 2021). We initiated a screening program to test analogues of ABM300 and other CB_1 allosteric modulators to identify high potency and metabolically stable molecules prior to *in vivo* testing in DATKO mice.

Methods: Novel CB_1 allosteric molecules were screened *in vitro* using a β-arrestin recruitment assay (Eurofins PathHunter® assay). Molecules with high efficacy in the primary screen were tested for *in vitro* metabolic stability using human and rat liver microsomes and compared to the allosteric effects mediated by ABM300 on agonist-induced β-arrestin recruitment, inhibition of cAMP and pERK1/2 phosphorylation in either PathHunter® CHO-K1 CNR1 β-Arrestin cells (Eurofins DiscoverX) or human CB_1-expressing CHO-K1 cells. Candidate high potency and metabolically stable molecules from the *in vitro* screen were tested for *in vivo* efficacy on psychosis-type behaviours in DATKO mice.

Results: *In vitro*, we identified potent and metabolically stable ABM300 analogues (ABM338, ABM436, ABM437, ABM440) and indole sulfonamide allosterics (ABD1085, ABD1055). These allosterics displayed similar *in vitro* signalling profiles to ABM300 with decreased CP-55,940 mediated β-arrestin recruitment (IC_{50} values 5-30 nM) and cAMP inhibition (IC_{50} values 181-476 nM). Despite displaying similar *in vitro* pharmacological profiles, we observed some striking differences *in vivo*. ABM338, ABM436 and ABM437 at 10 mg/kg decreased hyperactive exploratory phenotypes in the DATKO genetic model, while ABM440 (10 mg/kg) did not ameliorate this hyperlocomotive behaviour. ABD1085 (10 mg/kg), an allosteric with improved pharmacokinetic properties, also did not decrease hyperlocomotive behaviour in the DATKO pharmacological model.

Conclusions: We have characterized the *in vitro* signalling profiles and *in vivo* efficacies of novel CB_1 receptor allosterics with the aim of identifying allosterics with improved drug-like characteristics and *in vivo* efficacy. Despite displaying similar *in vitro* pharmacological profiles to ABM300 and superior metabolic stability and/or *in viv*o pharmacokinetic properties, we identified some molecules (ABM440, ABD1085) that lacked *in vivo* efficacy in our mouse model of hyperdopaminergia. Our results clearly indicate that *in vitro* screening strategies for identifying potential novel therapeutic compounds based on *in vitro* potency and metabolic stability do not necessarily translate into *in vivo* efficacy.

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

ACUTE USE OF LOW V. HIGH CONCENTRATION CANNABIS ON PSYCHOMOTOR PERFORMANCE AMONG ADULTS WITH DAILY V. MONTHLY USE

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Introduction: Acute cannabis use has been found to slow reaction time and affect working memory and spatial and psychomotor performance. These effects are important as they may increase the risk of motor vehicle crashes or other injury. However, these domains have not been well-studied among adults who use cannabis daily (who may have tolerance) or after use of high concentration products, which are now widely available in U.S. states with legalized use. Understanding the acute effects across a range of use frequencies and products has utility for understanding tolerance to cannabis and identifying impairment associated with recent use.

Methods: We recruited n=78 adults (ages 21-55) to complete a tablet-based assessment before and after observed *ad libitum* cannabis inhalation. Participants used cannabis less than 3 times a week ("monthly" n=21) or at least once a day ("daily" n=57); and were observed to smoke self-supplied cannabis flower (n=54) or inhale high concentration cannabis (n=24), for up to 15 minutes during data collection. Outcomes were measured before use (which was after 8+ hours of abstinence), and at approximately 60 and 120 minutes after use. Blood samples were collected at corresponding times. An additional n=29 completed protocols without using cannabis, to assess learning effects (total N=107). Change in performance from pre to post use was modeled using generalized linear models, with primary independent variables: frequency of use (tolerance) and amount used (operationalized by product concentration, blood THC levels, and self-reported drug effects). Age and gender were controlled for as potential confounders. (Additional data from recently recruited participants will be added to the final analysis for presentation, for a total of N=138.)

Results: In a task assessing visuomotor processing, participants with monthly use showed declines in performance, compared to the daily or no-use participants, particularly at 1 hour after use. Performance on the working memory task showed the most dramatic change from pre- to post-use, particularly for those with monthly use, who declined in performance as compared to the other groups. In two measures of spatial and psychomotor performance, those with occasional use declined in performance compared to those with daily use. There were few significant differences in performance on the reaction time task associated with acute use, regardless of frequency of use or acute use. There was no significant effect of blood THC, product concentration or self-reported drug effect. Effects were larger at 1 hour as compared to 2 hours after use. We also present findings by gender, and amount of cannabis used.

Conclusions: The findings are somewhat consistent with acquired tolerance to certain acute psychomotor effects and consistent with self-titration of high THC concentration products (rather than larger effects with higher concentration). Cognitive and psychomotor assessments may have utility for identifying impairment associated with recent cannabis use and could be applied in safety sensitive scenarios, such as it relates to motor vehicle safety, to prevent impaired driving.

Acknowledgements: Funded by the National Institutes of Health (R01DA049800).

REAL-TIME EFFECTS OF MEDICAL CANNABIS ON OLDER ADULTS WITH CHRONIC PAIN: EARLY RESULTS FROM A PROSPECTIVE COHORT WITH CONTROL

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Introduction: Rigorous data are lacking on the short- and long-term effects of medical cannabis (MC) on older adults with chronic pain. Mobile technologies such as smartphone and wearable sensors provide great potential to understand health behaviors/outcomes such as cannabis use and pain in patients' natural environment with real-time and fine-grained information. Embedding mobile technology-based assessments in a prospective cohort study allows researchers to collect the much-needed evidence on both real-time and long-term effects of MC.

Methods: The Study on Medical marijuana and Its Long-term Effects in older adults (SMILE Study) is an ongoing prospective cohort study that enrolls and follows older adults with chronic pain (≥50 years, target enrollment N = 328) as approximately half of them initiate MC while the other half do not. The study combines technology-based ecological momentary assessments (EMA) and in-person visits over 12 months to collect subjective (e.g., survey) and objective data (e.g., pain sensory test, blood test) of MC's effects on chronic pain and related health outcomes (e.g., mental health, physical activity). The smartphone-based EMA include several brief surveys (~3 minutes each) every day to capture detailed MC use patterns and self-reported real-time outcomes (e.g., momentary pain intensity, anxiety/depression). The EMA data are supplemented by objective data collected using a wearable sensor—Fitbit Charge 5 to track outcomes such as physical activity and sleep. Linear mixed effects modeling was used to examine changes in real-time health outcomes in the MC and control groups. As data collection is still in progress, this abstract is focused on the real-time data collected during first five weeks of the study.

Results: Data collected from 104 participants (mean age = 65± 8.9, 68% female, 61.5% MC group) were analyzed. At baseline, both MC and control group reported experiencing moderate to severe chronic pain for at least 6 months (6.1 vs. 6.5 respectively, out of a 0-10 scale). A total of 2273 daily and 7930 momentary EMA surveys were completed by participants during the first five weeks of study. By contrasting real-time outcomes with MC use vs. without MC use, MC use was associated with significant reductions in pain intensity ($\beta = -7.9$, $p < .05$), anxiety ($\beta = -0.33$, $p < .01$), hours in high-intensity pain ($\beta = -1.19$, $p < .01$), and significant increases in sleep hours ($\beta = 0.31$, $p < .05$). Fitbit data showed a significant increase in daily steps among the MC group over time ($\beta = 22.6$, $p < .01$), with a significant decrease in the non-MC group ($\beta = -16.6$, $p < .05$). However, Fitbit data showed no change in sleep hours for either group.

Conclusions: Our initial findings show that MC use was associated with lower real-time pain intensity and better physical and emotional functioning in older adults with chronic pain. Future data from this cohort with a full sample size and data extending beyond the first 5 weeks will further inform understanding of risks and benefits of MC use among this population.

Acknowledgement: This project is supported by National Institutes of Health (NIH/NIA) R01AG071729 (PI: Yan Wang).

INDIVIDUAL AND INTERACTIVE EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL (THC) AND ALPHA-PINENE

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Introduction: In recent years, the "Entourage Effect" hypothesis that various phytochemical constituents of cannabis contribute to its overall pharmacodynamic effects has gained traction despite a lack of empirical evidence in humans. This controlled human laboratory study evaluated whether the terpenoid alpha-pinene, which is commonly found in cannabis, mitigates acute impairment of working memory performance associated with administration of a high dose of delta-9-tetrahydrocannabinol (THC).

Methods: Healthy adults (n=16) completed six, double-blind, outpatient drug administration sessions. During each session, participants inhaled vaporized THC alone (30mg THC), vaporized alpha-pinene alone (15mg), vaporized THC and alpha-pinene in combination (30mg THC + 0.5mg pinene; 30mg THC + 5mg pinene; 30mg THC + 15mg pinene), or placebo in a randomized order. Outcomes assessed before and for 6 hours after drug administration included: working memory performance (Paced-Serial-Addition-Task, PASAT), encoding and retrieval of episodic memories via a delayed verbal recall test (International Shopping List Task), psychomotor performance (Digit-Symbol-Substitution-Task, DSST), and subjective drug effects (Drug Effect Questionnaire; DEQ).

Results: As expected, THC significantly impaired working memory performance and was associated with moderate to high self-reported drug effects, subjective ratings of increased impairment of memory, and difficulty performing routine tasks. Alpha-pinene did not significantly alter THC-induced subjective drug effects, self-reported trouble with memory or other subjective drug effects assessed by the DEQ at any dose tested. Co-administration of alpha-pinene with THC produced mild, nonsignificant attenuation of THC-induced working memory impairment that was most prominent in the 5mg alpha-pinene dose condition. Similarly, alpha-pinene only slightly attenuated THC-induced psychomotor deficits, albeit to a nonsignificant degree. When administered by itself, alpha-pinene did not produce any discriminable subjective drug effects or impact cognitive ability.

Conclusion: These data suggest that alpha-pinene does not appreciably alter THC-induced subjective drug effects or significantly attenuate THC-induced memory impairments when co-administered at or above doses typically present in cannabis. Though qualitative changes were in the direction of improved working memory performance when THC was combined with alpha-pinene at 5mg and 15mg, impairment of cognitive performance was still significant across dose conditions compared with placebo. This is in contrast to a recently completed study in our lab that showed a significant attenuation of THC-induced anxiety by the cannabis terpene d-limonene using the same laboratory model.

Support: National Institute on Drug Abuse (NIDA) Grants R01-DA043475 and T32-DA007209.

INHIBITION OF FATTY ACID BINDING PROTEIN 5 PREVENTS STRESS-INDUCED ANXIETY AND DEPRESSIVE-LIKE BEHAVIOURAL SYMPTOMS AND REVERSES STRESS-INDUCED INHIBITION OF HIPPOCAMPAL NEUROGENESIS

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Introduction: Adult neurogenesis is the process in which newly formed neural cells during adulthood are continuously added to the already existing neural network. Treatment with different classes of antidepressants and environmental insults such as chronic stress have been shown to increase and attenuate proliferation of newborn neurons, respectively, advocating for novel pharmacotherapeutic interventions to target adult neurogenesis for the treatment of neuropsychiatric conditions. The endocannabinoid (eCB) system modulates many biological processes including adult neurogenesis, emotional behaviour and signaling pathways. A chaperone protein in the eCB system, fatty acid binding protein 5 (FABP5), is responsible for the intracellular transport of eCB ligands like anandamide for its degradation. Here, using SBFI103, a potent inhibitor of FABP5, we aimed to investigate the effects of systemic FABP5 inhibition on: (1) anxiety- and depression-related behaviours, (2) key molecular targets that are heavily associated with anxiogenic and depressive-like behaviour, and (3) adult neurogenesis markers.

Methods: Following a 2-week long unpredictable stress paradigm, we assessed whether an acute intraperitoneal injection of SBFI103 could reverse stress-induced anxiogenic and depressive-like phenotype in Sprague-Dawley rats. Thereafter, we investigated mRNA expression levels and activity of signalling molecules and receptors in the eCB system within the limbic regions of the rat brain using RT-qPCR and Western blotting, respectively. Furthermore, we examined relevant markers of adult neurogenesis to explore the proliferation and differentiation of newborn neural cells in the subgranular zone of dentate gyrus using immunohistochemical (IHC) staining.

Results and Conclusion: Stress-induced anxiogenesis, tested by light-dark box and elevated plus maze, was ameliorated by both low dose (2mg/kg) and high dose (20mg/kg) SBFI103. Depression-like behaviour, tested by sucrose preference, novelty-suppressed feeding and forced swim, was restored by only high dose SBFI103. Neither dose of SBFI103 influenced cognition. SBFI103-induced behavioural alterations are substantiated by effects detected in the transcription of CB_2 and GPR55 receptors as well as by altered phosphorylation of Erk1-2, Akt and p70S6 kinase by SBFI103. IHC examinations revealed a reversal effect of SBFI103 on BRDU, Ki67 and doublecortin, markers of proliferation and differentiation of newborn neural cells, in both dorsal and ventral hippocampus. These findings reveal a very promising role for FABP-5 inhibition as a novel pharmacotherapy against various aspects of mood and anxiety disorders.

Acknowledgements: Funding provided by Artelo Biosciences and Mitacs Canada.

STUDYING THC-INDUCED IMPAIRMENT IN MICE: FROM NEW EXPERIMENTAL APPROACHES TO REAL TIME MONITORING OF 2-AG PRODUCTION AND AI ANALYSIS OF BEHAVIORS

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Introduction: THC induces a reduction in locomotor behavior such as distance travelled and average velocity. THC also increases specific behaviours in mice such as lying prone which is interrupted by episodes of spontaneous locomotion events. Here, we leveraged voluntary oral consumption of THC-gelatin, genetics and pharmacological approaches, fibre photometry monitoring of neuronal activity and of 2-AG production using $GRAB_{eCB2.0}$, as well as a newly developed linear track monitoring approach and unbiased AI analysis of mouse behaviour to elucidate the effect of THC on mouse spontaneous locomotion and other motor behaviours in mice. To unravel the neural basis of how THC induces these behavioural changes, we monitored neuronal activity and 2-AG dynamics in the prelimbic cortex (PrL), a brain structure involved in the planning and preparation of motor processes.

Methods: $GRAB_{eCB2.0}$ was expressed in Neuro2a (N2a) cells in culture and live-cell microscopy and 96-well plate reader were used to detect changes fluorescence. WT mice were injected with AAV5-hSyn-$GRAB_{eCB2.0}$, while VGAT-Cre and VGLUT1-Cre mice were injected with AAV5-DIO-GCaMP6f. Mice were implanted with optic fiber into the PrL. Activity was recorded while animals openly explored an operant chamber and a linear track. Behavioral identification of control and THC-induced changes were tracked using a machine learning pose estimation algorithm (SLEAP) and further classified with additional machine learning analysis.

Results: 2-AG synthesis by neurons is increased by neuromodulators that activate metabotropic receptors. We measured changes in 2-AG levels by N2a cells in culture using live-cell microscopy and detected increases in $GRAB_{eCB2.0}$ signal within seconds of activation of metabotropic receptors, a response that peaked at 2 min and slowly decayed over 10 min. This increase in 2-AG production was blocked by diacylglycerol lipase inhibitor DO34 (10 nM) and reduced by BAPTA-AM (30 μM).

In vivo recordings of mice showed that initiation of spontaneous locomotion in an operant chamber and in a linear track resulted in transient increases in glutamatergic and GABAergic activities and an increase in DO-34-dependent production of 2-AG in the PrL that lasted several seconds. Dose-dependent treatment of mice with THC moderately increased transient activity in VGLUT1 neurons and strongly increased transient activity in VGAT neurons, responses that preceded both spontaneous locomotion initiation and increased $GRAB_{eCB2.0}$ transient activity in the PrL. THC induced a robust, dose-dependent, reduction in spontaneous locomotion and motor behaviors (rearing, and grooming), and increased lying prone episodes. These changes in behaviors were tracked by simultaneously changes in neural activity and 2-AG signaling in the PrL.

Conclusion: THC differentially increases PrL glutamatergic and GABAergic activities at the initiation of spontaneous locomotion, a response followed by increases in 2-AG levels and CB_1R activation. Unbiased analysis of mouse behavior identifies previously unrecognized THC-induced changes in spontaneous locomotion patterns and behaviors controlled by changes in PrL neural activity.

Acknowledgements: Funded by NIH (DA051558 to NS and BL, DA026430 to NS; NS118130 to NS; DA056816 to NS & MRB; DA057186 to NS & MRB; AT011524 to BL & NS).

NEUROCHEMICAL AND BEHAVIORAL EFFECTS OF CANNABINOIDS

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Introduction: Despite having therapeutic applications, cannabinoids have multiple undesirable effects limiting their use, including notable abuse liability. Like most drugs of abuse, studies have reported cannabinoids to increase extracellular levels of the neurotransmitter dopamine (DA) in key reward-related brain regions, including the nucleus accumbens shell (nAcc shell). However, it is unclear how cannabinoid-induced changes in neurochemical dynamics in the nAcc shell impacts reward-related behavior.

Methods: Here, we first utilized *in vivo* microdialysis and liquid chromatography-mass spectrometry to quantify the effects of Δ^9-THC (0.1–3.2 mg/kg), the synthetic partial CB_1 agonists AM11101 (0.1–3.2 mg/kg), and the full CB_1 agonist AM8936 (0.01–1.0 mg/kg) on extracellular levels of dopamine (DA), glutamate (Glu) and GABA within the nAcc shell of mice. Next, using conditioned place preference (CPP), we determined the relationship between Δ^9-THC (0.1–1.0 mg/kg), AM11101 (0.1–1.0 mg/kg), and AM8936 (0.01–0.1 mg/kg) induced rewarding properties and changes in DA, GABA, and Glu levels.

Results: Results show that lower doses of all three cannabinoids (0.32 mg/kg Δ^9-THC and AM11101, 0.032 mg/kg AM8936) increase DA in the nAcc shell to 134–161% basal values, whereas administration of higher doses (0.1–1.0 mg/kg AM8936, 1.0–3.2 mg/kg AM11101 and Δ^9-THC) decrease DA levels to 59-70% basal values. Notably, the onset of these DA changes is immediate (i.e., 20 min) for Δ^9-THC and AM11101 but delayed by about 140 minutes after AM8936 administration. Interestingly, during the same time period, the doses of Δ^9-THC and AM8936 that increased DA also elevated GABA to 219% and 157% of basal values, as well as Glu to 133% and 136% of basal values (respectively). In contrast, the low doses of AM11101 that increase DA have little effect on GABA and Glu. Δ^9-THC produces no change in Glu or GABA at doses that decrease DA. However, high doses of AM11101 and AM8936 both decrease GABA to 54% and 58% basal values, as well as Glu to 70% and 73% basal values (respectively). Together, these data show a biphasic dose-response function on DA in the nAcc shell, but the effects on GABA and Glu are distinct among the three CB_1 drugs. Results from CPP studies demonstrate that Δ^9-THC (0.1–0.32 mg/kg) and AM8936 (0.032 mg/kg) produce increases in preference score, whereas AM11101 (0.1–1.0 mg/kg) did not induced CPP. Correlation analysis of grouped data from all CB_1 drugs revealed a strong positive relationship between increases in DA, GABA, and Glu in the nAcc shell and rewarding properties of Δ^9-THC, AM11101, and AM8936.

Conclusion: Together, these findings point to a complex neurochemical dynamic where elevations in DA, GABA, and Glu may be necessary to produce rewarding effects from CB_1 agonists.

Acknowledgement: Funded by the National Institutes of Health (P01DA009158).

DELTA-8-TETRAHYDROCANNABINOL PREVENTS COLLAGEN-INDUCED ARTHRITIC JOINT DEGENERATION AND PAIN-DEPRESSED BEHAVIOR

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Introduction: Major cannabinoids such as Δ^9-THC produce anti-inflammatory and antiarthritic effects in preclinical models, however little research has investigated the potential anti-arthritic effects of minor cannabinoids. The goal of the present study was to test the hypothesis that the minor phytocannabinoid delta-8-tetrahydrocannabinol (Δ^8-THC) attenuates morphological, behavioral, and immune effects of inflammatory arthritis in male mice. Female DBA/1J mice are reportedly resistant to developing collagen-induced arthritis (CIA). Thus, an additional goal was to assess whether CIA would develop in female mice using recently refined methods.

Methods: Adult male DBA/1J mice were inoculated with an emulsion of collagen and complete Freund's adjuvant injected (s.c.) into the tail. Twice daily injections of Δ^8-THC (3 or 30 mg/kg), the steroid dexamethasone (2 mg/kg), or vehicle were administered for two weeks following a second collagen treatment, 21 days after the initial immunization. Paw measurements and semiquantitative clinical arthritis scores were recorded daily. Latency to fall from an inverted grid was measured every two days to determine the progression of functional impairment. On the final day of testing, spontaneous wire-climbing behavior and temperature preference in a thermal gradient ring were measured to assess CIA-depressed and -conditioned behavior, respectively. To assess the sex-specific effects of our CIA preparation, a second group of female DBA/1J mice was subjected to the same methods as above, only without Δ^8-THC or dexamethasone treatment.

Results: The Δ^8-THC treatment (30 mg/kg) reduced paw swelling and clinical signs of arthritis. In histological assessments, Δ^8-THC reduced synovial inflammation and bone erosion in the ankle joint. CIA depressed climbing and induced a preference for a heated floor, and Δ^8-THC blocked each of these effects without producing locomotor deficits. Finally, Δ^8-THC attenuated CIA-induced levels of IL-1β, IL-6, and VEGF-A in whole hind paw homogenates. There were also no sex differences between mice subjected to CIA, indicating that females had developed arthritis to the same extent as males.

Conclusions: The minor phytocannabinoid Δ^8-THC not only blocked morphological changes but also prevented functional loss caused by collagen-induced arthritis. Paw function was quantified using automated test paradigms that are not confounded by traditional, subjective pain-induced behavioral tests. The demonstration that female DBA/1J mice are susceptible to CIA using these methods facilitates future experimentation exploring the sex-specific effects of antiarthritic treatments.

Acknowledgments: Research was supported financially by the National Institutes of Health (R01 AT010773) and the UConn CAMP Trainee Pain Research Grant.

COMPARISON OF THE ANTINOCICEPTIVE AND ANTIALLODYNIC EFFECTS OF PHYTOCANNABINOIDS ALONE VERSUS IN COMBINATION WITH THE KRATOM ALKALOID MITRAGYNINE IN MALE AND FEMALE MICE

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Introduction: As the opioid crisis continues in the US, those suffering from chronic pain look to perceived safer and more effective treatment options, including natural remedies. The scientific evidence, practice, and legislation surrounding the medical use of Cannabis have been steadily on the rise over the last several decades, with pain reduction and alleviation of anxiety as its top indications. In addition, Kratom, a coffee-like plant containing compounds that cause opioid and stimulant effects, has also gained popularity for the self-treatment of several symptoms, including chronic pain, prescription opioid dependence, and anxiety. A recent survey of Kratom users reported that the strongest predictor of Kratom use was Cannabis use, specifically cannabidiol (CBD). Our lab has previously demonstrated the antiallodynic effects of CBD as a successful preventative measure in rodent models of chemotherapy-induced peripheral neuropathy (CIPN), as well as the ability of the Kratom alkaloid mitragynine, to reverse pain-like behaviors associated with CIPN. In the present study, we compared the antinociceptive and anti-allodynic effects of phytocannabinoids alone and in combination with mitragynine in mouse models of acute thermal pain and CIPN-associated mechanical sensitivity.

Methods: Male and female C57BL/6 mice were used for these studies. For acute thermal antinociception, latency to withdrawal the hindpaw from a 55C^{0} hotplate was determined following administration of a range of THC, CBD, CBG, and mitragynine doses alone, or in combination. For prevention of CIPN symptoms, mechanical allodynia was measured at baseline and following paclitaxel exposure alone or with cannabinoids +/- mitragynine.

Results: For acute thermal antinociception, THC was the most efficacious at attenuating thermal sensitivity, followed by CBG. Neither CBD nor mitragynine showed antinociceptive effects across a range of doses. For the prevention of mechanical sensitivity associated with paclitaxel administration, both CBD and mitragynine dose-dependently prevented the development of paw sensitivity with equal potency and efficacy, while CBG was ineffective. Co-administration of mitragynine with THC or CBG decreased their antinociceptive effects.

Conclusion: Phytocannabinoids and mitragynine produce different effects in the acute versus neuropathic pain models. Importantly, this is the first report of prophylactic administration of mitragynine to prevent the development of paclitaxel-associated mechanical sensitivity; mechanisms of action should be further investigated. Co-administration of mitragynine with phytocannabinoids may decrease their potency or efficacy; however, this effect may be related to mitragynine decreasing the locomotor depressing effects of these phytocannabinoids. Other behavioral assays will be done to test animal reactions to locomotion, anxiety, etc.

Acknowledgments: Funding sources R01AT010778-01 (SJW), R21DA056729-01A01 (SJW).

CHARACTERISATION OF ANXIETY- AND DEPRESSION-RELATED BEHAVIOUR AND THE ENDOCANNABINOID SYSTEM IN THE RAT HIND LIMB ISCHEMIA-REPERFUSION MODEL OF CHRONIC WOUNDS

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Introduction: Ischemia-reperfusion injury can underlie the formation of chronic wounds, which are associated with a high incidence of comorbid anxiety and depression. The endocannabinoid system (ECS) has a role in ischemia-reperfusion injury and is involved in the modulation of both mood and anxiety. This study characterised anxiety- and depression-related behaviour in a rat model of hind limb ischemia-reperfusion (HLIR) injury and investigated alterations in the ECS in key brain regions associated with anxiety and depression.

Methods: Male and female Sprague-Dawley rats (220-350g, n=11-12/group) underwent HLIR or sham procedure on the left hind limb under isoflurane anaesthesia. Anxiety-related behaviour was assessed using the open field test on post-HLIR day 16, the light-dark box test on post-HLIR day 24 and the elevated plus maze on post-HLIR day 26. The sucrose preference and sucrose splash tests assessed depression-related behaviour on post-HLIR days 17-18 and post-HLIR day 20, respectively. Rats were euthanised on PSD 30, and brains were gross-dissected and snap-frozen. Quantification of endocannabinoids (2-AG and AEA) and *N*-acylethanolamines (PEA and OEA) was carried out by LC-MS/MS, while RT-qPCR was used to examine endocannabinoid-related gene expression (*Cnr1*, *Cnr2*, *Faah* and *Mgll*) in discrete brain regions.

Results: There was no effect of HLIR on anxiety- or depression-related behaviour. Female HLIR rats reared for longer than male HLIR rats in the open field test ($p<0.05$). 2-AG levels were lower in the amygdala of female HLIR rats compared to female shams ($p<0.05$), with no differences in AEA, PEA or OEA levels. Levels of endocannabinoids or *N*-acylethanolamines in the hippocampus, striatum or nucleus accumbens did not differ between groups. Higher levels of mRNA encoding *Faah* and *Mgll* were found in the amygdala of female HLIR rats compared to male HLIR rats. Higher levels of mRNA encoding *Cnr2* were found in the nucleus accumbens of male HLIR rats compared to female HLIR rats. There were no between-group differences in levels of mRNA encoding *Cnr1* in the amygdala, hippocampus, striatum or nucleus accumbens.

Conclusions: These results indicate sex differences in locomotor activity and the ECS in discrete brain regions following HLIR injury. Further work is required to determine the implications of lower 2-AG levels in the amygdala post-HLIR injury in female rats.

Acknowledgements: Funding was provided by the Irish Research Council Postgraduate Scholarship, Science Foundation Ireland and B. Braun Hospicare and is co-funded under the European Regional Development Fund under Grant Number 13/RC/2073-P2.

DYSREGULATION OF MONOACYLGLYCEROL LIPASE IN A HUMANIZED MOUSE MODEL OF SICKLE CELL DISEASE

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Introduction: Major comorbidities of sickle cell disease (SCD), a recessive mutation resulting in abnormal hemoglobin, include severe pain, premature organ failure, and reduced survival. While opioids alleviate severe SCD pain, they produce serious adverse effects. Thus, a great need exists for effective non-opioid, non-addictive analgesics to treat this severe pain. Drugs targeting different components of the endogenous cannabinoid (eCB) system (i.e., receptors and eCB regulating enzymes) show efficacy in a humanized mouse model of sickle cell disease (SCD). In particular, Khasabova et al. (Haematologica, 2023, 108:859-869, PMCID: PMC9973472) made an intriguing observation that the hyper-nociceptive phenotype of SCD is positively associated with plasma levels of 2-arachidonoylglycerol (2-AG). Accordingly, they reported that inhibition of diacylglycerol lipase-β (DAGL-β), a biosynthetic enzyme of 2-AG, reduced the upregulated levels of plasma 2-AG in SCD mice with a concomitant amelioration of the hyper-nociceptive phenotype. In the present study, we tested whether expression of the biosynthetic enzymes (i.e., DAGL-α and -β) and primary degradative enzyme (i.e., monoacylglycerol lipase; MAGL) of 2-AG becomes dysregulated in the SCD mouse pain model. Additionally, we examined whether a MAGL inhibitor ameliorates the hyper-nociceptive phenotypic of these mice.

Methods: Adult male and female HbSS-BERK (SCD) mice and HbAA-BERK (humanized normal hemoglobin control) mice served as subjects. Initial experiments used qRT-PCR assays to quantify relative expression levels of MAGL, DAGL-α and -β, and pro-inflammatory cytokines (i.e., IL-1β, IL-6, and TNF-α) in dorsal root ganglia (DRG). Additionally, we investigated whether the selective MAGL inhibitor MJN110 would ameliorate hypersensitivity to mechanical stimulation (von Frey assay), reduction of grip strength, and impaired nesting behavior in SCD mice. Subjects were also administered rimonabant (CB1R antagonist) and SR144528 (CB2R antagonist) to infer the role of these receptors. Finally, we examined whether the SCD mice and control mice would show differential effects to MJN110 in the conditioned place preference (CPP) paradigm.

Results: SCD mice showed a three-fold increase in expression levels of MAGL mRNA in DRG compared with control mice, but both genotypes displayed negligible differences in DAGL-α and -β expression. Moreover, IL-1β and IL-6, but not TNF-α, mRNA levels were significantly elevated in SCD mice. MJN110 significantly attenuated hyper-nociceptive responses of SCD mice in the von Frey assay, which was prevented by both CB1R and CB2R antagonists. MJN110 also normalized grip strength in SCD mice through a CB2R mechanism, but it failed to improve nesting behavior. While MJN110 did not produce a place preference or place aversion in control mice, it led to a significant place preference in SCD mice.

Conclusions: These results indicate that SCD mice show an upregulation of MAGL in DRG that may contribute to their pro-inflammatory and hyper-nociceptive phenotype. Accordingly, inhibition of this enzyme normalizes hypersensitivity to mechanical stimulation as well as normalizes a subset of functional behavior in the SCD mice. The results from the CPP paradigm suggest that MAGL inhibition lacks intrinsic reward properties in control mice but provides relief from an internal aversive state in SCD mice. Finally, multiple mechanisms of action (i.e., 2-AG stimulation of CB1Rs and CB2Rs, and possibly reduced production of the 2-AG metabolic product, arachidonic acid as well as its bioactive metabolites) mediate the pharmacological effects of MAGL inhibition.

Acknowledgements: P30 DA033934, R01 DA04357, T32 DA007027, 5TL1DK132771, HL147562, Southern Regional Education Board.

NEUROBIOLOGICAL UNDERPINNINGS OF CANNABIDIOL'S ACTION IN ATTENUATING OPIOID RELAPSE

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Introduction: Drug addiction is a chronic relapsing disorder characterized by cycling periods of compulsive drug use, abstinence, and relapse. Cannabidiol (CBD), a non-intoxicating cannabinoid, is currently under investigation as an anti-relapse treatment. Previously, our laboratory demonstrated that CBD attenuates cue-induced heroin-seeking in an animal model of relapse (Ren et al., 2009). Clinically, our group also showed that CBD attenuates craving and anxiety induced by drug-associated cues in abstinent individuals with heroin use disorder (Hurd et al., 2019). The exact mechanisms by which CBD exerts its anti-relapse effects are poorly understood. The objective of the current study was to assess the effects of CBD administration on heroin-seeking in conjunction with transcriptomic profiling in the basolateral amygdala (BLA).

Methods: Male Long Evans rats were trained to intravenously self-administer heroin over 15 days followed by 14 days of forced abstinence. Rats were acutely injected with either vehicle or CBD (5 or 10 mg/kg, i.p) 24 hours prior to a drug-seeking session. Blood was collected 1 hour after the CBD administration, and brains were extracted 1.5 hours following the drug-seeking session. Plasma was used to measure endocannabinoid and CBD levels. BLA tissue was dissected, and bulk RNA sequencing was performed.

Results: Both doses of CBD attenuated heroin-seeking during the drug-seeking test compared to vehicle controls. Acute CBD administration increased the levels of CBD, 7-OH-CBD, anandamide, and arachidonic acid. The BLA differential gene expression signature observed with heroin-seeking was normalized by CBD, particularly in processes relevant to morphine addiction, chemical synaptic transmission, and neuronal projection. Interestingly, a known CBD target, TRPV1, was amongst the normalized genes and correlated with active lever responding at the test.

Conclusions: These findings suggest that CBD reduces cue-induced drug-seeking behavior associated with normalizing BLA biological pathways impacted by heroin.

Acknowledgements: Funded by NIH DA048613 & FRQNT 285137.

MODULATION OF OPIOID-INDUCED BEHAVIORS BY PUTATIVE GPR55 RECEPTOR ANTAGONIST KLS-13019

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Introduction: Despite the addictive potential of opioids, these medications are the most effective pain reliever for many scenarios. Studies have shown that the adverse effects of opioid medications (tolerance, withdrawal) may be attributed pro-inflammatory pathways that are activated over the course of chronic administration. Cannabidiol (CBD) has shown anti-inflammatory effects in addition to mitigating the adverse effects of opioids. These effects may be mediated through the antagonism of G-protein coupled receptor 55 (GPR55), a putative cannabinoid receptor involved in inflammatory cascades. CBD is a weak antagonist of GPR55 with low bioavailability. While CBD has therapeutic properties, there is a need for the optimization of related compounds that maximize specificity, bioavailability, and potency. Our industry collaborators at Kannalife have synthesized a CBD analog, KLS-13019, with improved bioavailability and selectivity for GPR55. Considering the anti-inflammatory effects of KLS-13019 and findings with CBD regarding opioid effects, we tested the hypothesis that KLS-13019 would mitigate tolerance and withdrawal associated with chronic opioid administration in male C57Bl/6 mice.

Methods: To induce behavioral tolerance, C57BL/6 mice were treated every 12 hours with morphine sulphate (MS) at 10mg/kg for 5 days. They were then injected with KLS-01913 or vehicle and tested for latency in seconds while applying their paw to a hot plate to assess the maximum potential effect (MPE) of MS. For our withdrawal studies, C57BL/6 mice were given increasing doses of MS every day (2, 4, 6, 8, and 10mg/kg). Following the last MS dose, animals were given either KLS-01319 or vehicle. Two hours later, all animals were given naloxone to precipitate withdrawal, and placed immediately into plexiglass cages. Withdrawal behavior was assessed by visualizing the number of jumps over a 30min period.

Results: Our results show that KLS-01319 pretreatment reduced both behavioral tolerance (increased latency on the hotplate) and jumping behavior following naloxone-induced withdrawal vs both CBD and vehicle. Furthermore, the mice given KLS-01319 also have diarrhea, which may indicate an additional reversal of morphine-induced constipation.

Conclusions: In conclusion, our novel compound has shown improved efficacy in the attenuation of the adverse effects of chronic MS administration when compared to CBD. This work will be important for the development of specific compounds that allow for the therapeutic application of opioid pain medications without the adverse consequences that are associated with opioid administration.

Acknowledgements: This work was supported by R42 NS120548-02 (DB, SJW) and Internal funding granted to SJW by LKSOM.

EFFECT OF REPEATED MILD TRAUMATIC BRAIN INJURY DURING ADOLESCENCE ON THE ENDOCANNABINOID SYSTEM

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Introduction: Mild traumatic brain injuries (mTBI), like concussions, constitute 80% of brain injury cases worldwide, with ~23% occurring in adolescent populations. mTBI are characterized by rotational acceleration and deceleration of the brain within the skull that causes stretching, compression and shearing of white matter tracts, driving diffuse axonal injury. Adolescents experience high rates of mTBI, primarily from contact sports, which often occur in a repetitive manner (RmTBI). RmTBI can trigger secondary neuroinflammatory events, and ~30% of affected individuals have prolonged motor and cognitive deficits. Due to heterogeneous clinical symptoms, effective therapeutics are challenging to identify, placing a large burden on this population. The endocannabinoid (eCB) system is involved in neuroprotective effects following brain injury. eCBs like 2-Arachidonoylglycerol (2-AG) and anandamide (AEA) bind to cannabinoid receptors (CBR) located pre-synaptically on neurons and in glial cells to modulate neural activity and neuroinflammatory processes. The eCB system is primarily anti-inflammatory, with immune cells, including microglia, expressing CB1/2 receptors and secreting eCBs. Previous studies have shown that eCB expression increases following trauma and that activation of the eCB system is involved in repair mechanisms, thus providing a potential neural target for therapeutic intervention in adolescent RmTBI. The overall aim of this project is to assess the cognitive and motor effects of adolescent RmTBI in a translational rodent model and quantify the effects of RmTBI on eCB expression and signaling.

Methods: Adolescent (P34) male Sprague-Dawley rats were administered 5 mTBIs at 72-hour intervals to model RmTBI. We had 3 primary endpoints: 1) eCB levels assessed immediately following RmTBI, 2) eCB levels assessed 1-week post-RmTBI, and 3) neurobehavioral testing and neuroinflammatory profiling. mTBI were administered via the lateral impactor. Blood and brain tissue were harvested in both eCB cohorts to quantify endogenous 2-AG and AEA levels and plasma was processed for inflammatory cytokine analysis. Neurobehavioural assessments included the hanging bar task (motor), light-dark box (LDB; anxiety) and novel context mismatch (cognition). The hanging bar task was administered pre-RmTBI and 24 hours post-RmTBI. LDB was performed 1-week post-RmTBI and novel context mismatch was performed 2 weeks post-RmTBI. Following all behavioural analyses, brains were perfusion-fixed and processed for immunohistochemistry to quantify microglia and astrocyte (density, morphology and phenotype).

Results and Conclusions: Compared to sham animals, RmTBI significantly increased righting times (time it takes for rat to flip from prone position to supine; indicative of loss of consciousness/injury severity), when normalized to isoflurane time ($p>0.0001$). RmTBI animals significantly decreased time to hang compared to their base line prior to RmTBI ($p>0.001$). LDB, NCM, eCB analysis via mass spec and astrocyte analysis via immunohistochemistry are ongoing. This research has shown a validated concussion model and further investigates the role the eCB plays following injury. Future studies will investigate a therapeutic intervention to increase levels of 2-AG and AEA post injury to assess behavioural changes.

Acknowledgements: This research was supported by the CIHR Foundation Grant and MIST program to MN Hill and Branch Out Neurological Foundation to L Javorcikova.

UNRAVELLING SCHIZOPHRENIA SUSCEPTIBILITY INDUCED BY "*IN UTERO*" CANNABINOID EXPOSURE: EXPLORING LIPIDOMIC ALTERATIONS IN PATIENT-DERIVED CEREBRAL ORGANOIDS

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Introduction: Prenatal cannabinoid exposure (PCE) is implicated in disrupting fetal brain development and increasing susceptibility to neuropsychiatric disorders, including schizophrenia (SZ), cognitive, and mood/anxiety disorders. However, the underlying mechanisms remain poorly understood. Our research aims to elucidate these mechanisms using human-derived cortical organoids. Disturbances to the neurolipidome, representing alterations in the fatty acid and phospholipid composition of neural cells, are a hallmark of schizophrenia. Recent research on prenatal Δ9-tetrahydrocannabinol (THC) exposure has revealed persistent neurolipidomic abnormalities in rodents, alongside cognitive and emotional deficits resembling prodromal schizophrenia stages. As schizophrenia involves complex human-specific factors, we utilized patient-derived cerebral organoids to characterize lipidomic anomalies within schizophrenia and explore how prenatal cannabinoid exposure alters the neural lipidomic landscape.

Methods: Human cerebral organoids were derived from induced pluripotent stem cells. Organoids from healthy controls (n=4) and SCZ (n=4) patients were exposed to THC (100ng/ml), cannabidiol (CBD; 500ng/ml), and THC-CBD combination (100ng THC/500ng CBD) for 15 days, until organoids reach 1 month of development; a period resembling early cortical growth. Techniques included lipidomic analyses using MALDI IMS, gene expression via immunofluorescence, western blotting, quantitative PCR, and RNA sequencing. Electrophysiological characterization using a 3D brain microelectrode array and *in vivo* extracellular electrophysiology is ongoing.

Results: Preliminary characterization revealed expected neuronal markers in organoids at day 30 and Day 180 developmental stages. THC-CBD and THC exposure showed distinct lipidomic (mass spectrometry imaging) and metabolomic (RNAsec) profiles compared to CBD and VEH in control cell lines; this was far more pronounced in SCZ lines. SCZ organoids exposed to THC exhibited severe alterations in all assessed metrics. These changes resemble rodent model findings of prenatal THC exposure and lipidomic and molecular anomalies. RTqPCR showed differential expression of neuronal markers, particularly in THC+SZ organoids. Comprehensive RNA sequencing data comparing treatment groups will be presented, focusing on lipidomic pathways.

Conclusions: Our study highlights the critical role of lipidomic dysregulation in mediating the neurodevelopmental effects of prenatal cannabinoid exposure and elucidates novel pathways underlying schizophrenia susceptibility. By leveraging patient-derived cerebral organoids and advanced multi-omics approaches, we provide valuable insights into the complex interplay between cannabinoid exposure, lipid metabolism, and psychiatric disorders, paving the way for the development of targeted interventions for at-risk individuals.

Acknowledgements: No conflicts of interest. Funded by NSERC and CIHR.

CANNABIS USE AND METABOLIC DISEASE RISK: EFFECTS OF FLOWER CANNABIS USE (CBD VERSUS THC VERSUS CBD+THC) OR NONUSE ON INSULIN SENSITIVITY AND INFLAMMATION

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Introduction: Epidemiological data demonstrate that cannabis use may be associated with lower risk for type 2 diabetes, in part, due to lower body mass index (BMI) and better insulin sensitivity. However, much of this work is based on retrospective survey data so more rigorous designs are needed to better understand whether there is a mechanistic relationship between cannabis use and risk factors for metabolic disease.

Methods: The current study compared participants who used one of three cannabis flower products differing in their cannabinoid ratio (CBD dominant, THC dominant, CBD+THC) over four weeks of use to a non-user control group to assess changes in inflammatory biomarkers and insulin sensitivity. A total of 125 participants (average age 29.73 (sd=5.51), 59% female, 78% white) who were either did (n=97) or did not (n=28) use cannabis regularly were recruited for a study on cannabis use and insulin sensitivity. Participants who use cannabis were quasi-randomly assigned to purchase and use a cannabis flower product that was either THC-dominant, CBD-dominant, or contained approximately equal parts THC and CBD. Anthropometric data and blood were collected at baseline and a frequently sampled oral glucose tolerance test was undertaken to calculate the Matsuda Index; a measure of insulin sensitivity. Participants used their assigned cannabis product for 4 weeks, then returned to repeat all baseline measures and (for cannabis use groups) report frequency of study cannabis use.

Results: Groups were equivalent in BMI and all demographic characteristics. Pro-inflammatory biomarkers (TNF-α, IL-6, IL-1β., IL12, IFNG, and IL4) were examined as a composite and the chemokine MCP-1 was examined separately to estimate ambient inflammation. Glucose and insulin were sampled prior to and at 30, 60, 90 and 120 minutes post-consumption of 75g of glucose in the otherwise fasted state to calculate the Matsuda Index. Multilevel models assessing the effects of group (THC, CBD, THC+CBD, non-user), time, and the group by time interaction were estimated for each outcome. Controlling for sleep quality, energy balance, and alcohol use, there was a main effect of group on composite inflammation ($F(3,99) = 6.05$, $p<.001$), such that non-users had higher ambient inflammation than the THC group ($p<.001$), the THC+CBD group ($p<.001$) and the CBD group ($p<.05$). For MCP-1, there was a marginal group by time interaction ($F(3,69) = 2.68$, $p=.05$) such that there was no change non-users ($p=.53$) while there were significant increases in MCP-1 for both the THC ($p<.05$) and CBD (<.01) groups, and a smaller, non-significant increase for the THC+CBD group ($p=.18$). Controlling for BMI and inflammation, there were no significant effects of group, time, nor a group by time interaction on the Matsuda Index of insulin sensitivity.

Conclusions: Participants who use cannabis regularly exhibited less ambient inflammation as compared to those who do not use cannabis, though these differences were stable over time and seemed not to be influenced by the cannabinoid profile of the products that were used. Nevertheless, lower inflammation was not commensurate with greater insulin sensitivity in cannabis users.

Acknowledgements: Funded by the National Institute on Drug Abuse (1R01DA50515).

CHRONIC THC EXPOSURE DURING ADOLESCENCE PRODUCES PERSISTENT ECONOMIC DEMAND ABNORMALITIES IN 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 currently not well understood. To address this knowledge gap, our laboratory conducted longitudinal studies in nonhuman primates to examine the impact of chronic exposure during adolescence to delta-9-tetrahydrocannabinol (THC) 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 late 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, a touchscreen-based economic demand procedure was used to examine reward sensitivity by evaluating the extent to which these subjects, now adult, would defend consumption of a palatable food reinforcer, that varied across sessions in magnitude, in the face of escalating response requirements.

Results: All subjects exhibited demand functions that were well characterized by validated exponential equations. In drug-free control subjects, increasing reward magnitude produced orderly decreases in elasticity (alpha) that were well characterized by a simple linear regression (R^2=0.94). However, this fundamental magnitude/elasticity relationship was dose-dependently disordered in subjects treated with THC during adolescence (R^2 values =0.75 and 0.37 in, respectively, low and high dosage groups).

Conclusions: Taken together, chronic THC treatment during adolescence produced reward sensitivity deficits that persisted into adulthood, as assayed by a touchscreen-based economic demand procedure. These data are of particular concern in view of the ubiquitous role that basic reward processes play in everyday life.

Acknowledgements: Funded by the National Institute on Drug Abuse (Grant R01-DA047575).

BRIDGING THE SCHISM: CANNABINOID CB1 RECEPTORS REGULATE ODORANT-STIMULATED SALIVATION IN THE MOUSE

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Introduction: Salivation is required for essential tasks such as chewing and swallowing. Many important drug classes cause dry mouth as a side effect, contributing substantially to patient non-compliance. Over the medium-term losing the antibacterial properties of saliva results in greater rates of tooth decay and loss. The available treatments are mostly palliative. Cannabis user complaints of dry mouth point to likely cannabinoid regulation of salivation. We determined that cannabinoid regulation of basal salivation likely occurs via CB1 receptors on cholinergic neurons that innervate the submandibular gland. Basal salivation derives from the submandibular gland, while the adjoining parotid gland is responsible for stimulated salivation, the sort that occurs in response to olfactory or other cues. Almost no work has been done to examine cannabinoid regulation of parotid gland function or stimulated salivation. We investigated this with functional and protein-expression studies of stimulated salivation in mice.

Methods: Both male and females, as well as C57, CD1 and CB1-/- mice, between the ages 2-8 month were used in these experiments. Mice were exposed to odorant feed 3 times to establish associative learning. Mice them were tested with a saliva-collecting cannula, first to establish baseline salivation and then with concomitant exposure to an odorant to measure odorant-stimulated salivation. CP55940 was also used as a CB1 agonist. Mouse submandibular and parotid glands were collected for immunohistochemical analysis. Data were analyzed using one-way ANOVA with Bonferroni post-hoc tests, $p<0.05$ was considered significant.

Results: We observed a sex-dependence in stimulated responses to food-related odorants. We tested peanut butter and found that only male mice saw an increase in salivation in response to the odor. With a chocolate hazelnut Nutella and found that now only females increased salivation. In each case, we found that the CB1 receptor agonist CP55940 (0.5mg/kg, IP) reliably lowered baseline salivation, as shown previously, but also prevented the odorant-induced increase in salivation. And in each case, CB1 receptor knockout mice also saw no enhanced salivary response. Using immunohistochemistry we determined that CB1 receptors are expressed in neurons innervating the parotid gland, paralleling our findings in submandibular gland.

Conclusion: We find an interesting sex-dependence in responses to odorant cues, in both sexes, and CB1 receptor activation and deletion inhibit this odorant-stimulated salivation. The expression of CB1 receptors on neuronal inputs suggests that CB1 receptors inhibit two modes of salivation from distinct salivary glands via a similar mechanism.

SEX-SPECIFIC EFFECTS OF ADOLESCENT THC EXPOSURE ON mPFC MICROGLIA MORPHOLOGY, PRUNING, AND BEHAVIOR IN MICE

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Introduction: The use of cannabis during adolescence raises the risk of developing a psychotic disorder later in life. Adolescence marks a crucial developmental period for the prefrontal cortex (PFC), a region of the brain pivotal for decision-making and reward processing, as it undergoes final maturation and restructuring. While the developing PFC's flexibility allows it to adapt to environmental demands, it also offers a window of vulnerability to insults in the form of drugs and other negative environmental influences.

Microglia-mediated synaptic pruning, the process of eliminating inactive synapses, plays a significant role in the maturation and reorganization of the PFC. Any disruption in synaptic pruning can have adverse effects on neural circuits and behavior. Cannabis use during adolescence disrupts proper synaptic pruning and impairs connectivity within PFC circuits. Δ^9-tetrahydrocannabinol (THC), the primary psychoactive compound in cannabis, may interfere with PFC maturation by triggering microglia activation, leading to excessive pruning of synaptic terminals.

We hypothesized that impaired synaptic pruning in the PFC could be a mechanism through which THC negatively impacts behaviors.

Methods: In this study, we are focusing on the morphological changes in mPFC microglial cells that accompany the so-called activated state. Activated microglia show altered morphology e.g., decreased branching. We are employing Sholl analysis as well as other descriptive measures to determine the relative proportions of resting/activated microglia in brains from mice treated with THC or vehicle during adolescence (postnatal day 28-49) and adulthood (PND 90-111). To explore the pruning, we apply immunohistochemical approaches to quantify synapses within the PFC. We also explore the potential of co-treatment with CBD to mitigate these effects. Given the sex-specific nature of THC's impact on the brain, our study includes cohorts of both males and females. Furthermore, we evaluate anxiety-like, obsessive-like behaviors, and working memory to comprehensively assess the outcomes of altered microglial activation.

Results: In adolescent males, THC causes morphological changes of microglia and synaptic pruning. In adolescent females and in adult animals of both sexes THC treatment does not cause changes in microglial morphology.

Conclusions: Adolescent treatment with THC promotes sex-specific activation in microglia and altered pruning in PFC of male mice. Such changes may impact several behaviors including working memory, obsessive-like and anxiety like behaviors.

Acknowledgements: This work was supported by DA043982 and AT011162 grants.

EFFECTS OF POST-INJURY CBD ADMINISTRATION IN A NEW MODEL OF BRAIN DAMAGE OF INFLAMMATORY ORIGIN IN RAT NEWBORN

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Background: Perinatal infection is a well-recognized risk factor for cerebral palsy, long-term disability and mortality in term newborns. Nevertheless, so far anti-inflammatory treatments have been ineffective or exert unacceptable side effects. Therefore, Cannabidiol (CBD) has proven anti-inflammatory-based neuroprotective effects in other newborn rat models of brain damage.

Aims: to assess the neuroprotective effect of CBD in single and multiple doses in a new model of neonatal acquired brain damage of inflammatory origin in neonatal rats.

Methods: Inflammatory Hypoxic-Ischemic brain insult was induced by intraperitoneal (i.p) administration of 100 µg/kg lipopolysaccharide to P7-P10 newborn *Wistar* rats 4 h prior to hypoxia-ischemia (LPS-HI) [electrocoagulated carotid artery and 50 min of O_2 10% + N_2 90%]. 30 min after the end of the hypoxemic insult, vehicle (LPS-HI-VEH) or CBD 5 mg/kg was administered intraperitoneally (i.p.) in single dose (LPS-HI-CBD 1x) or multiple doses for 7 days (LPS-HI-CBD 7x). Similarly manipulated but without hypoxia-ischemia event rats served as controls (SHAM). At short (P14) and long time (P37), brain damage was assessed by magnetic resonance image, white matter injury (WMI) was analyzed by measuring the area of corpus callosum (CC) by hematoxylin-eosin staining, neurofunctional tests were performed (P14: motor behavior [negative geotaxis (coordination test), Grasp (fine reflex) test, Grip (gross reflex) test]; P37: Cylinder Rear Test (hemiparesis), Beam test (coordination), Novel Object Recognition (working memory), open field [distance (motor activity) and entries (anxiety-like behaviour)], For biochemical studies, samples were taken 24h, 7 and 30 days after brain injury. Western blot was performed to analyze neuroinflammation (TLR4, TNFa, IL1B markers) as well as apoptosis (Caspase-3 active) and oxidative stress, by determining protein nitrosylation (Oxyblot).

Results: Inflammatory/Hypoxic-Ischemic model reduced % brain volume and led WMI as shown by the reduction of CC. These were associated with functional damage observed both in the short and long term. Those effects were associated with neuroinflammation (observed by increased TLR4, TNFa and IL-1B), apoptosis (observed by increased Caspase-3 active) and oxidative stress (observed by increased in nitrosylated proteins). At short term a single dose of CBD administration prevented functional damage in spite of not being able to reduce the volume of brain damage but prevent white matter injury. At long term, a single dose of CBD prevented working memory impairment, but did not prevent impairment in coordination, development of hemiparesis. This was associated with no benefit in the reduction of brain volume damage, WMI as well as lack of protection against neuroinflammation, apoptosis and oxidative stress. On the other hand, at short- and long-term, multiple doses of CBD were able to increase brain volume (%) and reduce WMI. This was associated with the prevention of short- and long-term functional damage. According to biochemical assays, the CBD administration in multiple doses prevented the increase of inflammation, oxidative stress and cell death by apoptosis.

Conclusions: A single dose of CBD 30 min post-injury does not present sufficient therapeutic potency to prevent all the sequelae associated with brain damage of inflammatory origin as opposite to multiple doses of CBD. These results could be used as a basis for the study of different diseases whose therapeutic target is inflammation (i.e. meningitis or trauma).

ACEA SEX- AND DOSE- DIFFERENCES AND ITS RELIANCE ON CB1 AND 5-HT_{1A} in THE FORMALIN INFLAMMATORY PAIN AND TETRAD TESTS

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Introduction: The prevalence of chronic pain is rising and afflicted patients frequently receive significant reductions in their quality of life and productivity. Inflammatory pain is a key component of chronic rheumatic diseases, which represent the largest division of chronic pain causes, along with being implicated in acute infectious or traumatic causes of pain. Cannabis has a well-established history as an analgesic and recent sociopolitical and cultural changes have renewed interest in its potential benefit as an adjuvant or alternative treatment for pain. Although cannabinoids act at numerous receptors, there are traditionally two receptors that are considered cannabinoid receptors. Cannabinoid Receptor 1 (CB_1) is primarily, but not exclusively, located in the central nervous system and has been implicated in many of the psychoactive and antinociceptive effects of cannabis. Recently, the serotonin 1A receptor (5-HT_{1A}) has been demonstrated to have antinociceptive properties following its activation and has been associated with Cannabinoid Receptor 2. Arachidonoyl 2'-chloroethylamide (ACEA) is a selective CB_1 agonist that is frequently used in the evaluation of cannabinoid receptor activity. In this present study, we evaluated possible sex differences in ACEA analgesia in C57BL/6j mice in the formalin model of inflammatory pain. Furthermore, we assessed the effects of ACEA at the most effective dose in the classical tetrad test of cannabinoid activity: using the formalin test for assessing pain, the rotarod test to evaluate motor coordination, the open field test to evaluate anxiety, and rectal temperature. Finally, we confirmed that the effects of ACEA were due to activation of the CB_1 receptor via pretreatment with CB_1 inverse agonist AM251 and to evaluate a possible role for the 5-HT_{1A} in the mediation of these effects.

Methods: This study was performed using adult male and adult female C57BL/6j wild-type mice. Mice were pretreated via i.p. injection with either vehicle or ACEA (at doses 0.1, 0.25, 0.5, 0.75, 1, or 2.5 mg/kg) and then allowed to adapt for approximately 25 minutes. Mice were then injected s.c in their left hind paw with 10 µL of 2.5% formalin solution and their pain behavior was scored for the following hour, with pain behavior quantified by the composite pain score. Following the formalin test, mice were euthanized and brain and spinal cord tissue were collected. The most effective dose was then chosen (ACEA 0.5 mg/kg) and used for subsequent testing. A separate cohort of mice were then evaluated in the tetrad test following treatment with vehicle solution, treatment with ACEA, pretreatment with AM251 followed after 30 minutes by ACEA, or pretreatment with selective 5-HT_{1A} inhibitor WAY-100635, followed after 30 minutes by ACEA. Data was analyzed using one-way ANOVA with Bonferroni post-hoc, two-way ANOVA with Bonferroni post-hoc, or ANOVA with repeated measures with Greenhouse-Geisser correction, as appropriate ($p < 0.05$ was considered significant).

Results: ACEA provided antinociceptive effects in the formalin test during the acute phase in male mice only (at 0.75 mg/kg) and in the inflammatory phase in both male and female mice (at 0.5 mg/kg for both and 0.75 mg/kg in male mice only). No significant sex differences were noted in either phase; in both sexes, 0.5 mg/kg was the most effective dose in the inflammatory phase. ACEA produced no significant effects in the open field of anxiety; however, the group of female mice receiving pretreatment with AM251 prior to ACEA were noted to have an increase in anxiety associated behaviors. ACEA caused no significant alterations in motor coordination in male or female mice. ACEA caused an increase in rectal temperature in male, but not female, mice beginning at 90 minutes post-injection. Evaluation of the antinociceptive effects of pretreatment with either AM251 or WAY-100635 prior to ACEA injection are ongoing. RT-qPCR analysis for changes in gene expression of cannabinoid receptors, inflammatory markers, and cannabinoid tolerance markers will also be conducted.

Conclusion: This study demonstrates significant antinociceptive efficacy of CB_1 agonists in the formalin model of inflammatory pain. Further research is ongoing to better understand the effects of CB_1 agonism on gene expression and the mechanisms of CB_1 analgesia in the formalin model of inflammatory pain.

Acknowledgements: Funding via NIH R01 NIDA 044999-01A1S1 (JG), NIH R01 NIDA 044999-01A1 (JG), and Grant 121035 Texas Tech University (JG).

ASSESSING THE EFFECTS OF CANNABICHROMENE ON CHEMOTHERAPY-INDUCED NEUROPATHIC PAIN

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Introduction: Chemotherapy-induced peripheral neuropathy (CIPN) is a debilitating adverse effect experienced by roughly 30-40% of patients receiving chemotherapy treatment. Unfortunately, current therapeutic strategies require long-term treatment with limited efficacy, often requiring opioid-based medications. Studies have suggested the potential utility of cannabis-based medicines to alleviate neuroinflammation and subsequent pain. While multiple studies have explored delta^9-tetrahydrocannabinol and synthetic cannabinoids, a cannabinoid devoid of psychoactive effects would likely be a stronger candidate for drug development. Our current study utilizes cannabichromene (CBC), shown to have anti-inflammatory properties while devoid of psychoactive effects, evaluated the effectiveness of CBC and related derivatives on cisplatin and paclitaxel-induced neuropathic pain while also assessing if CBC can provide continued relief against these types of pain.

Methods: To assess the efficacy of CBC against CIPN, mice were subjected to either a cisplatin (2.3 mg/kg; 6 total injections) or paclitaxel (4 mg/kg; 4 total injections) dosing protocol. Following the administration protocols, single doses of CBC were administered 30 minutes prior to CIPN assessment. Mechanical sensitivity was assessed using an electronic Von Frey (eVF) to measure changes in response to mechanical stimulation of the hind paw at various stages (e.g., pre-chemotherapy, post-chemotherapy, and with treatment onboard). To determine the duration of relief of CBC against CIPN pain, mechanical sensitivity (i.e., utilizing the eVF) was assessed at 30 min, 4 hrs, 24 hrs, and 72 hrs.

Results: Acute administration of CBC in both male and female mice ablated the allodynia associated with cisplatin-induced neuropathy in a dose-dependent manner. Similar to oxycodone-treated controls, mice that received greater than 10 mg/kg CBC or CBC derivative showed withdrawal responses at the level of non-cisplatin treated control mice. However, CBC was not efficacious in attenuating paclitaxel-induced neuropathy. When examining the duration of relief, we see that CBC maintains its protective properties in male mice for up to 24 hours.

Conclusions: These data indicate that CBC can delay the onset of cisplatin-induced neuropathic pain and suggest its potential as a promising therapeutic for preventing or alleviating neuropathic pain. Further studies will explore the efficacy of CBC against other chemotherapies, such as oxaliplatin and vincristine. As well as, elucidate the effects of repeated dosing, sex-specific differences in pain and cannabinoid responsiveness, and determine the optimal therapeutic window of CBC.

Acknowledgments: P30GM122733.

EFFECTS OF CP55,940 ON STEP KINEMATICS DURING TREADMILL RUNNING IN MARKER-BASED 3D MOTION CAPTURE OF MICE

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Introduction: In our previous studies, we developed a marker-based 3D motion capture system to study fine kinematics in mice. We showed that CP55,940 at low doses significantly affects step kinematics in mice during vertical climbing, without affecting the total distance traveled. In this study, we aimed to test whether similar changes will be observed in mice during running on a treadmill at various speeds.

Methods: We used adult male C57BL/6 mice in a within-subject, randomized design (n=6-10). We recorded voluntary climbing behavior on a spoked mesh wheel, in mice treated with vehicle (1:1:18 EtOH, Kolliphor, saline) or a low dose of CP55,940 (0.3 mg/kg). 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) was used to track 3D trajectories and velocity of markers during voluntary locomotor tasks: climbing and running. Mice were recorded running for 30 s at increasing speeds (15, 20, 30 and 40 m/min) until failure.

Results: The maximum treadmill running speed achieved was significantly decreased by CP55,940 (0.3 mg/kg) from 28 ± 3.7 to 15 ± 3.9 m/min (mean ± SEM). In control conditions during treadmill running ankle swings had significantly higher mean and maximum speeds than during vertical climbing or open field exploration. CP55,940 reduced the height of the ankle swing during treadmill running, similarly as it does during climbing. CP55,940 did not reduce general locomotion during climbling but affected step kinematics decreasing step height and speed which is consistent with our previous observations.

Conclusions: Low dose CP55,940 impairs the ability to achieve higher speeds of voluntary treadmill running in mice. Moreover, it affects step kinematics even in conditions that do not inhibit general activity and decreases step height both during vertical and horizontal locomotion.

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

EXAMINING THE EFFECTS OF CHRONIC THC EXPOSURE ON AUDITORY FEAR CONDITIONING, FEAR EXTINCTION, AND SPONTANEOUS RECOVERY IN MALE AND FEMALE RATS

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Introduction: PTSD is characterized by increases in fear memory and impairments in fear extinction. Current treatments available include cognitive behavioural therapy (CBT), eye movement desensitization and reprocessing (EMDR) and prolonged exposure therapy. Often, these treatments take place in lab or clinical environments, which do not represent the context where the fear memory association took place**.** As a result, these treatments are not always effective, and individuals can experience a re-emergence of fear memory with the passage of time or spontaneous recovery. Research has shown that the eCB system plays a modulatory role in fear responses and memory for aversive stimuli. However, much of this research has investigated the effects of cannabis and tetrahydrocannabinol (THC) on fear memory has demonstrated mixed effects. Much of this research has focused on the effects of cannabinoids on fear extinction and retrieval, with their effects on conditioning (fear memory acquisition) studied less extensively. As inhalation is the primary form of cannabis consumption in humans, modelling this approach in rodents is important given the robust impact route of administration has on the pharmacokinetics and biodistribution of THC and its metabolites. Using a validated model of controlled passive THC inhalation, we can now fully examine the impact of chronic cannabis exposure on fear conditioning, extinction and spontaneous recovery.

Method: Sixteen male and sixteen female adult Sprague-Dawley rats were habituated to the vapour chamber for three days, following this, animals were randomly assigned to one of two vapour conditions: 1) vehicle (PEG) or 2) THC (10%) and subsequently exposed to vapour for 15-min once a day for ten days prior to auditory fear conditioning. Following ten days of vapour exposure, on day 11, all rats underwent fear conditioning where a conditioned stimulus (80dB, 4Hz tone) was paired with an aversive unconditioned stimulus (0.65mA foot shock) over seven trials. The following day, all animals underwent extinction training where the conditioned stimulus was no longer paired with the aversive outcome. Twenty-four hours and two-weeks later animals were re-exposed to the conditioned stimulus in the absence of the aversive foot shock. During all stages of fear conditioning, both passive (freezing) and active (darting) conditioned responses were quantified.

Results: In females, chronic THC exposure prior to conditioning had no impact on the acquisition of conditioned fear. During extinction training and the 24-hour extinction retrieval task, our results suggest that females exposed to THC extinguish fear memories at a faster rate during extinction training and show less active fear responses during a 24-hour retrieval task than females exposed to vehicle. Quantification of male behaviour is currently in progress.

Conclusion: These data suggest that chronic THC vapour exposure enhances fear extinction and fear memory retrieval in female rodents, highlighting the potential for cannabis as a treatment option for PTSD. However, further analysis is needed to elucidate these effects in males and examine any sex differences between males and females following chronic THC exposure.

INVESTIGATING THE EFFECTS OF VAPOURISED TETRAHYDROCANNABINOL (THC) ON FOOD REWARD VALUE

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Introduction: With many parts of the world legalising cannabis there is an urgent drive to assess if it's use is associated with certain health-related effects. It's well established that cannabis acutely drives food intake, commonly referred to as 'the munchies', and that tetrahydrocannabinol (THC) is mainly responsible for driving these feeding effects. Cannabis-induced feeding has been modelled in rodent and human studies, but it ultimately remains unknown how cannabis alters eating patterns. People anecdotally report that cannabis can trigger food craving and drive eating at times where food would not be otherwise be consumed, i.e., when satiated. Previous research shows that cannabis can increase food reward value, therefore, the aim of this project was to investigate if THC can promote food intake in conditions where there is no physiological drive to eat (e.g., with satiety or malaise) by increasing food motivation and reversing food reward devaluation.

Methods: Adult male and female Sprague Dawley rats were exposed to THC (100mg/ml in vehicle) or vehicle (polyethylene glycol) vapour for 15min (10s hit every 2min), to induce blood THC levels comparable to human cannabis use. To assess THC-driven feeding in regular and satiated conditions, rats were vaped, immediately given chow and intake measured. Satiety was induced by giving a 10% sucrose chow mash for 2hr prior to vapour exposure. To assess THC influence on food reward value in regular, satiated, and aversion conditions, rats were food restricted to 90% body weight and subjected to operant conditioning where they were trained to lever press on a progressive ratio (PR) schedule to acquire a food reward (45mg sucrose pellets) for 1-hr/day. On test days, rats were vaped and immediately subjected to operant conditioning. To induce satiety, 45mg sucrose pellets (same as used for conditioning) were given for 2hr prior to vapour exposure. To associate the operant task with aversion, rats were injected with lithium chloride (LiCl, 125mg/kg, IP) immediately after operant conditioning for 5 consecutive days. Revaluation index was calculated ([regular state lever presses - devalued state lever presses] / total lever presses) to assess strength of reward devaluation reversal.

Results: Irrespective of whether satiated or not, THC significantly increased chow intake in the first hour after exposure (both $p<0.05$). During PR operant responding in regular conditions, THC significantly increased the number of active lever presses (food reward paired), the number of food rewards acquired and the breakpoint (number of active lever presses met to earn last reward; all $p<0.05$). As expected, satiety and LiCl aversion alone significantly reduced operant responding (both $p<0.05$) demonstrating reward devaluation. Despite animals being in these devalued states, THC significantly increased the number of active lever presses, the number of food rewards and the breakpoint (all $p<0.05$). Revaluation index showed that in satiated conditions THC strongly reduced reward devaluation ($p<0.05$), and in aversion conditions THC had a more variable effect but still reduced reward devaluation (trend seen $p=0.059$). No significant sex effects were detected throughout.

Conclusions: We have shown that THC can disrupt homeostatic feeding patterns by promoting food intake when there is no physiological drive to eat. These feeding effects appear to be a result of THC increasing food motivation and reward value to reverse food reward devaluation. Further, these effects of THC are extremely robust, demonstrated by the ability of THC to reduce reward devaluation in different conditions (i.e., with both satiety and aversion). Work is currently underway to delve deeper into the underlying mechanisms of THC-driven feeding and motivation changes, research which is critical for fully understanding the physiological and behavioural effects of cannabis use.

ACUTE CANNABINOID-1 RECEPTOR BLOCKADE ATTENUATES THE MANIA-RELEVANT HYPEREXPLORATION INDUCED BY DOPAMINE TRANSPORTER INHIBITION

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Background: People with bipolar mania (BM) exhibit hyperexploratory behaviors and dysregulated dopamine function, including reductions in expression of the dopamine transporter (DAT). In rodents DAT inhibition reliably recreates such BM-relevant hyperexploration, which can be attenuated by BM medications (e.g., lithium), thus exhibiting predictive pharmacological validity. Given the adverse side-effects associated with treatments prescribed for BM, novel treatment options are needed. The endocannabinoid (eCB) system is also dysregulated in BM and is a promising target given its neuromodulatory role in dopamine signaling. Here, we examined the impact of altering eCB receptor activity via behavioral pharmacology on hyperexploration induced by pharmacological DAT inhibition by GBR12909.

Methods: Female and male C57BL/6J mice were tested in the behavioral pattern monitor (BPM) for 45 min once per week following systemic pretreatment with the eCB system modulators AM251 (cannabinoid-1 receptor antagonist [CB_1R], 1–3 mg/kg), AM630 (cannabinoid-2 receptor antagonist [CB_2R], 1–10 mg/kg), THC (0.3–3mg/kg), or CBD (0.3–3 mg/kg), alone, or in combination with the DAT inhibitor GBR 12909 (GBR; 0, 16 mg/kg). Repeated-measures ANOVAs were conducted to assess the main effects and interactions of pretreatments and GBR exposure on locomotor activity (distance travelled) and specific exploration (rearing) across time-binned data.

Results: GBR 12909 recreated the BM-relevant BPM hyperexploratory profile (increased locomotor activity and specific exploration). Pretreatment with the CB_1R antagonist AM251 (3 mg/kg) reduced distance travelled, but not rearing, in GBR-treated animals, with no effect in controls, specifically in blocks 2 and 3 (GBR*Pretreatment*Block: $F(6,238)=2.64$, $p=0.017$]. Pretreatment with acute AM630, THC, or CBD did not alter GBR-induced effects, though high-dose THC decreased locomotor and exploratory behavior of GBR-treated and control groups.

Conclusions: These data support: 1) reducing CB_1R, but not CB_2R, receptor function attenuates BM-relevant hyperlocomotion without attenuating BM-relevant increased specific exploration; 2) DAT function altered the role of the cannabinoid-1 receptor in locomotor patterns; and 3) neither THC nor CBD selectively affected BM-relevant hyperexploration. Thus, direct targeting of the cannabinoid-1 receptor may be suitable for attenuating BM-relevant hyperactivity.

Acknowledgements: Funded by R01-DA043535.

THE EFFECTS OF SHORT-TERM ORAL CANNABIDIOL (CBD) INGESTION ON INFLAMMATION, MUSCLE DAMAGE AND FUNCTIONAL RECOVERY FOLLOWING DOWNHILL RUNNING

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Introduction: Cannabidiol (CBD) is a naturally occurring compound from the Cannabis sativa plant. This major non-psychoactive phytocannabinoid has shown much promise for its immunomodulation and anti-inflammatory properties in mice models of arthritis and swelling.. It has gained a lot of attention for its proposed anti-inflammatory mechanisms in chronic inflammatory diseases. CBD protects function and integrity in human coronary artery endothelial cells via anti-inflammatory actions on reactive oxygen species (ROS) production, NF-κB activation, and migration and adhesion of monocytesThe primary objective of this research is to assess whether daily supplementation of a CBD medicinal cannabis oil product (cannabidiol (CBD), 150 mg or 400mg per dose) for 7 days prior to, on the day of, and 4 days following 45min of downhill running reduces systemic inflammation (12 days total supplementation) compared to a placebo in healthy physically untrained adults.

Methods: A total of 66 healthy participants will complete this 12-day (V1-V5), double blind, randomized, placebo-controlled parallel arm trial (ACTRN12619001120167). Over the 12-day treatment period, participants self-administered the treatment once a day (150mg CBD in 4ml oil per day; 400mg CBD in 4ml oil per day; or the equivalent of placebo 4ml oil per day). Following the downhill running protocol, serum markers of inflammation and muscle damage (IL-6, IL-8, IL-10, IL-12, TNFa, IL-1A, hsCRP, CK) will be assessed at 60-, 120-, and 180-minutes post-exercise following downhill running-induced muscle damage compared to a placebo. To assess the short-term effects of high and low dose CBD ingestion on serum markers of inflammation and damage (IL-6, IL-8, IL-10, IL-12, TNFa, IL-1A, hsCRP, CK) at 24-, 48-, 72- and 96-hours post-exercise following downhill running compared to a placebo.

Results: To date, 28 participants have completed all study procedures, with five more pending completion. Therefore, we will present data for all inflammatory outcomes for completed participants up until June 2024. Demographic data will be presented with summary statistics as appropriate. Inflammatory markers analyses will be via time series — on the acute and sub-acute time scales — to generate continuous inferred distribution kernels of inflammatory marker trajectories for the three trial arms. We will use a set of both parametric and non-parametric statistical tests (e.g. Bayesian, Kolmogorov-Smirnov and stochastic simulation methods) to compare the distribution kernels generated as described above in order to detect statistically significant differences between arms. All statistical analyses will be conducted with the use of SPSS 26.0 (SPSS Inc., USA), and tests will be two-tailed with a conventional level of significance of $p < 0.05$.

Conclusions: It is expected that the highest trajectory of systemic inflammatory markers identified by the time series analyses corresponds to the placebo arm. The lowest and second lowest are both statistically significant, corresponding to the 150mg and 450mg arms (not necessarily in dose order).

IS WHAT YOU SEE WHAT YOU GET? ACCURACY OF LABELED THC POTENCY ACROSS FLOWER AND CONCENTRATE PRODUCTS IN COLORADO

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Introduction: High delta-9-tetrahydrocannabinol (THC) cannabis products are quickly gaining market share across the U.S., and it is unknown whether labeled cannabinoid content accurately reflects true product potency. Prior work in this area has consisted of small studies and lacked broad representation of cannabinoids, potencies, and product types, such as high potency concentrates. This study aimed to assess discrepancies between labeled and observed cannabinoid content of flower and concentrate products, and if labeling accuracy varied by product type.

Methods: To test labeling accuracy, a representative sample of 277 products consisting of both cannabis flowers (n=178) and concentrates (n=99) were purchased and analyzed from dispensaries representing the geographical range across the state of Colorado. Cannabinoid potency analysis was performed in triplicate via HPLC-DAD in an ISO17025 accredited laboratory. Comparative analysis was used for observed versus labeled cannabinoid content. Accuracy (observed THC within ± 15% of labeled THC) by product type and presence of minor cannabinoids were also assessed. Data were analyzed using non-parametric tests (potency data was non-normally distributed) to compare labeled vs. observed values within the two product groups; chi square frequency tests were used to compare label accuracy between flower vs. concentrate products and the presence of minor cannabinoids; $p<.05$ was considered significant.

Results. In flower and concentrate products, observed THC potency was significantly lower than labeled potency ($U=18971$, $p=.001$; $U=6095$, $p=.003$). Labeling accuracy for THC content was significantly better in concentrates than flower ($X^2(2,n=277)=47.44$, $p<.001$), represented by 96.0% of concentrate products being within ±15% of labeled THC content, while only 56.7% of flower products fell within that same range. In these products, five minor cannabinoids, including cannabigerol (CBG), were found to be more abundant than cannabidiol (CBD).

Conclusions. Concentrate products were more likely to be accurately labeled than flower products. Further, when discrepancies did exist, they most often reflected lower observed THC content compared to the label. These novel data suggest standardized labeling practices in Colorado are effective for high potency concentrates. However, there may not be a one size fits all approach to testing and labeling standards for the full range of cannabis products available on state legal markets. Further, independent verification of product labels and testing procedures is needed to protect consumers and patients and ensure rigorous testing standards that apply across various types of cannabis products.

Acknowledgements. Funded by the State of Colorado's Institute of Cannabis Research (PIs Bidwell & Mackie).

THE PHARMACOKIENTICS AND PHARMACODYNAMICS OF ACUTE ORAL ADMINISTRATION OF A "FULL SPECTRUM" HEMP PRODUCT WITH A 1:1 CBD/CBDA RATIO

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Introduction: So-called "full-spectrum" cannabis/hemp products containing predominantly cannabidiol (CBD) and/or delta-9-tetrahydrocannabinol (THC), along with various other cannabinoids, are widely available. However, few clinical studies have carefully examined the pharmacokinetics (PK) and pharmacodynamics (PD) of full spectrum products. The purpose of this study was to examine the acute dose effects of a full spectrum hemp-derived CBD product that had been approximately 50% decarboxylated (i.e., ~1:1 ratio of CBD:CBDA and THC:THCA) and included the other minor cannabinoids CBDV, THCV, CBG, CBGA, CBN, D8-THC, CBC, and CBCA.

Methods: Healthy adults (n=15) completed three ascending-dose drug administration sessions and a randomized placebo control session. The study drug was an oral extract enclosed in soft gel tablets. Study doses were based on each individual participant's weight and included 1, 2, and 4mg/kg CBD (approx. 41.1, 73.4, 134.5 mg CBD; approx. 2.2, 3.9, 7.2 mg THC). PD measures (vital signs, subjective drug effects, cognitive performance) were obtained at baseline and for 8 hours post-dosing. Plasma specimens were collected during the same 8-hour sessions and at 24- and 48-hours post-dosing. PD outcomes were analyzed using 2-way ANOVAs with condition and time as repeated measures (n=15). Plasma samples from the first 12 participants were analyzed using LC/MS/MS and maximum concentrations (Cmax) and area under the curve (AUC) were derived for CBD, CBDA, THC, THCA, and relevant metabolites.

Results: Acute drug administration produced dose-orderly increases on subjective drug effect ratings and heart rate compared with placebo. The 4mg/kg dose significantly impaired performance on the Paced Auditory Serial Addition Task (a measure of working memory); performance on other cognitive tasks did not differ from placebo. Acute PD effects peaked 3-5 hours post-administration and returned to baseline by 8 hours. The 1, 2, and 4mg/kg doses produced mean plasma Cmax values of 11.7, 23.9, and 43.6ng/ml for CBD, and 1.4, 2.8, and 4.6ng/ml for THC, respectively. Plasma concentrations of CBDA and THCA were 18-24-fold higher than CBD and THC respectively, and had an earlier Tmax across all doses.

Conclusions: Acute administration of a "full-spectrum" CBD product produced dose-dependent PD effects. For PD measures, few differences from placebo were observed at 1mg/kg (41.1mg CBD/2.2mg THC). However, the 4mg/kg (134.5mg CBD/7.2mg THC) dose produced moderate subjective drug effects and significantly impaired working memory performance. Carboxylated cannabinoids exhibited substantially greater bioavailability compared with decarboxylated cannabinoids. Additional research is needed to directly compare carboxylated versus decarboxylated cannabinoids on therapeutic outcomes.

Acknowledgements: This project was funded by Cultivate Biologics.

IMPACT OF ADULT-USE CANNABIS LAWS: PERCEPTIONS OF REGISTERED MEDICAL CANNABIS PATIENTS

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Introduction: After the legalization of adult use (i.e., recreational) cannabis in Canada, some medical cannabis patients reported that they no longer had access to their preferred products due to changes in cannabis product manufacturers' priorities. Many US States have legalized adult use cannabis. The US Department of Health and Human Services has formally recommended that the Drug Enforcement Administration move cannabis from Schedule I to Schedule III and a DEA review is in progress. The effects of adult use cannabis legalization on the experiences of medical cannabis patients in the US is unknown. As Federal legalization of medical and/or adult use cannabis is anticipated, it is especially important to understand the impact of policies on those who are using cannabis therapeutically.

Methods: Researchers conducted the first known study to systematically examine the impact of adult use cannabis legalization on registered medical cannabis patients in the US. An anonymous, confidential Qualtrics survey asked a battery of questions comparing conditions before and after adult-use legalization. The survey was distributed through social media, press releases, newsletters, and email lists by LeafWell, a telehealth company operating in 35 States that connects potential medical cannabis patients with a qualified healthcare provider.

Results: Overall, participants (N = 505) rated the cost of all types of cannabis products significantly lower, and the quality and availability higher, after adult use legalization compared to before adult use legalization. Participants tended to agree that the products they were using work better for their symptoms after adult use legalization. However, a substantial minority of participants reported that adult use legalization made their medical use more problematic.
Many participants reported no longer having medical cannabis authorization because certification was too costly, they had to re-certify too frequently, medical cannabis was too costly, and/or visits to healthcare providers were too costly. Overall, participants were satisfied with their State's medical and adult use cannabis programs. Participants generally considered their own conditions to be better since adult use legalization, including comfort using cannabis, diversity of products available, overall health, legal concerns about cannabis use, symptoms, and the diversity of minor cannabinoids/terpenes in products available.

Conclusions: Overall, the expansion of cannabis markets with adult use legalization had beneficial impacts on medical cannabis patients registered prior to adult use legalization. However, experiences varied across participants and States, and problematic issues such as changes in medical system access were identified. These patterns may be informative for policy makers given the anticipated nationwide cannabis legalization.

WITHDRAWN

BEHAVIORAL AND BIOLOGICAL PREDICTORS OF VAPORIZED CANNABIS SELF-ADMINISTRATION IN RATS

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Introduction: Approximately 9% of first-time cannabis users will become dependent on cannabis, yet there are no FDA-approved pharmacotherapies for managing cannabis use disorder (CUD). This is in part due to flawed diagnostic nosology resulting in a lack of understanding of the mechanisms that give rise to CUD, as well as a conspicuous lack of translationally relevant animal models of cannabis use. To address these gaps, we have developed a model of cannabis self-administration that delivers vaporized cannabis extract in a response-contingent manner via the pulmonary route of administration that is most common in humans. We used this model to identify behavioral and biological predictors of motivation to self-administer vaporized cannabis in rats.

Methods: We conducted an extensive battery of behavioral assays in female and male Long Evans rats (N=48) prior to initiation of cannabis self-administration training and characterized endophenotypes using endpoints that correspond to the behavioral dimensions of the NIMH Research Domain Criteria (RDoC). We then used a series of linear regression analyses to determine whether behavioral and physiological parameters in the five RDoC dimensions (positive and negative valence systems, cognition, social processes, and arousal/regulatory systems) significantly predicted the number of cannabis vapor deliveries earned during a 3-hr progressive ratio test after four weeks of cannabis self-administration.

Results: The Arousal/Regulatory Systems model was significant, accounting for 59.3% of the variance in cannabis self-administration. Specifically, higher concentrations of basal corticosterone (CORT) predicted higher rates of cannabis vapor self-administration (p = .005), while lower concentrations of plasma anandamide (AEA) content predicted higher rates of self-administration (p = .008). The Cognition model was significant, accounting for 17.9% of the variance in cannabis self-administration. Specifically, better visual cue discrimination and poorer set shifting performance each predicted higher rates of cannabis self-administration (p's = .015 and .030, respectively). The Positive Valence model was also significant, accounting for 21.1% of the variance in cannabis self-administration, with greater motivation for sucrose reinforcement predicting higher rates of cannabis self-administration (p=.008). Finally, the Social Processes and Negative Valence models were not significant predictors of cannabis self-administration.

Conclusions: Our data indicate that basal CORT, circulating AEA content, high motivation for sucrose reinforcement, and greater reliance on visual cue-based strategies were all significant predictors of motivation to self-administer cannabis vapor in adulthood. Thus, these endophenotypes may precede the onset of problematic cannabis use, which could be leveraged to identify individuals with increased susceptibility for developing CUD.

Acknowledgements: Funded by NIH NIDA 5R21DA051689-02 awarded to RJM.

CB1-R NEGATIVE ALLOSTERIC MODULATION: CHARACTERIZING THE EFFECTS OF AMB300 ON HYPERDOPAMINERGIC PHENOTYPES

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Introduction: Negatively modulating the cannabinoid receptor 1 (CB1-R) has been shown to have several potential therapeutic applications. We have previously demonstrated the efficacy of the CB1-R negative allosteric modulator (NAM) ABM300 in alleviating psychosis-like phenotypes.[1] Despite promising outcomes, the pharmacokinetic profile of ABM300 reveals limited penetration into the brain (0.77 Kp, brain) raising questions about the mechanism underlying its antipsychotic effect. Here we aim to further characterize the behavioral effects of ABM300 on psychosis-like phenotypes, determining if the effects are mediated by centrally or peripherally located CB1-Rs.

Methods: We compared ABM300 with AM6545, a peripherally restricted CB1-R antagonist. We characterized their *in vitro* signalling profiles and their ability to modulate behaviours of psychosis and cannabimimetic behaviours *in vivo*. *In vitro*, both compounds were evaluated in their ability to inhibit CB1-R orthosteric agonist (CP-55, 940) signalling through arrestin recruitment and cAMP signalling utilizing the PathHunter® β-Arrestin assay well as the Eurofins HitFinder DiscoverX cAMP assay, respectively. Behaviorally, the effects of both ABM300 (10 mg/kg i.p.) and AM6545 (10 mg/kg i.p.) on the hyperlocomotive phenotype in the Dopamine Transporter Knockout (DAT-KO) genetic model as well as the amphetamine (AMPH) pharmacological model of psychosis-like behaviours have also been characterized. Finally, we plan to investigate whether these compounds can mitigate against agonist (CP-55, 940) stimulated cannabimimetic behaviours in wildtype C57B6/J mice measuring temperature, catalepsy and analgesia.

Results: *In vitro*, compared to ABM300 which demonstrated strong potency (arrestin IC_{50} 7.9 nM and cAMP IC_{50} 378 nM) but limited brain penetration *in vivo* (0.77 Kp), AM6545 similarly revealed an IC_{50} of 34.7 nM in the arrestin recruitment assay and IC_{50} of 39.8 nM in the cAMP assay. [1] Preliminary behavioural findings revealed that ABM300 effectively mitigates the hyperlocomotive in the pharmacological AMPH model, in addition to what has been seen in the genetic DAT-KO model.[1] Similarly, the peripherally mediated CB1-R antagonist AM6545 showed a reduction in the genetic model's hyperlocomotive phenotype, and a similar though less pronounced, reduction in the pharmacological model. Interestingly, AMB300 did not mitigate agonist-stimulated cannabimimetic behaviours in the tetrad assay. This is also in line with previous publications demonstrating that AM6545 does not affect agonist included tetrad behaviors. [2]

Conclusion: The results suggest that negatively targeting peripheral CB1-R, via either a NAM or an orthosteric antagonists, can reduce centrally medicated psychosis like behavior in both a genetic and pharmacological model.

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THC-DEPENDENT INCREASES IN NEURONAL ACTIVITY AND 2-AG SIGNALING IN MOUSE PREFRONTAL CORTEX AT THE INITIATION OF LOCOMOTION

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Introduction: THC triggers a dose-dependent reduction in spontaneous locomotion in mice that is characterized by general immobility interrupted by brief and stochastic locomotion events. Here, we leveraged fiber photometry, pose estimation behavioral classification, and genetic intervention to elucidate the neural basis of how THC modifies locomotor kinematic features by measuring neuronal activity and 2_AG dynamics within the medial prefrontal (mPFC) cortex in VEH and THC-treated mice.

Methods: WT mice were injected with AAV5-hSyn-$GRAB_{eCB2.0}$, while VGAT-Cre and VGLUT1-Cre mice were injected with AAV5-DIO-GCaMP6f and implanted with an optic fiber into the mPFC. Fiber photometric activity was recorded while animals openly explored a dual-view linear track chamber. Behavior was tracked using a convolutional neural network pose estimation algorithm (SLEAP). Positional data was fed into a second machine learning model to classify behaviors to reveal time-locked photometry activity and the further quantification of nuanced behavioral effects. To investigate the local network effect of THC in the mPFC, VGAT-Cre and VGLUT1-Cre mouse brains were infected with AAV5-DIO-CRISPR-Cnr1 to knockout the cannabinoid 1 receptor is respective neuron populations.

Results: mPFC glutamatergic and GABAergic activities increased concomitantly to the initiation of locomotion as indicated by increased GCaMP6f signal in VGLUT1-Cre neurons (Z-Score: 1.97 ± 0.24) and more pronouncedly in VGAT-Cre neurons (Z-Score: 8.64 ± 0.48) in response to THC treatment. We observed a gradual increase in $GRAB_{eCB2.0}$ activity linked to THC accumulation within minutes and discovered transient increases in activity associated with initiation of locomotion that increased as a function of THC concentration. Pharmacological treatment with MAGL inhibitor JZL-184 and DAGL inhibitor DO34 affected $GRAB_{eCB2.0}$ transient activities in opposite manner, suggesting the involvement of 2-AG. Behavioral changes induced by THC treatment included kinematic features (e.g. increased gait) as measured in the linear track and performing pose estimations. Some of these THC-induced impairing kinematic features were absent when deleting CB_1R in VGAT neurons.

Conclusions: These findings suggest THC differentially increases the activity of mPFC glutamatergic and GABAergic neurons, resulting in a pronounced increased of GABAergic interneurons at the initiation of locomotion compared to low activity in VEH-treated mice. Pharmacological intervention suggests 2-AG as the primary endocannabinoid released during walking events. Behavioral tracking and classification during neural recording allow to unravel THC-dependent locomotor impairment and emphasizes the nuanced kinematic impairment effects of THC beyond distance traveled. Modification of locomotor impairment by selective CB_1R-KO suggests importance of mPFC activity in regulated walk behavior and kinematic features during walk behavior.

Acknowledgements: Funded by F31 DA055448-01 and DA047626 to NS.

LONGITUDINAL STUDY OF CHANGES IN PAIN, POSITIVE AFFECT, AND NEGATIVE AFFECT AMONG PERSONS INITIATING MEDICAL MARIJUANA IN FLORIDA

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Introduction: Anecdotal evidence suggests that medical marijuana is helpful for persons with chronic pain, but there have been few prospective studies. It is possible that the mechanisms of improvement involve changes in more subjective feelings such as increases in positive affect and/or decreases in anger/irritability. We sought to assess changes in pain, pain interference, positive affect, and anger/irritability among adults initiating medical marijuana in Florida.

Methods: The Medical Marijuana and Me (M3) Study enrolled persons who were being certified for medical marijuana in Florida for one of 11 approved conditions in 2022-2023. Participants were recruited online and from various medical marijuana clinics across the state of Florida. They first completed an online baseline survey before starting medical marijuana, and then were asked to complete follow-up surveys at 3-months and 9-months. Questions included demographic characteristics, past/recent experience with marijuana, pain severity ratings in those with more than minor pain (4 items from BPI rating pain from 0 - 10), pain interference (4 items), PROMIS Positive Affect scale (15 items assessing momentary positive or rewarding affective experiences), and the PROMIS Anger instrument (5 items about self-reported angry mood and irritability over the past 7 days). We calculated means of pain severity scores, and standardized T-scores for pain interference, positive affect, and negative affect. For this analysis, we compared baseline scores with 3-month follow-up using follow-up using non-parametric paired tests.

Results: Of 602 persons who completed the baseline survey, 363 completed the 3-month follow-up and represent the sample for this analysis (mean age 42, 66% female, 85% White). At 3-months, there were statistically significant ($p<0.01$) reductions in the proportion of persons with any significant pain in the past 24 hours (48% vs 35%), reductions in average pain score on 4 BPI items from 2.50 to 1.76, and reduction in pain interference T scores from 57.2 to 52.3, all $p<0.001$. There were also overall significant improvements in positive affect T scores (37.8 to 42.2) and significant decreases in anger/irritability (58.7 to 54.2). No significant differences were seen between males and females.

Conclusions: These prospective data suggest overall improvements in pain, positive affect and reductions in anger/negative affect among persons initiating medical marijuana in Florida. Future subgroup analyses will assess whether these changes vary by clinical indications for medical marijuana and the amount of previous experience with marijuana.

Acknowledgement: This study was supported by funding from the Florida state legislature to the Consortium for Medical Marijuana Clinical Outcomes Research.

EFFECT OF CONCURRENT FAAH INHIBITION AND PROLONGED EXPOSURE THERAPY ON SYMPTOMOLOGY IN PEOPLE DIAGNOSED WITH POST-TRAUMATIC STRESS DISORDER

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Introduction: Abundant preclinical and clinical research implicates the endocannabinoid system in various stress-related conditions. Notably, anandamide (AEA), a principal eCB ligand, modulates the learning and consolidation of fear memories, processes believed to be dysregulated in post-traumatic stress disorder (PTSD). Building on this, we conducted a double-blind, placebo-controlled clinical trial to explore whether pharmacologically upregulating AEA signaling in PTSD patients enhanced the efficacy of prolonged exposure therapy.

Methods: In this trial, 101 participants with a PTSD diagnosis were randomly assigned to receive either 25 mg of the FAAH inhibitor JNJ-42165279 or placebo twice daily. After four weeks of taking the drug or placebo, participants underwent an 8-week internet-delivered cognitive behavioral therapy program concurrent to continuing the pharmacological regimen. PTSD symptom severity was assessed at trial baseline (week 0) and endpoint (week 12) using the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5). Additionally, after three to four weeks into treatment (prior to commencing the psychotherapy component), participants completed stress responsivity and fear memory tasks to assess symptomatology. At this time point, resting state and emotional conflict task fMRI data was also collected.

Results: Overall, there was a reduction in PTSD clinical scores on the CAPS-5 regardless of drug group. However, there was not a statistically significant difference in the change of CAPS-5 scores between FAAH inhibitor and placebo administered groups from baseline to week 12. Furthermore, there were no differences in stress-reactivity between drug groups. Lastly, there were no significant differences in the vmPFC-amygdala functional connectivity between treatment and placebo groups.

Conclusion: While all patients showed reduced CAPS-5 scores following prolonged exposure therapy, concomitant upregulation of AEA signaling with the FAAH inhibitor JNJ-42165279 had no further effect on clinical PTSD scores or symptomology.

CORTICOSTRIATAL NEURONS ARE DYNAMICALLY RECRUITED DURING CANNABIS VAPOR SELF-ADMINISTRATION IN RATS

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Introduction: Despite the widespread legalization and consumption of cannabis, the mechanisms underlying cannabis-seeking behavior remain enigmatic. Δ^9 tetrahydrocannabinol (THC), the principal psychoactive component of cannabis, induces its reinforcing effects through recruitment of the mesocorticolimbic reward circuit. The medial prefrontal cortex (mPFC), a key regulator of this circuit, mediates cannabinoid reinforcement in preclinical models in part via projections to the nucleus accumbens (NAc). However, the dynamic activity of mPFC→NAc projections during cannabis seeking has yet to be investigated. Thus, we used an *in vivo* wireless fiber photometry approach during cannabis vapor self-administration to characterize activity of mPFC neurons during different phases of cannabis-seeking behavior.

Methods: Adult rats received unilateral injections of a retrograde GCaMP construct (pGP-pAAVrg-syn-jGCaMP8f-WPRE) into the NAc core and a fiber optic cannula was implanted into the prelimbic area of the mPFC. Rats were trained to nosepoke for a 3-s puff of vaporized cannabis extract (150 mg/mL THC) or vehicle vapor (PEG-400) under a fixed ratio-1 reinforcement schedule in one-hour daily sessions for a minimum of 14 days. During this phase, recordings were conducted on days 1, 7, and 13. After an extended washout period, rats were switched to lever press pretraining to compare integrity of recordings to those obtained following nosepoke responding. After pre-training was completed (10 omissions or less in 2 consecutive sessions), rats began vapor self-administration in a lever-press system. During lever responding, recordings were conducted on self-administration days 1 and 10, as well as on extinction days 1 and 3, and finally, during a cue-induced reinstatement test.

Results: Preliminary data indicate that during the lever press experiments, rats trained to self-administer cannabis vapor displayed an increase in Ca^{2+} fluorescence at the onset of vapor delivery that was not observed following inactive lever presses or during extinction sessions when vapor was not available. Ongoing studies are adding more rats to explore the extent to which biological sex impacts cannabis-induced recruitment of mPFC→NAc projections and determine the pattern of corticostriatal activity during presentation of cannabis-associated cues during abstinence.

Conclusions: Our early results support the feasibility of conducting long-term fiber photometry recordings *in vivo* using a wireless system. Adopting this approach in future studies will allow us to investigate how different projections and cell types within relevant circuits/regions contribute to different phases of cannabis seeking, which can improve our understanding of how brain activity is related to CUD vulnerability in at-risk populations.

MICE EXPRESSING AN INTERNALIZATION-RESISTANT SIX POINT MUTATION FORM OF CB1R DISPLAY ALTERED RESPONSES TO CANNABINOIDS AND DIMINISHED RECOGNITION MEMORY

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Introduction: A variety of pain conditions show promising responses to the analgesic effects of cannabinoid treatment, but long-term cannabinoid use can lead to the development of tolerance to the antinociceptive effects, presenting a barrier to their chronic therapeutic use. Cannabinoid receptor 1 (CB_1R) internalization induced by agonist binding is an essential part of signal transduction regulation and the regulatory domain for internalization is the C-terminus tail of (CB_1R). The six point mutant (6PM) mice with a non-internalizing (CB_1R) express serine/threonine to alanine point mutations for six putative G protein-coupled receptor kinase (GRK) phosphorylation sites in the C-terminus tail of CB_1Rs that are required for efficient internalization, making this form of CB_1R unable to undergo internalization, trafficking, and resensitization. The goal of this study is to examine the effect of CB_1R internalization on sensitivity to the acute antinociceptive and hypothermic effects of cannabinoid agonists and measuring recognition memory and motor coordination in 6PM mice.

Methods: A cumulative dosing paradigm was used to assess the acute response to CP55,940, a strongly internalizing cannabinoid agonist. Antinociception was measured utilizing the tail-flick test and cannabinoid-induced hypothermia was examined by recording core body temperature. Motor coordination was evaluated using the rotarod test where the latency to fall was measured. Recognition memory was assessed using the novel object recognition (NOR) test where novel object recognition was determined by measuring time exploring a familiar versus a novel object placed in the test arena.

Results: The 6PM mice show decreased antinociceptive and hypothermic responses to the effects of CP55,940 compared to the wild-type littermate controls. 6PM mice also demonstrated a reduction in recognition memory by spending more time exploring the familiar object, rather than the novel object in the novel object recognition test relative to wild-type controls. There were no differences between wild-type and 6PM mice in the average time spent on the rotarod motor coordination test.

Conclusions: Data from this study demonstrate that the antinociceptive and hypothermic effects of CP55,940 were reduced in the 6PM mice suggesting that CB_1R internalization, trafficking, and recycling may play an important role in regulating the acute response to cannabinoids. These data also demonstrates that 6PM mice have a decrease in recognition memory, indicating a possible function for CB_1R internalization in learning and memory. Overall, these results suggest that internalization of CB_1R may be an important regulator of antinociceptive and hypothermic responses to cannabinoids as well as playing a role in memory formation and recall pathways.

Acknowledgements: Funded by NIH grant DA044999 and the NASA West Virginia Space Grant Consortium, Grant # 80NSSC20M0055.

THE BIDIRECTIONAL EFFECT OF 2-AG ON HYPERDOPAMINERGIC STATES: IMPLICATIONS OF THE CB2 RECEPTOR

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Background: Several serious, debilitating, and lifelong conditions, including psychosis in schizophrenia (SCZ), mania in bipolar disorder (BD), and ADHD, are often first diagnosed in young adults and relate to a known dysregulation in dopamine (DA) signalling (DA pathologies). The endocannabinoid system (ECS) is dysregulated in DA pathologies. Enzymes in the biosynthesis pathway of 2-arachidnoylglycerol (2-AG) are shown to be altered in SCZ; DAGL (2-AG synthesis) levels are decreased in patients with first episode psychosis and MAGL (primary 2-AG metabolism) expression levels are significantly lower in patients with SCZ. Elevated 2-AG is observed in individuals at high risk of psychosis. Despite this, the elevation of 2-AG is coveted in certain contexts, with clinical trials of MAGL inhibitors (MAGLi) currently underway for PTSD and Tourette's. However, evidence suggests increasing 2-AG may be detrimental in hyperDA pathologies. Previously, we've shown that increasing 2-AG in two models of hyperDA (amphetamine, AMPH; Dopamine Transporter Knockout, DATKO) led to an exacerbation of high dopamine states, mediated by Cannabinoid Receptor 1 (CB1). Interestingly, decreasing 2-AG presented opposite effects in both DATKO and AMPH models, highlighting a potential therapeutic avenue for novel, dopamine-indirect, treatments for SCZ. However, 2-AG is an agonist for both CB1 and CB2, with CB2 having a more direct link to DA modulation and SCZ; CNR2 (CB2 gene) is associated with SCZ development and mice lacking CB2 on striatal dopaminergic neurons exhibit a hyperDA tone, presenting as a hyperactive phenotype. Therefore, it is fitting for us to investigate CB2's role in responding to 2-AG levels in states of high DA.

Methods: Genetic (adult DAT-knockout (DATKO) and pharmacological (C57Bl/6J with amphetamine) models of hyperDA were treated acutely with a MAGL (MJN110, 5 mg/kg, i.p.) and tested on behavioural assays. Assessment of CB2 involvement was tested by administering a CB2 inverse agonist (AM630, 3mg/kg, i.p.) to block effects of MJN110 on high DA states. Data were analyzed with three-way ANOVA (behaviour; genotype, drug, sex), post-hoc Sidak's test.

Results: DATKO present with subcortical hyperDA; exploratory hyperactivity, impaired sensorimotor gating; increased 2-AG exacerbated hyperlocomotion. This was recapitulated in the AMPH model of high dopamine; increased 2-AG exacerbated psychostimulant responses. Acute inverse agonism of CB2, with AM630, saw a partial reversal of exacerbating 2-AG effects in both models of high dopamine. However, we observed that this effect is biphasic, dependent on the magnitude of synaptic dopamine and sex.

Conclusion: Preliminarily, it looks that while blockade of 2-AG's effects on CB1 put an absolute brake on hyperDA exacerbation, CB2 has a more nuanced role in moderating DA levels dependent on synaptic DA concentration. This avenue of therapeutic intervention could provide a more nuanced modulation of DA levels via targeting 2-AG's effects on CB2.

Acknowledgements: Funded by CIHR (PJT-173364).

OMEGA-3 POLYUNSATURATED FATTY ACIDS AS A THERAPEUTIC INTERVENTION FOR THE PSYCHIATRIC-LIKE CONSEQUENCES OF CHRONIC ADOLESCENT THC CONSUMPTION

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Introduction: Adolescent cannabis use is linked to an increased risk for psychiatric illness later in life, yet rates of adolescent cannabis use remain high. Exposure to the psychoactive component of cannabis, Δ9-tetrahydrocannabinol (THC), during this critical period of brain development can disrupt normal endocannabinoid system function, leading to long-term consequences. Indeed, we have previously shown in rats that adolescent oral consumption of THC edibles, a popular method of cannabis use in humans, produces sex-dependent effects on psychiatric-like behaviours in adulthood. These behavioural changes are associated with prefrontal cortex (PFC) hyperactivity and disrupted dopamine (DA) signalling in the ventral tegmental area (VTA)- nucleus accumbens (NAc) circuit. Currently, there are limited treatments to ameliorate the neurodevelopmental effects of THC; therefore, research is needed to identify effective intervention strategies. Diets deficient in omega-3 polyunsaturated fatty acids are also linked to neuropsychiatric conditions, and supplementation of omega-3s has been suggested as a therapeutic approach against the development and progression of psychopathology. Adequate omega-3 levels are crucial for proper DA regulation and are known to impact the endocannabinoid system and PFC inhibitory signalling. Thus, it is hypothesized that an omega-3-rich diet will interfere with the neurodevelopmental effects of THC by rescuing the disrupted PFC excitatory/inhibitory balance and DA signalling maladaptations.

Methods: Adolescent male and female rats were given edibles containing THC (increasing doses 1-5 mg/kg) mixed in Nutella® twice daily for 11 days during adolescence (Postnatal day; PND 35-45). Animals also received a dietary treatment of a control diet or a diet enhanced with omega-3s from the start of THC treatment for 21 days (PND 35-55). In adulthood (>PND75), rats underwent a battery of affective and cognitive behavioural tasks. *In vivo* electrophysiology was then carried out to determine changes in PFC glutamatergic and VTA dopaminergic activity.

Results & Conclusions: Rats in the omega 3 diet conditions consumed significantly more omega-3s than the control diet, confirming that they were successfully exposed to these essential fatty acids. Preliminary behavioural analyses revealed that edible THC during adolescence impaired temporal order novel object recognition and spatial working memory, which were prevented by omega-3 treatment in males and females. Further behavioural and electrophysiological analyses are underway to establish the impact of omega-3 dietary intervention on the effects of adolescent THC consumption on anxiety behaviour as well as PFC and VTA neuronal patterns.

Acknowledgements: This research is supported by the Natural Science and Engineering Research Council of Canada (NSERC), the Canadian Institute of Health Research (CIHR) and Canada First Research Excellence Fund (CFREF) to BrainsCAN at Western University.

ALTERED ENDOCANNABINOID SIGNALING MIGHT CONTRIBUTE TO OBESITY IN P62 KO MICE

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Introduction: Cannabinoid receptor 1 (CB1R) plays a crucial role in obesity by regulating energy metabolism, food intake, and fat accumulation. Recently, we have discovered an interaction between the endocannabinoid system and the adapter protein p62 [1]. P62 knockout (KO) mice exhibit obesity, insulin resistance, and leptin tolerance [2]. Loss of p62 leads to increased basal ERK activity, fostering increased adipogenesis, potentially explaining the obesity observed in p62 KO mice [2]. As a cargo protein involved in autophagy, p62 may facilitate the degradation of CB1 receptors via the autophagosomal-lysosomal pathway. This could lead to a reduction in CB1 receptor levels and subsequently affect cannabinoid signaling. Here, we have investigated whether hypothalamic CB1R expression or endocannabinoid levels are altered in p62 KO mice and thus could contribute to the development of high body weight.

Methods: We tracked the daily food intake of p62 KO and wild-type (WT) animals during the period when p62 KO mice typically become overweight. We monitored their home cage activity and measured their body weight. CB1R protein levels were assessed using Western blot, while hypothalamic 2-AG and AEA levels were measured using LC-MS/MS in p62 KO and control tissue samples. In WT cortical neurons, we quantified CB1R protein turnover after inhibiting autophagy.

Results:P62 KO animals become obese around 4 months of age, despite consuming a similar amount of food as WT animals. Interestingly, three weeks before obesity onset, KO mice reduced their activity, resembling CB1R activation. We therefore investigated whether increased endocannabinoid or CB1R levels contribute to this phenomenon. We found elevated 2-AG levels in the hypothalamus of p62 KO animals, with no differences in CB1R protein levels in brain tissues. However, in WT mouse neurons, we observed that inhibiting autophagy with bafilomycin significantly blocked CB1R degradation, demonstrating that CB1R protein turnover is mediated by autophagy.

Conclusions: The notable decrease in home cage activity predisposes the animals to obesity, even without increased food consumption at four months of age. Our findings suggest that alterations in the endocannabinoid system occur in p62 KO mice, potentially influencing the development of the obesity phenotype.

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ENDOCANNABINOID MODULATION OF DOPAMINE EFFECTS IS DEPENDENT ON SYNAPTIC LEVELS OF DOPAMINE: A NOVEL "AT-RISK" MODEL OF PSYCHOSIS

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Background: Psychotic disorders are a group of chronic and severe mental illnesses that are frequently linked with very poor quality of life, adverse long-term outcomes, and higher mortality rates. Schizophrenia (SCZ) is the most prevalent psychotic disorder, and its onset is believed to be driven by a combination of genetic and environmental factors. A common feature in SCZ patients is erroneous dopamine (DA) signalling. Beyond this, another pertinent emerging risk factor in the onset of SCZ are a disrupted endocannabinoid (eCB) tone, which may be triggered by external factors such as exogenous phytocannabinoids, or even stress. Enzymes responsible for the degradation (monoacylglycerol lipase, MAGL) and synthesis (diacylglycerol lipase, DAGL) of one of the principal eCBs, 2-arachidnoylglycerol (2-AG), are altered in SCZ. 2-AG levels are elevated in individuals at high risk of psychosis. Previously our group has characterized psychotic-like features in the hyperdopaminergic Dopamine Transporter Knockout (DAT-KO) transgenic mouse model and have observed that increasing 2-AG levels in DAT-KO mice via pharmacological inhibition of MAGL potentiates psychosis-related behaviours. However, this model presents with 5-fold higher synaptic dopamine levels as compared to WT, which can represent an extreme model of hyperdopaminergia that is not directly translatable to human presentations of hyperdopamine. Therefore, we propose to use a more clinically relevant model of increased synaptic dopamine (DAT-heterozygous, DAT-HET) to represent an "at-risk" phenotype, allowing us to evaluate a potential dose-dependent relationship between increased dopamine levels and sensitivity to 2-AG modulation.

Methods: Male and female adult DAT-HET mice were treated acutely with the selective MAGL inhibitor (MAGLi) (MJN110, 5 mg/kg, i.p.) alone, or co-administered with amphetamine (AMPH) (2 mg/kg, i.p.) and tested on behavioural assays. DAT-HET will further be used to evaluate environmental triggers of psychosis onset through exposure to psychological stress and exogenous cannabinoids (e.g., THC). Data were analyzed with three-way ANOVA (behaviour; genotype, drug, sex), post-hoc Sidak's test.

Results: DAT-HET mice, which have a two-fold increase in extracellular dopamine but lack phenotypic differences to their WT controls, did not show the same sensitivity to MAGL inhibition alone that was previously observed in our DAT-KO model. However, once synaptic dopamine levels were increased, DAT-HET mice showed sensitivity to increased 2-AG levels via MAGL inhibition, with an exacerbated amphetamine psychomotor response. Thus DAT-HET mice treated with a MAGLi showed trends toward exacerbation of locomotion and disrupted sensorimotor gating compared to vehicle treatment. DAT-heterozygous mice treated with both a MAGLi and AMPH likewise elicited higher locomotion compared to just those receiving AMPH alone.

Conclusion: While DAT-heterozygous display only mild trends toward psychotic-like behaviours, these data suggest that DAT-HET mice may exhibit "at-risk" psychotic features. Future studies will investigate the effect of additional 'hits' to the DAT-HET, for example moderate stress and exogenous phytocannabinoid use.

Acknowledgements: Funded by CIHR (PJT-173364).

EFFECTS OF PRESCRIBED MEDICAL CANNABIS ON DRIVING PERFORMANCE & COGNITIVE FUNCTION

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Introduction: Research has not yet established whether and to what extent prescribed medical cannabis impacts driving and/or cognitive function when used as directed under medical supervision. With cannabis laws rapidly shifting, this is crucial information for evidence-based health and road safety policy. This presentation will report on two semi-naturalistic trials (one completed and one ongoing) investigating driving performance and cognitive function in patients with a range of refractory health conditions.

Methods: In Study 1, 40 patients attended a single laboratory session and completed a range of driving and cognitive assessments before and after self-administering a standard dose of their prescribed cannabinoid medication. In Study 2, patients (n=8 completers so far) with chronic pain commencing treatment with medical cannabis attend 4 in-person laboratory sessions, including a baseline visit prior to treatment initiation (V1) and 3 subsequent visits at 1-, 2- and 3 months post-treatment initiation (V2-4). During V2-4, patients complete a range of driving and cognitive assessments before and after self-administering a standard dose of their prescribed cannabinoid medication. All data were analysed using linear mixed models with Bonferroni post-hoc tests, with $p < .05$ considered statistically significant.

Results: Study 1, which included 40 adults (55% male) aged between 23-80 years who had been prescribed medical cannabis for an average of 10.2 months, found no evidence of driving or cognitive impairment when comparing performance on a range of tasks before and after self-administration of THC-containing cannabinoid medication (mean THC dose for orally ingested oil = 9.61 mg; mean THC dose for inhaled flower = 37 mg). Lateral vehicular control was significantly worse ($p = .015$) and perceived driving effort significantly higher ($p = 0.43$) among patients using oils relative to flower, but this difference was stable over time and unaffected by THC administration. Participants showed some improvement on select tasks following medical cannabis self-administration, including a Multitasking Test and Rapid Visual Information Processing Test (both $p < 0.001$). Preliminary data from Study 2 (n=8) suggests no change in cognitive performance at 1-month post-treatment relative to pre-treatment, and a modest improvement on the Rapid Visual Information Processing Test ($p = 0.006$). Study 2 is currently ongoing and further data will be presented at the conference.

Conclusion: Data collected to date from 2 novel semi-naturalistic laboratory studies suggests that prescribed medical cannabis may have minimal effects on driving performance and cognitive function when used as directed, and potentially positive though subtle effects on select cognitive performance measures. It is important to note that these results are specific to patients with refractory conditions who have been prescribed a specific cannabinoid product by a physician within a regulated medical system.

ADULT RAT OFFSPRING BRAINS EXPOSED GESTATIONAL TO THC EXHIBIT DISTINCTIVE NEUROLOGICAL AND BIOMOLECULAR CHANGES IDENTIFIED BY FOURIER-TRANSFORM INFRARED SPECTROSCOPY

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Clinical findings suggest that children exposed to Cannabis in utero are at a higher risk of developing intellectual, psychiatric, and neurodevelopmental phenotypes in early life. Fetal developmental processes are extremely sensitive to Δ9-tetrahydrocannabinol (THC), where preclinical investigations show that prenatal exposure produces sex-specific changes in cortico-limbic circuits and behaviours in rodents. The present study aims to investigate potential neurological and biomolecular alterations in male and female adult rat brains of offspring exposed daily to THC throughout gestation using FTIR. FTIR characterizes regions rich in proteins, protein aggregates, lipids, and other essential biomarkers associated with oxidative stress, such as demyelination, lipid oxidation, and alterations in lipid and protein ratios.

Methods: Pregnant Sprague-Dawley rats were treated with *i.p.* injections of 3 mg/kg THC or vehicle (VEH) (1:1:18 Ethanol: Kolliphor: Saline) daily between gestational days (GD) 6 and 20. These animals were reared for behavioural analysis to assess social behaviours, learning and memory, prepulse inhibition and anxiety-like phenotypes. Following completion of behavioural analysis, 6 THC offspring (3 male and female), and 4 (2 male and female) VEH were euthanized, and brains were flash frozen and stored at -80°C. The left hemisphere of the brains was sectioned in the coronal plane in 20 µm slices airdried on CaF2 windows at -3.2 bregma using a cryo-microtome at -18°C. These sections are then stored at -80°C until immediately prior to analysis. Fourier-transform infrared spectromicroscopy (FTIR) was used to examine changes in the distribution of endogenous biomolecules in THC-exposed offspring. For data collection, an Agilent Cary Microscope was fitted with a 128x128 pixel Focal Plane Array detector and a 15x Objective with a scan parameter of 16 scans per pixel and 2x2 binning to yield excellent spatial resolution and adequate signal-to-noise ratio for the obtained spectra. The hyperspectral images were pre-processed and analyzed using Quasar Software. Two sections per animal were scanned and mean peak areas were examined. The data sets collected through this study will undergo statistical analysis according to k-means clustering and principle component analysis.

Results: Preliminary analysis indicates lipid oxidation and changes in lipid/protein ratios associated with THC exposure, indicating an increase in lipid content within the hippocampus and the surrounding grey and white matter. These data also show increased hippocampal lipid peroxidation, and a decrease in the ratio of Amide 1 to Amide 2 peak areas in THC-treated offspring in all regions of interest identified by K-means analysis, which suggest a change in secondary protein structure.

Conclusion: This type of investigation will identify emerging regions of interest and novel targets of exploration while offering validation of existing findings and aid in determining the biochemical changes associated with gestational THC exposure. In-depth analysis of peak area and shift, as well as secondary derivative peaks, examining metabolites such as glutamate, lactate and pyruvate, are ongoing. Complementary follow-up analysis using X-ray fluorescence Imaging (XFI) will highlight the presence of metal dysregulation, focusing on endogenous metals, including Cu, Fe and Zn and ions Cl- and K+, which are essential to brain development and function.

Acknowledgements: This work is supported by the Saskatchewan Research Chair in Drug Discovery and Development and Brain Canada Future Leaders Award RBL, and COMBRIDGE funding from the University of Saskatchewan College of Medicine to JGH. TB is supported by a CGS-D CIHR scholarship. This project was made possible by the Canadian Light Source.

THE *IN VIVO* INTOXICATION EQUIVALENCY OF Δ^8-TETRAHYDROCANNABINOL RELATIVE TO Δ^9-TETRAHYDROCANNABINOL IN MICE

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Introduction: There is an increase in commercially-available Δ^8-tetrahydrocannabinol (Δ^8-THC) products, particularly in the US; however, little is still known about its intoxicating effects relative to Δ^9-tetrahydrocannabinol (Δ^9-THC). Our lab has previously compared the relative cannabimimetic activities of Δ^9-tetrahyrdocannabipherol (Δ^9-THCP) and Δ^9-THC *in vitro* and in mice. This project aims to similarly compare Δ^8-THC to Δ^9-THC. Based on past *in vitro* data, we hypothesized that Δ^8-THC would produce an intoxicating effect that was lower than that of Δ^9-THC. The intoxication equivalency of Δ^8-THC relative to Δ^9-THC was assessed through pharmacokinetic time course experiments and pharmacodynamic dose-response experiments in mice.

Methods: Male and female C57Bl/6 mice were dosed with 10 mg/kg of Δ^8-THC or Δ^9-THC either orally or intravenously and blood samples were taken at 10 min, 1 hour, 3 hours, 6 hours, and 8 hours and cannabinoids and their metabolites were quantified using high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS). In addition, the tetrad assay was used to assess catalepsy, body temperature, nociception, and anxiolytic behaviour in response to oral or intravenous dosing at 1 – 10 mg/kg for each cannabinoid. Δ^8-THC's potency and efficacy were calculated at C_{max} and compared to Δ^9-THC's potency and efficacy at C_{max}.

Results: Experiments for this work are ongoing. Collection of blood samples for the time-course are pending analysis via HPLC-MS/MS to determine C_{max} and t_{max}. With these data we will then be able to compare Δ^8-THC and Δ^9-THC based on their potency, efficacy, and pharmacokinetic profiles.

Conclusions: In related studies, our laboratory has evaluated Δ^9-THCP, a phytocannabinoid that contains a seven-carbon alkyl side chain instead of the five-carbon alkyl side chain of Δ^9-THC. There we observed Δ^9-THCP was found at higher concentrations in the blood than Δ^9-THC when administered at the same dose, yet Δ^9-THCP-dosed animals exhibited less cannabimimetic activity than Δ^9-THC. Our work with Δ^8-THC will provide additional insight into the pharmacokinetic and pharmacodynamic differences between Δ^9-THC and other cannabinoids with similar chemical structures.

Acknowledgements: This work was funded by a Health Canada Research Contract from the Controlled Substances and Cannabis Branch.

REVIEW OF INTERVENTIONAL STUDIES WITH CANNABIS IN HUMANS FOR NON-THERAPEUTIC PURPOSES BY HEALTH CANADA

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Introduction: Since December 2022, Canadian researchers wanting to conduct interventional studies in humans with cannabis for non-therapeutic purposes can apply for a cannabis research licence under the new non-therapeutic research on cannabis (NTRC) framework. Health Canada implemented this risk-based framework to remove barriers faced by researchers wanting to conduct such studies under the conventional clinical trials framework.

Methods: The NTRC framework removed two main barriers under the clinical trials framework: the requirements to provide product-specific pre-clinical and clinical data in an investigator's brochure (IB) and to use cannabis produced under good manufacturing practices (GMP). Instead, supporting safety evidence is drawn from existing peer-reviewed literature and cannabis must meet the Canadian good production practices (GPP) quality standards that apply to cannabis products sold under the *Cannabis Act*. Under the NTRC framework, studies are categorized under three risk categories: category 1 (lower risk) for consumer preference studies, category 2 (medium risk) and 3 (higher risk) for safety and pharmacological studies. Application requirements vary based on the risk category: the higher the risk category, the more documents need to be submitted to Health Canada for review.

Results: Since implementation, Health Canada has received 32 applications for category 1, two applications for category 2 and five applications for category 3. Research topics for category 2 and 3 studies varied. Of the seven category 2 and 3 studies, five used inhalation as a mode of consumption, one used oral administration and one used both inhalation and oral administration. Almost the same number of category 2 and 3 studies assessed the effects of THC (3/7) and the effects of both THC and CBD (4/7). All studies recruited healthy participants between the ages of 19 and 65, but with different levels of experience consuming cannabis (from occasional to regular consumption), and assessed the acute effects of cannabis. For category 2 and 3 studies, two applicants were from the industry, while five were from academic institutions.

Conclusions: Although the NTRC framework has been successfully implemented, it is still a new framework. Health Canada expects an increase in the number of applications as researchers familiarize themselves with the framework and the application requirements and process. Health Canada will continue to promote this framework and engage with stakeholders to help facilitate applications to the NTRC framework.

Acknowledgements: Funded by the Government of Canada.

SAME-DAY SEDATIVE AND ANXIETY EFFECTS AND NIGHT-TIME SLEEP EFFECTS FOLLOWING LOW-DOSE COMBINED CANNABINOID FORMULATIONS

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Introduction: Low-dose, combined cannabinoid treatments of cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) are known to have anxiolytic and sedative properties, yet there is little consensus on the dosage and ratios that induce these effects in combined formulations. Considering the increased daytime use of prescribed combined cannabinoid treatments, this study examined the effect of differing doses of orally consumed treatments of combined CBD and THC oil on same-day sedation and anxiety post-dosing, in addition to exploring secondary night-time sleep effects that may follow an acute day-time dose.

Methods: A total of 31 healthy participants are expected to be recruited into a 5-week, double blind, randomized, placebo-controlled crossover trial (ACTRN12622001539729). Participants will attend five in-person experimental visits in total (V1-V5). Each participant will receive 1mL of orally administered oil consisting of an active treatment (2mg CBD/2 mg THC, 5mg CBD/5mg THC, 32mg CBD/2mg THC, 80mg CBD/5mg THC) or a placebo. The State-Trait Anxiety Inventory (STAI-S), Visual Analogue Scales (sedation) (VAS) and Karolinska Sleep Scale (KSS) will be used to measure state anxiety and sedation at approximately 40 minutes, 135 minutes and 4.5 hours post-doing during each visit. Sleep measures of total sleep time (TST), sleep onset latency (SOL) and number of awakenings after sleep will be collected over a 7-day wash-out period between doses using wrist-mounted actigraphy watches and sleep diaries. Separate linear mixed effect models will be applied to each outcome to explore and compare the effects of treatment on each measured outcome.

Results: Currently, four participants have successfully completed the study with two more participants pending completion. Therefore, the data presented will include STAI-S, VAS and KSS outcomes and sleep outcomes (TST, SOL, number of awakenings) of completed participants up until June 2024. The effects of time and condition, and the interaction of time and condition, will be presented and where a main effect has been observed, the outcomes of Bonferroni post hoc analyses will be included ($p<0.05$ considered significant for all).

Conclusions: It is hypothesized that higher doses of CBD:THC (32mg CBD/2mg THC and 80mg CBD/5mg THC) would produce increased feelings of same-day sedation and anxiolytic effects over time compared to lower doses (2mg CBD/2 mg THC, 5mg CBD/5mg THC) and placebo, in addition to having a protective effect on anxiety scores proportional to CBD dose. Resultantly, greater TST and SOL with fewer number of awakenings after sleep onset are hypothesized to be observed for higher doses of CBD:THC over the 7-day wash out period.

Acknowledgments: AH is supported by a Rebecca L Cooper Al and Val Rosenstrauss Fellowship (GNT: F2021894).

COMPARING KNOWLEDGE OF MEDICAL CANNABIS EFFICACY, RISKS, AND HARM REDUCTION AMONG PHYSICIANS, PATIENTS, CANNABIS ADVOCATES, AND UNDERGRADUATES

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Introduction: Cannabis has been used medicinally by humans for over 5,000 years and was popular for the treatment of a wide variety of ailments in the 19th and early 20th Centuries. In recent decades, many States have legalized medical and even recreational ("adult") cannabis use. The Department of Health and Human Services has formally recommended that the Drug Enforcement Administration move cannabis from Schedule I to Schedule III and a DEA review is in progress. Despite the large number of those in the US using cannabis for health and medical purposes, there is a lack of integration between the medical use of cannabis and mainstream health care. Many people use cannabis medicinally without the knowledge of or input from their primary health care provider and cite a lack of health care provider expertise as a reason for non-disclosure. This perception is not unfounded, as physicians receive little training on medical cannabis, giving evidence-based recommendations, or even the endocannabinoid system. This is the first known project to systematically compare medical cannabis knowledge among physicians, licensed medical cannabis patients, cannabis advocates, and undergraduates at a highly selective university in a state where medical cannabis has been legal for over a decade.

Methods: We combined data from assessments of medical cannabis-related knowledge collected among physicians (n=244), licensed patients at a medical cannabis dispensary with an education program and on-site physician (n=152), cannabis enthusiasts participating in a cannabis advocacy event (n=472), and undergraduates at a flagship public university (n=822).

Results: Physicians had the highest knowledge of cannabis' efficacy for treating medical conditions, followed in turn by undergraduates, medical cannabis patients, and cannabis advocates. Physicians and undergraduates had the highest knowledge of cannabis risks, medical cannabis patients had the lowest knowledge of cannabis risks. Undergraduates had the highest knowledge of effective cannabis harm reduction techniques, followed by in turn by physicians, and cannabis advocates (no data were available for patients). Cannabis advocates had the highest knowledge of cannabinoids, followed by undergraduates, followed by physicians (no data were available for patients).

Conclusions: Patterns of knowledge differed by content area; no group was consistently most knowledgeable. Our results highlight the mismatch between physician knowledge and medical cannabis policy. More concerted cannabis education efforts are needed at every level, especially for those in healthcare professions.

EYE MONITORING TECHNOLOGY TO DETECT CANNABIS-RELATED CHANGES IN DRIVING PERFORMANCE

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Introduction: Legal access to medical cannabis continues to increase, yet strategies to address the impact of cannabis-impaired driving remain underdeveloped. The introduction of novel vehicle safety systems and technologies, including eye-tracking devices, may support the proactive detection and management of impaired drivers before a collision can occur. Currently, their effectiveness for detecting cannabis-specific impairment is unclear. The aim of this work is to determine whether, and to what extent, medicinal cannabis products alter driving behaviour and if this can be effectively indexed and monitored through changes in eye movement and gaze behaviour.

Methods: A total of 31 healthy participants will complete this 5-week (V1-V5), double blind, randomized, placebo-controlled crossover trial (ACTRN:12622001539729). Under supervised administration, participants consume 1mL of a treatment dose at each session [i.e. active treatment(s): 2 mg delta-9-tetrahydrocannabinol (THC) / 2 mg Cannabidiol (CBD); 5 mg THC / 5 mg CBD; 2 mg THC / 32 mg CBD; 5 mg THC / 80 mg CBD; or placebo treatment]. Driving performance is assessed at approximately 2 hours and 4-hours post dosing using the Forum 8 driving simulator, comprising three scenarios that assess headway (distance to car in front), vehicle control (SDLP), speed maintenance, and response inhibition in standard highway driving environments. Eye movement behaviour is co-monitored during driving using a vehicle-mounted PC-Driver Monitoring System (PC-DMS). Gaze behaviour, expressed as gaze transition and stationary gaze entropy and blink characteristics, will be examined.

Results: To date, four participants have completed all study procedures, with two more pending completion. Therefore, we will present data for all driving and eye tracking outcomes for completed participants up until June 2024. Demographic data will be presented with summary statistics as appropriate. Separate Linear Mixed Models will be developed to assess differences in driving performance and ocular variables between treatments [THC:CBD combinations vs placebo) and across time (drive duration) examined in 10-minute bins]. Post hoc comparisons will be undertaken where significant condition or interaction effects are observed to determine the significance of differences as a function of time and treatment. All statistical analyses will be conducted with the use of SPSS 26.0 (SPSS Inc., USA), and tests will be two-tailed with a conventional level of significance of $p < 0.05$.

Conclusions: It is expected that when consumed at higher combined doses, medicinal cannabis products that contain THC will produce safety-relevant changes to driving performance, which can be effectively identified and indexed using novel eye tracking technologies.

Acknowledgments: AH is supported by a Rebecca L Cooper Al and Val Rosenstrauss Fellowship (GNT: F2021894). This project is directly supported by a grant awarded to AH by the Office of Road Safety, Department of Infrastructure of the Australian Federal Government.

PHYTOCANNABINOIDS IN COMBINATION: CHRONIC PAIN

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Introduction: Chronic pain, one of the most common reasons adults seek medical care, has been linked to restrictions in mobility and daily activities, dependence on opioids, anxiety, depression, sleep deprivation, and reduced quality of life. Unfortunately, the current treatment options for chronic pain are limited, often ineffective, and have associated side effects. There is a growing interest in the use of opioid-sparing analgesics, in particular medicinal cannabis, for pain management. It has also been suggested that compounds in the *cannabis* plant function more efficiently in concert with each other rather than alone - a concept known as the "entourage effect." This study aims to test the safety and efficacy of chronic administration of Beta-Caryophyllene (BCP) and cannabidiol (CBD) in chronic inflammatory pain.

Methods: In mice, we used a chronic inflammatory pain model (Complete Freund's Adjuvant, CFA) and two pain-like behavioral tests. We determined the analgesic effects of daily injections of the combination of CBD and BCP, and monitored for adverse effects.

Results: We found that daily injection of the combination of CBD and BCP maintained its analgesic effect without adverse effects on body weight, body temperature, locomotion, and liver toxicity.

Conclusions: The present data show that the combination of BCP and CBD maintains its analgesic effect with safety profiles in a chronic pain state. This suggests that this combination can serve as alternative analgesic therapy for chronic pain and support the entourage effect of cannabinoids.

CANNABINOID OIL FOR INTRACTABLE FACIAL PAIN PATIENT WITH PALATAL FISTULA

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Introduction: Oronasal fistula is rare condition after operation, we met the severe pain after plastic surgery.

Case: 40 years female came to pain center with severe maxillary and oral pain. She had received maxillary reductive plastic surgery 3 yrs prior and suffered from intractable pain following operation. From the facial bone 3D CT with toluidine blue staining, she was diagnosed with a oronasal fistula (Fig 1, 2). At the beginning, she complained of intraoral numbness and runny nose into oral cavity. She had difficulty sucking up fluids through a straw. Her suffering worsened, with persistent, severe facial pain at food intake, chewing, and brushing of teeth. Pain characteristics was diverse – from dull aching to burning, hot sensation, sometimes with breakthrough shock-like sensations. Her NRS score remained 7 to 10. She visited several hospitals, including a plastic surgeon, dentist, ENT specialist, pain specialist and psychiatrist. 2-3 times per week, she have to visit emergency room due to intractable pain. We prescribed various pain-killers, including acetaminophen, ketorolac, naproxen, carbamazepine, gabapentin, amitriptyline, pregabalin, – but she had only minimal response and often could not keep medication due to an array of side effects including severe drowsiness for carbamazepine and gabapentin, dyspepsia for acetaminophen, nausea for pregabalin, and palpitations with naproxen. Because of her severe pain, we finally prescribed several opioids – including Nefopam® and Nucynta® – to no significant effect. Jusnista® and Targin® made her severely drowsy with nausea/vomiting. Norspan® patch resulted in motion sickness, and Fentanyl patch brought on respiratory depression. For breakthrough pain, we prescribed Instanyl®, a nasal fentanyl spray, which also brought about respiratory depression. Fentora® (intra-buccal fentanyl) caused tongue paralysis. Further, intravenous ketamine, lidocaine, magnesium continuous infusion therapy were not effective and had unpleasant side effects. Finally, we recommend cannabinoid oil, and she tried this 2 drops under the tongue daily. At the beginning, she felt somewhat fishy smell, but there were no significant side effects. And she managed her pain with a ketorolac in the morning, and 2 drops cannabinoid oil. After 7 months, her daily NRS has decrease to 4, and she has not visited ER during this period.

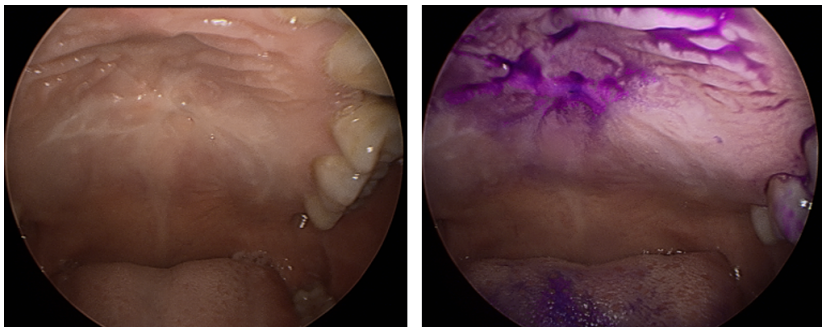

Fig 1. Toluidine blue staining for palatal fistula confirmation.

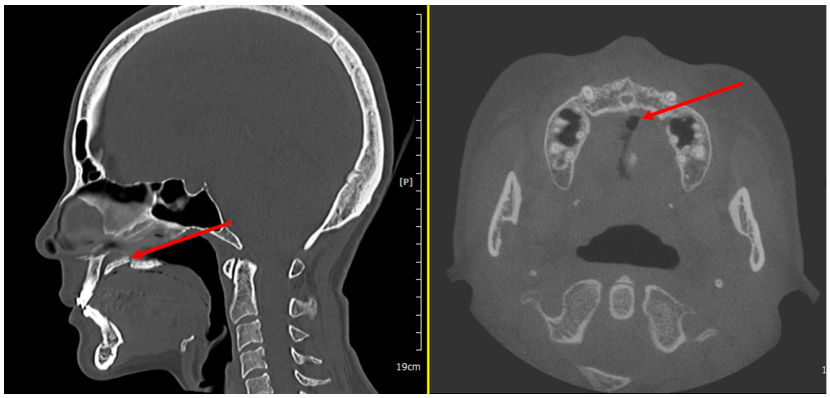

Fig. 2. Facial 3D CT. Arrow shows the fistula.